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Award Number: DAMD17-01-2-0038

TITLE: Novel Resuscitation from Lethal Hemorrhage - Suspended Animation for Delayed Resuscitation

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REPORT DATE: September 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2002	Final (15 Aug 01 - 14 Aug 02)

4. TITLE AND SUBTITLE
Novel Resuscitation from Lethal Hemorrhage - Suspended Animation for Delayed Resuscitation

5. FUNDING NUMBERS
DAMD17-01-2-0038

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

We have been working since the 1980s, for the past 4 years under DOD support, on novel ways to resuscitate "unresuscitable" trauma victims. We focus on combat casualties who exsanguinate internally within a few minutes to cardiac arrest. We have conceived and documented "suspended animation for delayed resuscitation" with the use of hypothermic saline flush into the aorta wihtin the first 5 minutes of no blood flow, using novel clinically relevant outcome models in dogs. With the use of saline flush we have achieved resuscitation with cardiopulmonary bypass to complete recovery after circulatory arrests of up to 90 min at 10°C. This is the report on year 4. In year 4, study I, with the use of novel solutions (Normosol, Unisol, Tempol), we achieved such success after up to 120 min no-flow. That had been so far with models not including severe tissue trauma. In study II of year 4 we added tissue trauma. The coagulopathy as a result of hemodilution, cold, ischemia, reoxygenation, and trauma, proved to be worse when tissue trauma was included. Nevertheless, we achieved complete recovery with trauma after up to 60 min no blood flow. In study III of year 4, we explored the potential use of suspended animation for unresuscitable normovolemic (civilian) sudden cardiac death. We documented a highly significant benefit derived from mild cooling (34°C) initiated already during cardipulmonary resuscitation basic and advanced life support, when that has to be extended for up to 1 hour in order to bridge the unresuscitable organism to prolonged cardiopulmonary bypass for more sophisticated treatment in the hospital. We also advised industries for novel devices which we will need to bring suspended animation to patients. We are planning clinical trials.

14. SUBJECT TERMS	15. NUMBER OF PAGES
resuscitation, exsanguinations, hypothermia, cardiopulmonary bypass, clinical death, cerebral ischemia, coagulopathy, traumatology	148
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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BAA 99-1 PROPOSAL

A. ABSTRACT (short version)

NOVEL RESUSCITATION FROM LETHAL HEMORRHAGE
Suspended Animation (SA) for Delayed Resuscitation

Keywords: Resuscitation. Hypothermia. Exsanguination. Combat Casualties. Cardiac Arrest. Cerebral Ischemia. Cardiopulmonary Bypass. First Aid.

This study of *suspended animation (SA)*, tentatively planned for 7 years (1998-2004), concerns military and civilian trauma-induced exsanguination cardiac arrest (CA) (near 100% "hopeless") and normovolemic sudden cardiac deaths resistant to standard CPR attempts (in 50% given up). *This proposal is only for year 4 (2001).* We will further develop and document, in modified dog outcome models, novel *field-to-hospital methods* for preservation of organ viability during CA of 1-2 h, for transport and repair, resuscitation, intensive care of 72 h or longer, and survival without brain damage. We are guiding (gratis) development of devices and planning of clinical trials to start in 2002. Our SA dog studies in years 1-3 (1998-2000) proved that aortic cold saline flush at the start of CA can achieve complete recovery after 90-120 min of CA at 10°C! However, there was no breakthrough benefit from 14 new or classical drugs. In 2001 we propose to conduct three studies: Study I, to explore novel cold flush solutions more preservative in smaller volume than saline, for portability by military paramedics in the field (albumin, albumin-tempol, Unisol). Study II, to document a protocol for clinical feasibility trials in emergency rooms; including *liver trauma,* packing, and *coagulation* studies. We will use open-chest aortic cold flush and post-CA cardiopulmonary bypass (CPB) compared to local warming and resuscitation with manual heart pumping. Study III, to study potential use of SA for CPR-resistant normovolemic VF-CA. We will compare bridging over 60 min CA (needed for transport and cannulation for prolonged CPB in the hospital) -- continued external CPR at normothermia, vs external CPR with mild hypothermia, vs CA (SA at 10°C). We will superimpose mechanism studies. *For years 5-7* (2002-2004) we have been invited to propose an expanded research *program* on "SA and other Novel Emergency Resuscitation Developments" (SANER), for presently unresuscitable military and civilian emergencies. Tentative plans, to be finalized jointly with the DOD, are for 4 *projects*: 1) Basic science research on the limits of resuscitability. 2) Pre-clinical outcome studies in dogs and pigs. 3) Novel devices developments with industries. 4) Multicenter clinical trials (in selected trauma hospitals) of: a) mild hypothermia for severe traumatic hemorrhagic shock; and b) profound hypothermic SA for exsanguination CA.

NOTHING ON THIS PAGE IS PROPRIETARY INFORMATION

ANNUAL RESEARCH REPORT FOR USAMRMC/TATRC
July 2001-September 2002

NOVEL RESUSCITATION FROM LETHAL HEMORRHAGE
Suspended Animation (SA) for Delayed Resuscitation
Project Year 4

INTRODUCTION

This research report for 2001/02 concerns our US Army funded research project on novel resuscitation from severe hemorrhage, "suspended animation (SA) for delayed resuscitation" (P.I.: Dr. Safar. Co-P.I.: Dr. Tisherman), project year 4 (academic year 2001/02, FY-01). This report will *not* include a smaller parallel project on hemorrhagic shock (HS) in rats and pigs, funded by the Office for Naval Research (P.I.: Dr. Tisherman. Co-P.I.: Dr. Safar). This report (SA) will, however, mention parallel developments, under our guidance (funded separately) of methods and devices aiming towards clinical trials planned for SA years 6-x. This SA project was carried out primarily in dogs, in *three studies* as originally proposed for year 4: 1) Novel solutions. 2) Adding trauma to exsanguination cardiac arrest. 3) SA potentials for normovolemic sudden cardiac death with ventricular fibrillation (VF). The 3 studies included *93 dog experiments*, almost all of which lasted about one week: insult experiments on Mondays and Tuesdays and life support through 72 or 96 h, euthanasia, necropsy of the whole organism, and brain histopathologic damage scoring. Two intact survivors to 72 h had normal cognitive function confirmed at 2 mo.

In this year 4, we continued using a systematic approach, aiming for a breakthrough in resuscitation attempts for the presently considered unresuscitable condition of traumatic exsanguination cardiac arrest of 2 hours no-flow. In year 1 (1998-99) we established the non-traumatic exsanguination CA model (1,2). In year 2 (1999-00), we explored pharmacologic flush strategies with portable flush volumes, achieving no breakthrough effect with any of 14 drugs (3). Some benefit came from antioxidant Tempol (4). In year 3 (2000-01) we pushed profound hypothermic preservation with aortic large-volume saline flush to tympanic temperature (Tty) 5-10°C; we achieved intact survival after CA no-flow periods of 60 min and 90 min at 10°C, and inconsistently after CA 120 min (5-7).

In year 4, study I, we first documented the *flush delay* limit of 5 min normothermic CA no-flow (8). This means that the exsanguinating soldier becoming pulseless will have to receive initiation of the aortic cold flush within 5 min. We then achieved intact survival after not only CA 90 min but also 120 min when we added novel flush solutions to the aortic cold flush to Tty 10°C (see below). In an adjunctive project, carried out at the end of the experiments, veno-venous cooling during spontaneous circulation lowered Tty much more rapidly (in 6 min from Tty 38°C to 34°C) than external cooling (9).

In study II, we explored for the first time *traumatic* exsanguination CA of 60 min no-flow and examined the associated coagulopathy which proved worse than with non-traumatic CA. With trauma, outcome was worse but brain HDSs were normal.

In study III, we explored whether SA might have benefit for normovolemic VF CA that is (temporarily) unresuscitable with external CPR. When hearts are unresuscitable, transforming protracted CPR BLS-ALS steps ABC into deliberate profound hypothermic CA for SA proved technically problematic; there were problems with volume shifts in the absence of exsanguination and with flushing of large volumes of iced solutions. Moreover, such a transformation of CPR steps ABC to CA at 10°C would probably be clinically unacceptable. We then studied VF-CA and continuance of steps ABC for up to 1 hour. Mild hypothermia through veno-venous extracorporeal shunt cooling during steps ABC was highly effective.

During year 4 we summarized in publications our SA results so far (10,11). In February 2002, the *New England Journal of Medicine* published two clinical breakthrough papers which confirmed our earlier discovery and development (in dog outcome models) of *mild* hypothermia *after* prolonged normothermic CA. These were positive randomized clinical trials by our alumni in Europe, and one in Australia. Safar and Kochanek wrote the invited editorial (12). Also, mild hypothermia doubled the golden hour of HS tolerance in our rat outcome models (13). Although mild hypothermia is not SA, these developments may lead in the autumn of 2002 to inclusion in national and international guidelines of mild hypothermia after CA. All this is giving other novelties in therapeutic hypothermia, such as SA, a boost.

BODY OF REPORT (year 4)

Study I: *Novel small volume solutions for hypothermic aortic flush* (14,15). (Figures 1 and 2, Table 1).

This study was without trauma, simulating internal exsanguination from a lacerated large vessel. Although we can reduce Tty by 3°C/min, the aortic flush of saline at 2°C, required for rapid induction of SA to achieve Tty 10°C, had to be with enormous volumes. Also, CA 120 min (our goal) we had not yet been able to consistently reverse to survival without brain damage. We therefore tried to overcome the underlying problems with use of flush solutions that are theoretically more effective than saline. Normosol (pH normalized Ringer's solution) is more physiologic than NaCl we used previously. Unisol-I (intracellular, for stasis) and Unisol-E (extracellular, for reperfusion) are therapeutic (Dr. Taylor). So is the antioxidant Tempol, which we added (8). We found the Synzyme preparation polynitroxylated albumin-Tempol to be effective in rats with hemorrhagic shock.

This dog study (14) was to investigate three different solutions compared to saline for aortic cold flush. Methods: Dogs (20-25 kg) were exsanguinated over 5 min to CA of 30 min no-flow (2). At CA 2 min, the dogs received an aortic flush of 25 mL/kg at 2°C over 1 min, using saline (n=5), albumin 5% or 25% (n=6), Unisol-UHK (organ preservation solution) (n=5), or polynitroxylated albumin plus tempol (an antioxidant) (PNA-T) (n=5). The flush was through a balloon-tipped catheter inserted via the femoral artery into the abdominal aorta. Resuscitation was by closed-chest cardiopulmonary bypass, followed by assisted circulation for 2 h, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to 72 h, when outcome was evaluated as overall performance category (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=death); neurologic deficit scores (NDS 0-10%=normal, 100%=brain death); and total brain histologic damage scores (HDS 0=normal, >40=moderate damage, >100=extensive damage). Results: Lowest tympanic temperature during CA was 32°C in all dogs. Unisol resulted in pharmacologic defibrillation during CA, PNA-T in lowest NDS and HDS. Outcome at 72 h as OPC, NDS, and HDS, see "Results" (below). Conclusions: Three aortic flush solutions, physiologically more rational than saline, may not give a breakthrough

effect as moderate hypothermia, but Unisol might be beneficial for the heart and PNA-Tempol for the brain.

Results:

	Saline	Albumin	Unisol	PNA-T	p-value
OPC	3,3,3,3,3	3,3,3,3,4,4	3,3,3,4,5	3,3,3,3,3	0.4
NDS%	47 (38-58)	49 (38-90)	54 (48-97)	35 (31-44)	0.01*
HDS	88 (37-128)	132 (82-290)	132 (89-200)	68 (38-96)	0.02*

Data are median (range). *post hoc: $p<0.05$ Unisol vs PNA-T

In another study with the same novel solutions (15) we achieved intact survival after CA 120 min no-flow in a reproducable manner. Methods: Male dogs (20-26 kg) were exsanguinated over 5 min to CA of 120 min no-flow. At CA 2 min, the dogs received arterial cold flush, using a catheter inserted into the femoral artery, until Tty reached 10°C; then flush was continued from the femoral artery until rectal temperature reached 20°C. Resuscitation was by closed-chest cardiopulmonary bypass (CPB), followed by assisted circulation for 2 h, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to 72 h, when outcome was evaluated as overall performance category (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=death) neurologic deficit score (NDS 0-10%=normal, 100%=brain death); and total brain histologic damage scores (HDS 0=normal, >40=severe damage, >100=extensive damage). The controls' flush was with saline at 2°C, 1 L/min; optimized flush was with Normosol at 2°C, 2 L/min from the femoral artery plus Unisol-I (organ-preservation solution) plus tempol (antioxidant) at the end of the flush; CPB was primed with Unisol-E (without K) instead of dextran/Ringers. Results: In the historic (plus concurrent) saline control group (n=6), OPC 1 was achieved in 2 dogs, OPC 2 in 1 dog, OPC 3 in 1 dog, and OPC 4 in 2 dogs. In the optimized flush group (n=6), OPC 1 was achieved in 5/6 dogs, and OPC 2 in 1 dog (p=0.06). Median (range) NDS was 26% (0-91) vs 1% (0-9) (p=0.09). HDS was 21 (10-172) vs 38 (12-98) (p=0.7). The flush volume for Tty 10°C was the same as with saline; i.e., ≤ 700 ml/Kg. Conclusion: an optimized single large volume cold aortic flush at start of CA can achieve intact survival with only minor histologic brain damage, after a no-flow time of 120 min.

Fig. 1

NOVEL SOLUTIONS FOR INTRA-ISCHEMIC AORTIC COLD FLUSH FOR PRESERVATION DURING 30 MIN CARDIAC ARREST IN DOGS.

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*Organ Recovery Systems Inc, **Synzyme Technologies

Objectives:

In exsanguination cardiac arrest (CA), conventional resuscitation attempts are futile. Therefore we introduced the use of cold aortic saline flush at start of CA to rapidly induce preservative hypothermia during prolonded CA for "suspended animation for delayed resuscitation". This study was to investigate, in dogs, three different solutions compared to saline for aortic cold flush.

Methods:

Dogs (20-25 kg) were exsanguinated over 5 min to CA of 30 min no-flow. At CA 2 min, the dogs received an aortic flush of 25 mL/kg at 2°C over 1 min, using saline (n=5), albumin 5% or 25% (n=6), Unisol-I (organ preservation solution with K^+) (n=5), or polynitroxylated albumin plus tempol (an antioxidant) (PNA-T) (n=5). The flush was through a balloon-tipped catheter inserted via the femoral artery into the abdominal aorta. Resuscitation was by closed-chest cardiopulmonary bypass, followed by assisted circulation for 2 h, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated as overall performance categories (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=death); neurologic deficit score (NDS 0-10%=normal, 100%=brain death); and brain histologic damage score (Total HDS 0=normal, >40=severe damage, >100=extensive damage).

Results:

Lowest tympanic temperature during CA was 32°C in all dogs. At 72h, all dogs achieved poor overall performance (OPC 3-5) (figure 1); Unisol resulted in pharmacologic defibrillation during CA; PNA-T in lowest NDS (figure 2) and HDS (figure 3).

	Control (n=5)	Albumin (n=6)	Unisol (n=5)	PNA-T (n=5)
OPC 5 (brain death)			●	
OPC 4 (coma)		●●	●	
OPC 3 (severe disability)	●●●●●	●●●●	●●●	●●●●●
OPC 2 (moderate disability)				
OPC 1 (normal)				

Figure 1. Overall performance categories (OPC) at 72 h after resuscitation, p=0.4.

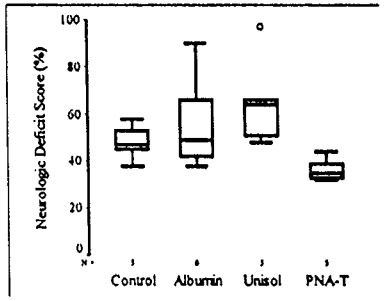

Figure 2. Neurologic Deficit Scores at 72 h after resuscitation, p=0.01 (post hoc: p<0.05 Unisol vs PNA-T)

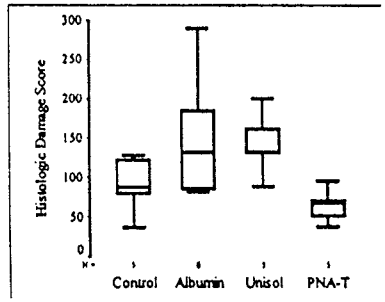

Figure 3. Total Brain Histologic Damage Scores at 72 h after resuscitation, p=0.02 (post hoc: p<0.05 Unisol vs PNA-T).

Conclusion:

Three aortic flush solutions, physiologically more rational than saline, do not give a breakthrough effect at moderate hypothermia, but Unisol might add benefit for the heart and PNA-Tempol for the brain.

(Supported by U.S. Department of Defense)

Fig. 2

INTACT SURVIVAL OF 120 MIN CARDIAC ARREST AT 10°C IN DOGS. CEREBRAL PRESERVATION BY COLD AORTIC FLUSH. NOVEL SOLUTIONS

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*Organ Recovery Systems Inc

Objectives:

In exsanguination cardiac arrest (CA), conventional resuscitation attempts are futile. Therefore we introduced the use of cold aortic saline flush at start of CA to rapidly induce preservative hypothermia during prolonged CA, for "suspended animation" for hemostasis followed by resuscitation. In previous dog studies, saline flush to tympanic temperature (Tty) 10°C resulted in normal survival after CA 90 min, but not consistently after CA 120 min. This study was to explore an optimized aortic flush, to consistently achieve normal outcome after CA 120 min.

Methods:

Male dogs (20-26 kg) were exsanguinated over 5 min to CA of 120 min no-flow. At CA 2 min, the dogs received an aortic cold saline flush at 2-4°C, using a balloon-tipped catheter inserted via the femoral artery into the thoracic aorta, until Tty reached 10°C; then flush was continued from the femoral artery, until rectal temperature reached 20°C. The optimized flush was first with Normosol 650 mL/kg at 2-4°C, 2 L/min, to Tty 10°C , then at the end of the flush Unisol-I (with K^+) 50 mL/kg (organ-preservation solution) plus tempol 300 mg/kg (antioxidant). Resuscitation was by closed-chest cardiopulmonary bypass (CPB), primed with dextran/ringers in the saline flush group, and with Unisol-E (without K^+) in the optimized flush group. Then we used assisted circulation to 2 h, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated as overall performance category (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=brain death); neurologic deficit score (NDS 0-10%=normal, 100%=death); and brain histologic damage scores (Total HDS 0=normal, >40=severe damage, >100=extensive damage).

Results:

Flush volume was 14.6 ± 1.7 L in the historic (plus concurrent) saline flush group, and 15.5 ± 4.0 L in the optimized flush group (p = 0.9). Lowest Tty during flush was 6.7 ± 0.6°C in the saline flush group vs 8.4 ± 0.5°C in the optimized flush group (p = 0.004) (figure 1). OPC ranged from normal to coma in the saline flush group, while in the optimized flush group 5/6 dogs achieved normality (OPC=1, figure 2). NDS and HDS varied greatly in the saline flush group compared to the optimized flush group, without a statistically significant difference (figures 3 and 4).

	Saline flush (n=6)	Optimized flush (n=6)
OPC 5 (brain death)		
OPC 4 (coma)	••	
OPC 3 (severe disability)	•	
OPC 2 (moderate disability)	•	•
OPC 1 (normal)	••	•••••

Figure 2. Overall performance categories (OPC) at 72 h after resuscitation, p=0.06.

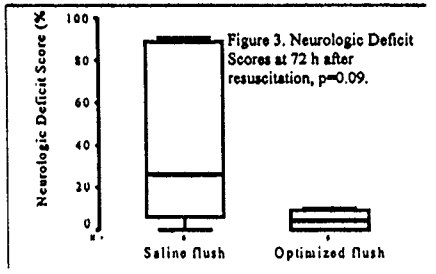


Figure 3. Neurologic Deficit Scores at 72 h after resuscitation, p=0.09.

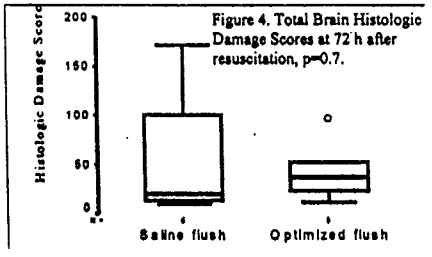


Figure 4. Total Brain Histologic Damage Scores at 72 h after resuscitation, p=0.7.

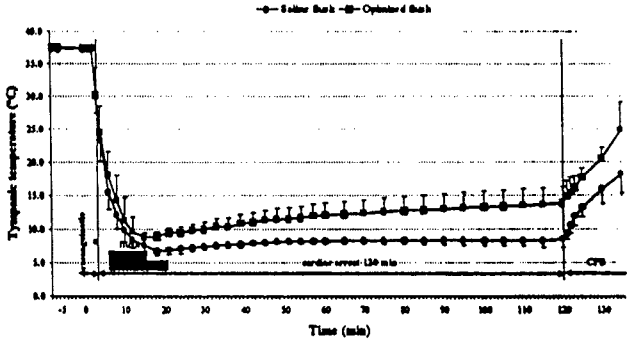


Figure 1. Tympanic membrane temperature (Tty) during exsanguination cardiac arrest (CA) of 120 min. Aortic flush was in the saline flush group via a balloon catheter with 1000 mL/min first into the thoracic aorta until Tty 10°C, continued into the femoral artery until Trectal 20°C and in the optimized flush group via cannula with 2000 mL/min into the femoral artery. The dog's head was put into ice-water during no-flow in the saline flush group starting 5 min after begin of CA. Area under the temperature curve during no-flow, p=0.002. Optimized flush gave better outcome although Tty was higher.

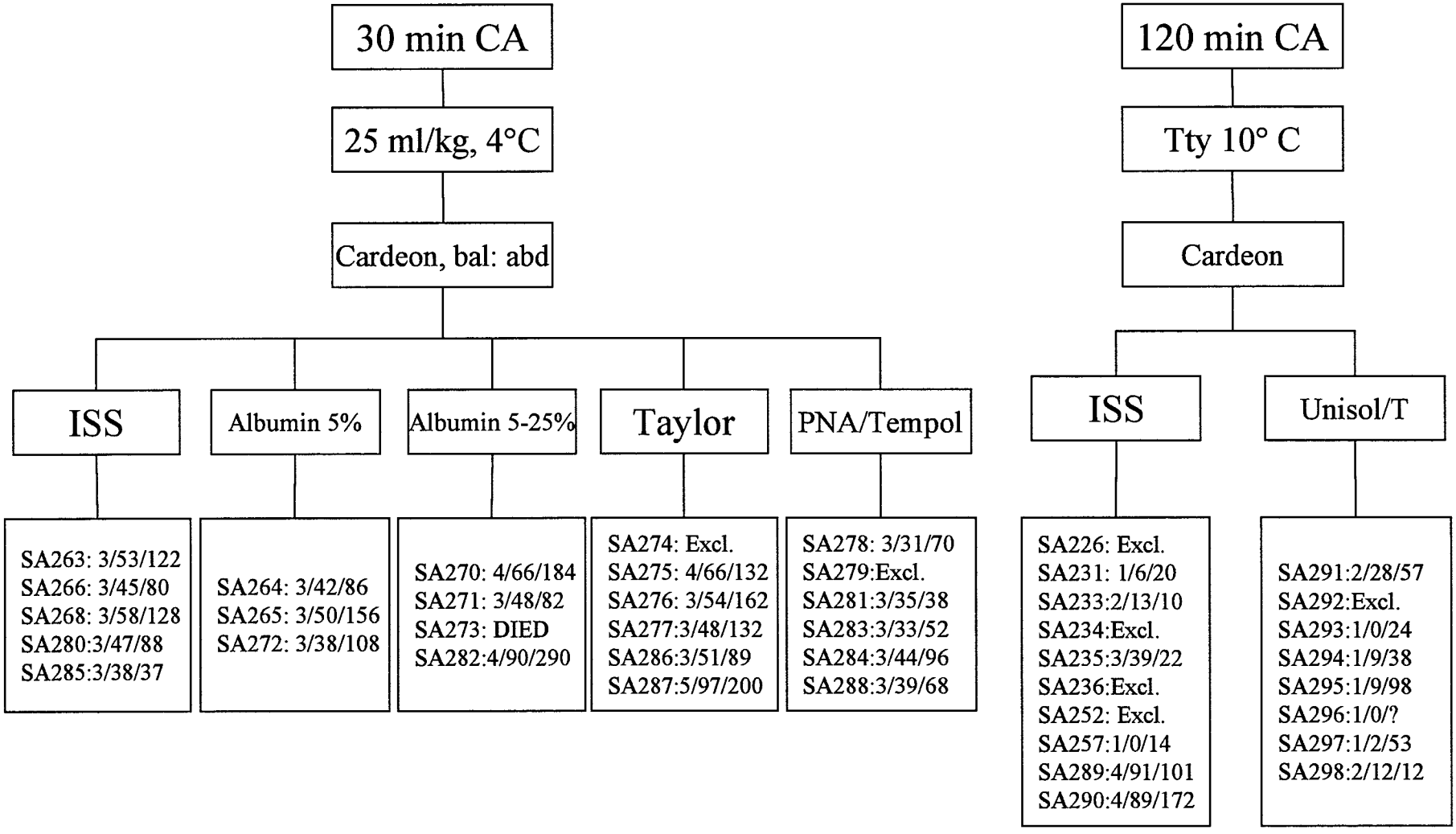
Conclusion:

An optimized single large volume cold aortic flush at start of CA can achieve normal survival with minor histologic damage after a no-flow time of 120 min. A field method for rapid vessel access and fluid cooling should be developed.

(Support by U.S. Department of Defense)

Each number represents
one dog experiment

Table 1
Year 4, Study I
Suspended Animation with Novel Solutions



Study II, SA by emergency thoracotomy for traumatic exsanguination (16,17) (Table 2)

Resuscitation attempts after traumatic exsanguination cardiac arrest (Exs-CA no-flow) almost never succeed because vital organs suffer permanent ischemic damage during the time required to control the bleeding site. In dogs of study I with non-traumatic Exs-CA of 90 min (some even 120 min), we had achieved intact survival by aortic cold flush of saline or novel solutions at CA 2 min to tympanic temperature (Tty) <10°C. Using 16 pilot experiments we defined the trauma, hemorrhage and flush and found CA 90 or 120 min plus trauma not (yet) resuscitable. Our goal in study II was to achieve survival without brain damage with SA after *traumatic* exsanguination cardiac arrest of 60 min in dogs (16). In the present study, we explored the hypothesis that additional trauma would worsen the chance of intact survival.

Methods. The definitive study was with no trauma (n=6), vs a *trauma* group (n=8) which received at start of CA standardized laparotomy, spleen transection, and thoracotomy; and during CA a splenectomy. In both groups, starting at CA 2 min, flush of saline at 2°C into the femoral artery was initiated and continued until Tty of 10°C. Restoration of spontaneous circulation and assisted circulation were with cardiopulmonary bypass (CPB) to 2 h (with heparin bonded system), and mild hypothermia (Tty 34°C) to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated as overall performance categories (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=death); neurologic deficit scores (NDS 0-10%=normal, 100%=brain death); and 72 h perfusion fixation, necropsy, and determination of total and regional brain histologic damage scores (HDS). Hematocrit was kept above 25%, if needed with donor blood.

Results. All 14 dogs survived to 72 h. The 6 non-trauma control experiments resulted in prompt resuscitation and intact survival (OPC 1), NDS 1% (range 0-13%) (normal) and total HDS 11 (4-22) (near normal). In 3/8 trauma dogs controlled ventilation was needed beyond 20 h because of airway edema, hypoventilation, cardiovascular complications, renal failure and neurologic deficit. 4/8 trauma dogs achieved final OPC 1, one OPC 2, one OPC 3, and two OPC 4; NDS was 13% (0-87) and brain HDS was zero. Blood loss in the trauma group ranged widely (up to 1300 mls) and was associated with poor outcome. Coagulation studies revealed in both groups, after resuscitation, transient initial hypocoagulation with coagulation factors

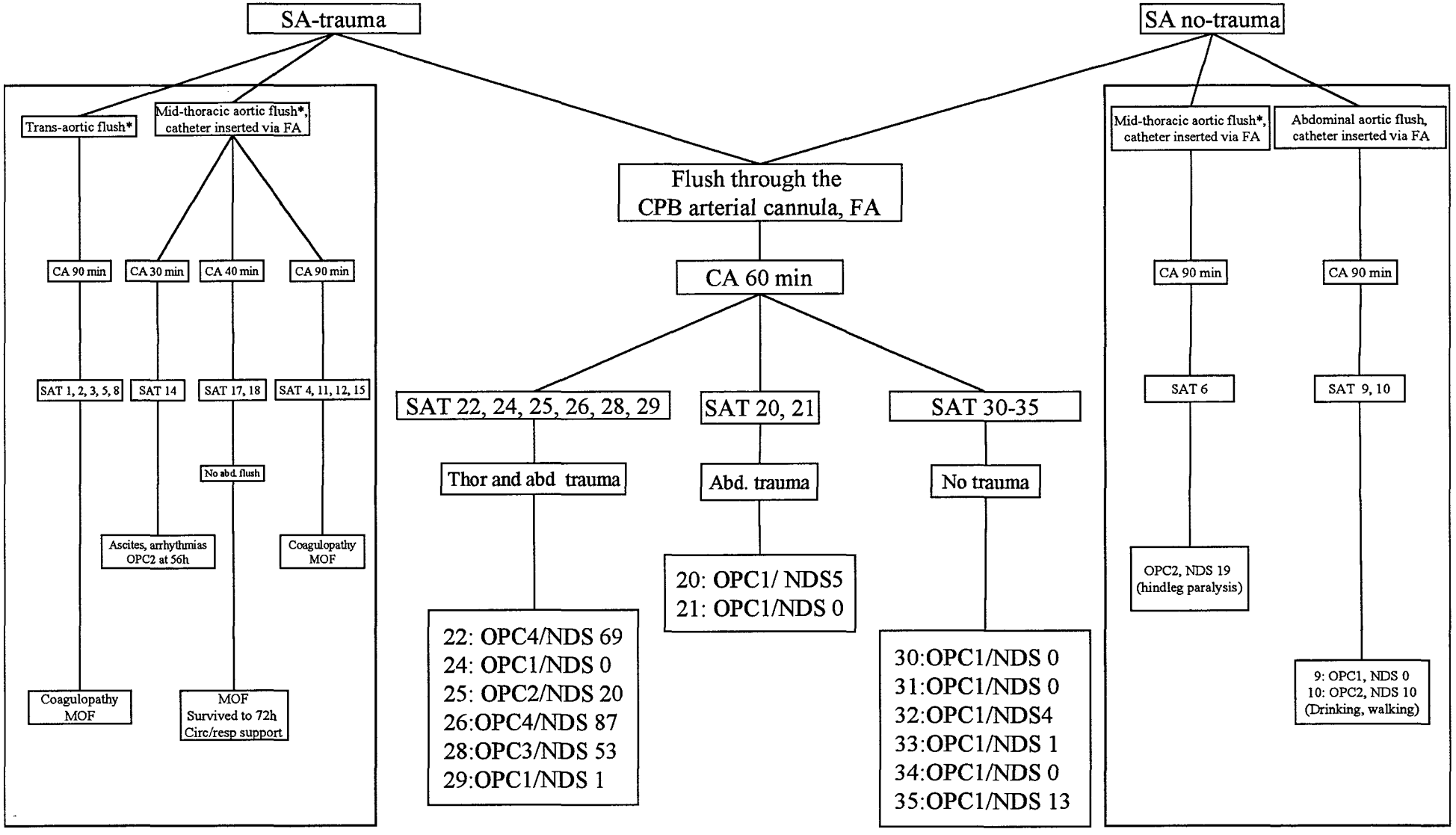
consumption, and fibrinolysis activation. This was followed by delayed hypercoagulation. There was no evidence of sustained DIC. Platelet count decreased to 50% baseline at 1 h after resuscitation, without normalization by 24 h. Plasma concentrations of plasminogen activator inhibitor peaked at 6-9 h after the insult. All changes occurred in both groups, but were numerically worse in the trauma group.

Coagulopathy and *multiple organ failure* were studied in detail in these 14 dogs. Hematocrit was essentially normalized in both groups with initially shed blood. In the trauma group fresh donor blood was added. All non-trauma dogs survived without neurologic deficits or extracerebral organ complications. In 3 trauma dogs, cardiovascular- pulmonary complications and renal failure occurred. Blood loss in the trauma group was 0-1300 ml and associated with poor outcome. After CA, in both groups, thromboelastograms (TEG) indicated severe hypocoagulation at 1 h of recirculation with narrowed alpha angle ($\propto$), prolonged reaction time (r) and reduced maximum amplitude (MA). Prothrombin time (PT) and partial thromboplastin time (PTT) were prolonged and factors II, V, VIII and fibrinogen levels were reduced. Antithrombin III (AT-III) levels were reduced and remained so until 24 h in the control group and until 72h in the trauma group. Platelet levels were 50% baseline at 1 h and did not normalize in both groups. Plasminogen activator inhibitor (PAI) increased 6x at 6-9 h, with higher levels in the trauma group; it gradually decreased thereafter and was followed by a delayed hypercoagulation toward 72h, with wide $\propto$, short r and high MA in the TEG curves. At 72h, PT, PTT and clotting factors had normalized, but plasmin, antiplasmin and fibrinogen were increased.

Conclusions. The rapid induction of profound hypothermia (Tty 10°C) (suspended animation) can enable survival without brain damage after Exs-CA of 60 min no flow *even in the presence of trauma*, although with worse extracerebral organ failure than without trauma. Coagulopathy and possibly a thrombotic microangiopathy, as a result of ischemia, reactive oxygen species, CPB, hemodilution, and hypothermia are worsened by trauma. Extracerebral organ complications and thrombotic microangiopathy were observed specially when trauma was added to this model. After trauma, resuscitation from 90 or 120 min no-flow remains a challenge.

Each number represents
one dog experiment

Table 2
Year 4, Study II
SA after Exsanguination CA with vs. without Trauma



Excluded: SAT 7- flush complications (air emboli)
Blood donors: SAT 13, 16, 19, 23, 27
*= Flush until Tty 10oC, then lower aortic flush until 700mL/kg or Tperitoneal 20oC

22: Severe general edema, some spont. resp., but inefficient, BP↓ when ETCO2
26: Extub at 48h, airway obstr. Inefficient resp. at 72h. ARF.
28: Moderate edema, Extub at 70h – collapsed, reintubated

Study III, Normovolemic VF CA. "SA" with mild hypothermia during CPR (18,19) (Table 3).

About 50% of out-of-hospital CPR attempts fail to restart heart beat and patients are given up. We know that many of these hearts are capable to resume adequate function if given rest or if repaired. In 4 "pilot experiments" (see below) we found normovolemic CA to be not suitable for aortic cold flush induction of SA unless enormous venous fluid volumes are discarded or recirculated. Moreover, discussions with clinicians revealed that it would be unacceptable to replace continued CPR-ALS ABC attempts with deliberate hypothermic CA (i.e., SA), since nobody can predict when the heart starts beating, even if late. We do need, however, a bridge of sustained viability to long-term cardiopulmonary bypass. We decided to simulate a temporarily "unresuscitable" CA with CPR steps ABC to VF 40 or 60 min using mild hypothermia (34°C) *during* closed chest CPR (18). We had shown earlier that mild hypothermia (34°C) *after* normothermic CA improves cerebral outcome. We hypothesized that mild or moderate hypothermia (30°C) *during* prolonged closed chest CPR steps ABC would further improve outcome. We achieved preservation during CPR not only of the brain but also of the heart.

Methods: Twenty-four dogs were subjected to VF, normothermic no flow of 3 min, BLS of 7 min, and ALS for unsuccessful ROSC attempts of 10 min. They were then randomized to 4 groups: group 1 (n=7) with continued normothermic CPR-ALS-ABC. Group 2 (n=6) with hypothermic i.v. flush (20 ml/kg normal saline at 2°C) and veno-venous extracorporeal shunt cooling to tympanic temperature (Tty) 26-28°C. Group 3 (n=6) same as group 2 but veno-venous shunt to Tty 34°C during CPR. Group 4 (n=5) normothermic flush and veno-venous shunt. After VF 40 min, reperfusion was with cardiopulmonary bypass. Intensive care was to 96 h. Outcome was evaluated as overall performance categories (OPC 1=normal, 5=death); neurologic deficit scores (NDS 0-10%=normal, 100%=brain death); and 96 h perfusion fixation, necropsy, and determination of total and regional brain histologic damage scores (HDS).

Results: Of the normothermic CPR dogs, all in group 4 and all but one in group 1 (which remained comatose) died within 58 h, because of malignant arrhythmias and respiratory failure or vasopressor resistant shock. At necropsy there was macroscopic evidence of hemorrhagic tissue trauma to heart, lungs, and liver, even in the survivors. Suspected are mechanical trauma from prolonged chest compressions, electric injury, ischemia, reactive oxygen species, and catecholamines. These morphologic changes (using a new myocardial damage scoring system) were present in both groups, but worse in the normothermic groups. In mildly hypothermic groups 2 and 3, all dogs survived to 96 h (see outcome table, below); morphologic changes in the brain were absent or minimal.

Outcome at 96 h

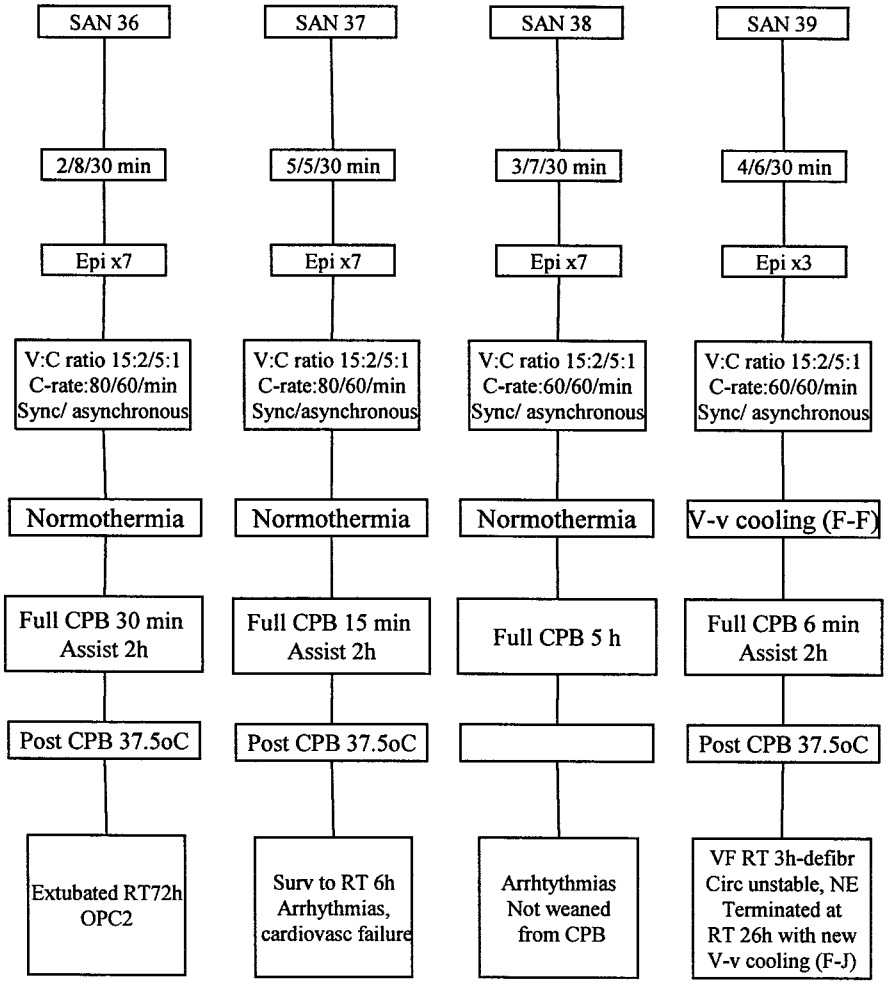
	Gr 1: 37.5°C	**Gr 2: 27°C**	**Gr 3: 34°C**	**Gr 4: 37.5°C**
OPC	6/7 died at 4-58 h 1/7 OPC 4	1,1,1,1,1,4	1,1,1,2,2,2	Died < 24h
NDS	6/7 died 1/7 NDS 92	1 (0-92)	1 (0-11)	Died < 24h
HDS	0, 26, 78	1 (0-66)	1 (0-4)	46

Conclusions Mild or moderate hypothermia *during* prolonged closed-chest CPR preserves viability of organs, without risk of complications, and improves outcome.

In summer 2002, to be completed at the end of September 2002, we have added another CPR study, a series of dog experiments to evaluate an extended CPR steps ABC to 60 min, comparing immediate vs delayed induction of mild hypothermia by veno-venous shunt cooling as in the previous study, during CPR ABC for normovolemic unresuscitable CA. The results are dramatic and comparable to those of the previous study, namely, immediate cooling achieved survival vs delayed cooling did not. This study will be reported in detail next year

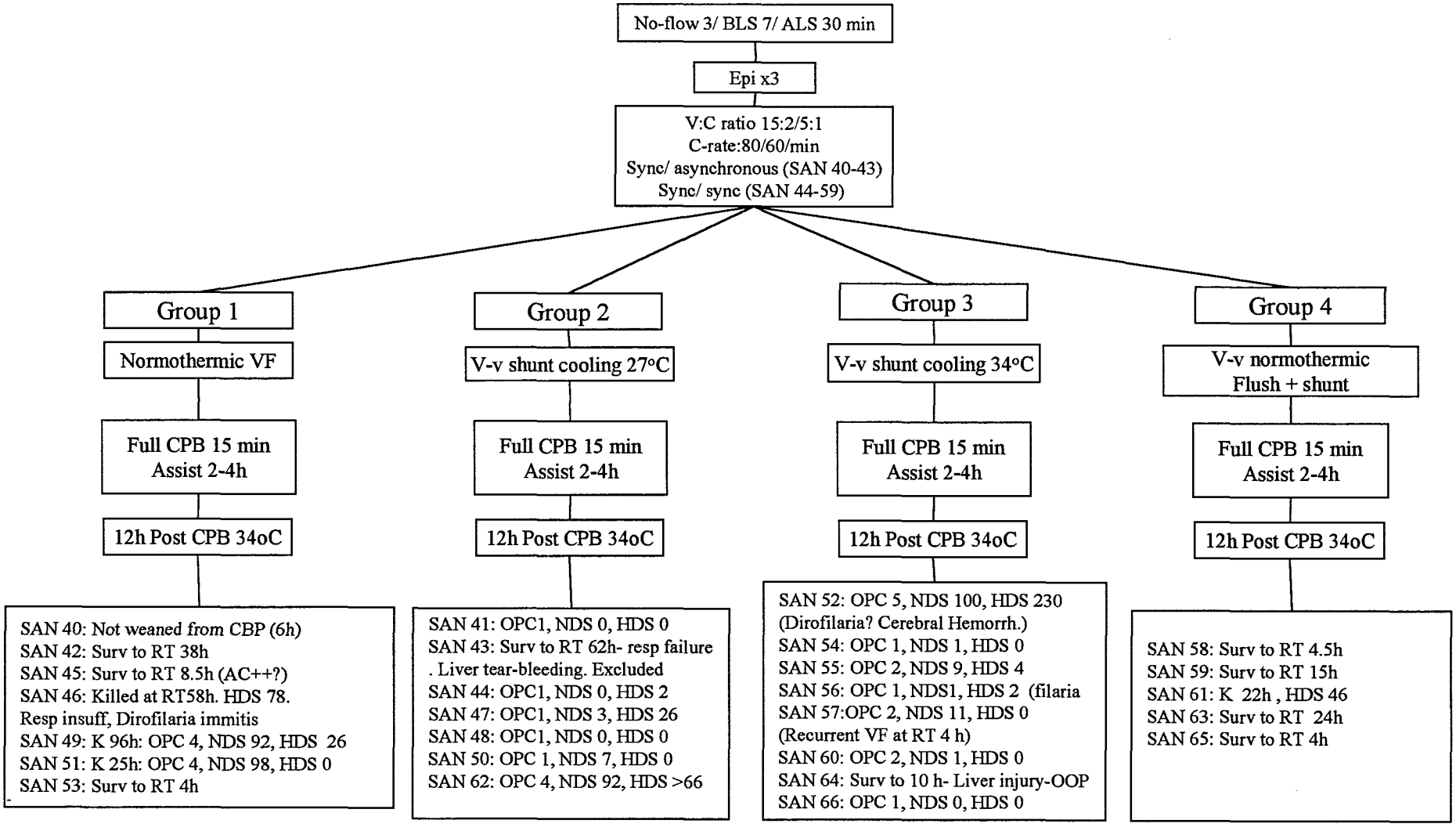
Each number represents
one dog experiment

PILOT EXPERIMENTS



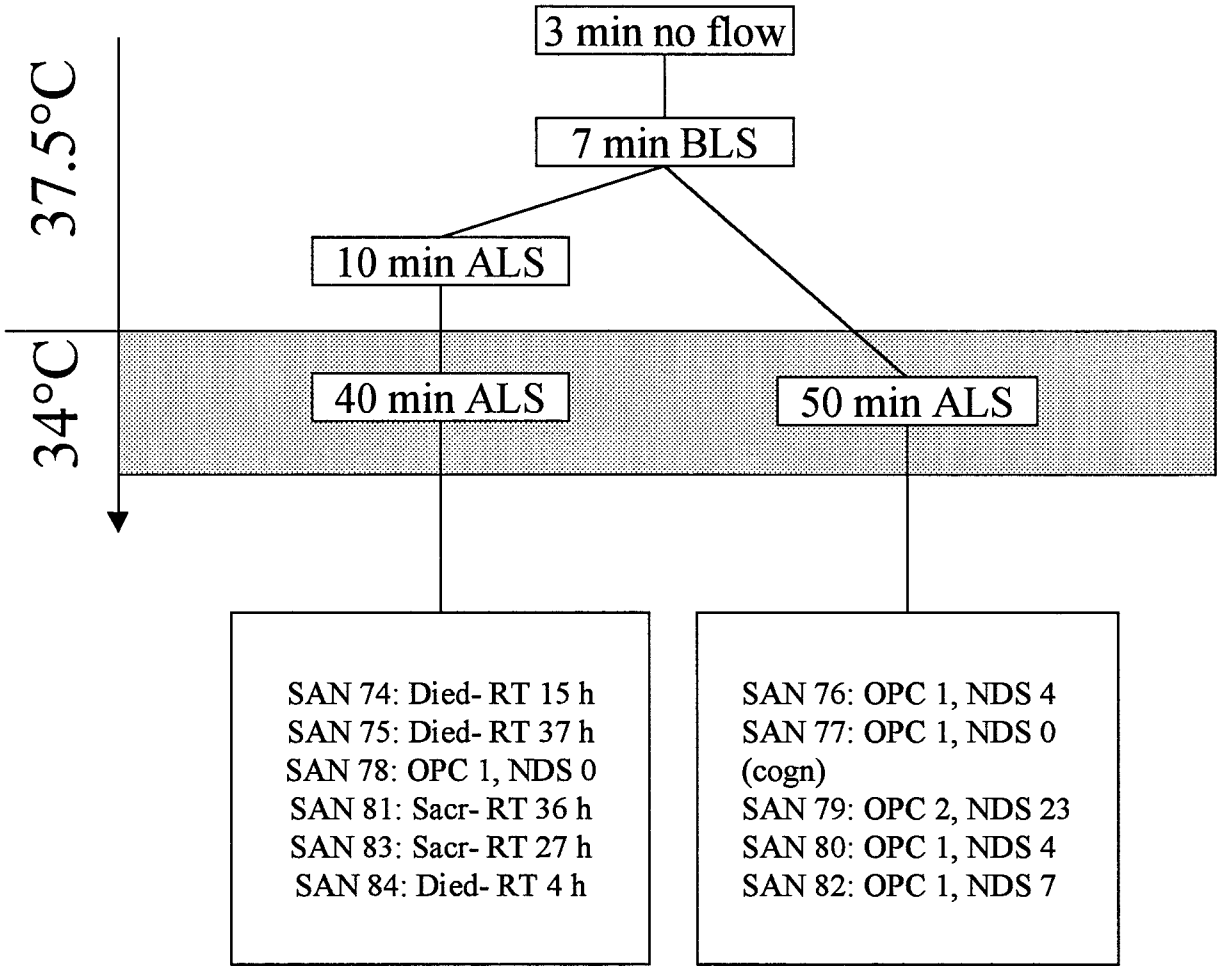
Each number represents
one dog experiment

Table 3
Year 4, Study III-a
"Suspended Animation" with mild hypothermia during CPR
Normovolemic VF CA (SAN) 40 min



Each number represents
one dog experiment

Table 3 continued
Year 4, Study III – b
"Suspended Animation" with hypothermia for Normovolemic VF CA (SAN) 60 min.



Devices developments.

In the mid-1990s, Safar-Klain-Stezoski and the University of Pittsburgh had received 2 patents -- on a portable heart-lung machine (CPB) with cooler (heat exchanger) and single or double balloon aortic catheters for emergency hemostasis, aortic flush for SA, and temperature controls. These patents the University licensed to the Cardeon Co. in California. Starting in 1995, Safar presented and discussed with SCRR associates other potentially patentable ideas: portable cooler-pump for blood cooling; iced solution reservoir for ambulances for SA by aortic flush; miniaturized tympanic thermometers; "smart" catheters (with Dr. Yaffe); transthoracic insertion; trans-thoracotomy balloon catheter; and others. No patent applications have yet been submitted.

We established a steering committee with Dr. Lyn Yaffe as administrative chairman, to coordinate laboratory results from this Army project, developments of methods and devices, and planning clinical trials of mild hypothermia for traumatic hemorrhagic shock and profound hypothermic aortic flush SA for exsanguination CA. This steering committee includes the Pittsburgh team (Safar, et al, for SA, and Tisherman, et al, for HS [ONR]), ITTRI-Dr. Yaffe (smart catheter project), the Biocontrol Co. (portable cooler project), a still frustrated search for a maker of portable CPB, and other industries; Dr. Tisherman, et al, for planning clinical trials. That steering committee met throughout the year in conference calls every week, and in person with Dr. Yaffe visiting Pittsburgh about once per month. The project of ITTRI-Dr. Yaffe includes Dr. Klain, and as advisors, Dr. Safar, Dr. Tisherman, and Mr. Stezoski.

Our dog euthanasia experiments were also used for testing of adjunctive methods and devices, before euthanasia, to save extra dog lives. These efforts have led to prototypes of aortic balloon catheters which were tested in our dog experiments and are now being improved. The Biocontrol project promises to have a prototype of a portable cooler in our hands by March 2003. The Cardeon Co. provided regular and special aortic balloon catheters for use in dogs; and is planning to honor our long-standing request for industries to make prototypes of portable heart-lung machines with coolers.

Miscellaneous in 2001/02.

Dr. *Safar's* illnesses required operations for a spine problem in October 2001, a nephrectomy in November 2001, and a pelvic exenteration for cancer in May 2002. He is now on chemotherapy. All this removed him from fulltime work only for 3x 2 weeks. He continued the leadership for this project throughout.

For clinical trials, Dr. *Tisherman* identified and communicated with 6 potentially participating major trauma hospital groups.

Dog experiments were carried out by a big team on a weekly basis. In July 2001, the dog lab was closed for cleaning and team vacations. There was during the year a period of interruption due to laboratory renovations demanded by the Animal Care and Use Committee to meet state and national requirements. The weekly lab meetings continued throughout.

Ala Nozari, M.D., Ph.D., assistant professor of anesthesiology at the University of Upsala Sweden, started with us in summer 2001. His predecessor as team leader, Wilhelm Behringer, M.D. of the University of Vienna, overlapped with Dr. Nozari for an undisturbed continuance of these difficult dog experiments. Dr. Behringer returned to his institution in Vienna on August 31, 2001 and prepared grant applications to donors in Europe (with our help) for support of another large animal research intensive care unit (primarily for pigs) in Vienna, to be used in communication and collaboration with Pittsburgh. This was one reason for Dr. Safar to visit Vienna in April 2002.

John Williams, M.D. became the new chairman of our department of anesthesiology. Our department's critical care medicine program received departmental status, under Dr. *Mitchell Fink* as successor of Dr. *Ake Grenvik* (1971-) and Dr. *Safar* (1961-71).

Dr. Safar lectured twice at the Cleveland Clinic in support of hypothermia research, attended the American Society of Anesthesiologists' (ASA) meeting in October 2001 together with our Army projects' research fellows who presented abstracts. Dr. Safar presented at the ASA meeting the present status of many years of research on suspended animation, which aroused national attention. Two TV stations interviewed us (Discovery; ABC). Drs. *Safar* and *Tisherman* attended and presented at the DOD Combat Casualty Care meeting in Fort Walton Beach, Florida, September 9-10, 2001, and experienced the Attack on America on September 11, 2001 immediately upon return to Pittsburgh. That disaster of terrorism prompted cancellation of

the resuscitation presentations scheduled at the International History of Anesthesiology Congress in Spain in September 2001.

Dr. Safar (accompanied by Dr. Nozari) presented our DOD results among other topics at the Academy of Neurology congress in Denver in April 2002. That was also the time when we submitted the Army proposal for year 5 (2002/03), which was positively reviewed by the AIBS and the USAMRMC. Dr. Safar had to cancel his presence at the International Trauma Anesthesia and Critical Care Society meeting in Stavanger Norway, where, however, other team members discussed some of our topics. Dr. Safar was invited to have some of his life-long research materials (also of US Army research in the 1950s and 60s) in the archives of the University of Pittsburgh. Dr. Safar received an honorary doctor's degree from the Charles University of Prague. Our DOD research group was represented at the Society of Critical Care Medicine meeting in San Diego 2002 with 7 abstracts and other presentations. Dr. Safar could not go.

With Dr. Safar's initiative, the long standing desire to help the lay public acquire *life supporting first aid* (LSFA) skills, was enhanced by the realization that terrorist attacks may call for even greater vigilance than before for life saving by bystanders. Chemical and blast weapons are more LSFA relevant than biologic weapons. Dr. Safar initiated communication with DOD, and a chemical agent Army researcher of the 1950s (J. Clements, MD), on questions of LSFA for casualties of chemical weapons. Our research team launched several leadership group meetings throughout the year to help the Save A Life Foundation, in collaboration with other national organizations, to start in Western Pennsylvania with novel training programs for school children, to be extended for adults later. LSFA includes CPR BLS plus basic trauma life support. Dr. Safar has been aiming for a revival of the self-training systems he and Mr. Laerdal developed in the 1970s, which proved superior to "courses" (see LSFA review by Eisenburger and Safar in *Resuscitation* 1999).

Dr. Safar conducted multiple discussions with the new American Heart Association (AHA) CPR-ECC committee chairman, Dr. Nadkarni, urging a combination in training programs between cardiac and trauma life support. Drs. Safar, Bircher, Mossesso, and Hickey of the SCRR will participate in October 2002 in the AHA guidelines meeting.

Dr. Tisherman a(Co-P.I.) lectured at the International Surgical Society in Brussels on "exteme resuscitation"; at the American Association of Neurologic Surgeons in Chicago (on

suspended animation research); at the Society of Air Force Clinical Surgeons in Las Vegas (on hypothermia); at the SERF forum in Baltimore (on instruments for evaluating crisis management skills); and on several occasions in Pittsburgh concerning our research on HS and SA.

In February 2002 the *New England Journal of Medicine* published the first results of randomized clinical trials (one in Europe by our alumni; one in Australia) with an editorial by Drs. *Safar* and *Kochanek*, concerning mild hypothermia after cardiac arrest. Both studies showed statistically a higher chance for survival without brain damage in the treatment group. This revelation caused much publicity. It documented in patients what we had discovered and initiated in dogs since 1987. Although this is not SA, it gave the therapeutic hypothermia programs a great boost.

Dr. *Kochanek* presented his and Dr. Safar's views at the World Congress on Drowning in Amsterdam in June 2002. Dr. *Kochanek* (co-investigator) has been a widely sought lecturer worldwide, including topics of this Army project.

Dr. Carcillo, in preparation for our Army proposal SA year 5, presented to us for discussion his preliminary data on plasma exchange for microangiopathy with coagulopathy in septic conditions of children. For year 4 study II on trauma, we attracted *Dr. Bontempo* and his associates as co-investigators. Dr. Bontempo is an internationally recognized expert on coagulopathy.

Dr. Jenkins, invited by Dr. Safar, obtained preliminary data on proteomics in the hippocampus of rat brains during prolonged clinical death without reperfusion. This also is in preparation for year 5.

USAMRMC/TATRC. On July 25, 2002, we (Drs. Safar, Tisherman, Yaffe, Kochanek, Klain, Nozari, ITTRI, and Biocontrol) followed an invitation from Dr. Calcagni, project officer of our Army grant, to participate in presentations to the at Fort Detrick concerning our current research and plans for therapeutic hypothermia research. We presented for 2 hours the conglomerate of our studies, with emphasis on SA.

REPORTABLE OUTCOMES

See the body of the report.

KEY RESEARCH ACCOMPLISHMENTS AND CONCLUSIONS

1. In study I of SA year 4 (8-15), using a sophisticated exsanguination cardiac arrest intensive care outcome model in dogs, *without trauma*, we showed that profound hypothermic suspended animation (preservation) of the organism (with emphasis on brain and heart), by aortic cold flush, started within 5 min of circulatory arrest, is more effective with the use of novel solutions (specifically, Normosol plus Unisol plus Tempol) than merely using saline solution. We had shown in year 3 that hypothermic strategies are highly effective for preservation, while over 14 pharmacologic strategies are not. With novel solution flush we extended the longest period of cardiac arrest no flow at 10°C which can be reversed to survival without brain damage, using (portable emergency) cardiopulmonary bypass – from 90 min no flow (with saline flush) to 120 min no flow (with novel solution flush)!

We *conclude* that the solutions tested should replace saline in future explorations and that even potentially more effective pharmacologic solutions be explored with the goal to enable a reduction in the volumes of cold fluid required. This is to make the suspended animation approach more readily feasible for initiation in the field.

2. In study II of SA year 4 (16,17), using a similar exsanguination cardiac arrest model in dogs as above, but now *with tissue trauma*, we found that the longest period of cardiac arrest no flow at 10°C which can be reversed to survival without brain damage is not 120 min, but closer to 60 min no flow. The reasons include the coagulopathy (due to ischemia, hemodilution, CPB, hypothermia, reoxygenation injury, trauma, and massive transfusion), which we found present also without trauma, is much worse with trauma. In addition, the trauma-induced blood loss and need for donor blood and the above derangements have a tendency to worsen the postarrest multiple organ failure. The coagulopathy, which we tested in terms of numerous sophisticated variables, has a consistent pattern of early postarrest hypocoagulability leading to late postarrest hypercoagulability.

We *conclude* that some innovative methods (for year 5), such as fresh whole blood and plasma exchange therapy, should be tried to mitigate the coagulopathy, microangiopathy, and multiple organ failure caused by tissue trauma plus exsanguination cardiac arrest.

3. In study III of SA year 4 (18,19), we used a different model in dogs -- namely normovolemic ventricular fibrillation cardiac arrest, simulating temporarily unresuscitable hearts -- a major civilian public health problem. We are seeking the best bridging method to maintain viability of the organism, starting with the beginning of arrest to about 1 hour later, when in civilian practice sudden cardiac death patients can be rushed to hospital emergency rooms for (portable emergency) cardiopulmonary bypass; this can be prolonged until the heart recovers from stunning, is repaired or replaced, or the brain declared "dead" for organ donation. We discovered and documented that the suspended animation idea we developed for exsanguination cardiac arrest would be neither physiologically suitable nor clinically acceptable for patients undergoing prolonged CPR steps ABC. However, we then discovered and documented that ventricular fibrillation of 40 min duration, with most of this time covered by CPR basic and advanced life support steps ABC continued (low flow) under normothermia, cannot be survived, even with bypass resuscitation attempts; whereas it can be consistently survived intact, when mild hypothermia is induced *during* CPR. This we accomplished with rapid induction of mild hypothermia (Tty 34°C) by veno-venous shunt cooling, a method which should become feasible for use by ambulance paramedics. When we extended the ventricular fibrillation period to 60 min, only very early induction of mild hypothermia achieved intact survival.

We *conclude* that mild hypothermia (which we had shown earlier in dogs and patients to reduce brain damage when induced *after* normothermic cardiac arrest) is even more potent as a preservative-resuscitative tool if induced as early as possible *during* initiation of CPR.

4. Research accomplishments prior to this reported year 4, concerning new approaches to therapeutic hypothermia in general, for various indications, have been elaborated on during year 4, beyond the SA topic.

APPENDICES

PUBLICATIONS

1. Behringer W, Prueckner S, Safar P, Radovsky A, Kentner R, Stezoski SW, Henchir J, Tisherman SA: Rapid induction of mild cerebral hypothermia by cold aortic flush achieves normal recovery in a dog outcome model with 20-minute exsanguination cardiac arrest. Acad Emerg Med 7:1341-1348, 2000.

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15. Behringer W, Safar P, Nozari A, Wu X, Kentner R, Tisherman SA, Radovsky A: Intact survival of *120 min* cardiac arrest at 10°C in dogs. Cerebral preservation by cold aortic flush (*and novel solutions*). Crit Care Med 29/12:A71, 2001. (Abstract #225). [SCCM meeting 2002]. Manuscript in preparation.

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17. Nozari A, Bontempo F, Safar P, Wu X, Stezoski SW, Tisherman S: Coagulopathy and multiple organ failure after *traumatic* exsanguination cardiac arrest (CA) of 60 min in dogs. *Submitted* to SCCM meeting 1/2003.

18. Nozari A, Safar P, Tisherman S, Wu X, Stezoski SW: Hypothermia induced *during* cardiopulmonary resuscitation (BLS steps ABC) increases intact survival after prolonged normovolemic cardiac arrest in dogs. Anesthesiology 96 (Suppl):A417, 2002. (Abstract). [ASA meeting 2002]. Manuscript in preparation.

19. Nozari A, Safar P, Wu X, Stezoski SW, Tisherman S: Intact survival in dogs after cardiac arrest (CA) of 40 min with mild hypothermia (34°C) *during* closed chest CPR: myocardial and cerebral preservation. *Submitted* to SCCM meeting 1/2003. Manuscript in preparation.

BASIC INVESTIGATIONS

Rapid Induction of Mild Cerebral Hypothermia by Cold Aortic Flush Achieves Normal Recovery in a Dog Outcome Model with 20-minute Exsanguination Cardiac Arrest

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Abstract. **Objectives:** Resuscitation attempts in trauma victims who suffer cardiac arrest (CA) from exsanguination almost always fail. The authors hypothesized that an aortic arch flush with cold normal saline solution (NSS) at the start of exsanguination CA can preserve cerebral viability during 20-minute no-flow. **Methods:** Twelve dogs were exsanguinated over 5 minutes to CA of 20-minute no-flow, resuscitated by cardiopulmonary bypass, followed by post-CA mild hypothermia (34°C) continued to 12 hours, controlled ventilation to 20 hours, and intensive care to 72 hours. At CA 2 minutes, the dogs received a 500-mL flush of NSS at either 24°C (group 1, $n = 6$) or 4°C (group 2, $n = 6$), using a balloon-tipped catheter inserted via the femoral artery into the descending thoracic aorta. **Results:** The flush at 24°C (group 1) decreased tympanic membrane temperature [mean (±SD)] from 37.5°C (±0.1) to 35.7°C (±0.2); the flush at 4°C (group 2) to 34.0°C (±1.1) ($p = 0.005$). In group 1, one dog achieved overall performance category (OPC) 2 (moderate disability), one OPC 3 (severe disability), and four OPC 4 (coma). In group 2, four dogs achieved OPC 1 (normal), one OPC 2, and one OPC 3 ($p = 0.008$). Neurologic deficit scores (0–10% normal, 100% brain death) [median (25th–75th percentile)] were 62% (40–66) in group 1 and 5% (0–19) in group 2 ($p = 0.01$). Total brain histologic damage scores were 130 (62–137) in group 1 and 24 (10–55) in group 2 ($p = 0.008$). **Conclusions:** Aortic arch flush of 4°C at the start of CA of 20 minutes rapidly induces mild cerebral hypothermia and can lead to normal functional recovery with minimal histologic brain damage. The same model with aortic arch flush of 24°C results in survival with brain damage in all dogs, which makes it suitable for testing other (e.g., pharmacologic) preservation potentials. **Key words:** cardiac arrest; cerebral ischemia; exsanguination; trauma; hemorrhage; cerebral preservation. ACADEMIC EMERGENCY MEDICINE 2000; 7:1341–1348

THE MAJORITY of civilian trauma victims who die from their injuries, as well as the majority of "killed-in-action" combat casualties, without lethal head trauma, die rapidly in the field or in the emergency department (ED) as a result of uncontrollable intrathoracic or intra-abdominal exsanguination.[1] In such cases, fluid resuscitation attempts fail, and a completely new approach to resuscitation must be found. Consequently, Bellamy and Safar in 1984 introduced the concept of "suspended animation,"[1] which is "preservation of the organism during very prolonged circulatory arrest, to enable transport and repair during pulselessness, to be followed by delayed resuscitation."

In sudden normothermic cardiac arrest (CA), cerebral oxygen availability approaches zero in 10–20 seconds[2]; glucose and ATP approach zero in 5 minutes.[3,4] Until recently, the 5-minute limit for reversible normothermic CA no-flow was accepted.[5] It has been established that the lower the brain temperature prior to CA (protection) and during CA (preservation), the longer can viability of the brain be maintained. Complete cerebral recovery in dogs or patients has been achieved with protec-

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Received March 15, 2000; revision received June 27, 2000; accepted July 3, 2000. Presented at the SAEM annual meeting, Boston, MA, May 1999 (best basic science oral presentation award).
Supported by grant #N00014-97-1-1064 of the U.S. Navy, Office of Naval Research (ONR).
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tion/preservation by mild hypothermia (34–36°C) during CA up to 15 minutes[6]; moderate hypothermia (28–32°C) during CA up to 20 minutes[7]; deep hypothermia (11–27°C) during CA up to 30 minutes[8,9]; and profound hypothermia (6–10°C) during CA up to 45 minutes[10]—or 60 minutes, even when preceded by normothermic hemorrhagic shock of 60 minutes.[11]

In the field, the induction of systemic hypothermia has not yet been possible within the 5-minute limit of cardiac arrest. Therefore, there is a need to find a tool for induction of mild to moderate cerebral hypothermia within seconds in the field. This could be accomplished by an aortic arch cold flush via balloon-tipped catheter to preserve the brain and heart with mild or moderate hypothermia until hemostasis can be achieved or until more prolonged preservation with deep or profound hypothermic circulatory arrest[11,12] can be induced and reversed by cardiopulmonary bypass (CPB).[13] The optimal rapid vessel-access method for use in the field is to be determined. The ultimate, clinically relevant suspended animation scenario would be: exsanguination over 5 minutes; CA of 20 or 30 minutes; aortic arch flush (by paramedic or physician in the field) at start of CA to induce mild to moderate cerebral hypothermia; initiation of portable CPB during CA to lower temperature to profound or ultraprofound level to extend preservation of the organism in pulselessness to 1 hour or longer for transport and repair; followed by reperfusion and rewarming with CPB and survival without brain damage.

Aortic arch cold flush in dogs for preservation during CA of 15 minutes has been evaluated.[14] In the current study, the duration of exsanguination CA was extended to 20 minutes. We hypothesized that aortic arch flush with normal saline solution (NSS) 500 mL at 24°C at the start of CA of 20 minutes rapidly induces minimal cerebral hypothermia and results in survival *with* brain damage, while the same flush at 4°C can result in 72-hour survival with functionally and histologically normal brains.

METHODS

Study Design. This study was approved by the Institutional Animal Care and Use Committee and followed national guidelines for the treatment of animals. Fourteen male custom-bred hunting dogs, 8–12 months old, with a body weight of 20–25 kg, were used. Briefly, the dogs were exsanguinated to CA of 20-minute no-flow, resuscitated by closed-chest CPB, had controlled ventilation to 20 hours, and intensive care to 72 hours. At CA 2 minutes, the dogs received a flush into the aortic arch using a balloon-tipped catheter inserted via the femoral artery. In group 1 (n = 7), the flush was at 24°C and in group 2 (n = 7), at 4°C. All experiments were performed by the same team within four months, in mixed sequence without randomization, as part of a systematic exploration of aortic flush preservation potentials.

Animal Subjects and Preparation. After premedication with ketamine (10 mg/kg IM) and orotracheal intubation, the dogs were mechanically ventilated under anesthesia with titrated halothane 0.5–1.5% and N_2O:O_2, 50:50%. Temperature probes were inserted for measuring tympanic membrane (Tty), esophageal (Tes), and rectal temperatures (Tr). Via a peripheral IV cannula (18 g), dextrose 5% in 0.45% NaCl, 100 mL/h, was administered. A polyethelene 90 catheter was inserted into the left femoral artery via sterile cutdown for monitoring of arterial pressure and for blood sampling. A pulmonary artery catheter (7.5 Fr) for pressure monitoring, cardiac output determination, temperature measurements (Tpa), and blood sampling was advanced via the left femoral vein. A prototype balloon catheter (8 Fr), with one hole at the tip of the catheter (Cardeon Corp., Cupertino, CA) was advanced via the right femoral artery into the thoracic aorta for arterial bleeding and for the aortic flush. The right external jugular vein was cannulated with a multiple-holed 18-Fr cannula, which was advanced to the level of the right atrium, for venous bleeding and for venous return to the CPB system.

Arterial and central venous pressures and electrocardiographic activity were continuously recorded on a polygraph (Grass Model 7D Polygraph, Quincy, MA). Pulmonary artery pressure, pulmonary artery occlusion pressure, cardiac output, arterial and mixed venous blood gases, hemoglobin, hematocrit, sodium, potassium, glucose, and lactate were measured at regular intervals. Just before start of the insult, Tty was controlled at 37.5 ± 0.1°C by heating blanket and lamp.

Study Protocol.

Insult. After two baseline measurements, heating devices, IV fluids, and halothane were discontinued, while the dogs were weaned to spontaneous breathing of N_2O:O_2, 75%:25% via a T-tube. When the canthal reflex returned, hemorrhage was initiated. Over a 5-minute period the dogs were bled via the arterial and venous cannulae, and the blood was collected in bags with sodium citrate for later reinfusion. The hemorrhage was controlled to achieve a mean arterial pressure (MAP) of 40 mm Hg at 2 minutes, 30 mm Hg at 3 minutes, and 20 mm Hg at 4 minutes. At 5 minutes, to assure zero blood flow, ventricular fibrillation (VF) was induced with a transthoracic

shock of 110 volts AC, 60 Hz, for 2 seconds, repeated as needed. Total arrest time (no-flow) was 20 minutes.

Aortic Arch Flush. Two minutes after the onset of CA, the balloon of the aortic catheter was inflated with NSS 1.5 mL to occlude the aorta. NSS 500 mL, at exactly 24°C (group 1) or 4°C (group 2), was then flushed into the aortic arch over 60 seconds, using a roller pump. After the flush, during CA, the aortic catheter was replaced by a short arterial CPB cannula (7 or 8 Fr) to allow better flow than can be achieved via the narrow lumen of the long balloon catheter.

Resuscitation. Reperfusion after CA was with CPB, because standard cardiopulmonary resuscitation cannot reliably achieve restoration of spontaneous circulation (ROSC) from CA of 20 minutes.[6] The circuit was primed with 400 mL of dextran 40 10% plus Ringer's solution (50:50%). Sodium bicarbonate (2 mEq/kg) and heparin (1,500 units) were added. Just before the start of CPB, additional heparin (1,500 units) and sodium bicarbonate (2 mEq/kg) were injected into the circuit. The dogs were paralyzed with pancuronium (0.1 mg/kg). Cardiopulmonary bypass was started with a flow of 100 mL/kg/min, and reinfusion of the shed blood was titrated to achieve a central venous pressure (CVP) of 10–15 mm Hg. Repetitive doses of epinephrine (0.01 mg/kg) were given if necessary to increase the MAP to 100 mm Hg. Starting at approximately 2 minutes, defibrillation attempts were with external DC countershocks of 150 J, increased by 50 J for repeated shocks. Oxygen flow through the oxygenator was adjusted to keep $PaCO_2$ at 30–35 torr. Controlled ventilation at a rate of 8–10 breaths/min was resumed to prevent atelectasis and maintain $PaCO_2$ at 30–35 torr and $PaO_2 \geq 100$ torr after ROSC. The IV fluids were restarted at 100 mL/h. A base deficit of >6.0 mEq/L was treated with sodium bicarbonate. When ROSC was established, a norepinephrine infusion was titrated IV to achieve a brief hypertensive bout of MAP > 150 mm Hg, followed by MAP controlled at 90–150 mm Hg. The CPB flow rate was reduced to 75 mL/kg/min at 60 minutes, to 50 mL/kg/min at 90 minutes, and was stopped at 120 minutes. During CPB, activated clotting times were maintained at >300 seconds with additional heparin as needed.

Intensive Care. After weaning from CPB, controlled ventilation was continued to 20 hours with $N_2O:O_2$, 50:50%. The dogs were paralyzed with pancuronium. For suspected pain (mydriasis, tachycardia, hypertension), fentanyl 5–10-μg/kg boluses were given IV. Hypotension (MAP < 90 mm Hg) was treated with Ringer's solution IV or with titrated norepinephrine. Once analgesia was assured, severe hypertension (MAP > 150 mm Hg) was controlled with boluses of labetalol (0.25–0.5 mg/kg) or hydralazine (0.1–0.2 mg/kg). At 20–24 hours, paralysis was reversed with neostigmine (0.05 mg/kg) plus atropine (0.025 mg/kg) IV, the dogs were extubated, the catheters were removed, and the dogs were transferred to a stepdown intensive care unit for continuous observation and life support by technicians and critical care physicians to 72 hours. Seizures, running movements, or opisthotonos were controlled with diazepam (0.2–0.3 mg/kg IV per bolus). Tympanic membrane temperature was controlled at 34°C with external cooling and warming for the first 12 hours after the start of CPB, and at 37.5°C until 72 hours.

Outcome Evaluation. The methods used in this study for the evaluation of function and cerebral morphologic changes have been described.[15] Overall performance was evaluated according to overall performance categories (OPC 1 = normal; 2 = moderate disability; 3 = severe disability; 4 = coma; and 5 = death). Neurologic function was evaluated as neurologic deficit scores (NDS 0–10% = normal, 100% = brain death). The OPC and NDS were evaluated every eight hours after extubation. The final evaluations at 72 hours were independently recorded and agreed upon by two team members to diminish potential observer bias when subjective assessments of behavior are performed. If necessary, sedation was reversed with flumazenil (0.1 mg, repeated if needed).

After final outcome evaluation, for morphology studies, the dogs were reanesthetized and sacrificed by infusing approximately 2 liters of paraformaldehyde (3%, pH 7.4) into the thoracic aorta via a left thoracotomy. We performed a complete necropsy, including macroscopic staging of damage in gut and heart (mild—moderate—severe hemorrhage—necrosis). The brain was removed after one hour of fixation.[15] After cutting 3-mm thick slices, the same six slices of each brain were paraffin-embedded, cut into sections 4 microns thick, and stained with hematoxylin-eosin-phloxine. Using light microscopy, a pathologist, unaware of treatment assignments, then scored 19 distinct anatomic brain regions for severity and extent of ischemic neuronal changes, infarcts, and edema, as described previously.[15] The total numeric histologic damage score (HDS) was the sum of all area scores. An HDS of >30 represents significant damage, and >100 represents severe damage.

Data Analysis. The dogs that did not follow protocol or died from extracerebral causes were excluded. Brain death was included as cerebral outcome. Data are given as mean ± standard deviation (±SD) or the median and interquartile

TABLE 1. Physiologic Variables at Baseline and Six Hours after Restoration of Spontaneous Circulation (ROSC) for Group 1 (24°C Aortic Arch Flush, $n = 6$) and Group 2 (4°C Aortic Arch Flush, $n = 6$)*

	Baseline				6 Hours after ROSC			
	Group 1		Group 2		Group 1		Group 2	
Heart rate (beats/min)	140	(120–154)†	110	(100–130)†	145	(128–155)†	105	(98–113)†
Mean arterial pressure (mm Hg)	108	(98–118)	96	(92–106)	148	(134–158)	148	(134–151)
pH	7.37	(7.35–7.38)	7.37	(7.36–7.39)	7.38	(7.36–7.42)	7.38	(7.35–7.41)
PaO_2 (torr)	264	(258–290)	265	(254–273)	301	(218–309)	285	(272–292)
$PaCO_2$ (torr)	33	(31–35)	35	(34–40)	35	(33–40)	39	(36–41)
Hematocrit (%)	36	(33–44)	34	(32–37)	36	(35–40)†	29	(28–31)†
Base excess (mEq/L)	−5.5	(−6.7–−3.0)	−2.8	(−5.2–−1.6)	−2.4	(−3.6–−0.9)	−1.6	(−2.1–−0.7)
Serum sodium (mmol/L)	146	(144–151)	145	(143–148)	153	(149–154)	151	(150–152)
Serum potassium (mmol/L)	3.5	(3.3–3.7)	3.8	(3.6–3.9)	2.9	(2.7–3.6)	3.5	(3.3–3.9)
Blood glucose (mg/dL)	168	(139–202)	155	(150–203)	218	(190–244)	172	(148–263)
Blood lactate (mmol/L)	3.1	(1.7–3.9)	3.2	(1.8–3.9)	4.5	(3.7–5.4)	3.8	(3.3–4.7)

*Data are given as median and IQR (interquartile range).
†$p < 0.05$ comparing group 1 and group 2.

TABLE 2. Resuscitation Requirements for Restoration of Spontaneous Circulation (ROSC) for Group 1 (24°C Aortic Arch Flush, $n = 6$) and Group 2 (4°C Aortic Arch Flush, $n = 6$)*

	Group 1		Group 2		p-value
Countershocks, total number	3	(1–7)	1	(1–2)	0.18
Countershocks, total energy (J)	475	(150–1,525)	150	(150–350)	0.18
ROSC (min after start of CPB)	7	(5–9)	3	(3–5)	0.03
Total epinephrine (mg)	0.5	(0.4–0.7)	0.6	(0.3–1.1)	0.82
Total norepinephrine (mg)	2.4	(1.5–3.7)	1	(0.8–1.6)	0.02
Total bicarbonate (mEq)	142	(129–155)	123	(100–129)	0.02
Hypertensive bout: peak MAP (mm Hg)	183	(168–196)	176	(164–186)	0.59
Hypertensive bout: duration† (min)	3	(2–11)	2	(1–20)	0.49

*Data are given as median and IQR (interquartile range); CPB = cardiopulmonary bypass; MAP = mean arterial pressure.
†Duration of bout = time with MAP >150 mm Hg.

range (IQR = the difference between the 25th and 75th percentiles) unless otherwise specified. We used the independent-samples t-test or the Mann-Whitney U test for the comparison of continuous variables and the chi-squared test for trend to test proportions between groups. To account for the change of temperature over time, we calculated the area under the (temperature) curve (AUC). All data were computed with SPSS for Windows, release 8.0 (Chicago, IL). A p-value < 0.05 was considered statistically significant.

In a previous dog study, a sample size of six dogs in each group achieved 100% power to detect an absolute difference in NDS of 40% (41 ± 12%, which represents severe damage, vs 1 ± 1%, which is considered as normal) with a significance level (alpha) of 0.05 using a two-sided two-sample t-test. Therefore, a sample size of seven dogs in each group was considered to be sufficient for this study.

RESULTS

Of the 14 dogs, two had to be excluded from outcome evaluation. One dog in the 24°C group 1 died with irreversible pulmonary edema at 24 hours. One dog in the 4°C group 2 had to be excluded because of human error with aortic flush. Six dogs in each group survived to 72 hours. The dogs in group 1 weighed 24 kg (IQR 23–25); those in group 2 weighed 24 kg (IQR 23–26) ($p = 1.0$). There was no group difference in extracerebral physiologic variables important for cerebral recovery, either at baseline or at 6 hours after ROSC, except heart rate, which was higher in group 1 at baseline and at 6 hours, and hematocrit, which was higher in group 1 at 6 hours (Table 1).

For resuscitation from CA of 20 minutes (Table 2), there was no difference in required number and energy of countershocks, and total epinephrine dose. The time to achieve ROSC was longer in group 1. Total norepinephrine and bicarbonate requirements were higher in group 1. The peak MAPs and durations of the hypertensive bout (time with MAP > 150 mm Hg) were similar between the two groups.

The tympanic membrane temperature just prior to the insult was 37.5°C (SD ± 0.1) in both groups. The 24°C flush in group 1 decreased Tty during CA to 35.7°C (SD ± 0.2), whereas the 4°C flush in group 2 decreased Tty to 34.0°C (SD ± 1.1) ($p = 0.005$). The Tty changes over time (AUCs) dur-

ing arrest were different between the two groups (Fig. 1). The lowest Tty during CA for each dog is shown in Table 3. The lowest Tpa during arrest was 32.0°C (SD ± 1.3) in group 1 vs 27.6°C (SD ± 1.8) in group 2 (p = 0.003). The lowest Tes was 35.7°C (SD ± 2.0) in group 1 vs 31.2°C (SD ± 2.2) in group 2 (p = 0.005). The Tr did not vary from baseline values during arrest in either group.

At normothermia, brain temperature is well reflected by Tty.[16] When deep hypothermia is rapidly induced during CPB, large temperature gradients between the brain and Tty are found, from 3.9°C below to 6.7°C above brain temperature.[17] In pilot experiments, the 24°C aortic arch flush used in this study decreased brain temperature (measured with a tissue probe) to approximately 34°C (Tty 36°C), whereas the 4°C flush decreased brain temperature to approximately 30°C (Tty 34°C). In the 72-hour outcome series we did not measure brain temperature directly because of the risk of bleeding into the brain, particularly during anticoagulation.

Outcome. All 12 dogs could be weaned from CPB at 2 hours and extubated at 24 hours. Final OPCs at 72 hours were better in group 2, with 4°C aortic arch flush, than in group 1, with 24°C aortic arch flush (p = 0.008) (Table 3). Four of the six dogs after 24°C flush remained comatose (OPC 4), whereas four of the six dogs after 4°C flush achieved normality (OPC 1).

TABLE 3. Final 72-hour Outcomes after Exsanguination Cardiac Arrest of 20-minute No-flow for Each Dog

	Tty (°C)*	OPC†	NDS (%)‡	Total HDS§
Group 1¶				
Dog 1	35.6	2	8	56
Dog 2	35.4	3	51	136
Dog 3	35.9	4	61	64
Dog 4	35.8	4	63	136
Dog 5	35.9	4	65	140
Dog 6	35.4	4	67	124
Group 2¶				
Dog 1	34.2	1	0	4
Dog 2	33.7	1	0	16
Dog 3	33.8	1	5	12
Dog 4	32.2	1	5	32
Dog 5	35.6	2	19	64
Dog 6	34.4	3	49	52

*Tty = lowest tympanic membrane temperature during cardiac arrest (p = 0.005).
†OPC = overall performance category (1 = normal, 5 = brain death) (p = 0.008).
‡NDS = neurologic deficit score (0–100%) (p = 0.01).
§HDS = total brain histologic damage score (p = 0.008).
¶Group 1: 24°C aortic arch flush (*n* = 6); group 2: 4°C aortic arch flush (*n* = 6).

Final NDSs at 72 hours were also better in group 2 than in group 1 [5% (IQR 0–19) vs 62% (IQR 40–66), p = 0.01]. Four dogs in group 2 vs one in group 1 had NDS 0–10% ("normal"); only two dogs in group 2 had NDS 0% (Table 3).

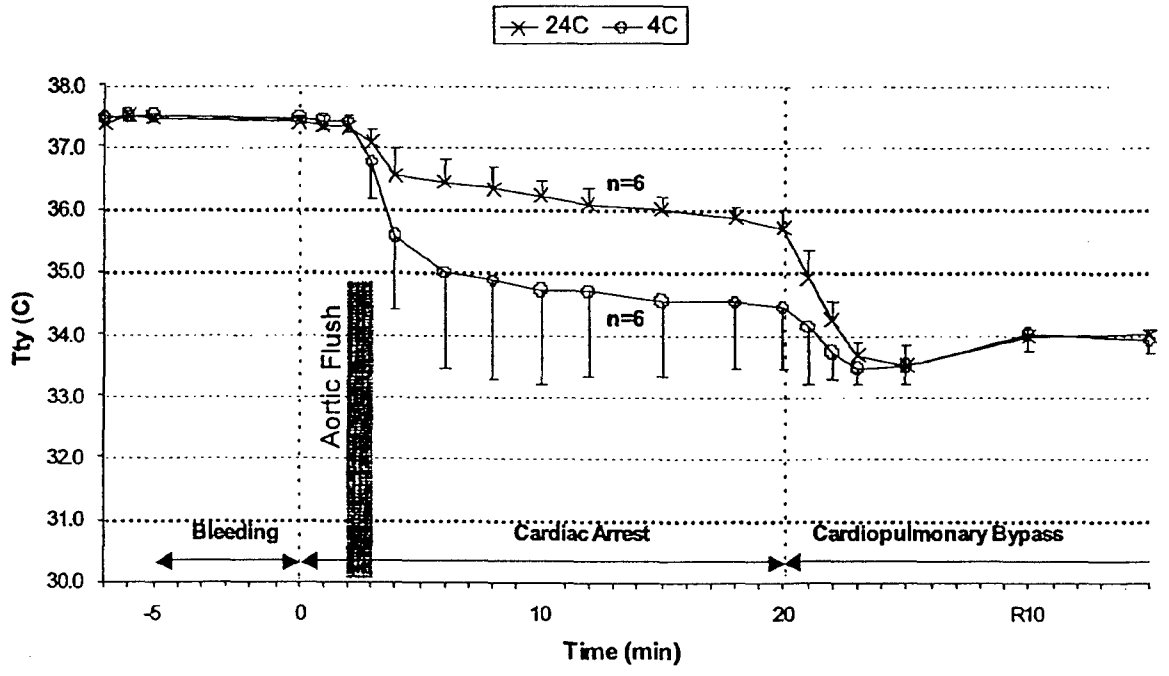

Figure 1. Tympanic membrane temperature (Tty) during exsanguination cardiac arrest and resuscitation with cardiopulmonary bypass. Aortic flush was with 500 mL normal saline solution at 24°C vs 4°C. R = resuscitation time. Temperature areas under the curve during arrest are significantly different between groups (p = 0.037).

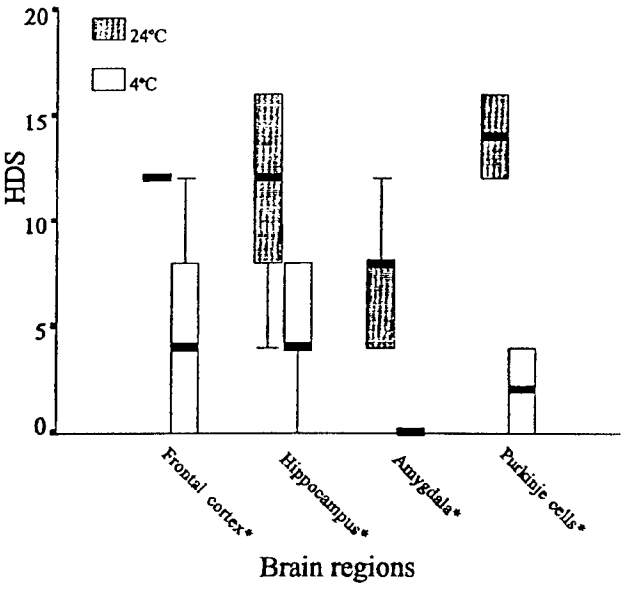

Figure 2. Regional histologic damage score (HDS). The box represents the interquartile range. The line across the box indicates the median. The whiskers are the highest and lowest values, excluding outliers; *$p < 0.05$; (frontal cortex in the 24°C group: all values are 12; amygdala in the 4°C group: all values are 0).

At necropsy, all the animals in the 4°C flush group 2 were macroscopically normal, except one dog, which had areas of mild hemorrhage in the gut mucosa. In the 24°C flush group 1, all dogs had areas of mild to moderate hemorrhage in the gut, and pale or hemorrhagic foci in the epicardial and endocardial surfaces of the heart. Total brain HDSs were higher in group 1 than in group 2 [130 (IQR 62–137) vs 24 (IQR 10–55), $p = 0.008$]. Three of the six dogs in group 2 had near-normal histologic findings. None had histologically entirely normal brains; i.e., total HDS = 0 (Table 3). Regional brain HDSs in the frontal cortex, hippocampus, dentate gyrus, caudate nucleus, putamen, amygdala, thalamus, and Purkinje cells were significantly ($p < 0.05$) higher in group 1 than in group 2 (partly shown in Fig. 2). The globus pallidus, midbrain, substantia nigra, pons, medulla, and dentate nucleus did not have histologic damage in either group.

DISCUSSION

This study is part of a U.S. Navy-sponsored systematic program to ultimately document—in dog outcome models—suspended animation of 2 hours,[1,11,12] rapidly induced with a method feasible in the field.[14] The results of this study document the feasibility of inducing preservative (mild) hypothermia in dogs via aortic arch flush within 1–2 minutes of the start of prolonged CA. The group 2 aortic arch flush of 4°C with a fluid volume of 500 mL in dogs (equivalent to 2 L in an adult human) induced mild cerebral hypothermia within 2 minutes, and thereby achieved near-normal functional outcome after 20-minute CA. The flush volume used could be carried by a medic in the field. A lightweight device for cooling to 4°C and a technique for rapid vascular access remain to be developed.

With exsanguination CA of 15 minutes in dogs, a 24°C NSS flush gave functionally normal outcome with some histologic brain damage, whereas a 4°C aortic arch flush led to normal survival with histologically normal brains.[14] In the current study the arrest time was extended to 20 minutes, an insult that is not survivable at normothermia without brain damage.[6,18] This study documented that this model is appropriate for cerebral resuscitation research; i.e., the control group survives to 72 hours with brain damage. Using the aortic arch flush at 24°C as in group 1, we are currently evaluating use of preservation drugs for potentially replacing or augmenting cooling.

The objectives of suspended animation include: 1) helping to save victims of temporarily uncontrollable (internal) traumatic exsanguination (combat casualties[18] and civilian trauma victims[19]) without severe brain trauma, by enabling evacuation and resuscitative surgery during circulatory arrest, to be followed by delayed resuscitation; 2) helping in everyday emergency medical services (EMS) to save some seemingly unresuscitable victims of nontraumatic sudden death[20]; and 3) enabling selected elective surgical procedures to be performed that are feasible only during a prolonged state of no blood flow.[21] In 1988–1994, six outcome studies in dogs were conducted with severe hemorrhagic shock (HS) followed by suspended animation, induced and reversed by CPB with plasma substitute, using deep (10–20°C) or profound (5–10°C) hypothermic circulatory arrest (DHCA, PHCA).[11,12] In one dog study of HS of 60 minutes followed by suspended animation with PHCA of 60 minutes, survival without brain damage was achieved.[11] In another study, HS of 30 minutes and PHCA of 120 minutes were followed by survival with brain damage.[12]

Inducing mild hypothermia before or during arrest, usually not possible clinically, can be expected to offer greater benefit than induction after reperfusion.[22] The 4°C flush decreased Tty to about 34°C, while the 24°C flush decreased Tty only to about 35.7°C. This small but statistically significant difference in Tty during arrest seems to be sufficient to preserve the brain during 20-minute no-flow, as reflected by significantly improved OPC, NDS, and HDS in the 4°C flush group. This confirms earlier findings that small changes in in-

tra-ischemic brain temperature can markedly improve histologic damage after cerebral ischemia.[23]

With CA no-flow of 20 minutes or longer, the heart needs to be protected in order to support long-term survival.[6,13,24] With the 24°C flush, four of six dogs had mild to moderate hemorrhagic damage of the heart. In contrast, with the 4°C flush, all dogs had macroscopically normal hearts. The macroscopic damage in group 1 was reflected by a greater need for norepinephrine to maintain MAP after ROSC. We also observed moderate hemorrhagic damage of the gut in four dogs in the 24°C flush group 1. We doubt that there was direct hypothermic protection of the gut, since the balloon was inflated above the splanchnic vessels and since, in all dogs, rectal temperature did not deviate from baseline during arrest. We suspect that the protective effect of the colder flush for the intestines was due to improved hemodynamics with a lower requirement for norepinephrine to maintain MAP.

The median time to achieve ROSC after start of CPB was 7 minutes (IQR 5–9) in group 1 and 3 minutes (IQR 3–5) in group 2 ($p = 0.03$). An MAP > 100 mm Hg was achieved within 1 minute after start of CPB in both groups. We doubt that the longer time to achieve ROSC had influence on neurologic outcome, since the no-flow and low-flow times were similar and we know from clinical experience that prolonged CPB does not result in neurologic deficit. Group 2 also required a higher dose of norepinephrine to keep MAP above 100 mm Hg during CPB, and more bicarbonate to keep base excess in the normal range (Table 2). A deleterious effect on histologic damage by norepinephrine and bicarbonate cannot be ruled out, although this is unlikely.

From 24 hours to 72 hours, all the dogs in group 1 received diazepam to treat seizures, running movements, and opisthotonos due to brain damage; the median amount was 154 mg (IQR 78–194). In group 2, only one dog received a total diazepam dose of 70 mg. There is no hint in the literature that diazepam adversely affects cerebral outcome after cardiac arrest. Sedation was reversed before final evaluation.

Clinical Relevance

The goal for induction of suspended animation is to buy time until CPB can be initiated in the field or in the hospital ED.[25] Induction of suspended animation could be initiated by medics in the field by flushing a cooled solution into the aorta in hopes of achieving this goal. Therefore, the use of an aortic balloon catheter was introduced in our study. With the balloon inflated in the descending thoracic aorta during flush, the two organs most vulnerable during cardiac arrest—the brain and the heart—can be selectively perfused. Our study suggests that this method can be applied quickly and provide normal neurologic recovery with minimal histologic damage.

Limitations and Future Questions

Although, in the present study, a 4°C flush achieved good NDS, good OPC 1, and only mild histologic brain damage, a limitation of this study was the lack of testing of cognitive function, such as spatial learning and memory. We cannot rule out subtle brain damage or even delayed loss of neurons after 72 hours. In future definitive studies of suspended animation, by flush and ultraprofound hypothermic arrest, we plan to perform sophisticated cognitive function tests in two-month survivors.

The most significant limitation of this work is the clinical scenario on which it is modeled. In the field, reliable access to the central arterial circulation within 2 minutes is challenging. Cannulation of the femoral artery percutaneously has been shown to be feasible and rapid.[26,27] For rapid vessel access in the field, we are exploring better approaches to the femoral vessels, a thoracotomy approach, and a new parasternal approach. Portable CPB is not yet available in the field. We are urging industry to develop a portable CPB system that could be used outside the hospital; a prototype is used for our experiments.[25] However, the first clinical feasibility trials of a cold aortic flush are being considered in major trauma centers for patients arriving in the ED pulseless from exsanguination. Resuscitation of these patients often includes a thoracotomy, which could provide quick access to the aorta. The Shock–Trauma Center in Baltimore receives around 80 patients/year who could possibly benefit from suspended animation (Champion H, personal communication, 1999).

The complete functional recovery achieved in the current study required Tty 34°C, and was not possible with Tty decreased to only approximately 36°C with the NSS flush at ambient temperature (24°C). The latter would be more feasible in combat situations. The 4°C solution could be available in civilian EMS, but large volumes are required to provide preservation for arrest of more than 20 minutes' duration, as shown in another dog study, in which a 4°C aortic flush with 100 mL/kg could preserve the brain for a no-flow duration of 30 minutes.[28]

Conclusions

We conclude that, in dogs, aortic arch flush at the start of exsanguination CA of 20-minute no-flow,

using NSS 500 mL at 4°C, induces mild cerebral hypothermia within 2 minutes, which can result in normal functional recovery with minimal histologic brain damage. Using the same flush at ambient temperature (24°C), achieving survival without histologic brain damage after CA 20 minutes or longer will require additional pharmacologic strategies. This study's 24°C flush model, which achieves consistent survival *with* brain damage, is suitable for testing pharmacologic preservation–resuscitation potentials.

Patrick Kochanek, MD, made valuable suggestions. Rochelle Hans, Carol Korbanik, Jason Stezoski, and Sherman Culver helped with ICU life support. Howell Sasser, PhD, advised on statistical analyses. Patricia Boyle helped with editing. Valerie Sabo helped with preparation of the manuscript. The Cardeon Corp. (Cupertino, CA) provided the flush catheter.

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Anesthesiology 2000; 93:1491-9

Rapid Hypothermic Aortic Flush Can Achieve Survival without Brain Damage after 30 Minutes Cardiac Arrest in Dogs

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Background: **Neither exsanguination to pulselessness nor cardiac arrest of 30 min duration can be reversed with complete neurologic recovery using conventional resuscitation methods. Techniques that might buy time for transport, surgical hemostasis, and initiation of cardiopulmonary bypass or other resuscitation methods would be valuable. We hypothesized that an aortic flush with high-volume cold normal saline solution at the start of exsanguination cardiac arrest could rapidly preserve cerebral viability during 30 min of complete global ischemia and achieve good outcome.**

Methods: **Sixteen dogs weighing 20–25 kg were exsanguinated to pulselessness over 5 min, and circulatory arrest was maintained for another 30 min. They were then resuscitated using closed-chest cardiopulmonary bypass and had assisted circulation for 2 h, mild hypothermia (34°C) for 12 h, controlled ventilation for 20 h, and intensive care to outcome evaluation at 72 h. Two minutes after the onset of circulatory arrest, the dogs received a flush of normal saline solution at 4°C into the aorta (cephalad) via a balloon catheter. Group I (n = 6) received a flush of 25 ml/kg saline with the balloon in the thoracic aorta; group II (n = 7) received a flush of 100 ml/kg saline with the balloon in the abdominal aorta.**

Results: **The aortic flush decreased mean tympanic membrane temperature (Tty) in group I from 37.6 ± 0.1 to 33.3 ± 1.6°C and in group II from 37.5 ± 0.1 to 28.3 ± 2.4°C ($P = 0.001$). In group I, four dogs achieved overall performance category (OPC) 4 (coma), and 2 dogs achieved OPC 5 (brain death). In group II, 4 dogs achieved OPC 1 (normal), and 3 dogs achieved OPC 2 (moderate disability). Median (interquartile range [IQR]) neurologic deficit scores (NDS 0–10% = normal; NDS 100% = brain death) were 69% (56–99%) in group I *versus* 4% (0–15%) in group II ($P = 0.003$). Median total brain histologic damage scores (HDS 0 = no damage; >100 = extensive damage; 1,064 = maximal damage) were 144 (74–168) in group I *versus* 18 (3–36) in group II ($P = 0.004$); in three dogs from group II, the brain was histologically normal (HDS 0–5).**

Conclusions: **A single high-volume flush of cold saline (4°C) into the abdominal aorta given 2 min after the onset of cardiac arrest rapidly induces moderate-to-deep cerebral hypothermia and can result in survival without functional or histologic brain damage, even after 30 min of no blood flow. (Key words: Cardiopulmonary bypass; cerebral preservation; hemorrhage; ischemia; resuscitation.)**

NORMOTHERMIC cardiac arrest (CA; *i.e.*, temporary complete global brain ischemia) lasting 5 min or longer and reversed by standard resuscitation is almost invariably followed by brain damage.[1-5] Hypothermia induced before arrest (protection) is more likely to mitigate postischemic brain damage than when induced after arrest (resuscitation).[2,4,6] This study explored hypothermia induced during arrest (preservation). After normothermic CA of longer than 10 min in dogs, restoration of spontaneous circulation requires emergency cardiopulmonary bypass (CPB).[7] We suspect that many military or civilian victims of traumatic exsanguination[8] and presently unresuscitable patients with normovolemic sudden cardiac death[9] could be saved with rapid preservation of brain and heart to buy time for transport, repair, and resuscitation with CPB. About one half of out-of-hospital cardiopulmonary-cerebral resuscitation attempts for normovolemic CA fail to restore heartbeat,[9] and many long-term survivors suffer permanent brain damage.[2] For sudden cerebral ischemia, the rapid loss of energy,[5] disappointing pharmacologic cerebral resuscitation trials,[2,4] and the benefits from even mild preservative and resuscitative cerebral hypothermia[2,3,6,10-12] have been reported.

In 1984, Bellamy and Safar,[8] considering combat casualties killed in action, recommended research into rapid induction of preservation of the organism for transport and surgical hemostasis without pulse, to be followed by delayed resuscitation to survival without brain damage. Using dog outcome models of exsanguination CA and CPB for the induction of profound hypothermia (5–10°C), we achieved cerebral preservation during complete circulatory arrest of 60 min,[13] but not 120 min.[14] We have recently documented effective rapid induction of cerebral hypothermia without CPB, using aortic arch cold flush to induce hypothermic preservation within 5 min of no blood flow for CA 15 min[15,16] or CA 20 min.[17]

We hypothesized[18] that flushing the aorta (and, hence, the brain) with normal saline at 4°C immediately after the onset of CA of 30 min can achieve survival without brain damage, and that the cold flush must include the spinal cord and abdominal viscera, which cannot tolerate normothermic ischemia of longer than 20 min.[17]

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Received from the Safar Center for Resuscitation Research of the University of Pittsburgh, Pittsburgh, Pennsylvania. Submitted for publication January 28, 2000. Accepted for publication August 10, 2000. Supported by grant No. N00014-99-1-0765 from the United States Office of Naval Research, Arlington, Virginia. Presented in part at the annual meeting of the American Society of Anesthesiologists, Dallas, Texas, October 9 through 12, 1999, and at the 72nd scientific session of the American Heart Association, Atlanta, Georgia, November 7–10, 1999.

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Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh, Pittsburgh, Pennsylvania, and followed United States national guidelines for the treatment of animals. Sixteen male custom-bred hunting dogs, 8–12 months of age and with a body weight of 20–25 kg, were used. All experiments were performed by the same team within 1 y in mixed sequence without systematic randomization.

Preparation

The dogs were fasted overnight with free access to water. After premedication with 10 mg/kg ketamine intramuscularly, anesthesia was induced with 50%:50% N_2O:O_2 and 2–4% halothane *via* mask. After tracheal intubation, the dogs were mechanically ventilated with tidal volumes of 15 ml/kg and positive end-expiratory pressure of 5 cmH_2O, without paralysis. Anesthesia was continued with nitrous oxide and 0.5–1.5% halothane titrated to sustain normotension. The ventilatory rate was adjusted to achieve normocapnia (arterial partial pressure of carbon dioxide [$Paco_2$], 35–40 mmHg), with an end-tidal carbon dioxide level of 4–5%. Electrocardiogram electrodes were attached to the extremities, and a pulse oximeter probe was placed on the tongue. Gastric and bladder catheters were inserted. Temperature probes were inserted for measuring tympanic membrane (Tty), esophageal (Tes), and rectal temperatures (Tr). Dextrose, 5%, in 5 ml · kg^{-1} · h^{-1} NaCl, 0.45%, was administered *via* a peripheral intravenous line to maintain central venous pressure at a level higher than 3 mmHg.

A PE 90 catheter (Becton, Dickinson Co., Parsippany, NJ) was surgically inserted into the left femoral artery for monitoring of arterial pressure and blood sampling. A 7.5-French balloon catheter (Intellicath Continuous Cardiac Output Thermodilution Catheter; Baxter Co., Irvine, CA) was inserted *via* the left femoral vein into the pulmonary artery for pressure monitoring, continuous cardiac output determination (Vigilance Monitor software 4.42, Baxter Co., Irvine, CA), temperature measurements (Tpa), and blood sampling. The right femoral artery was cannulated with a prototype 8-French catheter with one hole at the distal end (Cardeon Corp., Cupertino, CA) and an inflatable balloon 1 cm from the tip which, when inflated with 1.0–1.5 ml saline, occluded the aorta (as determined by disappearance of femoral artery pressure). The catheter had an internal diameter of 2.24 mm. To verify that the balloon was placed in the abdominal or descending thoracic aorta, the length was marked prior to insertion. The right external jugular vein was cannulated with a multiple-holed, spiral-reinforced, 18-French plastic cannula which was advanced to the level of the right atrium. This was used for venous bleeding and for venous return to the CPB system.

Arterial and central venous pressures and electrocardiography were continuously recorded on a polygraph (Grass Model 7D Polygraph; Astro-Med Inc., West Warwick, RI). Pulmonary artery pressure, pulmonary artery occlusion pressure, cardiac output, arterial and mixed venous blood gases, hemoglobin, hematocrit, sodium, potassium, glucose, and lactate were measured at regular intervals. Blood gases were measured at 37.5°C without correction for body temperature. Just before start of the insult, Tty was controlled at 37.5 ± 0.1°C using a heating blanket and lamp.

Cardiac Arrest

After two baseline measurements, heating devices, intravenous fluids, and halothane were discontinued while the dogs were weaned to spontaneous breathing of N_2O:O_2 75%:25% *via* an endotracheal tube. When the canthal reflex returned, hemorrhage was initiated. Over a 5-min period the dogs were bled *via* the arterial and venous cannulae into bags containing citrate. The hemorrhage was controlled to achieve mean arterial pressure (MAP) of 40 mmHg at 2 min, 30 mmHg at 3 min, and 20 mmHg at 4 min. At 5 min, ventricular fibrillation (VF) was induced with a transthoracic shock of 95–105 V alternating current at 60 Hz for 2 s, repeated as needed. Total no-flow time was 30 min.

Preservation by Aortic Flush

The flush strategies chosen were based on previous results[15–17] and pilot experiments (see Discussion). Two min after the onset of VF, the balloon of the aortic catheter was inflated with 1.0–1.5 ml saline to occlude the aorta. The aorta was flushed with normal saline solution using a roller pump. In group I, with the balloon in the thoracic aorta, 25 ml/kg of 4°C saline was infused over 1 min. In group II, with the balloon in the lower abdominal aorta, 100 ml/kg of 4°C saline was infused over 4 min. (We had found in a pilot experiment that mean carotid artery pressure during this kind of aortic arch flush is about 100 mmHg). The venae cavae were allowed to drain into bags during the aortic flush. After the flush, during CA, the aortic catheter was replaced by a short, 7- to 8-gauge arterial CPB cannula to optimize flow for later resuscitation by CPB.

Resuscitation

After CA 30 min, reperfusion was achieved with CPB.[3,7] The CPB system included a centrifugal pump (Biomedicus, Eden Prairie, MN), a hollow-fiber membrane oxygenator, and a heat exchanger (Medtronic, Anaheim, CA). The circuit was primed with 400 ml of Dextran 40 10% in saline plus Ringer's solution, 50%: 50%. Sodium bicarbonate, 2 mEq/kg, and 1500 U heparin were added. Just before start of CPB, an additional

1500 U of heparin and 2 mEq/kg sodium bicarbonate were injected into the circuit. The dogs were paralyzed with 0.1 mg/kg intravenous pancuronium. CPB was started with a flow of 100 ml · kg^{-1} · min^{-1}, and enough shed blood was reinfused to achieve a central venous pressure of 10–15 mmHg. Repetitive doses of 0.01 mg/kg epinephrine were given into the femoral artery, if necessary, to increase the CBP-generated MAP to 100 mmHg. After CPB of 2–5 min with vigorous VF, defibrillation was attempted with an external direct-current counter-shock of 150 J. If necessary, shocks were repeated at 200 J, and a maximum of 300 J. Gas flow through the oxygenator was adjusted to keep $Paco_2$ at 30–35 mmHg and arterial partial pressure of oxygen (Pao_2) greater than or equal to 100 mmHg. Controlled ventilation at a rate of 8–10 breaths/min was resumed to prevent atelectasis. CPB controlled Tty at 34°C from reperfusion to 12 h. The intravenous fluids were restarted at 100 ml/h. A base deficit of more than 6 mEq/l was corrected with sodium bicarbonate. When restoration of spontaneous circulation was established, CPB was continued for assisted circulation to 120 min, with 100 ml · kg^{-1} · min^{-1} for 60 min, 75 ml · kg^{-1} · min^{-1} for 30 min, and 50 ml · kg^{-1} · min^{-1} for 30 min. During CPB, the activated clotting time was maintained at greater than 300 s with additional heparin if needed. After restoration of circulation, norepinephrine by intravenous drip was titrated to achieve initially a brief hypertension with MAP greater than or equal to 150 mmHg, followed by MAP controlled at 90–150 mmHg. The remaining shed blood was gradually reinfused, avoiding central venous pressure higher than 15 mmHg.

Intensive Care

Controlled ventilation was continued to 20 h with N_2O:O_2 50%:50%. The dogs remained paralyzed with pancuronium until 20 h to assure a steady state of cardiovascular–pulmonary variables. For analgesia in case of suspected "stress" (mydriasis, tachycardia, hypertension), fentanyl boluses of 50 or 100 μg per dog were titrated intravenously to maintain the pupils at small size and to help reverse severe hypertension (MAP ≥ 150 mmHg) and tachycardia. More potent anesthesia was avoided because it can influence ischemic brain damage. In the dogs of this study, there were no painful stimuli during the 20 h paralysis after CA. There was also residual postischemic cerebral depression evident in all dogs at 20 h (evident after stopping all anesthesia and paralysis). Nevertheless, to be certain that this anesthetic regimen was sufficient,[19] two dogs without ischemia and without paralysis were intubated under brief, light halothane and were then ventilated with N_2O:O_2 50:50%. Whenever movement, reaction to the endotracheal tube, or widening of pupils occurred, intravenous boluses of 100 μg fentanyl were given. As expected, the required doses were greater in normal dogs than in the post-CA dogs: namely, 100 μg/dog about every 15 min. The canthal reflex also remained active. Nevertheless, there was no purposeful escape behavior and no movement on paw pinch.

After resuscitation, hypotension (MAP < 90 mmHg) was treated with intravenous Ringer's solution or titrated norepinephrine. After analgesia was assured by the presence of small pupils, severe hypertension (MAP > 150 mmHg) was also controlled with boluses of 0.25–0.5 mg/kg labetalol or 0.5–1.0 mg/kg hydralazine. For infection prophylaxis, the dogs received 250 mg cefazolin intravenously every 8 h. Respiratory care included rotation, suctioning, and "sighing" at regular intervals. At 20–24 h after resuscitation, paralysis was reversed with 0.05 mg/kg neostigmine plus 0.025 mg/kg atropine administered intravenously. Weaning to spontaneous breathing was accomplished *via* endotracheal tube. The dogs were extubated when they were able to maintain normal Pao_2 and $Paco_2$ with spontaneous breathing, upper airway reflexes had returned, and circulation was stable. When dogs appeared awake, the catheters were removed under brief, light nitrous oxide–halothane anesthesia by mask, and the dogs were transferred to a stepdown area in the intensive care unit for continuous monitoring and life support by technicians and critical care physicians. Suspected discomfort (howling, restlessness), seizures, running movements, or opisthotonos were controlled with titrated intravenous 0.2-to 0.3-mg/kg boluses of diazepam. For diazepam requirements, see Results. Tty was controlled with external cooling and warming at 34°C to 12 h[12] and at 37.5°C from 12–72 h.

Outcome Evaluation

Function. The methods used for the evaluation of outcome in terms of function until 72 h and morphology at 72 h have been described elsewhere.[20–23] Performance was evaluated according to overall performance categories (OPC) 1–5, where OPC 1 = normal; 2 = moderate disability; 3 = severe disability; 4 = coma; and 5 = brain death or death. Neurologic function was evaluated as neurologic deficit scores (NDS) 0–100%, where NDS 0–10% = normal and 100% = brain death. NDS included level of consciousness, breathing pattern, cranial nerve function, sensory and motor function, and behavior. Beginning 24 h after resuscitation, OPC and NDS were evaluated in dogs weaned from paralysis and fentanyl, and evaluation was continued every 8 h. The final evaluations at 72 h were independently recorded and agreed upon by two team members. Attempts were made to discontinue any sedation at least 4 h prior to final evaluation. If necessary, diazepam effect was reversed with 0.1 mg flumazenil intravenously, repeated as needed.

Morphology. After functional outcome evaluation, the dogs were reanesthetized in the same manner as

initially. The left hemithorax was opened, and the proximal descending aorta was ligated. A large-bore cannula was inserted proximal to the ligature. The dogs were then euthanized by infusing approximately 2 l of paraformaldehyde 3% (pH 7.4) with a roller pump with the right atrium opened, until clear fluid returned from the vena cava. A complete necropsy was performed. Macroscopic damage in gut and heart was estimated (dark hemorrhages, pale necroses), and samples of extracerebral organs were taken for histologic examination. After 1 h of brain fixation *in situ,* the skull was sawed open and the brain was removed.[23] Each brain was cut into 3-mm-thick coronal sections, which were immersed in paraformaldehyde for further fixation. Six selected slices were paraffin-embedded, cut into sections 4 μm thick, and stained with hematoxylin–eosin–phloxine. Using light microscopy, the same pathologist (Dr. Radovsky), who was unaware of treatment assignments, scored 19 distinct anatomic brain regions (see Results) for severity and extent of ischemic neuronal changes (shrunken, eosinophilic neuron with pyknotic nucleus), infarcts, and edema.[23] For each region examined, the severity and extent of these lesions were assessed as HDS on a four-point scale, where minimal = 1+; moderate = 2+; severe = 3+; and maximal = 4+. The points were then multiplied by a weighting factor depending on the type of lesion (infarction 4×, neuronal necrosis 2×, and edema 1×). A maximal HDS of 56 per region was obtained if all three types of lesions occurred with maximal extent. Total HDS for the entire brain were obtained with a maximal possible score of 1,064 for all 19 regions combined. A total HDS of more than 100 represented extensive damage and had previously correlated with severe final NDS.[10–12,14,20–23] In two pilot experiments (see Discussion), the spinal cord was also removed and macroscopically abnormal sections were processed, stained with hematoxylin–eosin, and evaluated by light microscopy.

Statistical Analysis

Dogs that did not follow protocol or that died from extracerebral causes were excluded from analysis. Brain death was included as a cerebral outcome. Data are given as mean ± SD or the median and IQR (the difference between the 25th and 75th percentiles) unless otherwise specified. We used the independent-samples *t* test or the Mann–Whitney U test for the comparison of continuous variables (physiologic variables, final NDS, and final HDS). We used the chi-square test for trend to determine group differences of final OPC. Since our endpoint was final outcome (at 72 h), changes in OPC, NDS, and HDS over time were not statistically analyzed. To account for the change in temperature over time during arrest, we calculated the area under the temperature curve. All data were computed using SPSS for Windows, release 8.0 (SPSS Inc., Chicago, IL). A *P* value less than 0.05 was considered statistically significant.

Results

Of the 16 dogs exsanguinated to CA, three had to be excluded from outcome evaluation: in group I, one of the eight dogs died 9 h after restoration of spontaneous circulation as a result of unrecognized airway obstruction, and one died 36 h after restoration of spontaneous circulation with heart failure caused by heartworms. This left six dogs in the protocol. In group II, one of the eight dogs developed severe oropharyngeal edema of unknown cause post-CA, which made extubation and evaluation of OPC and NDS impossible. This left seven dogs in the protocol. In group I, three of the six dogs included in the protocol developed an increasing need for large doses of norepinephrine; to obtain their brains for histologic evaluation, their OPC and NDS were determined after brief weaning from controlled ventilation prior to anticipated severe hypotension, with MAP still within protocol parameters. They were then reanesthetized at 42 h, 52 h, and 60 h, respectively, for perfusion-fixation and morphologic evaluation. In group II, all seven dogs survived to 72 h.

There were no group differences in extracerebral variables important for cerebral recovery (table 1) at baseline and at 6 h after resuscitation, with the exception of central venous pressure, which was higher in group II at 6 h; lactate, which was higher in group I at 6 h; and O_2 extraction ratio, which was higher in group II at 6 h. Hematocrit immediately after resuscitation showed no significant intergroup difference (table 2).

There was no difference between groups in the required number and energy of defibrillating countershocks, total epinephrine doses, and bicarbonate requirement (table 2). The time to achieve restoration of spontaneous circulation was significantly longer and total norepinephrine requirements significantly higher in group I. The beginning of the induced brief hypertension was significantly earlier and the peak MAP significantly higher in group II *versus* group I (table 2). The duration of the hypertension (time with MAP >150 mmHg) varied greatly without intergroup difference.

Tty (fig. 1) just prior to the insult was 37.6 ± 0.1°C in group I *versus* 37.5 ± 0.1°C in group II ($P = 0.1$). Saline flush rapidly decreased Tty during CA to a minimum of 33.3 ± 1.6°C in group I *versus* 28.3 ± 2.4°C in group II ($P = 0.001$). Tty change over time (area under the curve) during CA was significantly different between the two groups (fig. 1). The lowest Tpa during CA was 28.2 ± 1.4°C in group I *versus* 20.9 ± 2.8°C in group II ($P < 0.001$). The lowest Tes was 32.9 ± 4.2°C in group I *versus* 21.1 ± 5.5°C in group II ($P = 0.002$). Tr remained

Table 1. Physiologic Variables at Baseline and 6 h after Resuscitation

	Baseline		6 h after ROSC	
	Group I	Group II	Group I	Group II
Heart rate (beats/min)	110 (108–141)	130 (120–130)	145 (120–153)	105 (90–140)
Mean arterial pressure (mmHg)	95 (94–96)	100 (90–115)	138 (125–141)	140 (130–155)
Blood glucose (mg/dl)	181 (123–288)	210 (178–221)	179 (156–298)	173 (171–195)
Pao_2 (mmHg), F_{IO_2} 0.5	270 (249–286)	279 (272–286)	309 (288–511)*	280 (218–293)
$Paco_2$ (mmHg)	36 (34–40)	34 (33–38)	37 (34–39)	38 (35–40)
Arterial pH	7.35 (7.34–7.38)	7.34 (7.31–7.37)	7.35 (7.30–7.39)	7.35 (7.34–7.39)
Blood lactate (mM)	4.1 (2.1–4.3)	3.2 (2.6–3.8)	5.9 (4.5–7.5)†	3.4 (3.0–4.3)†
Base excess (mEq/l)	−4.0 (−5.1–−2.8)	−5.2 (−5.9–−4.7)	−4.5 (−6.1–−2.8)	−2.9 (−3.9–−1.5)
Serum sodium (mM)	144 (143–147)	145 (144–147)	153 (148–157)	153 (149–164)
Serum potassium (mM)	3.5 (3.1–3.7)	3.5 (3.3–3.6)	3.0 (2.9–3.2)	2.9 (2.7–3.2)
Hematocrit (%)	37 (34–42)	34 (33–37)	43 (31–47)	34 (27–35)
Cardiac index ($l \cdot min^{-1} \cdot m^{-2}$)	4.5 (3.3–6.9)	3.4 (3.0–5.6)	3.1 (2.6–5.2)	2.3 (3.0–3.1)
Oxygen extraction ratio (%)	15 (9–19)	20 (16–27)	15 (11–22)†	28 (21–32)†

Aortic flush at the start of the arrest was in group I with 25 ml/kg saline at 4°C (n = 6) and in group II with 100 ml/kg saline at 4°C (n = 7). Data are given as median and interquartile range.

* This high 75th-percentile arterial oxygen tension (Pao_2) value, above the expected value for a fractional inspired oxygen tension (F_{IO_2}) of 0.5, was caused by measuring the value for one dog by error under an F_{IO_2} of 1.0. † $P < 0.05$ comparing group I *versus* group II.

ROSC = restoration of spontaneous circulation; $Paco_2$ = arterial carbon dioxide tension.

normothermic and did not differ from baseline values during CA.

Outcome

OPC and NDS between 24 and 72 h improved less in group I than in group II. Final OPCs were better in group II, with 100 ml/kg low aortic flush, than in group I, with 25 ml/kg high aortic flush ($P = 0.001$; table 3).

Final NDS (table 3) was 69% (IQR, 56–99%) in group I (including the three dogs that had to be killed before 72 h) *versus* 4% (IQR, 0–15%) in group II ($P = 0.003$). Four dogs in group II with OPC 1 had NDS 0–10% (*i.e.*, were normal). The slightly higher NDS in the other three dogs in group II reflected hind leg weakness and difficulty in walking or standing; however, they showed normal cerebral function and behavior.

The total dose of diazepam administered in each dog is shown in table 3. Dogs 1, 2, and 3 of group I received 0.2 mg flumazenil intravenously before evaluation of final OPC and NDS. Dogs 4, 5, and 6 of group I remained intubated under fentanyl and hence received no diazepam. In these dogs, fentanyl was reversed with naloxone shortly before euthanasia for evaluation of final OPC and NDS.

At necropsy, all dogs in group II were macroscopically normal. In group I, all dogs had moderate to severe (widespread) hemorrhagic areas in the gut mucosa and over the epicardium and endocardium.

On brain histology at 72 h, total brain HDS was 144 (IQR, 74–168) in group I *versus* 18 (IQR, 3–36) in group II ($P = 0.004$). Two dogs in group II had essentially normal brains on light microscopic examination, with total HDS of 0 and 4, respectively (table 3). Regional brain HDS (fig. 2) in frontal, parietal, occipital, and temporal cortices, hippocampus, dentate gyrus, putamen, amygdala, and thalamus were higher in group I than in group II ($P < 0.05$; fig. 2). In group II, no ischemic

Table 2. Resuscitation and Restoration of Spontaneous Circulation

	Group I	Group II	*P* Value
Countershocks, total number	1 (1–3)	3 (2–3)	0.2
Countershocks, total energy (J)	150 (150–550)	500 (300–500)	0.2
ROSC (min after start of CPB)	5 (5–7)	4 (3–4)	0.02
Total bicarbonate (mEq)	210 (158–295)	170 (130–215)	0.3
Total epinephrine (mg)	0.9 (0.5–1.7)	0.7 (0.6–1.2)	0.8
Total norepinephrine (mg)	39.3 (12.1–105.3)	1.3 (1.3–1.6)	0.004
Brief hypertension			
Start (min)*	9 (6–15)	4 (3–4)	0.005
Duration (min)†	4 (2–25)	8 (3–8)	0.6
Peak MAP (mmHg)	179 (167–194)	210 (205–220)	0.003
Hematocrit immediately after start of CPB (%)	19 (17–21)	15 (14–19)	0.08

Aortic flush at start of arrest was in group I with 25 ml/kg saline at 4°C (n = 6) and in group II with 100 ml/kg saline at 4°C (n = 7). Data are given as median and interquartile range.

* Start of hypertensive bout = time after start CPB. † Duration of hypertensive bout = time with mean arterial pressure (MAP) greater than 150 mmHg.

ROSC = restoration of spontaneous circulation; CPB = cardiopulmonary bypass.

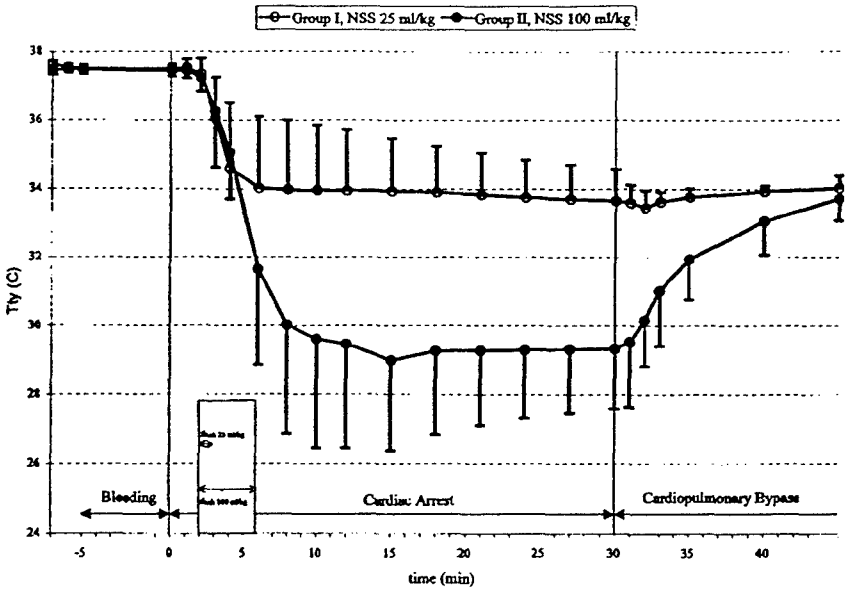

Fig. 1. Tympanic membrane temperatures (Tty) during exsanguination cardiac arrest of 30 min no-flow and resuscitation with cardiopulmonary bypass. Aortic flush with balloon catheter was with 25 ml/kg saline into the aortic arch in group I and with 100 ml/kg saline into the abdominal aorta in group II; in both cases, the saline was at a temperature of 4°C. Temperature areas under the curve during arrest were significantly different between groups ($P = 0.003$). NSS = normal saline solution.

changes were found in the most vulnerable neurons, *i.e.*, in the hippocampus (three dogs) and in the cerebellar Purkinje neurons (four dogs). In group I, the insular cortex was damaged in one dog, and the midbrain and substantia nigra were damaged in another dog. The medulla and dentate nucleus did not show any histologic damage in either group (these regions are not shown in fig. 2). Two dogs in group I and one dog in group II showed small infarcts. Spinal cord lesions in two pilot experiments are discussed later (see Discussion).

Table 3. Final 72-h Outcomes after Exsanguination and No Flow of 30 min for Each Dog

Dog No.	Tty* (°C)	Diazepam (mg)	OPC*	NDS† (%)	HDS‡
Group I					
1	33.7	50	4	54	68
2	34.7	20	4	56	160
3	33.9	45	4	60	192
4	33.4	0§	4 (60 h)	78	132
5	30.1	0§	5 (52 h)	97	76
6	33.9	0§	5 (42 h)	100	156
Group II					
1	26.5	0	1	0	NA
2	25.8	0	1	0	16
3	31.3	0	1	1	4
4	29.6	2.5	1	4	0
5	25.2	10	2‖	14‖	46
6	30.2	0	2‖	15‖	20
7	29.6	0	2‖	18‖	32

Exceptions were dogs 4, 5, and 6 of group I, which had to be terminated at 60, 52, and 42 h (see text). Dog 1 of group II was allowed to survive, was adopted, and is normal at 1 yr. Aortic flush at start of arrest was in group I with 25 ml/kg saline at 4°C (n = 6), with the tip of the balloon catheter in the thoracic aorta, and in group II with 100 ml/kg saline at 4°C (n = 7), with the tip of the balloon catheter in the abdominal aorta.

* $P = 0.001$. † $P = 0.003$. $P = 0.004$. § No diazepam required because anesthetized with fentanyl until final evaluation (overall performance category [OPC; 1 = normal, 5 = brain death] and neurologic deficit score [NDS; 0–100%] after fentanyl was reversed). ‖ Weakness of the hind legs (cerebral performance was normal).

Tty = lowest tympanic membrane temperature during cardiac arrest; diazepam = total dose required; HDS = total brain histologic damage score; NA = not available.

Discussion

This study documents that a single flush of 100 ml/kg cold saline (4°C) into the abdominal aorta (not into the thoracic aorta) *via* balloon catheter at the start of 30 min CA can allow survival without brain damage. To achieve similar results in a human, about 7 l of cold saline would be required. The mechanism is presumably related to the rapid induction of moderate to deep cerebral hypothermia (group II) at a rate much faster than could be achieved with surface cooling. This study also documents that in CA of 30 min, from which the healthy dog heart is unresuscitable under normothermia,[3,24,25] mild hypothermia (group I) is sufficient to preserve the heart's ability to beat, although severely damaged, but is insufficient for cerebral preservation. Aortic arch flush in group I left the nonflushed normothermic spinal cord and the viscera damaged.

Elective, slow, protective, pre-CA cooling has been practiced since the 1950s.[26,27] This study provides the first documentation of rapid preservative cooling during arrest. The degree of cerebral preservation seemed better than that seen previously with cooling to mild[2,3] or moderate hypothermia[26–28] induced before normovolemic CA. Normothermic aortic arch flush provided no significant preservation in dogs using the same model.[15] After normothermic VF-CA of 12.5 min, deep hypothermia by CPB does not result in better outcome than mild post-CA cooling.[10]

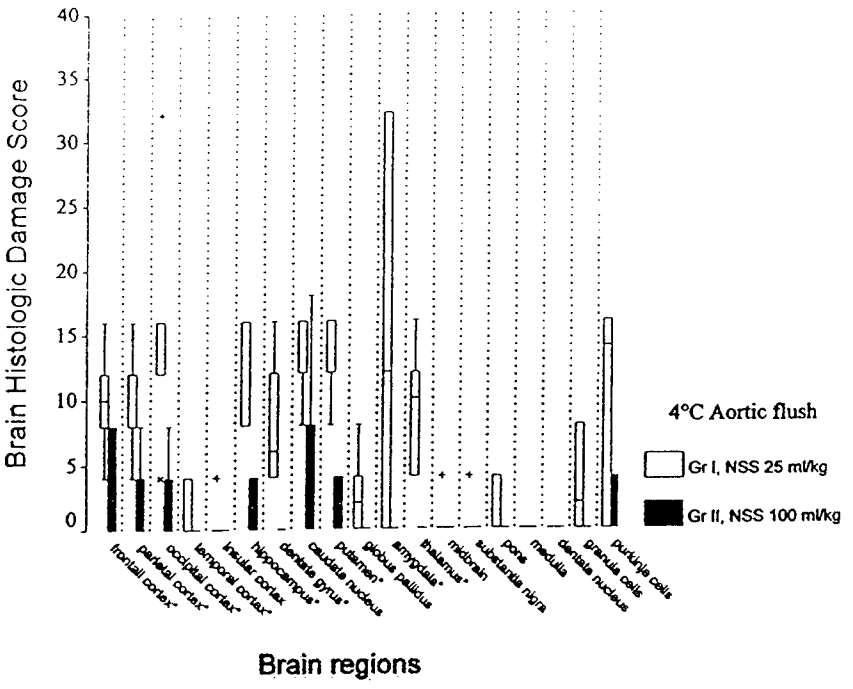

Fig. 2. Regional histologic damage scores (HDS). The box represents the interquartile range. The line across each box indicates the median. The whiskers indicate the highest and lowest values. The x indicates outliers (values between 1.5 and 3 box-lengths from the upper or lower edge of the box). The + indicates extremes (values >3 box-lengths from the upper or lower edge of the box). *$P < 0.05$. Gr = group; NSS = normal saline solution.

Pilot Experiments on Flush

This study was preceded by five pilot experiments in dogs to explore flush volume and catheter balloon position at start of CA 30 min to outcome at 72 h. Aortic arch flush with 50 ml/kg saline at 4°C resulted in one dog at 72 h with OPC 3, NDS 50%, and HDS 172 and with severe hemorrhagic areas in the duodenal mucosa, and in two dogs with normal cerebral function but with OPC 2 because of spastic paralysis of the hind legs (NDS 32% and 29%; HDS 20 and 40, respectively). Hearts and intestines were macroscopically normal. Two other dogs, flushed with 100 ml/kg saline at 4°C with the balloon at the level of the diaphragm, achieved normal cerebral function but with OPC 2 because of spastic paralysis of the hind legs (NDS 29% and 29%; HDS 16 and 12, respectively). Hearts and intestines were macroscopically normal. In these two dogs, the lumbar spinal cords showed histologic evidence of extensive degenerative changes, primarily in the ventral gray columns. This was characterized by neuronal degeneration with swelling and chromatolysis, neuronophagia, and prominent gliosis, including occasional glial nodule formation. Because of these pilot experiments, we decided for the final study group II to place the tip of the balloon catheter into the lower abdominal aorta.

Adjunctive Study of DNA Damage

All dog brains in the study were also stained using the TUNEL method.[29] Evidence of DNA fragmentation was found predominantly in neurons which, on regular staining, appeared shrunken and had condensed nuclei. A new DNA damage scoring method was developed and revealed that these scores correlated with HDS.

Dietrich *et al.*,[30] had shown that brief (4 h) postarrest mild hypothermia after normothermic incomplete forebrain ischemia in rats postpones but does not permanently salvage hippocampal neurons at 2 months. This is not relevant for our study, which produced moderate intraischemic (preservative) hypothermia, which in the Dietrich study gave lasting salvage of neurons at 2 months. Additionally, we used prolonged (12 h) post-CA mild hypothermia which, in a forebrain ischemia rat study by Colbourne *et al.*,[31] gave permanent benefit. Our dog 1 from group II (table 3) is functionally normal 1 y after CA.

Attempts at cerebral resuscitation with drugs have so far been disappointing.[2,4,32] In the mid-1980s we resumed research on resuscitative moderate hypothermia (30°C) after normothermic CA.[2,33] Breakthrough effects in dogs on outcome, however, were documented only when mild hypothermia (34–36°C), which is simpler and safer than moderate hypothermia, was discovered to improve cerebral outcome when induced before VF-CA up to 15 min,[2,3,7] and even when induced after normothermic VF-CA of 10–12.5 min.[2,10–12] Mild resuscitative hypothermia essentially normalized cerebral outcome after VF-CA of 11 min when combined with cerebral blood flow–promoting measures.[12] Thus, protective-preservative hypothermia, induced and reversed by CPB, has been shown to preserve the brain and whole organism for VF-CA up to 15 min at about 35°C,[3,7] for CA up to 20 min at about 30°C,[2,7,26] for CA up to 30 min at about 20°C (deep hypothermia),[28] and for CA up to 60 min at about 10°C (profound hypothermia).[13] For transport and repair in exsanguinated trauma victims, it

is assumed that at least 30 min of preservation is needed until CPB can be initiated.

In a steady state, brain tissue temperature seems well reflected in Tty.[34] However, when deep hypothermia was rapidly induced during CPB, large temperature gradients between brain temperature and Tty were found.[35] In pilot experiments with the same dog model, we found that brain tissue may have been transiently 4–10°C below Tty. This might explain the unexpected complete cerebral preservation achieved in group II during CA of 30 min at Tty 28°C.

With CA of 30 min or longer, the heart needs to be protected to enable restoration of stable circulation.[24,25] With aortic arch flush of 25 ml/kg saline (group I), all six dogs had macroscopically severe hemorrhagic damage of the heart. In contrast, with flush of 100 ml/kg saline (group II), all seven dogs had macroscopically normal hearts. In group I, with the low-volume flush and high balloon position, the intestines also showed severe hemorrhagic damage. There was also a greater need for norepinephrine after return of spontaneous circulation to maintain normotension. In group II, abdominal mild hypothermia seemed to have protected the abdominal viscera and spinal cord; this was for tissue supplied by the superior mesenteric artery. Rectal temperature did not deviate from baseline during CA in both groups. The protection of intestines by flush into the abdominal aorta in group II could also have been the result of lower norepinephrine requirement and better overall hemodynamics.

For group I, we debated whether to exclude from outcome evaluation the three of six dogs that had cardiovascular failure requiring increasing amounts of norepinephrine postarrest. To obtain brain tissue, these three dogs were weaned from paralysis and fentanyl early to determine OPC and NDS; they were then euthanized for brain histologic examination at 42, 52, and 60 h rather than at 72 h (table 3, group I, dogs 4, 5, and 6). With comparable damage, earlier HDS would show fewer ischemic neurons than at 72 h. Our exclusion criteria of extracerebral organ failure with cardiovascular–pulmonary variables out of protocol did not apply, because we could maintain normotension, normoxia, and other critical variables until euthanasia. A deleterious effect on the histologic damage by norepinephrine cannot be ruled out. The two dogs that developed brain death (with dilated, fixed pupils) did so despite normotension; they could not be weaned to spontaneous breathing. Group I results confirm that CA 30 min, even with mild cardiac hypothermia, is too severe an insult to expect cardiovascular resuscitability.[3,7,24,25]

The broader objectives of this preservation study include: (1) Helping to save victims of temporarily uncontrollable (internal) traumatic exsanguination, such as combat casualties and civilian trauma victims without severe brain trauma[8]; (2) helping emergency medical services save some nontraumatic cases of normovolemic, normothermic, sudden cardiac death who are unresuscitable by standard cardiopulmonary resuscitation[2,9]; and (3) enabling selected elective surgical procedures that are feasible only during a prolonged state of no blood flow.[36] The last example would not require high-speed preservative cooling. With large fluid volumes and CPB, profound hypothermic asanguinous trickle flow could extend the tolerated preservation time to longer than 3 h.[36]

Various clinically feasible methods for the induction of cerebral hypothermia have been tested in animals and patients since the 1950s.[2,26] None is as rapidly effective as aortic cold flush[15–18] or CPB with heat exchanger.[10–14] Initiation of CPB, however, takes more time than the brain can tolerate under normothermic CA. The percutaneous Seldinger technique, with or without minor cutdown, available for femoral vessels and other vessels, has been shown to be feasible and rapid[37] but has not been explored for empty vessels in exsanguination. Almost as rapid as CPB cooling would be intracarotid cooling.[38,39]

For civilian emergency care, the methods described in this report should be feasible now, in the hands of physician-staffed teams of mobile intensive care unit ambulances and in hospital emergency departments. There, CPB should be available to continue with profound hypothermic total circulatory arrest[13] or trickle flow[36] to achieve preservation of at least 60 min. We are exploring novel methods for access to the aorta with[40] and without thoracotomy.[2] For field resuscitation by combat medics, rapid access to the aorta without thoracotomy, without CPB, and with small fluid volume at ambient temperature are needed. We therefore systematically explored the preservation achievable in the same dog model with six pharmacologic strategies, using aortic arch flush of 25 ml/kg saline at 24°C, which achieved a Tty of 36°C.[32] Thus far, moderate-to-deep hypothermia[15–18] proved much more preservative than any of the 14 drugs tested.[32]

We conclude that, in dogs, a single high-volume flush of cold saline (4°C) into the abdominal aorta at the start of exsanguination CA of 30 min rapidly induces moderate-to-deep cerebral hypothermia and can allow survival without functional or histologic brain damage. Future research should explore flush preservation for even longer arrest times, using profound hypothermia plus pharmacologic adjuncts.

Patrick Kochanek, M.D., (Director of the Safar Center for Resuscitation Research and Professor of Anesthesiology/Critical Care Medicine and Pediatrics, University of Pittsburgh) and Lyn Yaffe, M.D., (US Navy Medical Research and Development Command) made valuable suggestions. Robert Wagner, V.M.D., (Central Animal Facility, University of Pittsburgh School of Medicine) helped with assessing spinal cord damage. Rochelle Hans, Carol Korbanik, Jason Stezoski, and Sherman Culver, technicians (Safar Center for Resuscitation Research, Department of Anesthesiology/Critical Care Medicine, University of Pittsburgh) helped with intensive care unit life support. The Cardeon Corp. (Cupertino, California) provided the flush catheter. Howell Sasser, Ph.D., (Carolinas

Medical Center, Department of Research Planning and Evaluation, Charlotte, North Carolina) advised on statistical analyses. Patricia Boyle (Department of Anesthesiology/Critical Care Medicine, University of Pittsburgh) helped with editing. Valerie Sabo (Safar Center for Resuscitation Research, University of Pittsburgh) helped with preparation of the manuscript.

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EXPLORATION OF PHARMACOLOGIC AORTIC ARCH FLUSH STRATEGIES FOR RAPID INDUCTION OF SUSPENDED ANIMATION (SA) (CEREBRAL PRESERVATION) DURING EXSANGUINATION CARDIAC ARREST (EXCA) OF 20 MIN IN DOGS

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Objectives: ExCA can rarely be survived by conventional resuscitation attempts. This is an overview of systematic explorations of methods for rapid initiation of SA (preservation for transport and resuscitative surgery) with mild hypothermic aortic arch flush. We hypothesized that flush with drugs at start of 20 min ExCA, can achieve normal functional recovery, as did saline flush at 4°C without drug. **Methods:** 43 dogs (20-25 kg) were exsanguinated over 5 min to CA of 20 min no-flow, resuscitated by closed-chest cardiopulmonary bypass. Controlled ventilation was to 20 h, and intensive care to 72 h. At CA 2 min, the dogs received a flush of 500 ml saline at 24°C into the aortic arch via a balloon catheter. Added to the flush were drugs for different strategies: delaying energy failure (2Chloroadenosine [Ad], Thiopental [Th], Fructose-Bi-Phosphate [FBP]); protecting membrane integrity (MK801; Nimodipine; Phenytoin with Th [Ph/Th]); preventing apoptosis (Cycloheximide [Cyclo]); blocking intracellular Ca^{2+} (Ca^{2+} calmodulin antagonist [W-7]). **Results:** See table. Overall performance category (OPC) 1 (normal function at 72 h) was achieved only by 2 dogs with Th and 1 dog with Ph/Th. Neurologic deficit score (NDS) 0-10%=normal, 100%=brain death. Brain histologic damage scores will be presented. **Conclusion:** Seeking a breakthrough effect - consistant normal recovery (OPC 1)- was not achieved with any of these pharmacologic approaches. Combination treatments of drugs with relatively "better" outcomes should be tested. (Supported by US Navy MRDC-ONR)

	Control	Ad	Th	FBP	MK801	Nimod	Ph/Th	Cyclo	W-7
OPC 1	0/7	0/2	2/9	0/5	0/5	0/2	1/7	0/3	0/2
NDS (%)	56 (29-65)	50,43	52 (22-57)	55 (39-63)	50 (33-55)	33,66	55 (38-59)	50,39, 42	66,48

OPC data are given as n with good outcome from n total.
NDS data are given as single values or median (interquartile range).

Journal of Cerebral Blood Flow & Metabolism
22:105–117 © 2002 The International Society for Cerebral Blood Flow and Metabolism
Published by Lippincott Williams & Wilkins, Inc., Philadelphia

Antioxidant Tempol Enhances Hypothermic Cerebral Preservation During Prolonged Cardiac Arrest in Dogs

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Summary: The authors are systematically exploring pharmacologic preservation for temporarily unresuscitable exsanguination cardiac arrest in dogs. They hypothesized that the antioxidant Tempol improves cerebral outcome when added to aortic saline flush at the start of cardiac arrest. In study A, no drug (n = 8), Tempol 150 mg/kg (n = 4), or Tempol 300 mg/kg (n = 4) was added to 25 mL/kg saline flush at 24°C (achieving mild cerebral hypothermia) at the start of 20-minute cardiac arrest. In study B, no drug (n = 8) or Tempol 300 mg/kg (n = 7) was added to 50 mL/kg saline flush at 2°C (achieving moderate cerebral hypothermia) at the start of 40-minute cardiac arrest. Cardiac arrest was reversed with cardiopulmonary bypass. Mild hypothermia lasted for 12 hours, controlled ventilation was sustained to 24 hours, and intensive care was provided for up to 72 hours. In study A, overall performance category 1 or 2 (good outcome) was achieved in all eight dogs treated with Tempol compared with three of eight dogs in the control group ($P = 0.03$). In study B, good outcome was achieved in all seven dogs treated with Tempol versus only two of 8 dogs in the control group ($P = 0.007$). In both studies, neurologic deficit scores were significantly better in the Tempol group, but not total histologic damage scores. At 72 hours, electron paramagnetic resonance spectroscopy of Tempol revealed direct evidence for its presence in the brain. Single- and double-strand DNA damage, nitrotyrosine immunostaining, total antioxidant reserve, and ascorbate acid levels were similar between groups, and thiol levels were decreased after Tempol in study B. The authors conclude that when added to aortic saline flush at the start of prolonged cardiac arrest, the antioxidant Tempol can enhance mild or moderate hypothermic cerebral preservation in terms of improved functional outcome. The mechanisms involved in this beneficial effect need further clarification. **Key Words:** Cerebral ischemia—Cardiopulmonary resuscitation—DNA damage—Hemorrhage—Outcome—Reperfusion injury.

Attempts to resuscitate patients from intrathoracic or intraabdominal exsanguination cardiac arrest (CA) before control of bleeding have failed (Bellamy et al., 1996). In the search for a needed new approach, Safar and Bellamy (1984) recommended research into "suspended animation for delayed resuscitation," to preserve brain and organism during prolonged CA. This approach would enable transport and repair during pulselessness.

Hypothermia during CA induced and reversed by cardiopulmonary bypass (CPB) preserves brain and organism during up to 15-minute CA no-flow during mild hypothermia (33–36°C) (Safar, 1988), up to 20-minute CA during moderate hypothermia (28– 32°C) (Bigelow et al., 1950), up to 30-minute CA during deep hypothermia (16–27°C) (Livesay et al., 1983), and up to 60-minute CA during profound hypothermia (5–15°C) (Capone et al., 1996). In normothermic CA, the brain tolerates a no-flow time of only approximately 5 minutes (Safar, 1988). Therefore, to avoid the loss of viability of cerebral neurons, a suspended animation strategy must be induced within 5 minutes of CA onset. Because CPB is not immediately available in the field, we introduced and explored cold saline flush with large flush volumes into the aorta via a balloon tipped catheter to rapidly preserve cerebral viability in dogs during 15-minute (Woods et al., 1999), 20-minute (Behringer et al., 2000*a*), 30-minute (Behringer et al., 2000*b*), and 60-, 90-, or in some dogs even 120-minute CA (Behringer et al., 2001*a*). For suspended animation in the field, the

Received May 25, 2001; final revision received September 26, 2001; accepted September 26, 2001.

Supported by the U.S. Department of Defense and the U.S. Office of Naval Research grants N00014-97-1-1064 and N00014-99-0765.

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aortic flush solution would need to be delivered using a portable small volume at ambient temperature. An aortic arch flush of approximately 25 mL/kg saline at 24°C at the start of 20-minute CA induced mild cerebral hypothermia (tympanic membrane ≈ 36°C) and resulted in survival with brain damage (Behringer et al., 2000*a*). We then hypothesized that adding a drug with cerebral preservation potential to this aortic arch saline flush would achieve functional and histologic normality. Using this 20-minute CA model, we systematically explored 14 pharmacologic cerebral preservation potentials (Behringer et al., 1999). Drugs were selected for the following six pharmacologic strategies: (1) delaying energy failure, (2) protecting membrane integrity, (3) preventing structural degradation, (4) regulating protein synthesis, (5) preventing re-oxygenation injury, and (6) preserving mitochondria. Concerning strategies one and two, adenosine (Woods et al., 2000), fructose-1,6-bisphosphate (Behringer et al., 2001*b*), the *N*-methyl-D-aspartate antagonist MK801 (Behringer et al., 2001*b*), and thiopental plus phenytoin (Behringer et al., 2001*c*) were not effective.

This report concerns strategy five. We added the cell-permeable antioxidant 4-hydroxy-2,2,6,6,-tetramethyl-piperidine-1-oxyl (Tempol) to the saline flush. Tempol has been shown to benefit rats with hemorrhagic shock (Kentner et al., 2000) or splanchnic artery occlusion (Mota-Filipe et al., 1999), traumatic brain injury (Beit-Yannai et al., 1996; Zhang et al., 1998), or focal brain ischemia (Beaulieu et al., 1998; Rak et al., 2000), and gerbils with global brain ischemia (Cuzzocrea et al., 2000). Tempol was never investigated in a large (higher) animal species and a clinically relevant model of prolonged CA with evaluation of long-term outcome. In this study, adjunctive biochemical and immunohistologic observations were also made to explore suspected mechanisms of the action of Tempol. Using our exsanguination CA dog model, we hypothesized that Tempol added to aortic saline flush at 24°C at the start of a 20-minute no-flow CA enhances the documented benefit of mild cerebral hypothermia (ambient temperature flush for use in the field); and that Tempol added to aortic saline flush at 2°C with a larger volume at the start of 40-minute no-flow CA enhances the documented benefit of moderate cerebral hypothermia (feasible in hospitals). We expect that for both CA durations, Tempol by flush results in normal functional recovery without histologic brain damage.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and the Department of Defense, and followed national guidelines for the treatment of animals. All experiments were conducted by the same team. Study A was conducted within 9 months in mixed sequence and without randomization as part of a systematic exploration of 14 pharmacologic aortic flush-preservation potentials (Behringer et al., 1999), whereas study B was conducted within 3 months with randomization. Briefly, the protocol called for exsanguination to 20-minute no-flow CA (study A) or 40-minute no-flow CA (study B), resuscitation with CPB, mild hypothermia to 12 hours, controlled ventilation to 20 hours, intensive care to 72 hours, and outcome evaluation. In study A, after 2 minutes CA, the dogs received a flush over 1 minute into the thoracic aorta with 25 mL/kg saline at 24°C without the drug (control group, n = 8) or with 150 (n = 4) or 300 mg/kg Tempol (n = 4) added. In study B, after 2 minutes CA, the dogs received a flush over 2 minutes into the abdominal aorta (necessary to include preservation of the spinal cord when CA exceeds 20 minutes) with 50 mL/kg saline at 2°C, either without the drug (control group, n = 8) or with 300 mg/kg Tempol (n = 8).

Preparation

A detailed description of the model was published previously (Behringer et al., 2000*a,b*). Thirty-two male custom-bred hunting dogs (body weight, 18–26 kg; age, 8–12 months) were premedicated with intramuscular ketamine 10 mg/kg, anesthetized with halothane (2–4%) and a 1:1 ratio of nitrous oxide to oxygen via a cone mask. After tracheal intubation, the dogs were mechanically ventilated (Harvard Piston Ventilator model 613, Harvard Apparatus, South Natick, MA) with tidal volumes of 15 mL/kg and a positive end-expiratory pressure of 5 cm H_2O without paralysis. Temperature probes were inserted for monitoring tympanic membrane, esophageal, and rectal temperatures. Fluid maintenance was with dextrose 5% in 0.45% sodium chloride at 5 mL · kg^{-1} · $hour^{-1}$. A PE 90 arterial catheter was inserted for pressure monitoring and blood sampling, and a 7.5-Fr pulmonary artery catheter (Intellicath Continuous Cardiac Output thermodilution catheter, Baxter Co., Irvine, CA) was inserted for continuous monitoring of pulmonary artery pressure, temperature, and cardiac output (Baxter Vigilance Monitor, software 4.42). An 8-Fr prototype balloon catheter with one hole at the tip (Cardeon Corp., Cupertino, CA, U.S.A.) was inserted for arterial bleeding and aortic flush, and a multiple-hole 18-Fr transjugular vena cava catheter was inserted for venous bleeding and venous return to the CPB system. Tympanic membrane temperature was controlled at 37.5 ± 0.1°C with a heating blanket and lamp.

Cardiac arrest and flush

After two baseline measurements at tympanic membrane temperature 37.5°C, heating devices, intravenous fluids, and halothane were discontinued while the dogs were weaned to spontaneous breathing of a 3:1 nitrous oxide to oxygen mixture via a T-tube. When the canthal reflex returned, hemorrhage was initiated to achieve a mean arterial pressure (MAP) of 40 mm Hg at 2 minutes, 30 mm Hg at 3 minutes, and 20 mm Hg at 4 minutes. At 5 minutes, to assure zero blood flow, ventricular fibrillation was induced with a 60-Hz transthoracic shock of 110 V (alternating current) for 2 seconds, and was repeated as needed. Total arrest time (no-flow) was 20 minutes in study A and 40 minutes in study B.

Two minutes after the onset of CA, the balloon of the aortic catheter was inflated to occlude the aorta and saline was flushed using a roller pump, as described previously. After the flush and during CA, the aortic catheter was replaced by a short arterial CPB cannula (7 or 8 Fr).

Resuscitation

Reperfusion after CA was achieved with CPB using a centrifugal pump (Biomedicus, Eden Prairie, MN, U.S.A.) and

hollow-fiber membrane oxygenator with heat exchanger (Medtronic, Anaheim, CA, U.S.A.) primed with 400 mL dextran 40 (10% in saline) and Ringer solution (1:1) including sodium bicarbonate (2 mEq/kg) and heparin (1,500 U). Just before the start of CPB, additional heparin (1,500 U) and sodium bicarbonate (2 mEq/kg) were given intravenously. Cardiopulmonary bypass began with a flow of 100 mL/kg per minute, and reinfusion of the shed blood was titrated to achieve a central venous pressure of 10 to 15 mmHg and a MAP greater than 100 mm Hg. If necessary, epinephrine (boluses of 0.01 mg/kg) was administered intravenously. For defibrillation we used external direct-current countershocks of 150 J, and repeated shocks were increased by 50 J. Controlled ventilation was resumed with 100% oxygen at a rate of 8 to 10 inflations per minute. A base deficit of greater than 6.0 mEq/L was corrected with intravenous sodium bicarbonate. When restoration of spontaneous circulation was established, a norepinephrine infusion was titrated intravenously to achieve a brief hypertensive bout of MAP greater than 150 mm Hg, after which MAP was maintained at 90 to 150 mm Hg (Sterz et al., 1990). Shed blood was gradually reinfused into the CPB system to maintain a central venous pressure of 8 to 15 mm Hg and a hematocrit greater than 30%. During CPB, the activated clotting time was maintained at more than 300 seconds with heparin. Cardiopulmonary bypass flow for assisted circulation was reduced to 75 $mL \cdot kg^{-1} \cdot minute^{-1}$ at 60 minutes and 50 $mL \cdot kg^{-1} \cdot minute^{-1}$ at 90 minutes, and was stopped at 120 minutes.

Intensive care

After weaning dogs from CPB assist at 2 hours, controlled ventilation was continued to 20 hours with a 1:1 mixture of nitric oxide and oxygen. Paralysis was maintained with doses of intravenous pancuronium (0.1 mg/kg, repeated as needed). To prevent "stress" (mydriasis, hypertension), fentanyl was titrated intravenously in boluses of 5 to 10 μg/kg. Hypotension (MAP < 90 mm Hg) was managed with intravenous titrated Ringer solution or norepinephrine. Severe hypertension (MAP > 150 mmHg) was controlled with intravenous boluses of labetalol (0.25–0.5 mg/kg) or hydralazine (0.1–0.2 mg/kg). At 20 to 24 hours, paralysis was reversed to spontaneous breathing with neostigmine (50 μg/kg) plus atropine (25 μg/kg) and the dogs were extubated. Thereafter, seizures, running movements, opisthotonos, or spontaneous tachypnea were controlled with titrated doses of diazepam (0.2–0.3 mg/kg intravenously) as needed. Tympanic membrane temperature was controlled at 34°C with external cooling and warming for the first 12 hours after the start of CPB, and at 37.5°C until 72 hours.

Preservation with Tempol

Tempol was purchased from Sigma-Aldrich (Milwaukee, WI, U.S.A.) and dissolved in isotonic saline solution. The Tempol solutions were filtered with a 0.22-μm filter (Fisherbrand; Fisher Scientific, Pittsburgh, PA, U.S.A.) before the aortic flush. In study A, we explored the addition of Tempol 150 (n = 4) or 300 mg/kg (n = 4) to the aortic flush after 2 minutes of cardiac arrest. These doses were chosen based on the literature (Beit-Yannai et al., 1996; Zhang et al., 1998; Mota-Filipe et al., 1999) and pilot experiments. In study B, we chose the addition of a larger dose of Tempol (300 mg/kg) (n = 8) to the aortic saline flush after 2 minutes of cardiac arrest because of the longer duration of ischemia.

General outcome evaluation

Performance was evaluated according to overall performance categories (OPC) (1 = normal, 2 = moderate disability, 3 = severe disability, 4 = coma, and 5 = death) (Leonov et al., 1990). Neurologic function was evaluated as neurologic deficit scores (NDS) (0–10% = normal, 100% = brain death) (Radovsky et al., 1995). Scores were evaluated every 8 hours after extubation until final evaluations at 72 hours. Attempts were made to discontinue any sedation at least 4 hours before final evaluations. If necessary, sedation was reversed with flumazenil (0.1 mg intravenously, repeated if needed).

Brain histopathology

For morphologic studies, the dogs were reanesthetized after the final outcome evaluation with ketamine 10 mg/kg intramuscularly, followed by 0.5 to 1.5% halothane with a 1:1 mixture of nitric oxide and oxygen via tracheal tube and controlled ventilation. After left thoracotomy, the dogs were killed by infusing paraformaldehyde (4%, pH 7.4) into the aortic arch using a roller pump at a pressure of approximately 100 mm Hg, with the right atrium opened, until clear fluid returned (usually 2 L).

Light microscopic scoring. The brain was removed after 1 hour of fixation. After cutting 3-mm thick slices, the same six slices of each brain were embedded in paraffin, cut into 4-μm thick sections, and stained with hematoxylin-eosin-phloxine. Using light microscopy, the same pathologist (AR), unaware of treatment assignments, scored 19 distinct anatomic brain regions according to severity and the extent of ischemic neuronal changes, infarcts, and edema, as described previously (Radovsky et al., 1995). The total brain histologic damage score (HDS) was the sum of all area scores. A score of more than 40 represents significant damage, and more than 100 represents severe damage.

Nitrotyrosine immunohistochemistry (study B). Nitrotyrosine was detected in the hippocampus immunohistochemically at 72 hours, indicating of the presence of peroxynitrite and other nitrosating agents (Whalen et al., 1999). Tissue sections were removed of paraffin, rehydrated, and processed by a high-temperature antigen-retrieval technique. Sections were incubated in a 1:200 dilution of antinitrotyrosine antibody (Upstate Biotechnology, Lake Placid, NY, U.S.A.) diluted in phosphate-buffered saline, followed by incubation in appropriate secondary antibody. Sections were then washed in phosphate-buffered saline and incubated with an avidin-biotin complex (ABC Standard kit; Vector Labs, Burlingame, CA, U.S.A.) and then reacted with diaminobenzidine (DAB, Vector Labs). Nitrotyrosine immunoreactivity was evaluated by one observer (RSBC) who was blinded to experimental groups as follows: 0 = no increase in immunoreactivity above background, 1+ = few immunoreactive cells, 2+ = moderate immunoreactivity in cells in < 4 200X fields, 3+ = moderate immunoreactivity in cells in ≥ 4 200X fields, and 4+ = marked immunoreactivity in cells and prominent dendritic labeling in ≥ 4 200X fields.

Double-strand DNA damage (study B). TUNEL (terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate-biotin nick end labeling) was performed at 72 hours (Clark et al., 2001). Briefly, 5-mm thick paraffin-embedded coronal sections containing hippocampus were removed of paraffin and incubated in 1 μM proteinase K (Boeringer Mannheim, Indianapolis, IN, U.S.A.). Sections were then incubated in 300 U/mL terminal deoxynucleotidyl transferase and 20 nmol/mL biotin-16-deoxyuridine (Boeringer Mannheim). TUNEL was viewed using ABC and DAB. The extent of DNA damage in the hippocampus for each brain section was evaluated by one observer (RSBC) who was blinded to experimental groups as follows: 0 = no TUNEL-positive cells; 1 = 1 to 10 TUNEL-positive cells in > 3 400X fields, 2 = 11 to 20 TUNEL-positive cells in > 3 400X fields,

3 = 21 to 30 TUNEL-positive cells in > 3 400X fields, 4 = 31 to 40 TUNEL-positive cells in > 3 400X fields, and 5 = > 41 TUNEL-positive cells in > 3 400X fields.

Single-strand DNA damage (study B). The DNA PANT (polymerase I-mediated biotin deoxyadenosine triphosphate nick translation) labeling was also performed at 72 hours (Clark et al., 2001), and was optimized for the *in situ* detection of cells with increased single-strand DNA breaks after cerebral ischemia. Briefly, deparaffinized sections were incubated in 1 μmol/L proteinase K (Boeringer Mannheim). Sections were then incubated in 40 U/mL DNA polymerase I and 29 μmol/mL biotin-14-deoxyadenosine triphosphate (both from Life Technologies, Gaithersburg, MD, U.S.A.) in buffer. The slides were incubated in ABC and DNA strand breaks viewed with DAB. Slides were coverslipped for light-microscopic analysis and evaluated by one observer (RSBC) who was blinded to experimental groups.

Biochemical mechanisms

Ascorbate radicals and Tempol. Electron paramagnetic resonance (EPR) spectroscopy was used to measure ascorbate radical and Tempol in brain homogenates at 72 hours, and in plasma samples. We used a JEOL-RE1X spectrometer (Kyoto, Japan) at 25°C in gas-permeable Teflon tubing (0.8-mm internal diameter and 0.013-mm thickness from Alpha Wire Corp (Elizabeth, NJ, U.S.A.). The tube (approximately 8 cm long) was filled with 60 μL mixed sample, folded into quarters, and placed in an open 3-mm internal diameter EPR quartz tube in such a way that all of the sample was within the effective microwave irradiation area. The spectra were recorded at a 3,355-G center field, 20-mW power; 0.79-G field modulation, 50-G sweep width, 4000 receiver gain, and 0.1-second time constant. Spectra were collected using EPRware software (Scientific Software Services, Bloomington, IL, U.S.A.).

Total antioxidant reserve. The total antioxidant reserve in brain homogenates at 72 hours in both studies was assayed by chemiluminescence produced in the presence of luminol and peroxyl radicals, as described previously (Tyurina et al., 1995). A water-soluble azoinitiator (AAPH) was used to produce peroxyl radicals at a constant rate. Oxidation of luminol (400 μmol/L) by AAPH-derived peroxyl radicals in 50 mmol/L disodium phosphate buffer (pH 7.4) at 37°C was started by the addition of AAPH (50 mmol/L). A delay in the chemiluminescence response, which is caused by interaction of endogenous antioxidants with AAPH-derived peroxyl radicals, is observed upon addition of brain homogenate (0.1 mg protein/mL). Based on the known rate of peroxyl radical generation by AAPH, the amount of peroxyl radicals scavenged by endogenous antioxidants was determined. A luminescent plate reader (ML 1000; Dynatech Laboratories, Billingshurt, U.K.) was used for determinations.

Ascorbic acid. High-pressure liquid chromatography was used to determine ascorbic acid levels in brain homogenates at 72 hours in both studies. We used an ODS Hypersil column (200 x 4.6 mm, 5 μm) (Hewlett Packard). The supernatant obtained by precipitation of proteins by 10% ethanol acid and sedimentation (10,000 g × 10 minutes) was used. The Shimadzu high-pressure liquid chromatography system (Kyoto, Japan) was used with an LC-600 pump and SPD-M10A diode array detector (detection by absorbance at 264 nm). The eluant was methanol:water (1:24 by volume) adjusted to pH 3.0 by ethanol acid at a flow rate of 1 mL/minute. Under these conditions, the retention time for ascorbic acid was 3 minutes. Acquired data were exported from detectors using Shimadzu EZChrom software.

Thiols. The concentrations of low molecular weight thiols and protein thiols were determined in brain homogenates at 72 hours in both studies. We used ThioGlo-1 (CalBiochem), a maleimide reagent, which produces a highly fluorescent product upon reaction with sulfhydryl groups. To homogenates of brain tissue containing 15 to 30 μg protein/mL, ThioGlo-1 was added to a final concentration of 10 μmol/L (in dimethyl sulfoxide solution). Low molecular weight thiol content was estimated by an immediate fluorescence response observed upon addition of ThioGlo-1 to the brain homogenate. A standard curve was established by addition of low molecular weight thiols (glutathione 0.04–4.0 μmol/L) to 50 mmol/L disodium phosphate buffer (pH 7.4) containing 10 μmol/L ThioGlo-1. Total protein thiols were determined as an additional fluorescence response after addition of sodium dodecyl sulfate (4 mmol/L) to the same homogenate. A Cytofluor 2350 fluorescence plate reader (Millipore, Bedford, MA, U.S.A.) was used in the assay using excitation filter 360 ± 40 nm and emission filter 530 ± 25 nm.

Determination of proteins. Protein concentration were determined with the Bio-Rad Protein Assay kit. A standard curve was established by addition of bovine serum albumin to the Bio-Rad assay kit, and protein content was calculated.

Statistical analysis

Dogs that did not meet protocol criteria or died from extracerebral causes were excluded. Brain death as an outcome was included if the experiment was performed according to protocol. Data are given as mean and SD, if normally distributed, or as median and interquartile range (IQR; difference between the 25th and 75th percentiles). We used the independent-samples *t*-test or the Mann-Whitney Test for the comparison of continuous variables (physiologic variables: NDS, HDS, ascorbate, thiols, and antioxidant reserve in brain), and the Fisher exact test for differences in proportions between groups (OPC 1, 2 = good outcome vs. 3–5 = bad outcome). To quantify the change of temperature over time during arrest, we calculated the area under the temperature curve. All data were computed with SPSS for Windows, release 8.0 (Chicago, IL, U.S.A.), or NCSS for Windows (Keysville, UT, U.S.A.). A P value < 0.05 was considered statistically significant.

RESULTS

For both studies, a total of 32 dogs were exsanguinated to CA. In study A, all 16 dogs survived to 72 hours in protocol (control group, n = 8; Tempol group, n = 8). In study B, one dog in the Tempol group was excluded because of a technical mistake in the aortic flush. A total of 15 dogs survived to 72 hours in protocol (control group, n = 8; Tempol group, n = 7).

Resuscitation

In studies A and B, there were no group differences in extracerebral variables important for cerebral recovery at baseline (Table 1) and 6 hours after resuscitation (Table 2). In both studies, there were no differences in the required number and energy of defibrillating countershocks, in the time needed to achieve restoration of spontaneous circulation, and in bicarbonate requirement. Total epinephrine doses required were significantly higher with Tempol in both studies (Table 3). The total norepinephrine requirements were the same for both groups in study A, but significantly higher in the Tempol

TABLE 1. *Physiologic variables at baseline*

	Study A (20 min ExCA)		Study B (40 min ExCA)	
	Control (n = 8)	Tempol (n = 8)	Control (n = 8)	Tempol (n = 7)
Heart rate (beats/min)	120 (110–146)	118 (103–120)	110 (103–120)	120 (110–140)
Mean arterial pressure (mm Hg)	98 (90–108)	95 (91–106)	100 (91–108)	95 (90–105)
Blood glucose (mg/dL)	175 (162–222)	177 (154–191)	182 (152–195)	172 (161–205)
Pao_2 (mm Hg) Fio_2 0.5	269 (239–288)	272 (231–300)	277 (226–285)	250 (241–274)
$Paco_2$ (mm Hg)	35 (33–38)	33 (30–35)	37 (34–41)	38 (31–41)
Arterial pH	7.36 (7.34–7.39)	7.36 (7.33–7.40)	7.33 (7.29–7.35)	7.33 (7.29–7.34)
Blood lactate (mmol/L)	3.2 (1.9–4.6)	2.9 (1.9–3.9)	2.7 (1.8–3.9)	4.1 (2.5–4.8)
Base excess (mEq/L)	−3.9 (−5.7—2.3)	−5.5 (−6.2—4.3)	−6.1 (−7.8—2.4)	−4.8 (−5.3—3.7)
Serum sodium (mmol/L)	145 (145–147)	147 (144–147)	147 (145–148)	146 (145–149)
Serum potassium (mmol/L)	3.7 (3.4–3.7)	3.3 (3.3–3.5)	3.5 (3.2–3.6)	3.5 (3.3–3.5)
Hematocrit (%)	34 (34–39)	38 (36–44)	36 (31–41)	37 (34–40)

Data are given as median and IQR (interquartile range). No statistically significant differences between groups.

ExCA, exsanguinations cardiac arrest; Pao_2, arterial oxygen pressure; Fio_2, fraction of inspired oxygen; $Paco_2$, partial pressure of carbon dioxide (arterial).

group in study B (Table 3). The induced brief hypertension had the same duration, with a MAP greater than 150 mm Hg and the same peak MAP in both groups of both studies, but the beginning of hypertension was significantly delayed in the Tempol groups in both studies (Table 3). Cardiac index varied greatly between dogs, and postarrest values were often doubled compared with baseline values, but there were no differences between groups.

Temperatures

Tympanic membrane temperature just before exsanguination was 37.5 ± 0.1°C in all groups (Fig. 1). In study A (Fig. 1-I), the 25 mL/kg saline flush at 24°C decreased tympanic membrane temperature during CA within 2 minutes to a minimum of 35.4 ± 0.4°C in the control group versus 35.4 ± 0.3°C in the Tempol group ($P = 0.9$). Rectal temperature remained normothermic and did not differ from baseline values during CA. In study B (Fig. 1-II), the 50 mL/kg saline flush at 2°C decreased tympanic membrane temperature during CA within 5 minutes to a minimum of 27.5 ± 1.4°C in the control group versus 28.4 ± 0.6°C in the Tempol group ($P = 0.1$). Rectal temperature decreased from 37.8 ± 0.2°C to 37.2 ± 0.5°C ($P = 0.03$) in the control group and from 37.7 ± 0.2°C to 37.0 ± 0.7°C ($P = 0.02$) in the Tempol group during CA.

Methemoglobinemia

In the first dogs administered Tempol, we observed that the blood in the bypass tubing remained dark colored after passing through the oxygenator. Dogs receiving 150 mg/kg Tempol showed methemoglobinemia peak levels of 2.5% to 4.3% at 30 minutes of reperfusion, whereas those receiving 300 mg/kg Tempol showed 4.5% to 13.4% levels at 1 to 2 hours of reperfusion. Twelve hours after Tempol administration, methemoglobin levels were below 1%.

TABLE 2. *Physiologic variables at 6 hours after start of resuscitation*

	Study A (20 min ExCA)		Study B (40 min ExCA)	
	Control (n = 8)	Tempol (n = 8)	Control (n = 8)	Tempol (n = 7)
Heart rate (beats/min)	110 (85–120)	115 (93–124)	100 (91–108)	140 (90–160)
Mean arterial pressure (mm Hg)	150 (135–154)	128 (114–146)	133 (126–150)	150 (135–150)
Blood glucose (mg/dL)	232 (192–300)	178 (144–215)	213 (181–237)	191 (172–245)
Pao_2 (mm Hg), Fio_2 0.5	266 (232–293)	278 (229–304)	287 (254–297)	276 (240–296)
$Paco_2$ (mm Hg)	38 (35–42)	35 (32–40)	41 (35–46)	45 (34–48)
Arterial pH	7.38 (7.35–7.43)	7.41 (7.38–7.47)	7.33 (7.31–7.35)	7.32 (7.30–7.38)
Blood lactate (mmol/L)	4.7 (3.7–6.9)	4.1 (3.2–4.7)	5.6 (4.2–7.0)	4.3 (3.8–5.9)
Base excess (mEq/L)	−2.0 (−4.0–0.0)	−0.8 (−1.6–0.6)	−5.2 (−6.5—2.7)	−2.1 (−5.7—1.8)
Serum sodium (mmol/L)	151 (148–154)	153 (151–156)	153 (153–157)	156 (154–159)
Serum potassium (mmol/L)	3.4 (3.1–3.6)	3.1 (3.0–3.3)	3.0 (2.9–3.3)	3.0 (2.6–3.2)
Hematocrit (%)	31 (28–34)	29 (26–32)	34 (32–37)	31 (28–34)

Data are given as median and IQR (interquartile range). No statistically significant differences between groups.

ExCA, exsanguinations cardiac arrest; Pao_2, arterial oxygen pressure; Fio_2, fraction of inspired oxygen; $Paco_2$, partial pressure of carbon dioxide (arterial).

TABLE 3. *Requirements for restoration of spontaneous circulation (ROSC)*

	Study A (20 min ExCA)		Study B (40 min ExCA)	
	Control (n = 8)	Tempol (n = 8)	Control (n = 8)	Tempol (n = 7)
Countershocks, total number	4 (2–5)	3 (0–7)	2 (1–4)	2 (2–3)
Countershocks, total energy (J)	700 (275–850)	400 (38–1350)	300 (188–650)	300 (300–500)
ROSC (min after start of CPB)	5 (3–5)	4 (3–7)	3 (3–4)	3 (3–4)
Total bicarbonate (mEq)	130 (125–170)	143 (130–150)	178 (150–184)	165 (150–180)
Total epinephrine (mg)	0.7 (0.5–0.8)*	1.1 (0.9–1.4)	0.7 (0.5–0.7)*	1.6 (1.4–1.8)
Total norepinephrine (mg)	2.1 (1.5–2.6)	3.7 (2.1–6.9)	1.2 (0.7–1.9)*	4.2 (2.2–5.9)
Hypertensive bout				
peak MAP (mm Hg)	165 (160–175)	160 (155–164)	170 (158–186)	160 (155–170)
Start (min)†	7 (6–7)*	10 (8–11)	5 (3–8)*	13 (9–18)
Duration (min)‡	4 (2–8)	3 (2–5)	2 (1–5)	4 (1–5)

Data are given as median and IQR (interquartile range). ExCA, exsanguinations cardiac arrest; CPB, cardiopulmonary bypass; MAP, mean arterial pressure.

* $P < 0.05$, Tempol versus respective control; †start of hypertensive bout = time after start CPB; ‡duration of hypertensive bout = time with MAP > 150 mm Hg.

General outcome evaluation

In both studies, OPC at 72 hours were significantly improved in the Tempol groups compared with the control groups (Table 4). In study A, OPC 1 was achieved in three of four dogs receiving 150 mg/kg Tempol and in two of four dogs receiving 300 mg/kg Tempol. In study B, dogs receiving Tempol listed as OPC 2 showed normal cerebral function, and were rated as OPC 2 because of severe weakness in all extremities resulting in the inability to sit, walk, or stand. Histologic evaluation of the spinal cord in four of these dogs revealed no damage under light microscopy, except in one dog with a small focal area of spongiform changes in the dorsal horn of the gray matter in the thoracic segment.

In both studies, final NDS at 72 hours were significantly better in the Tempol groups than in the control groups (Fig. 2). In study A, six of eight dogs in the Tempol group had an NDS below 10% (i.e., were normal). In study B, all seven dogs in the Tempol group had NDS ranging between 24% and 31% (reflecting the weakness in all extremities described previously, with seemingly normal cerebral function and behavior) compared with NDS ranging between 22% and 57% in the eight control dogs.

Brain histopathology

Light microscopic scores. In both studies, there were no differences in total brain HDS between Tempol and control groups at 72 hours (Fig. 3). None of the dogs in either study had a normal brain on light microscopic examination. All HDS were scattered ischemic neuronal scores; scores for macroinfarcts and edema were zero. No microinfarcts, hemorrhages, edema, or lesions were detected in white matter at 72 hours. In study A, regional brain HDS in the temporal cortex, hippocampus, and amygdala were significantly higher in the Tempol group, and HDS in the dentate gyrus were significantly lower in this group (Fig. 4-I). In study B, regional brain HDS in the dentate gyrus was significantly lower in the Tempol group (Fig. 4-II).

Nitrotyrosine immunohistochemistry (study B). Nitrotyrosine brain damage scores at 72 hours were not different between groups [median 3 (IQR, 3–4) in the control group vs, 3 (IQR, 2–4) in the Tempol group, $P = 0.2$]. Increased nitrotyrosine immunoreactivity in both groups was detected in cells with the morphologic appearance of neurons, glia, and endothelium; in some sections, prominent dendritic labeling was seen, and immunoreactive cells with the morphologic appearance of oligodendrocytes were observed in white matter regions of all slides examined. No staining was observed in slides incubated without primary antibody.

DNA damage (study B). The DNA damage scores by TUNEL staining showed no difference between groups [median 2 (IQR, 1–4) in the control group vs. 4 (IQR, 2–5) in the Tempol group, $P = 0.2$]. The PANT stain for single-strand DNA damage was not detected in either group.

Biochemical mechanisms

Ascorbate radicals and Tempol. In brain tissue homogenates at 72 hours, EPR signals of ascorbate radicals (Fig. 5-I) were detected in all dogs. Because Tempol can be reduced by ascorbate to the EPR-silent Tempol-hydoxylamine (Tempol-OH), we used the oxidant potassium-ferricyanide to convert Tempol-OH to its EPR-detectable radical form Tempol (Fig. 5-II). The Tempol signal was detected in study A in one of three available brains of dogs treated with 150 mg/kg Tempol, in all three available brains of dogs treated with 300 mg/kg Tempol, and in study B in five of seven brains treated with 300 mg/kg Tempol. The addition of potassium-ferricyanide to brain homogenates from dogs that did not receive Tempol produced no EPR signal of Tempol. In study A, the amplitude of the Tempol signal in plasma

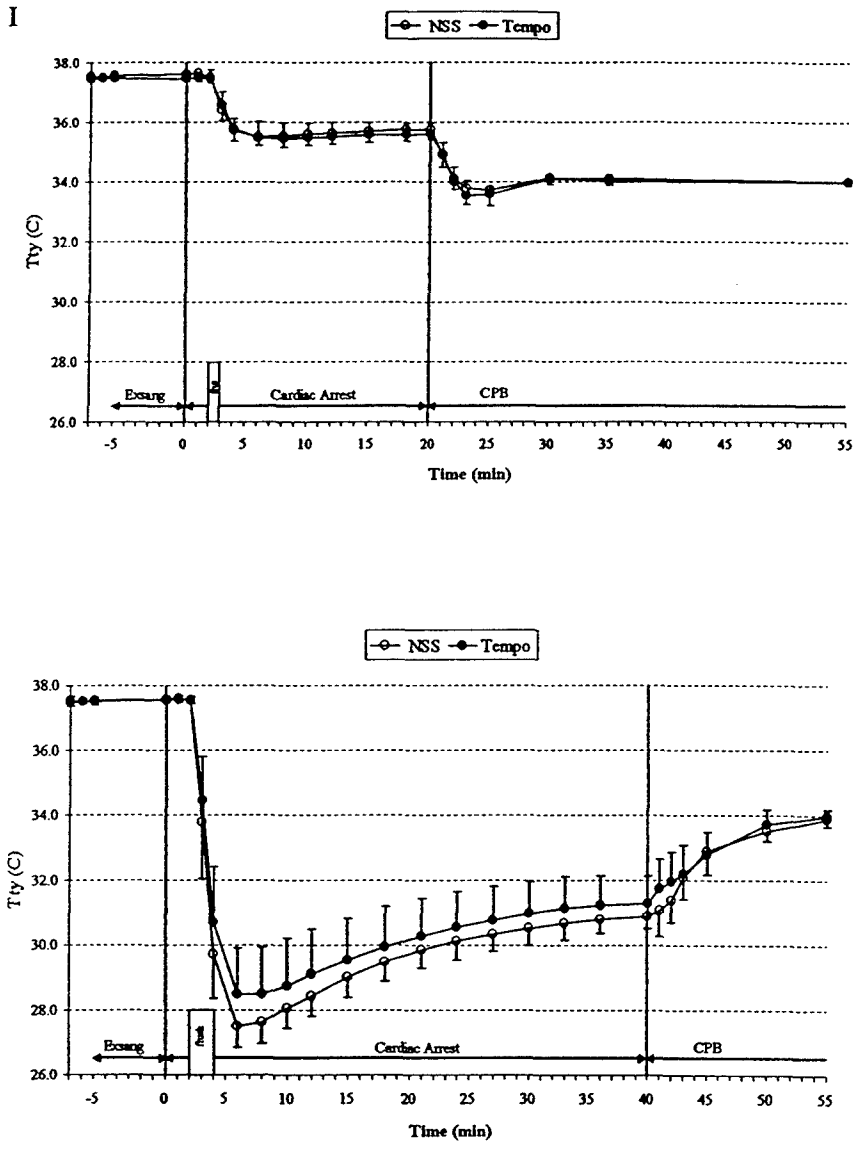

FIG. 1. Tympanic membrane temperatures during exsanguination cardiac arrest of 20 (study A, I) and 40 minutes no-flow (study B, II). Resuscitation with cardiopulmonary bypass. Aortic flush with balloon catheter was into the aortic arch in study A (saline, 25 mL/kg at 24°C) and into the abdominal aorta in study B (saline, 50 mL/kg at 4°C). Temperature areas under the curve during arrest were not different ($P = 0.5$ in I, and $P = 0.2$ in II).

was highest at 1 minute of reperfusion and was detectable until 8 hours of reperfusion in dogs treated with 150 mg/kg Tempol, and until 36 hours in dogs treated with 300 mg/kg Tempol. The addition of potassium-ferricyanide did not change the amplitude of the signal in measurements at 1 or 30 minutes and 1 hour. The ascorbate radical signal of control dogs showed a threefold increase above baseline at 1 minute of reperfusion, was not detectable at 30 minutes and 1 hour, and slowly returned to baseline values at 72 hours of reperfusion. In study B, EPR signals were measured only at 1 minute and at 3 and 72 hours of reperfusion; the Tempol signal was detectable at 1 minute and at 3 hours, but not at 72 hours of reperfusion, The addition of potassium-ferricyanide increased the amplitude of the Tempol signal 10-fold at 1 minute and 100-fold at 3 hours.

TABLE 4. *Outcome in terms of final overall performance categories (OPC 1–5) at 72 hours after exsanguinations cardiac arrest (ExCA)*

	Study A (20 min ExCA)		Study B (40 min ExCA)	
OPC	Control	Tempol	Control	Tempol
5 (brain death or death)				
4 (coma)			•	
3 (severe disability)	•••••		•••••	
2 (moderate disability)	••	•••	••	•••••••
1 (normal)	•	•••••		
P value	0.03		0.007	

Each dot represents one dog.

Total antioxidant reserve. The total antioxidant reserve in brain frontal cortex at 72 hours was not different between groups in both studies (Fig. 6-I) and was only numerically lower than in sham experiments without CA.

Ascorbic acid. Ascorbate levels in brain frontal cortex at 72 hours were not different between groups in both

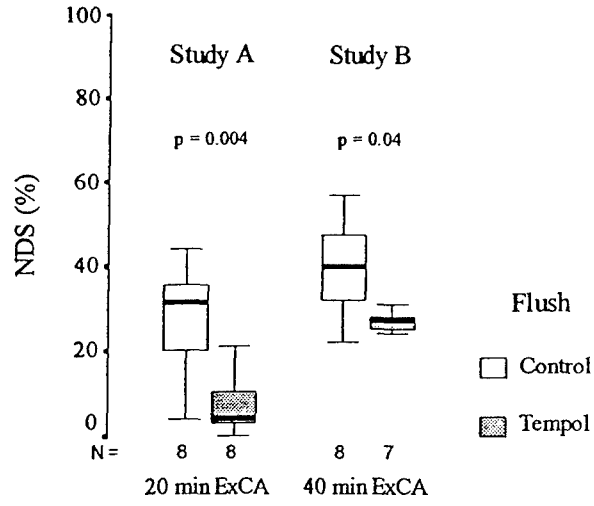

FIG. 2. Neurologic deficit scores 72 hours after exsanguination of cardiac arrest of 20 (study A) and 40 minutes (study B). Boxes represent interquartile ranges. The line across each box indicates the median, and the whiskers (⊥T) are the highest and lowest values.

studies (Fig. 6-II) and were similar to that in dogs without CA (Fig. 6).

Thiols. Protein sulfhydryl content at 72 hours in brain frontal cortex in study A showed a trend toward slightly higher concentrations of protein thiols in the Tempol group, and toward decreased concentration of protein thiols in the Tempol group in study B (Fig. 6-III). Low molecular weight thiols in brain frontal cortex were not different in study A, but were significantly lower in the study B Tempol group (Fig. 6-IV). Values were similar to those in dogs without CA.

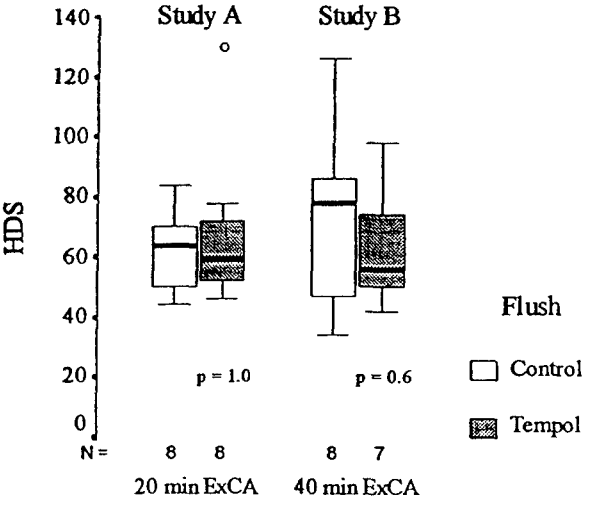

FIG. 3. Total brain histologic damage scores with light microscopy 72 hours after exsanguination cardiac arrest of 20 (study A) and 40 minutes (study B). Boxes represent interquartile ranges. The line across each box indicates the median, and the whiskers (⊥T) are the highest and lowest values. The o indicates outliers (values between 1.5–3 box-lengths from the upper or lower edge of the box).

DISCUSSION

In this study, Tempol in an aortic flush at the start of prolonged CA improved NDS and OPC after CA compared with saline flush, augmenting the preservation effect of mild hypothermia (tympanic membrane temperature, 35.5°C) for a 20-minute CA and of moderate hypothermia (tympanic membrane temperature, 28°C) for a 40-minute CA. This is the first documentation of mitigation of functional deficit by an antioxidant in a clinically relevant CA outcome model in dogs. However, Tempol did not prevent overall histologic brain damage.

The discrepancy between the beneficial effects of Tempol on functional deficits but not on histologic damage is puzzling. In our previous CA dog studies to 72- to 96-hour outcomes, a normothermic VF-CA no-flow time of 10 minutes followed by normothermic resuscitation resulted in the same total HDS found in this study [i.e., 75 ± 15 (Sterz et al., 1991) or 106 ± 44 (Vaagenes et al., 1984)], but functional outcome (NDS) was invariably poor. One of several possible explanations might be found in a selective preservation of synaptic structure and function by Tempol in neurons not considered doomed in this study (not shrunken and eosinophilic with pyknotic nuclei). Tempol could reduce reactive oxygen species (ROS), which can impair *N*-methyl-D-aspartate antagonist receptors and various mechanisms necessary for signal transduction between surviving neurons. Recovery of integrative function despite cellular damage is possible (Bothe et al., 1986). Because Tempol reduced histologic damage in both studies in the dentate gyrus, the least vulnerable region of the hippocampal formation, it may be more likely to detect a preservative effect of a treatment in this region. The possibility that longer life support could have resulted in further recovery of control dogs is unlikely, because life support for more than 4 to 7 days did not show improvement beyond the NDS at 48 hours. The final outcome evaluation at 72 hours, though it could not be blinded, was agreed upon by at least two team members to diminish potential observer bias. The detailed components of OPC and NDS called for objective recording.

In this study, we again demonstrated the feasibility of inducing preservative hypothermia during CA within a few minutes, using a cold saline flush through a balloon catheter into the aorta. Our study also shows that Tempol, a water-soluble, inexpensive, commercially available compound that improves cerebral function after CA, deserves additional investigation because it penetrates the blood-brain-barrier (Mitchell et al., 1990). That aspect is important because temporary complete, normothermic global cerebral ischemia up to 30 minutes does not grossly disrupt the blood-brain-barrier (Schleien et al., 1990), as does focal ischemia or brain trauma. Another novel finding is that the beneficial effect of

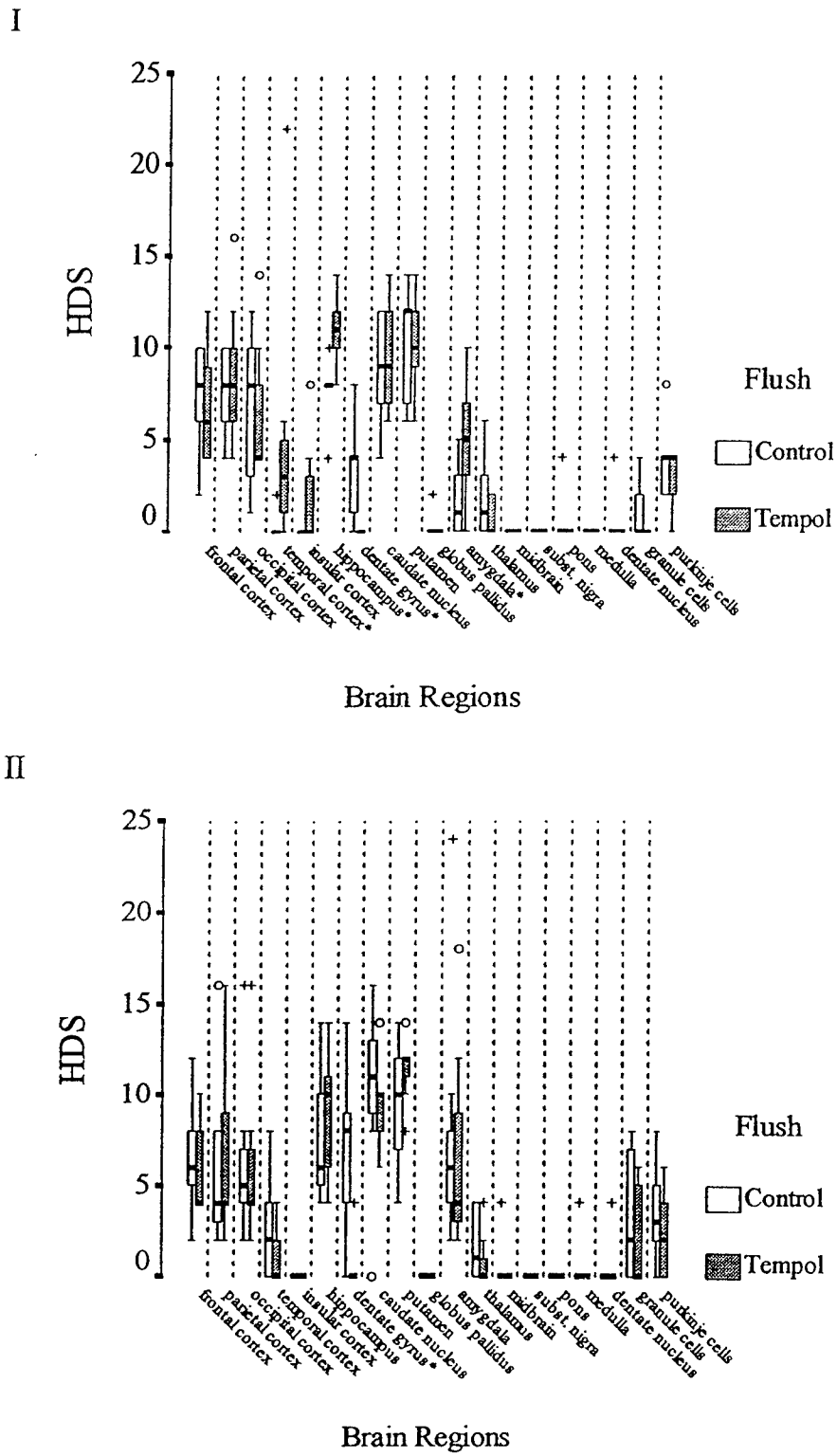

FIG. 4. Regional brain histologic damage scores 72 hours after exsanguination of cardiac arrest of 20 (study A, I) and 40 minutes (study B, II). Boxes represent interquartile ranges. The line across each box indicates the median, and the whiskers (⊥T) are the highest and lowest values. The o indicates outliers (values between 1.5–3 box-lengths from the upper or lower edge of the box). The + indicates extremes (values > 3 box-lengths from the upper or lower edge of the box). $^*P < 0.05$.

moderate or mild hypothermia, which itself mitigates free-radical reactions (Lei et al., 1994), can still be augmented using a pharmacologic antioxidant. Ideally, cerebral preservation should be induced before anoxic depolarization (e.g., within 3 minutes of CA). From another dog study with 30-minute exsanguination CA (Behringer, 2001, unpublished data) we learned that cold aortic flush with 100 mL/kg saline at 2°C at 2 minutes of CA (as in this study) lead to normal outcome and minimal histologic brain damage. The same flush delayed to

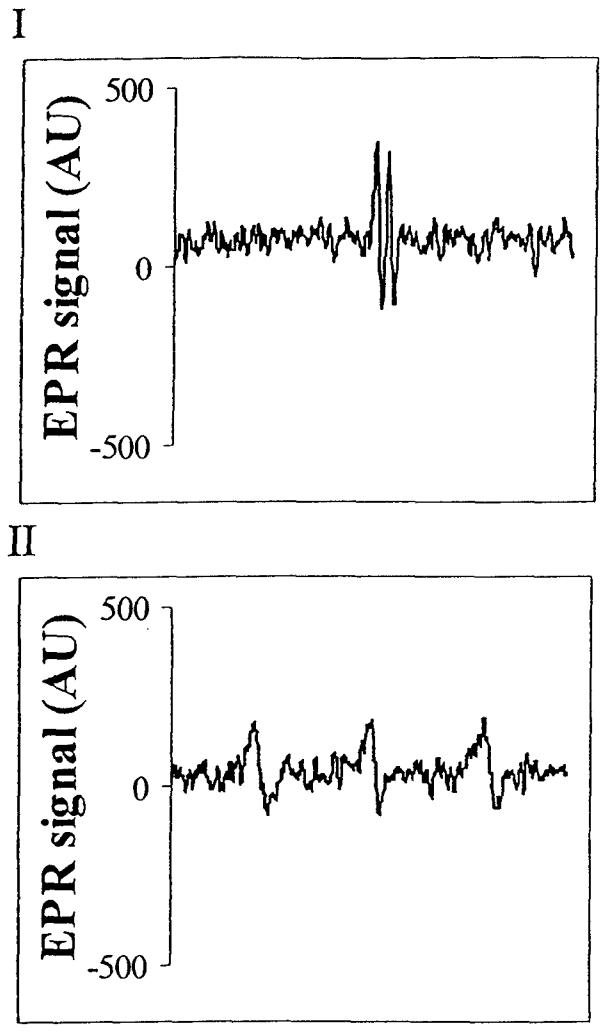

FIG. 5. Electron paramagnetic resonance spectroscopy of brain frontal cortex 72 hours after exsanguination cardiac arrest in one dog in study B. I indicates representative typical doublet signal of the ascorbate radical; II, representative typical triplet signal of Tempol after adding the oxidant potassium-ferricyanide to the sample.

5 minutes of CA lead to normal outcome with moderate histologic brain damage, and delay to 8 minutes lead to poor outcome with severe histologic brain damage. Delayed administration of Tempol may reduce its efficacy as well.

The objectives of suspended animation for temporarily unresuscitable conditions have been discussed elsewhere (Tisherman et al., 1991; Capone et al., 1996; Bellamy et al., 1996; Behringer et al., 2000*b*) and include (1) helping to save military or civilian trauma victims without severe brain trauma with presently unresuscitable exsanguination CA, (2) helping to save persons experiencing seemingly unresuscitable, nontraumatic sudden cardiac death, and (3) enabling selected elective surgical procedures to be performed that are feasible only during a prolonged state of no blood flow.

Reactive oxygen species seem to be important pathophysiologic mediators of postischemic anoxic encephalopathy. Glutamate release and increases in intracellular calcium activate several free-radical pathways during brain ischemia, which markedly increase during reperfusion, leading to neuronal death. Based on the various sources of free radicals, there exist different mechanisms to inhibit ROS-induced brain damage (Hall, 1997). Indirect-acting antioxidants that inhibit formation of eicosanoids include ibuprofen and indomethacin; xanthine oxidase inhibitors include allopurinol. Enzymatic agents include superoxide dismutase and catalase. Spin-trapping compounds like PBN (n-tert-butyl-α-phenyl-nitrone) scavenge hydroxyl radical; NOS inhibitors, or a peroxy nitrite scavenger reduce the generation or effects of peroxy nitrite. αTocopherol and 21-aminosteroid tirilazad mesylate inhibit lipid peroxidation, and deferoxamine chelates iron. However, intravenous administration of these compounds does not assure adequate delivery to brain tissue because of the blood-brain barrier.

Tempol, a stable nitroxide radical, readily penetrates the blood-brain barrier and enters cells (Mitchell et al., 1990), which should permit the molecule to scavenge both intracellular and extracellular ROS. Nitroxides are universal antioxidants, combining some of the previously mentioned mechanisms, and have direct radical scavenging effects. They act as superoxide dismutase mimetics by removal of $O_2^{-}\cdot$ in a catalytic process. Nitroxides are first reduced by $O_2^{-}\cdot$ to a hydroxylamine intermediate that can be oxidized by another $O_2^{-}\cdot$ to the initial nitroxide, allowing the molecule to act as a self-replenishing antioxidant (Samuni et al., 1990). Nitroxides oxidize Fe^{2+} to preempt the Fenton reaction (Mitchell et al., 1990).

The mechanisms of Tempol effects are not thoroughly explored. In study B, immunostaining for nitrotyrosine was used to determine if Tempol administration prevented peroxynitrite-mediated nitrosylation of proteins (Cuzzocrea et al., 2000). We did not find a difference in immunostaining for nitrotyrosine between controls and Tempol-treated dogs. In our model, Tempol may not influence the peroxynitrite pathway. We used TUNEL and PANT staining to elucidate the effect of Tempol on downstream aspects of the cascade of ischemic cell death. Nitroxides were shown to prevent free-radical–induced DNA oxidative damage *in vitro* (Damiani et al., 2000). In our study, TUNEL staining revealed double-strand DNA damage in both groups at 72 hours, without group differences. The lack of protection against double-strand DNA damage by Tempol was unanticipated because free-radical damage to the mitochondria is known to be a major initiator of apoptotic pathways (Chan, 2001). One possible explanation is that enzymatic rather than oxidative cleavage is the major source of DNA damage in our model. TUNEL staining, however, is not specific for apoptosis. It is consistent with, but not confirmative of, apoptosis. We did not detect any PANT

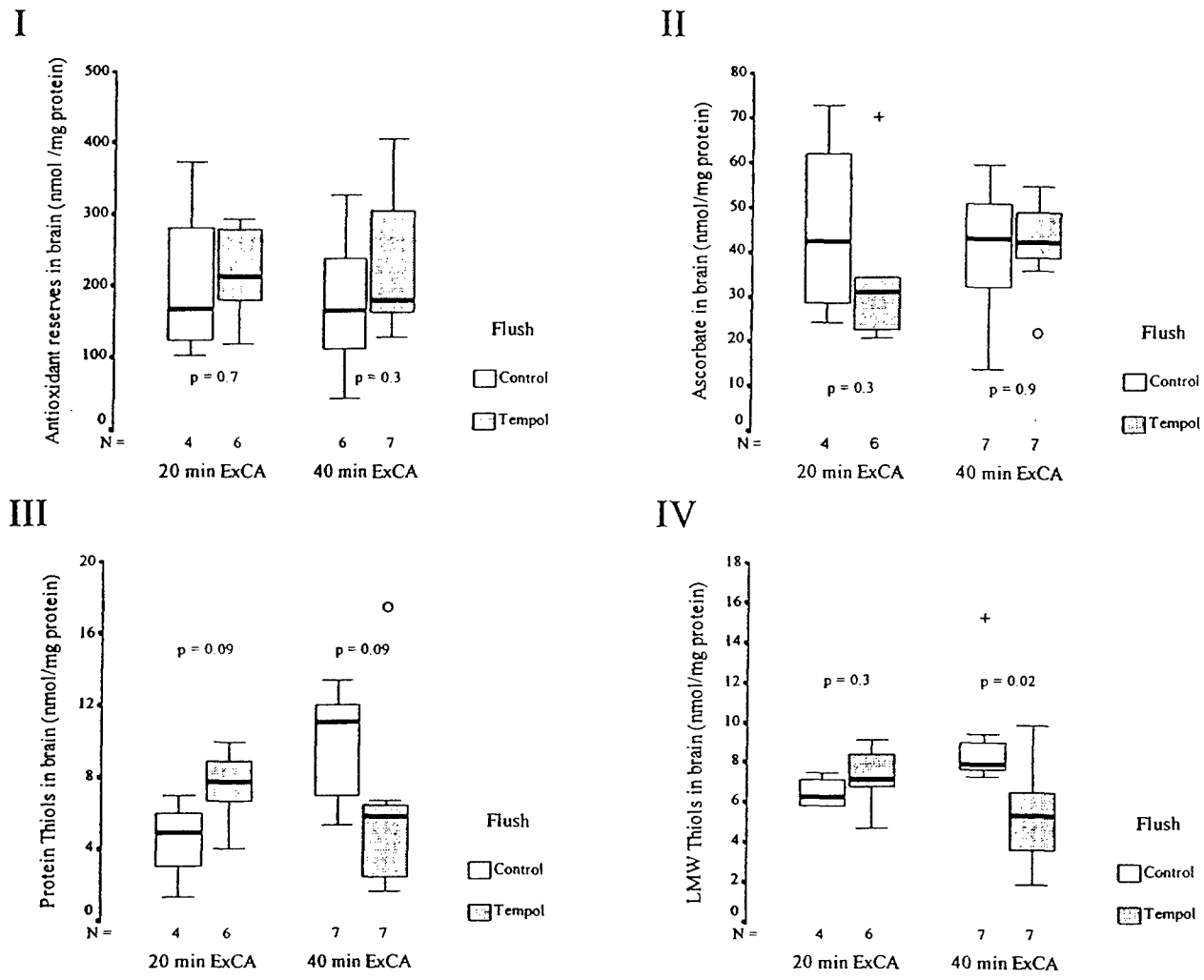

FIG. 6. Total antioxidant reserve (I), ascorbate (II), protein thiols (III), and low molecular weight thiols (IV), in brain frontal cortex 72 hours after exsanguination cardiac arrest of 20 (study A) and 40 minutes (study B). Boxes represent interquartile ranges. The line across each box indicates the median, and the whiskers (⊥T) are the highest and lowest values. The o indicates outliers (values between 1.5–3 box-lengths from the upper or lower edge of the box). For reference, in shams (n = 4) without cardiac arrest or Tempol, brain values were 202 ± 60 nmol/mg protein (n4) for antioxidant reserve, 45 ± 11 for ascorbate, 4.5 ± 1.2 for protein thiols, and 6.4 ± 0.4 for low molecular weight thiols.

staining at 72 hours in controls or in the Tempol group. Most likely, 72 hours after the insult is too late to detect single-strand DNA damage. At this point, cells have recovered from the insult, or damage to the cell structure is too severe to be detected by PANT staining.

The EPR Tempol signal in the brain was detected at 72 hours in almost all dogs treated with 300 mg/kg Tempol. This finding is consistent with a sustained effect of the drug, even when given only with the flush at the start of CA. The EPR Tempol signal in plasma was highest at the start of reperfusion and was detectable for up to 36 hours. This finding shows that Tempol was delivered and present in the brain. After 40-minute but not 20-minute CA, the addition of the oxidant potassium-ferricyanide to plasma samples early at reperfusion increased the Tempol signal, probably because reducing equivalents (in part $O_2^{-}\cdot$), which reduce Tempol to the EPR-silent Tempol-OH, accumulate more during 40-minute CA. The addition of potassium-ferricyanide converts Tempol-OH back to its EPR-detectable radical form Tempol.

We assume that ischemia-reperfusion causes a free-radical attack in the brain that consumes the antioxidant reserve. Only total antioxidant reserve in brains of four dogs without CA were slightly higher, whereas thiols and ascorbate levels were similar. Ascorbate is the primary water-soluble antioxidant in the brain, where the EPR detectable ascorbate radical intermediate reflects its interactions with oxidative stress-inducing free-radical species. We would anticipate detecting a greater antioxidant reserve in dog brains treated with Tempol. When ROS are generated in the brain, these radicals will be likely scavenged by Tempol and Tempol-OH, thereby protecting other targets from oxidative damage. Total antioxidant reserve in the brain frontal cortex 72 hours

after CA was not different between groups (Fig. 6-I). As a recycling antioxidant, Tempol is reduced to Tempol-OH at the expense of other intracellular reductants, such as ascorbate and thiols. One can easily envision a cascade in which the radical form of Tempol is reduced to Tempol-OH at the expense of ascorbate oxidation. Thiols, in turn, can recycle ascorbate from its oxidation products at the expense of their own oxidation. This idea is in line with our observation of unchanged ascorbate levels (Fig. 6-II) and decreased levels of thiols (Figs. 6-III and 6-IV) in study B dogs treated with Tempol. Consumption of thiols yields increased amounts of Tempol-OH, which is a strong electron-donating antioxidant. The finding that the total antioxidant reserve in brain at 72 hours did not differ between groups suggests that Tempol preserves other antioxidants, and is finally recycled at the expense of thiols. Obviously, thiols are not the major components of the total antioxidant reserve in the brain because the decrease in thiols was not reflected in a decrease in total antioxidant reserve. Measurements of biochemical markers of the oxidative pathways only at 72 hours after CA limit the conclusion. Measurements at different times would be desirable, but were not feasible in this outcome-oriented study.

Antioxidants are expected to be beneficial when administered during reperfusion. In two pilot experiments with the same model, we added 100 and 150 mg/kg Tempol to the flush at start of CA and 50 and 150 mg/kg added during reperfusion in the two dogs, respectively. With these preservative doses, which are lower than those in the present study, both dogs remained as severely brain damaged, as did the controls. This finding suggests that reoxygenation injury triggers occur during ischemia. Indeed, there is an increase in free-radical formation during ischemia (Nelson et al., 1992). In rats with hemorrhagic shock, Tempol given early but not late improved outcome (Kentner et al., 2000). Although mild or moderate hypothermia in itself is beneficial through synergism of multiple mechanisms, adding Tempol for an antioxidant effect apparently adds to that of hypothermia. Further improvement in preservation potency might necessitate broader combination treatments.

The introduction of Tempol to clinical resuscitation medicine might be problematic. Tempol (but not Tempol-OH) can oxidize the iron moiety of hemoglobin, which can lead to the methemoglobinemia seen in this study. Clinically, the maximal values of 4.5% to 13.4% methemoglobin seen at 1 to 2 hours of reperfusion (after 300 mg/kg Tempol) causes only minor symptoms (Wright et al., 1999). At 12 hours, methemoglobin levels were below 1% (presumably harmless). An alternative approach would be to use Tempol-OH instead of Tempol because our results suggest that the reducing environment during and after CA favors accumulation of Tempol-OH. Recovery of Tempol from Tempol-OH is achieved after adding the oxidant potassium-ferricyanide.

In a swine model, Tempol produced dose-related hypotension accompanied by reflex tachycardia, agitation, somnolence, and seizures (Hahn et al., 1999). In our model, the mild hypotensive effect of Tempol was easily manageable with higher doses of epinephrine to restore spontaneous circulation, and norepinephrine after restoration of spontaneous circulation to maintain normotension (Table 2). We did not observe agitation, somnolence, or seizure activity in the dogs treated with Tempol after anesthesia was discontinued after 24 to 72 hours.

We conclude that in dogs, the antioxidant Tempol enhances the beneficial effect of mild or moderate cerebral hypothermia on functional outcome when induced at the start of prolonged CA by aortic flush. Tempol in the doses used seems to produce no major complications. The mechanism of the beneficial effect on neurologic dysfunction without the mitigation of histologic damage is complex and needs further clarification. Future research exploring optimal preservative and resuscitative strategies for CA should include investigation of Tempol and other brain-penetrating nitroxides.

Acknowledgments: The authors thank Drs. Lyn Yaffe, Carleton Hsia, and Larry W. Jenkins for their valuable suggestions. Keri Janesko and Paula Nathaniel for preparing tissue slides for histologic evaluation, Sherman Culver, Nikolas Dedousis, Yuichi Sakai, William Stezoski, and Jason Stezoski for helping with intensive care unit life support, and Patricia Boyle for helping with the editing. The Cardeon Corporation provided the flush catheter.

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SURVIVAL OF 60 MIN CARDIAC ARREST IN DOGS WITH 15°C VS 20°C CEREBRAL PRESERVATION BY COLD AORTIC FLUSH. STUDY I.

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Introduction: Exsanguination or prolonged normovolemic cardiac arrest (CA) can rarely be survived with conventional resuscitation attempts. A new approach must be found to preserve the brain and heart until surgical hemostasis can be achieved or cardiopulmonary bypass (CPB) can be initiated ("suspended animation"). We are exploring in dogs **Methods** to preserve the organism during prolonged CA. In this study, we investigated whether intact survival after hypothermic CA of 60 min requires brain temperature of 15°C or 20°C as delivered by a single cold aortic flush (AF) at the start of CA. **Methods:** Male dogs (~20 kg) were exsanguinated over 5 min to CA of 60 min no-flow. At CA 2 min, the dogs received a saline AF (~1 L/min) at 0–4°C until tympanic temperature (Tty) reached 15°C (n=3) or 20°C (n=3), using a balloon-tipped catheter inserted via the femoral artery into the abdominal aorta. After the AF, the head was immersed in ice-water. Resuscitation was by closed-chest CPB. Post-CA mild hypothermia was to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated at 24–72 h in terms of overall performance category (OPC 1=normal, 5=brain death), and neurologic deficit score (NDS 0–10%=normal, 100%=brain death); and at 72 h with total and regional histologic damage scores (HDS). **Results:** All 3 dogs with Tty 15°C survived and achieved functional normality (OPC 1 and NDS 0%). All 3 dogs with Tty 20°C achieved OPC 2 (moderate disability) with NDS 22%, 27%, and 19% due to various degree of disabilities in the hindlegs. The flush volume and duration needed to reduce Tty from baseline 37.5°C to 15°C was 7.6 L (8:08 min), 5.2 L (5:10 min), and 5.6 L (6:15 min); to Tty 20°C 3.0 L (3:15 min), 3.2 L (3:30 min), and 2.9 L (3:18 min). HDS and spine histology are pending. **Conclusion:** A single large volume cold saline flush into the abdominal aorta at start of CA induces rapidly profound cerebral hypothermia of 15°C, preserving total body viability for a no-flow time of 60 min. A lower flush volume might preserve the brain but not the spinal cord.

(Supported by the US Office of Naval Research)

128/M34

INTACT SURVIVAL OF 60, 90, AND 120 MIN CARDIAC ARREST IN DOGS WITH 10°C CEREBRAL PRESERVATION BY COLD AORTIC FLUSH. STUDY II.

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Introduction: Exsanguination or prolonged normovolemic cardiac arrest (CA) can rarely be survived with conventional resuscitation attempts. A new approach must be found to preserve the brain and heart until surgical hemostasis can be achieved or cardiopulmonary bypass can be initiated ("suspended animation"). We are exploring in dogs methods to preserve the organism during prolonged CA. In this study, we investigated the longest duration of profound hypothermic CA reversible to intact survival after a single cold aortic flush (AF) at the start of CA. **Methods:** Male dogs (~20 kg) were exsanguinated over 5 min to CA of 60 min (n=3) or 90 min (n=3) no-flow. At CA 2 min, the dogs received a saline AF (~1 L/min) at 0–4°C until tympanic temperature (Tty) reached 10°C, using a balloon-tipped catheter inserted via the femoral artery into the abdominal aorta. After the AF, the head was immersed in ice-water. In one additional dog, no-flow time was 120 min with the balloon of the AF-catheter deflated and pulled back below the aortic bifurction after reaching Tty of 10°C, continuing the flush until rectal temperature reached 20°C. Resuscitation was by CPB. Post-CA mild hypothermia was to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated at 24–72 h in terms of overall performance category (OPC 1=normal, 5=brain death), and neurologic deficit score (NDS 0–10%=normal, 100%=brain death); and at 72 h with total and regional brain histologic damage scores (HDS). **Results:** All dogs with 60 and 90 min CA survived and achieved functional normality (OPC 1 and NDS 0%). The flush volume needed to reduce Tty to 10°C ranged from 8.9 to 14.6 L, and flush duration from 9:11 to 15:58 min. The one dog with 120 min CA achieved OPC 1, but showed mild disability in the hindlegs (NDS 6%), with a flush volume of 16.4 L over 16:39 min. HDS are pending. **Conclusion:** A single large volume cold AF at start of CA induces rapidly profound cerebral hypothermia of 10°C, preserving cerebral and myocardial viability for a no-flow time of up to 120 min. A clinically feasible method for rapid aortic access should be developed. (Supported by the US Office of Naval Research)

Revision R1 of Manuscript CCM-00220-2002

To Crit Care Med
Final
First submission 2/02
Revision submitted 8/16/02

Survival Without Brain Damage After Clinical Death of 60 – 120 min in Dogs Using Suspended Animation by Profound Hypothermia

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Funding: Supported by US Department of Defense, i.e., the Office of Naval Research (ONR), grant #N00014-97-1-1064; and the US Army's MRMC/TATRC, grant #N00014-99-1-0765.

Key words: Cardiac Arrest. Hypothermia. Hemorrhage. Resuscitation. Cerebral ischemia. Cardiopulmonary bypass.

ABSTRACT:

Objectives: This study explored the limits of good outcome of brain and organism achievable after cardiac arrest (no blood flow) of 60 - 120 min, with preservation (suspended animation) induced immediately after the start of exsanguination cardiac arrest (CA).

Design: Prospective experimental comparison of three arrest times, without randomization.

Setting: University research laboratory.

Subjects: 27 custom-bred hunting dogs (17 – 25 kg).

Interventions: Dogs were exsanguinated over 5 min to CA no-flow of 60 min, 90 min, or 120 min. At CA 2 min, the dogs received, via a balloon-tipped catheter, an aortic flush of isotonic saline at 2°C (at a rate of 1 L/min), until tympanic temperature (Tty) reached 20°C (for 60 min CA), 15°C (for 60 min CA), or 10°C (for 60, 90, or 120 min CA). Resuscitation was by closed-chest cardiopulmonary bypass, post-CA mild hypothermia (Tty 34°C) to 12 h, controlled ventilation to 20 h, and intensive care to 72 h.

Measurements: Overall performance categories (OPC 1 = normal, 2 = moderate disability, 3 = severe disability, 4 = coma, 5 = death), neurologic deficit scores (NDS 0 – 10% = normal, 100% = brain death), and regional and total brain histologic damage scores at 72 h (total HDS > 0 – 40 = mild; 40 – 100 = moderate; > 100 = severe damage), and morphologic damage of extracerebral organs.

Results: *For CA 60 min* (n = 14), Tty 20°C (n = 6) was achieved after flush of 3 min, and resulted in 2 dogs with OPC 1, and 4 dogs with OPC 2; median NDS = 13% (range 0 – 27%); and median total HDS = 28 (range 4 – 36). Tty 15°C (n = 5) was achieved after flush of 7 min, and resulted in all 5 dogs with OPC 1; NDS = 0% (0 – 3%); and HDS = 8 (0 – 48). Tty 10°C (n = 3) was achieved after flush of 11 min, and resulted in all 3 dogs with OPC 1; NDS = 0%; and

HDS = 16 (2 – 18). *For CA 90 min* (n = 6), Tty 10°C was achieved after flush of 15 min, and resulted in all 6 dogs with OPC 1, NDS = 0%, and HDS = 8 (0 – 37). *For CA 120 min* (n = 7), 3 dogs had to be excluded. In the 4 dogs within protocol, Tty 10°C was achieved after flush of 15 min. This resulted in one dog with OPC 1, NDS 0%, and total HDS 14; one with OPC 1, NDS 6%, and total HDS 20; one with OPC 2, NDS 13%, and total HDS 10; and one with OPC 3, NDS 39%, and total HDS 22.

Conclusions: In a systematic series of studies in dogs, the rapid induction of profound cerebral hypothermia (Tty 10°C) by aortic flush of cold saline immediately after the start of exsanguination CA (which is "unresuscitable" with current methods), can achieve survival without functional or histologic brain damage, after CA no-flow of 60 or 90 min, and possibly 120 min. The use of additional preservation strategies should be pursued in the 120 min arrest model.

INTRODUCTION

In considering resuscitation from severe hemorrhage, one must differentiate between hemorrhagic shock, which is low flow and common; and exsanguination cardiac arrest (CA) which is no-flow and rare. CA is the topic of this study. Civilian trauma patients and military combat casualties with penetrating (often repairable) trunkal injuries exsanguinate rapidly to CA. Conventional resuscitation attempts are futile, and survival rates are near zero (1-4). For such unresuscitable conditions, since 1984, Safar and Bellamy have recommended research into "suspended animation for delayed resuscitation." This they have defined as "induction of preservation of the organism within the first 5 min of CA (no-flow) for transport and surgical hemostasis during clinical death, to be followed by delayed resuscitation to survival without brain damage" (4).

Treatment induced before arrest (*protection*) and maintained during arrest (*preservation*) is more likely to mitigate post-ischemic brain damage than when induced after arrest (*resuscitation*) (5,6). Suspended animation is preservation-resuscitation with use of drugs or hypothermia. Using systematic studies of exsanguination CA in a reproducible dog outcome model with induction of preservation by aortic flush at 2 min CA, of saline at 24°C, via a balloon-tipped catheter (7-10), we obtained disappointing outcome results with a series of mechanism-specific pharmacologic therapies (11-15). The exception was the anti-oxidant Tempol, which improved functional outcome (15). In contrast, lowering the temperature of the flushed saline to 2°C and progressively increasing the flush volume, starting the flush at 2 min of normothermic exsanguination CA, we could decrease brain (tympanic membrane) temperature to around 34°C, which preserved brain viability during CA of 15 min (7) and 20 min (8), and to around 28°C, which preserved brain viability for 30 min (9). The present study is an extension

of these systematic efforts to maximize the duration of CA (no-flow) from which resuscitation to survival can be achieved without vital organ systems damage (10). Attempting to extend this maximal CA period from 30 min to 120 min is called for by the fact that transport and surgical haemostasis in patients with traumatic exsanguination to CA would need such prolonged preservation, particularly in military combat scenarios.

Protective-preservative hypothermia, induced and reversed with cardiopulmonary bypass (CPB), is clinically used for some elective operations on heart or brain, but has not been evaluated yet for emergency scenarios as in this study. Elective therapeutic hypothermia has been shown to protect the brain and whole organism in animals or patients for up to 15 min CA at brain temperature of about 35°C (mild hypothermia) (16,17), for up to 20 min CA at about 30°C (moderate hypothermia) (18), for up to 30 min CA at about 20°C (deep hypothermia) (19), for up to 60 – 150 min CA at 5 - 10°C (profound hypothermia) (20-27), and perhaps even for longer CA with ultraprofound hypothermia (28-30). The normal brain is not damaged by temperatures lowered to 5-10°C (31), but can be damaged by temperatures below 5°C (32,33). In most of the above mentioned studies of protective-preservative hypothermia for elective prolonged CA (23-30), induction of hypothermia was with CPB, *before* induction of CA and without total exsanguination; also, evaluation of cerebral function and histology was not quantitative as in our present study.

The experiments reported in this paper are the first systematic explorations of emergency measures aimed at maximizing the reversible CA no-flow duration. The method should ultimately be inducible for patients also outside hospitals. Ours is the only group experimenting with this suspended animation approach, which includes early and rapid induction of preservation with aortic flush and intensive care life support for 72 h to give the ischemic anoxic

encephalopathy time to mature. The objective of this study was to simulate the scenario of rapid exsanguination to death from a laceration in the aorta or vena cava, and to determine for the first time, the longest no-flow period from which resuscitation to complete recovery can be accomplished with the aid of CPB. Among the firsts of this study is also the use of a single aortic saline flush immediately *after* the start of CA (no-flow), to include preservation and long-term intensive care to outcome evaluation in terms of function and semi-quantitative histologic brain damage. We *hypothesized* (10) that preservation during CA 60, 90 or 120 min (no-flow) requires Tty 10°C to achieve intact survival without histologic brain damage.

MATERIAL AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and the Department of Defense and followed national guidelines for the treatment of animals. All experiments were conducted by the same team between May 2000 and January 2001, in mixed sequence, without randomization. The protocol called for exsanguination to CA -- in study A with 60 min no-flow, comparing three preservative levels of hypothermia (Tty 20, 15, or 10°C); and in study B with 90 min vs 120 min no-flow at Tty 10°C. Resuscitation was with CPB, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to final outcome evaluation at 72 h. At CA 2 min, the dogs received a flush into the aorta with saline at 2°C—in study A until Tty reached 20°C (n = 6), 15°C (n = 5), or 10°C (n = 3) for CA 60 min; and in study B until Tty reached 10°C for CA 90 min (n = 6), or 120 min (n = 7, of which 3 had to be excluded [see results]).

Preparation

We studied 27 custom-bred hunting dogs (17 – 25 kg body weight; age 8 – 12 months, simulating young healthy trauma victims). Details of preparation and model have been described (7-9,13-15). Briefly, after premedication with ketamine, orotracheal intubation, anesthesia with halothane and N_2O:O_2 (1:1), temperature probes were inserted for measuring Tty and esophageal (Tes) and rectal temperatures (Tr). In pilot experiments during flush, brain tissue temperature decreased more rapidly than Tty, but the two equilibrated rapidly within ± 1°C. Intravenous maintenance fluid was with dextrose 5% in 0.45% NaCl. The left femoral artery was cannulated for monitoring of arterial pressure. A pulmonary artery catheter was inserted for monitoring of pressure, cardiac output, and temperature (Tpa). A prototype balloon catheter (8 Fr), with one hole at the tip of the catheter, was advanced via the right femoral artery into the aorta for arterial

bleeding and for the aortic flush. The right external jugular vein was cannulated with a multiple-holed cannula (18 Fr), which was advanced to the level of the right atrium, for venous bleeding and for venous return to the CPB system. Arterial and central venous pressures and the electrocardiogram were continuously recorded. Arterial and mixed venous blood gases, hemoglobin, hematocrit, sodium, potassium, glucose, and lactate were measured at regular intervals. Blood gases were controlled as measured at normothermia (-alpha-stat strategies). Samples for liver function (GOT, GGTP, and bilirubin in serum) and kidney function (creatinine in serum and urine) were taken at baseline, 24 h, and 72 h. Just before start of the insult, Tty was controlled at 37.5° ± 0.1°C by heating blanket and lamp.

Insult

After two baseline measurements, heating devices, i.v. fluids, and halothane were discontinued, while the dogs were weaned to spontaneous breathing of N_2O:O_2 (2:1) via a T-tube. When the canthal reflex returned (as an indication of very light anesthesia), hemorrhage was initiated. Over a 5-min period the dogs were bled via the arterial and venous cannulae (simulating traumatic laceration), and the blood was collected in bags with sodium citrate anticoagulant, for later reinfusion. Hemorrhage was controlled to MAP 20 mmHg at 4 min. At 5 min, to assure zero blood flow, ventricular fibrillation (VF) was induced with one or more subcutaneous transthoracic shocks of 110 volts AC, to fully control the onset of circulatory arrest. Total arrest. (no-flow) time was 60 min in study A, and 90 or 120 min in study B.

Aortic flush

Two min after the onset of CA, the balloon of the aortic catheter, placed in the abdominal aorta, was inflated with 1.5 mL saline, known to occlude the aorta (9). Saline at 2°C was then flushed into the aorta at a rate of 1 L/min, using a roller pump. In study A, the flush was stopped

when Tty reached 20°C (n = 6), 15°C (n = 5), or 10°C (n = 3). In study B, for CA 90 min, the flush was stopped when Tty reached 10°C (n = 6). For CA 120 min, a pilot experiment had shown the hind legs with rigor mortis at start of resuscitation, and the rectum was necrotic at necroscopy. Therefore, in study B, for CA 120 min, the balloon was first placed in the thoracic aorta until Tty reached 10°C and then deflated and pulled back into the femoral artery, continuing the flush until Tr reached 20°C (n = 7). After the flush, during CA, the aortic catheter was replaced with a short arterial CPB cannula (7 or 8 Fr).

During CA, the head and neck were emperically immersed in ice-water to prevent the spontaneous rewarming by 3-5°C we have seen previously during CA of 60 – 120 min without ice water immersion. In two pilot experiments, laryngeal-pharyngeal edema occurred after the whole neck was immersed in ice water longer than 1 h; therefore, in study B only the head was immersed in ice water and neck edema did not occur.

Resuscitation

After CA no-flow of 60, 90, or 120 min, reperfusion was with CPB, because standard CPR cannot reliably achieve restoration of spontaneous circulation (ROSC) from CA longer than 12 min no-flow (6,16,17). The CPB circuit was primed with 400 mL of Dextran 40 10% plus Ringer's solution (1:1). Sodium bicarbonate (2 mEq/kg) and heparin (1500 units) were added. Just before the start of CPB, additional sodium bicarbonate (1 mEq/kg) was injected into the circuit. The dogs were paralyzed with pancuronium (0.1 mg/kg i.v.). The temperature of the water bath of the CPB heat exchanger was set to 5°C above Tty, until Tty reached 34°C. CPB was started with a flow of 50 mL/kg/min for Tty < 20°C, increased to 75 mL/kg/min for Tty 21°C – 30°C, and increased to 100 mL/kg/min for Tty > 30°C. Reinfusion of all shed blood was titrated to achieve a central venous pressure of 10 – 15 mmHg. Repetitive doses of epinephrine

(0.01 mg/kg) were given intra-arterially as necessary to increase MAP to 60 mmHg during Tty < 20°C, to 80 mmHg during Tty 21°C – 30°C, and to 100 mmHg during Tty > 30°C. When Tpa reached 32°C, defibrillation attempts were with external DC countershocks of 150 J, increased by 50 J for repeated shocks. Oxygen flow through the oxygenator was adjusted to keep $PaCO_2$ at 30 – 35 mmHg and $PaO_2 \geq 100$ mmHg. During CPB of 2 h, controlled ventilation was resumed with 100% O_2 at a rate of 8 – 10 inflation/min. The i.v. fluids were restarted with a flow of 100 mL/h. A base deficit of > 6.0 mEq/L was corrected with sodium bicarbonate. When ROSC was established, a norepinephrine infusion was titrated i.v. to achieve a brief hypertension of MAP ≥ 150 mmHg (9). We are including in standard protocols hypertensive reperfusion which improves cerebral blood flow and outcome (36). Thereafter, MAP was controlled at 90 – 150 mmHg. The CPB flow rate for assisted circulation, was reduced to 75 and 50 mL/kg/min, and stopped at 120 min. During CPB, activated clotting times were maintained at > 300 sec with additional heparin as needed.

Intensive Care

After weaning from CPB assist at 2 h, controlled ventilation was continued to 20 h with N_2O:O_2, 1:1 for analgesia. Paralysis was maintained with intermittent doses of pancuronium. To prevent stress, fentanyl boluses (5 – 10 μg/kg) were given i.v. whenever mydriasis, tachycardia, or hypertension occurred. In pilot experiments without paralysis, there were no escape movements with this regimen (9). Hypotension (MAP < 90 mmHg) was treated with titrated i.v. infusion of Ringer's solution or norepinephrine. Severe hypertension (MAP > 150 mmHg) was controlled with titrated i.v. boluses of labetalol or hydralazine. Standard intensive care included airway suctioning, periodic deep lung inflations, and position change (rotation). The dogs received cefazolin every 8 h for infection prophylaxis. At 20 – 24 h, paralysis was

reversed to spontaneous breathing with neostigmine plus atropine. The dogs were extubated when they were able to maintain normal blood gas values during spontaneous breathing and after upper airway reflexes had returned. The catheters were then removed under brief light N_2O-halothane anesthesia by mask. When awake and stable, the dog was transferred to a step-down ICU to 72 h, with O_2 by mask, continuous monitoring of pulse rate and arterial O_2 saturation by technicians and critical care physicians. Seizures, running movements, opisthotonos, or spontaneous tachypnea were controlled with titrated doses of diazepam (0.2 – 0.3 mg/kg i.v.) as needed. Tty was controlled at 34°C with external cooling and warming for the first 12 h after start of CPB, and at 37.5°C until 72 h. The maintenance i.v. fluid was dextrose 5% in NaCl 0.45% until 24 h, and dextrose 10% in NaCl 0.45% thereafter for supply of energy -- until the dog was able to eat and drink. Mild hyperglycemia is not harmful in patients (34) and might even be beneficial (35).

Outcome evaluation

Function and cerebral morphologic changes were evaluated as described before (6,36-40). Briefly, performance was evaluated according to overall performance categories (OPC 1 = normal; 2 = moderate disability; 3 = severe disability; 4 = coma; and 5 = death or brain death). Neurologic function was evaluated as neurologic deficit scores (NDS 0 – 10% = normal; 100% = brain death). OPC and NDS were evaluated every 8 h after extubation. Final evaluations (72 h) were independently determined and agreed upon by two team members. Attempts were made to discontinue any sedation at least 4 h prior to final evaluations. If necessary, sedation was reversed with flumazenil.

After final outcome evaluation at 72 h, for morphologic studies (37), the dogs were re-anesthetized as before, the left hemithorax opened, and the proximal descending aorta ligated. A

large-bore cannula was inserted proximal to the ligature. The dogs were then killed by infusing into the aortic arch approximately 2 liters of paraformaldehyde (4%, pH 7.4). A complete necropsy was performed and samples of extracerebral organs were taken for histologic examination. Macroscopic scoring was performed of damage in gut and heart (mild vs moderate vs severe hemorrhage; any necrosis).

One hour after perfusion fixation, the brain was removed. After cutting 3-mm thick slices, the same six slices of each brain were paraffin-embedded, cut into sections 4 microns thick, and stained with hematoxylin-eosin-phloxine (37). Using light microscopy, the same pathologist (AR), unaware of treatment, group assignments, and hypotheses, scored 19 distinct anatomic brain regions for severity and extent of ischemic neuronal changes (shrunken eosinophilic neurons with pyknotic nuclei), infarcts, and edema, as described previously (37,38). The total brain histologic damage score (HDS) was the sum of all area scores. A total HDS between zero and about 40 has usually correlated with normal or minimally impaired function; about 40 – 100 represented moderate damage; and total HDS > 100 indicated severe damage (37,38). Extracerebral variables were monitored as described in Results.

For exploration of *cognitive function* recovery, 3 dogs with OPC = 1 and NDS 0 – 10% (normal) at 72 h -- one of study A after CA 60 min at Tty 20°C, one of study B after CA 90 min at Tty 10°C, and one normal dog without CA -- were evaluated over 6 months for their ability to learn a spatial version of a successive reversal learning task, modified from Head et al (41). The dogs were initially habituated to a test chamber. On one wall of the chamber were two head holes that could be covered or opened by a sliding door. Outside of each head hole was a block that covered the reward. The dogs were trained to find a food reward based on the spatial position (left vs right), that was the reverse of the preceding trial. The reward was alternatively

hidden under the left or right block and a correct response was counted if the dog chose the side with the reward. We measured the number of sessions, of 10 consecutive trials each, needed to twice achieve 8 of 10 correct responses.

Statistical analysis

Dogs that either did not meet protocol criteria or died from extracerebral causes before 72 h were excluded from outcome analysis. Brain death as an outcome was included if the study process satisfied protocol. Data are given as mean and standard deviation (SD), if normally distributed, otherwise as median and range. This study was an exploratory one not depending on statistical group differences, and in study A, we did not perform any statistical group comparisons, since one group consisted of only 3 dogs. Merely for the purpose of complete information, we used in study B the Independent-Samples T test or the Mann-Whitney U Test for the comparison of continuous variables (physiologic variables; NDS; HDS), and the chi-squared test for trend to test for differences in proportions of OPC values between groups. All data were computed with SPSS® for Windows, release 8.0 (Illinois, USA), or NCSS® for Windows (Utah, USA). A p-value < 0.05 was considered statistically significant.

RESULTS

For both studies, a total of 27 dogs were exsanguinated to CA. In *study A* with CA 60 min (n = 14), and in *study B* with CA 90 min (n = 6), all dogs survived to 72 h in protocol. In *study B* with CA 120 min (n = 7), three dogs failed to survive to 72 h and had to be excluded because one developed pulmonary edema during CPB, hemorrhagic diarrhea, anemia, and hemorrhagic lungs and was euthanized at 23 h; one had the flush delayed due to technical pump error; and one died at 25 h unrecognized in the stepdown unit after premature extubation due to human error. Thus, only 4 of the 7 dogs in the CA 120 min group survived to final evaluation at 72 h. One dog of study A after CA 60 min at Tty 20°C, and one dog of study B after CA 90 min at Tty 10°C, although within protocol, provided only functional outcome data because they were kept alive for cognitive function testing at 6 mo, leaving 22 of the 27 dogs for morphologic evaluation at 72 h.

Heart rate, mean arterial pressure, and arterial PO_2, PCO_2, hematocrit, base excess, sodium, potassium, glucose, and lactate --at baseline and at resuscitation time 6 h -- were within normal ranges, except in *study B* at 6 h, where the blood glucose values in the CA 120 min group were statistically higher (median 316 mg/dL, range 289 – 363) than in the CA 90 min group (median 227 mg/dL, range 107 – 253) ($p = 0.01$).

Flush and temperatures

Flush volumes and durations needed to achieve the target Tty are given in table 1. After stopping the aortic flush, Tty decreased spontaneously slightly further, in *study A* with CA 60 min to a lowest Tty of 18.3 ± 1.8°C (range 15.1 – 20.0) in the 20°C group; to 14.4 ± 0.3°C (14.2 – 14.9) in the 15°C group; and to 10°C, 9.7°C, and 9.8°C in the 10°C group (figure 1A). In *study B* with CA 90 min the lowest Tty was 9.2 ± 1.0°C (7.5 – 10.0) (figure 1B); and with CA 120 min

the lowest Tty was 6.9 ± 0.8°C (5.9 – 7.9) (figure 1B). Lowest Tes (core T) ranged between 15.1 and 24.8°C in the Tty 20°C group, between 16°3C and 22.8°C in the Tty 15°C group, and between 6.1 and 15.3°C in the Tty 10°C groups. Lowest Tr was approximately 35 – 37°C, except for the CA 120 min dogs (with cold flush into the femoral artery), in which lowest Tr ranged between 20.0 and 24.6°C. In one dog, the flush was stopped when 15 L flush volume was consumed, with Tr 24.6°C.

Resuscitation

During reperfusion with CPB, the time required to increase core temperature (Tpa) to 32°C depended on the depth of hypothermia at the end of CA (table 1). In both studies, once Tpa reached 32°C, ROSC was achieved after 1 – 3 countershocks (table 1). In *study A*, one dog in the 20°C group spontaneously developed QRS complexes at the start of CPB and ROSC at 5 min after the start of CPB. The amount of epinephrine required to keep MAP in protocol during CPB did not differ between groups, nor did the amounts of norepinephrine and bicarbonate required after ROSC (table 1). There were no significant group differences in the highest MAP and the duration of the brief induced hypertension, which ranged between 1 and 14 min (table 1).

Complications

In addition to the complications that caused 3 exclusions (see above), there were complications in 4 of the included dogs: One of the five included dogs with CA 60 min at 15°C developed increased pulmonary artery wedge pressure and pulmonary edema while on controlled ventilation. The pulmonary edema was managed with increasing PEEP; arterial PO_2 remained above 200 mmHg. One dog after CA 60 min developed fever in the step-down unit at 32 h, which was reversed with acetaminophen by mouth. One dog of this group had hemorrhagic diarrhea during the observation phase. One dog, after CA 90 min at 10°C, had hemorrhagic

diarrhea during the observation phase; and one dog of the same group developed tachycardic atrial fibrillation 1 h after start of CPB, which was resistant to amiodarone and diltiazem, but could be terminated with one synchronized countershock (50 J) at 24 h.

Overall and Cerebral Outcome

OPC, NDS, and total brain HDS at 72 h are shown in figure 2; regional HDS in figure 3.

In *study A*, after CA 60 min at Tty 20°C, 2 dogs achieved OPC 1 with NDS 0% and 1% (normal). In one of these dogs, total brain HDS was 28, and the other dog was kept for cognitive function testing. Four dogs achieved OPC 2 due to various degrees of motor impairment of the hind legs, including inability to stand and walk, which resulted in NDS 6 – 27%; they showed normal cerebral performance. Their total brain HDS was 4 – 36. Histologic evaluation of the spinal cord did not show any pathology. In the Tty 15°C and 10°C groups, all dogs achieved OPC 1 and NDS 0% (except that one in the 15°C group had NDS 3% due to weak hind legs). Total brain HDS was below 40 in all dogs in the 15°C and 10°C groups, except one in the 15°C group with worse HDS. Histologically normal brains were seen in 1 dog in the Tty 20°C group (HDS 4), 2 dogs in the 15°C group (HDS 0 and 6), and 1 dog in the 10°C group (HDS 2).

In *study B*, after CA 90 min at Tty 10°C (n = 6), all dogs were functionally normal (OPC 1 and NDS 0%), with total brain HDS = 8 (0 – 37) (n = 5). One dog had HDS zero. After CA 120 min (n = 4), functional outcomes varied. One dog achieved OPC 1 with NDS 0% and total HDS 14. One dog achieved OPC 1 with NDS 6% due to mild disability in the hind legs, and HDS 20. One dog achieved OPC 2 with NDS 13 due to weakened hind legs, and HDS 10. One dog achieved OPC 3 (poor outcome) with NDS 39, and HDS 22. Total brain HDS were below 40 in all dogs after CA 90 or 120 min, also in the one dog with OPC 3.

Regional brain HDS showed the same distribution in all groups. Therefore, the results are summarized for all 22 dogs in which HDS was evaluated (figure 3). In 17 of the 19 regions, the median HDS was zero. Putamen and caudate nucleus seemed to be the most vulnerable regions in this model, as was the case in this model also with moderate hypothermia. All scores were the result of scattered ischemic neurons, except for one dog with CA 90 min at 10°C that showed also edema in the thalamus and dentate nucleus, and another dog of the same group that had also an infarcted area in the dentate nucleus.

Cognitive function testing in the one dog with OPC 1 after CA 60 min at Tty 20°C, and in the one dog with OPC 1 after CA 90 min at 10°C, demonstrated that both were able to meet the criteria for successfully learning the cognitive task at 3 – 6 months after CA. Neither of these two CA dogs performed worse than the normal dog without CA.

Extracerebral Outcome

Extracerebral malfunction was transient and morphologic changes at 72 h were not severe in both studies (table 2). Variables reflecting gross cardiovascular-pulmonary function were restored to normal during controlled ventilation, except for the 4 included dogs with complications described above. At 24 h there were no major increases of alveolar-arterial PO_2 gradients; no dog was hypoxemic. Liver function test values were transiently impaired in all 72 h survivors; serum GOT values increased from baseline to 24 h, and then decreased, but remained above normal until 72 h. Serum GGTP and bilirubin were within the normal ranges at baseline, 24 h, and 72 h. Kidney function seemed to be preserved; urine flow ceased during and for 1 – 2 h after CA, then at 120 min after ROSC, achieving normal (baseline) serum creatinine levels at 24 h and 72 h. Creatinine clearance varied greatly between dogs, independent of group assignment.

Necropsy after CA 60 min at Tty 20°C, revealed moderately hemorrhagic areas in the gastric mucosa in only one dog. In the Tty 15°C group, hemorrhagic consolidation in one lung lobe was found in 3 dogs; moderate hemorrhagic areas on the liver surface in 2 dogs; and mild to moderate hemorrhagic areas in the mucosa of the small intestines in 2 dogs. In the 10°C group, the one dog with fever had lobar pneumonia, and the dog with hemorrhagic diarrhea had mild hemorrhagic areas on the surface of the liver and the rectal mucosa. Necropsy after CA 90 min at Tty 10°C, revealed mild to moderate hemorrhagic areas on the surface of the liver in 3 of 5 dogs, hemorrhagic gallbladders in 2 of 5, and mild hemorrhagic areas in the mucosa of the rectum in the one dog with hemorrhagic diarrhea. Necropsy after CA 120 min at 10°C, revealed moderate hemorrhagic areas on the surface of the liver and gallbladder in 2 of 5 dogs, mild hemorrhagic areas in the gut mucosa in 2 of 4 dogs, and moderate hemorrhagic areas in the gastric mucosa in one dog. None of the 22 necropsies revealed macroscopically necrotic ileum, as is often seen after severe shock states.

DISCUSSION

"Suspended animation for delayed resuscitation" is being researched to buy time for patients with temporarily unresuscitable CA and give them a chance to survive without brain damage (4,5). This clinically relevant exploratory study in dogs is one of a series of systematic studies of exsanguination CA. Trauma surgeons would like a preservation time of at least 60 min for transport and surgical hemostasis. In study A, we determined the lowest temperature needed to preserve the organism for 60 min. In study B, the longest possible duration of preservation with Tty 10°C was determined. This study is the first demonstration of profound hypothermic preservation induced after the onset of no-flow of 60 – 120 min, under simulated emergency conditions and with intensive care to 72 h. Complete recovery was documented quantitatively in terms of overall performance (OPC) and cerebral function (NDS), gross morphology of all organs, and total brain histologic damage scores (HDS). Interpretation of the results has limitations because the model is difficult and -- although clinically relevant -- is clinically not fully realistic. Brain histologic damage was evaluated by one pathologist who was blinded to hypotheses and group assignments.

Our results demonstrate the following: 1) It is possible to rapidly induce preservative deep and profound hypothermia via a single large-volume cold saline flush into the aorta after the onset of CA, without the need for CPB or heat exchanger. Requirements for use of this approach in the field, still to be worked out, include rapid access to aorta and vena cava, several liters of fluid ready in cold storage, and a portable cooling and pumping device. 2) An aortic cold flush to Tty 15°C, starting 2 min after onset of CA, can preserve viability of the organism, including the brain, for a no-flow time of up to 60 min; all 5 dogs achieved OPC 1, whereas Tty 20°C resulted in 4 of 6 dogs with hind leg weakness (OPC 2). 3) An aortic cold flush to Tty

10°C can preserve viability of the organism, including the brain, reproducibly for a no-flow time of up to 90 min, and in some cases for a no-flow time of even 120 min. 4) To achieve preservation during CA 60 – 120 min, distribution of the cold flush must include the spinal cord and abdominal viscera (see later). 5) For CA 120 min no-flow, many unknown variables need to be clarified to explain the variable outcomes; theoretically optimized pharmacologic solutions at 2°C should be explored in comparison with saline used so far (42,43). 6) With our model's ICU life support we are encouraged by the fact that the cooled extracerebral organs recovered fully with respect to function after CA 120 min, perhaps because the endothelium of the microcirculation was protected by cold *plus* washout of blood prior to stagnation or thrombosis. 7) This non-traumatic model and systemic heparinization for CPB used in this study are clinically *relevant* for exsanguination from a lacerated large vessel, but not for major diffuse tissue trauma which causes a major inflammatory response and coagulopathy in addition to that due to hemodilution, hypothermia, and ischemia (44). Recently, adding trauma to our Tty 10°C model, without systemic heparinization, use of a heparin-bonded CPB circuit, and use of fresh whole blood transfusion, we have achieved intact survival after CA of 60 min, but not yet after longer no-flow (44).

Hypothermia exerts its beneficial effects not merely by a reduction of O_2 requirement (uptake) (18,45), but through the synergism of multiple mechanisms, such as preservation of ATP; and reduction of excitotoxicity (47), edema (48), free radical reactions (49), and inflammation (50,51). Protective-preservative profound or ultra-profound hypothermia during CA 60 – 180 min, induced with CPB *before* the insult, in animals, has been reported previously by us (20-22) and others (23-30). In one study, esophageal temperature was reduced to near 0°C and CA extended to 180 min; 3 of 12 dogs survived (30). When in this study esophageal

temperature was reduced to only 3°C, and life support was optimized, 5 of 7 dogs survived without histologic brain damage (30). Details of histologic evaluation are critical. We have learned since the 1970s (38) that proof of "survival without brain damage" requires histologic search for selectively vulnerable ischemic neurons, in many selectively vulnerable regions, using systematic, semiquantitative examination and scoring of lesions throughout the entire brain, as in this study (37). Hind-leg weakness can recover to normality after 3 – 15 days (26,29). Since in our study all dogs with hind-leg weakness were euthanized at 72 h, and the spinal cord did not reveal any histopathology, it is unclear if this deficit would have eventually recovered completely.

Flush to Tty 10°C seems to enable preservation of the organism for up to CA 120 min, but not reliably (figure 2); the one dog that achieved only OPC 3 was conscious but not alert, and surprisingly had only very mild histologic brain damage. Longer observation might have resulted in functional recovery. In previous studies with shorter CA and mild hypothermia, no improvement of outcome was observed after 72 h (36-40). Possible explanations of variable outcomes after CA 120 min include inhomogeneous, multi-focal lack of cold flush perfusion. Future considerations for enhancing preservative hypothermia might include: adding a vasodilator to enhance homogenous fluid distribution, increasing flush pressure, and using a more physiologic flush solution than saline (42,43,52-55) and new drugs (15).

The significance of minimal to mild histologic brain damage at 72 h, seen in the majority of dogs in our study, is uncertain. We previously established significant correlations between total HDS and NDS at 3 – 4 days after normothermic ventricular fibrillation CA of 5-20 min no-flow (6,16,17,36-40). Less clear-cut correlations after longer CA can be explained by extracerebral organ failure (poor OPC) and morphologically normal brain, as in this study, or

improved cerebral function in the presence of unmitigated morphologic damage in "silent" brain regions (15). In a previous study by others (27), dogs after CA 105 min at 5 – 10°C showed microscopic changes in the brain although cognitive function seemed normal. It is encouraging that in our study cognitive function tested in two dogs 6 months after CA 60 or 90 min, was the same as in one control dog without CA. These explorations were carried out during the development of a new method of assessing cognitive function in dogs.

Further development of the potentials of "suspended animation for delayed resuscitation" for presently unresuscitable exsanguination CA (3,4) must go beyond the pathophysiologic and pharmacologic outcome studies performed so far: 1) Comparison of outcome with simulated present standard care is not necessary, because we simulated clinical scenarios in which thoracotomy, laparotomy, and fluid infusion have not been clinically effective. 2) We are planning clinical feasibility trials in emergency departments of trauma centers for exsanguinating trauma patients arriving pulseless (or becoming pulseless). Such patients, typically with penetrating truncal injuries, usually receive emergency thoracotomy (3,54,55). Rapid thoracotomy and aortic cannulation under vision, with available catheters (54) could preserve viability during CA until hemostasis is achieved, to be followed by reperfusion and slow re-warming by CPB. The right atrium or vena cava will have to be drained for decompression. The flush and drainage catheters can later serve CPB. Our results in large dogs suggest that in humans at least 18 L of flush fluid at 2°C would be needed to achieve Tty 15°C. This would preserve the organism for CA of up to 1 h. A cooling container for storing such a large volume of cold fluid and a pump are needed. Continued asanguinous low-flow by CPB, at profound hypothermia, can preserve the organism for more than 2 h (52). The sites of large-vessel injuries will influence where the balloon of the aortic catheter is to be placed, as inserted via the femoral

artery or via thoracotomy. 3) Ideally, CPB for cooling, reperfusion-rewarming and prolonged cardiopulmonary support should be available in the emergency departments of major trauma hospitals (4,17,56,57). For emergency departments under austere conditions, however, resuscitation from 10°C without CPB, using manual heart pumping and intrathoracic warm saline, should be explored. 4) For combat casualty care (4,58), access to the aorta without thoracotomy, perhaps by a still-to-be-developed "smart catheter" and a portable cooling/pumping device are needed. 5) Recently we have seen improved prevention beyond that achieved with cold saline flush, using a more physiologic brain preservation solution (53), without or with the antioxidant tempol (42,43). 6) Normovolemic CA, i.e., normothermic sudden cardiac death outside hospitals, resists attempts at ROSC by CPR in 50% of cases (59,60). It is possible that some of these deaths could be prevented with some still to be determined modification of "suspended animation," to bridge viability until the initiation of prolonged CPB. This and other novel possibilities for the suspended animation concept deserve exploration in animal models.

CONCLUSIONS

We conclude that the rapid induction of profound cerebral hypothermia by aortic flush of cold saline to Tty 10°C, immediately after the start of exsanguination CA (which is "unresuscitable" with current methods), can achieve survival without functional or histologic brain damage, after CA no-flow of 60 or 90 min, and possibly 120 min. The 120 min CA needs additional preservation strategy. ~~This non-traumatic model revealed no gross evidence of coagulopathy.~~

We recommend clinical feasibility trials of suspended animation for delayed resuscitation in cases of exsanguination CA from penetrating ~~truncal~~ injury, treated with emergency thoracotomy in trauma hospitals' emergency departments. More research and development are needed for the suspended animation approach in cases of blunt tissue injuries with coagulopathy, and for out-of-hospital scenarios requiring percutaneous vessel access and a portable cooling-pumping device. ~~and for possible benefit in CPR resistant normovolemic sudden cardiac deaths.~~

ACKNOWLEDGEMENTS:

Edwin Klein, VMD, performed histologic evaluation of the dogs' spinal cord. Sherman Culver, Nikolas Dedousis, Jeremy Henchir, Yuichi Sakai, William Stezoski, Jason Stezoski, and Murugan Subramanian helped with ICU life support. Alan Abraham helped with cognitive function tests. The Cardeon Corp. provided the flush catheter. Patricia Boyle helped with editing the manuscript.

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Table 1. Aortic flush volume and duration needed to achieve target tympanic temperature. Requirements for restoration of spontaneous circulation (ROSC) and hypertensive bout.

	Study A (60 min CA)			Study B (Tty 10°C)	
	Tty 20°C (n = 6)	Tty 15°C (n = 5)	Tty 10°C (n = 3)	90 min (n = 6)	120 min (n = 4)
Flush volume (mL/kg)	159 (144 – 228)	306 (258 – 373)	430, 469, 546	578 (535 – 736)	666 (598 – 755)
Flush duration (min:sec)	3:30 (3;15 – 5:30)	7:10 (5:19 – 8:40)	9:11, 10:55, 12:15	14:33 (11:50 – 15:58)	15:17 (13:45 – 16:39)
Time to reach Tpa 32°C (min)	22 (11 – 23)	20 (13 – 55)	31, 32, 33	44 (24 – 56)	43 (29 – 62)
Countershocks, total number	1 (0 – 2)	1 (1 – 3)	1, 1, 1	1 (1 – 1)	1 (1 – 2)
Countershocks, total energy (J)	150 (0 – 300)	150 (150 – 500)	150, 150, 150	150 (150 – 150)	150 (150 – 300)
Total bicarbonate (mEq)	205 (165 – 235)	185 (155 – 270)	200, 200, 230	220 (190 – 280)	250 (150 – 290)
Total epinephrine (mg)	0.9 (0.2 – 2.4)	1.0 (0.2 – 1.8)	0.6, 1.0, 1.5	1.6 (0.4 - 3.4)	1.3 (1.1 - 1.8)
Total norepinephrine (mg)	0.96 (0.80 – 1.76)	1.12 (0.96 – 2.24)	1.76, 1.92, 2.88	3.12 (0.8 - 7.68)	1.20 (1.12 - 1.28)
Hypertensive bout: peak MAP (mmHg)	175 (160 – 200)	165 (150 – 200)	150, 155, 160	165 (150 – 175)	165 (150 – 170)
Hypertensive bout start (min)[a]	22 (9 – 29)	30 (19 – 63)	40, 41, 43	54 (28 – 64)	49 (36 – 67)
Hypertensive bout duration (min)[b]	5 (3 – 7)	4 (3 – 5)	2, 4,6	4 (1 – 14)	4 (3 – 4)

Data are given as median (range), or as single values. CA, cardiac arrest. Tty, tympanic temperature. Tpa, pulmonary artery temperature. CPB, cardiopulmonary bypass. MAP, mean arterial pressure. [a]Start of hypertensive bout = time after start of CPB. [b]Duration of hypertensive bout = time with MAP > 150 mmHg.

Table 2. Parameters for liver and kidney function. Normal values in dogs: serum GOT = 10 – 50 IU/L; serum GGTP = 0 – 6 IU/L; serum bilirubin = 0.0 – 0.3 mg/dL; serum creatinine = 0.7 – 1.5 mg/dL; creatinine clearance = 2.9 – 4.5 ml/min/kg.

	Study A (60 min CA)										Study B (Tty 10°C)					
	Tty 20°C (n = 6)			Tty 15°C (n = 5)				Tty 10°C (n = 3)			90 min (n = 6)			120 min (n = 4)		
Dog	1	2	3	1	2	3	4	1	2	3	1	2	3	1	2	3
Serum GOT (IU/L)																
BL	Na	26	46	26	29	23	29	26	26	19	33	22	29	44	41	27
24 h	545	545	1717	495	1227	1083	1005	2827	625	227	738	593	246	571	680	1596
72 h	230	184	1234	169	350	262	453	509	na	41	217	147	277	392	279	1009
Serum GGTP (IU/L)																
BL	Na	9	10	7	6	5	5	6	6	6	8	5	8	5	13	7
24 h	6	10	9	11	16	6	7	7	6	5	8	11	8	8	10	8
72 h	8	11	11	9	7	9	12	7	na	6	9	8	8	7	11	17
Serum bilirubin (mg/dL)																
BL	Na	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.6	0.1
24 h	0.2	0.2	0.1	0.4	1.1	0.3	0.1	0.2	0.2	0.2	0.2	0.4	0.1	0.3	0.3	0.2
72 h	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.2	na	0.2	0.2	0.2	0.2	0.3	0.3	0.5
Serum creatinine (mg/dL)																
BL	Na	0.8	0.7	1.5	0.9	0.8	1.5	0.7	0.6	0.6	0.6	1	0.9	1	0.8	0.7
24 h	0.6	0.5	0.6	1.3	0.5	0.8	0.9	0.6	0.5	0.3	0.5	0.8	0.8	0.7	0.8	0.7
72 h	0.6	0.6	0.6	1.5	0.4	0.9	0.8	0.8	na	0.8	0.7	0.9	0.9	0.7	0.9	0.8
Creatinine clearance (ml/min/kg)																
24 h	6.4	5.6	2	1.8	5	5.1	2.7	6.7	6.1	6	7.1	2.8	3.2	4.4	1.5	4.4

FIGURE LEGENDS:

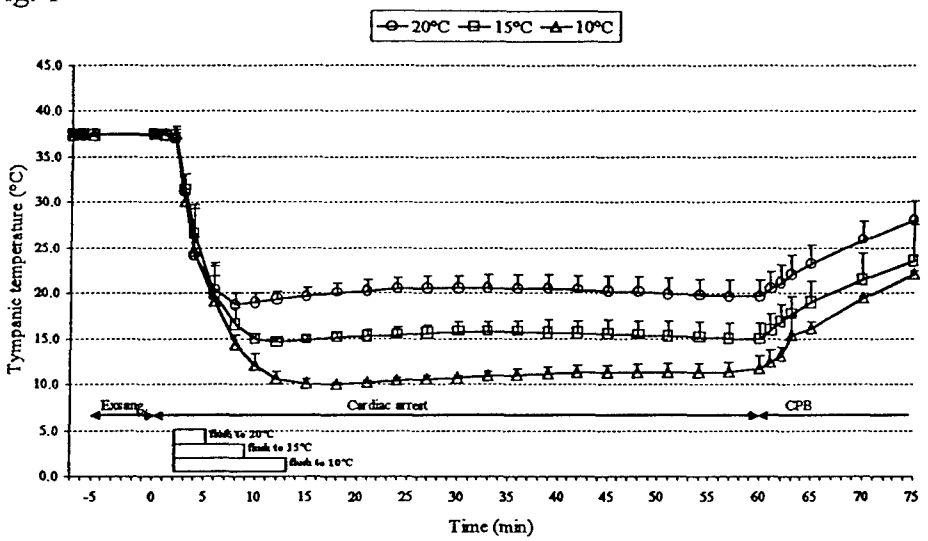
Figure 1: Tympanic membrane temperatures (Tty) during exsanguination cardiac arrest of 60 min no-flow in study A (A); and 90 – 120 min no-flow in study B (B). Resuscitation was with cardiopulmonary bypass (CPB). Data are given as mean and standard deviation.

Figure 2: Outcome in terms of final overall performance categories (OPC 1 - 5) at 72 h after exsanguination cardiac arrest of 60 min no-flow in study A; and cardiac arrest of 90 – 120 min no-flow in study B. Each dot represents one dog. NDS = neurologic deficit scores (1-100%). HDS = total brain histologic damage scores (0-40 no or mild damage; 40-100 moderate damage; > 100 severe damage). $^{*}n = 4$ and $^{\dagger}n = 5$ (one dog each was maintained for cognitive function testing).

Figure 3: Regional brain histologic damage scores (HDS) at 72 h after exsanguination cardiac arrest of 60 - 120 min. Boxes represent interquartile ranges. The line across each box indicates the median. The whiskers (⊥T) are the highest and lowest values. The o indicates extremes (values more than 3 box-lengths from the upper or lower edge of the box).

Fig. 1

A


20°C
15°C
10°C
Tympanic temperature (°C)
Time (min)
Exsang.
Cardiac arrest
CPB
flush to 20°C
flush to 15°C
flush to 10°C

B

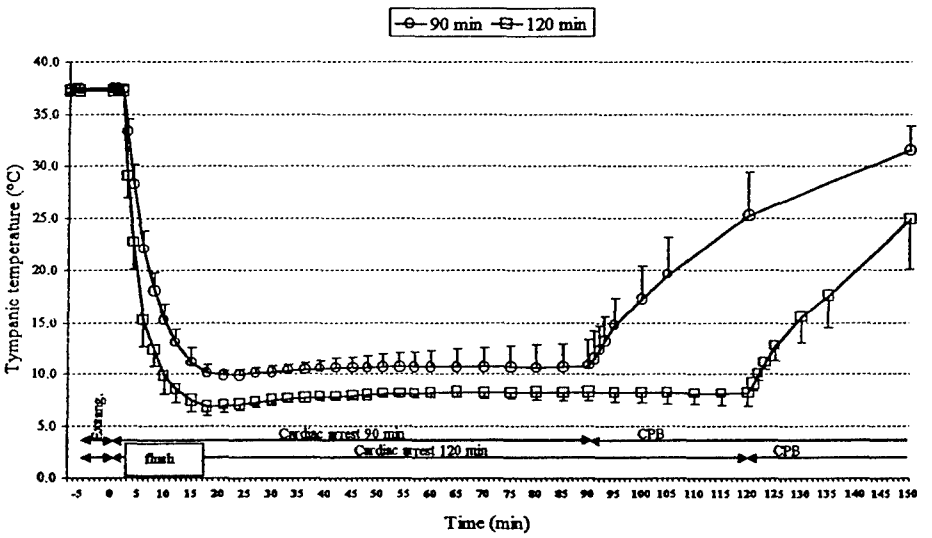
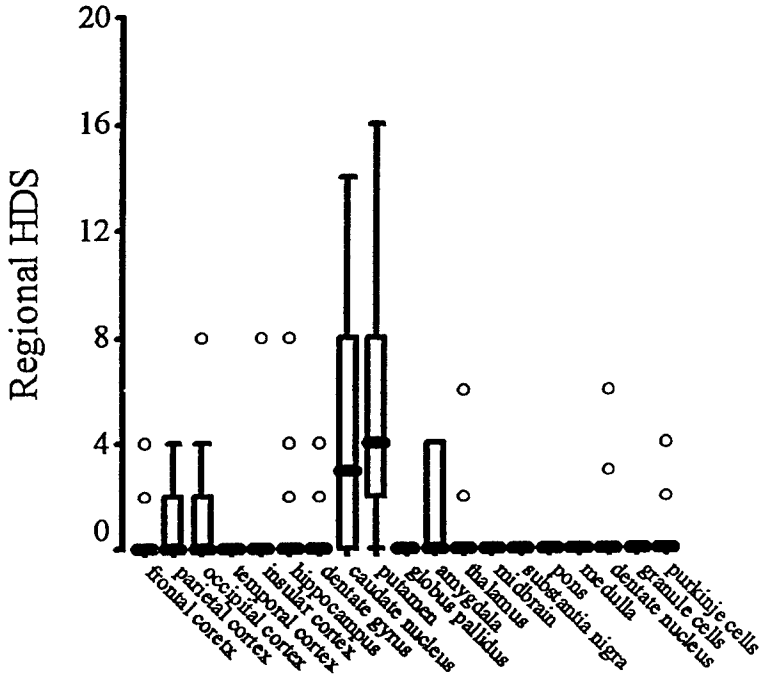

90 min
120 min
Tympanic temperature (°C)
Time (min)
Exsang.
Cardiac arrest 90 min
CPB
flush
Cardiac arrest 120 min
CPB

Fig. 2

	Study A (60 min CA)			Study B (Tty 10°C)	
	Tty 20°C (n = 6)	Tty 15°C (n = 5)	Tty 10°C (n = 3)	90 min CA (n = 6)	120 min CA (n = 4)
OPC 5 (death or brain death)					
OPC 4 (coma)					
OPC 3 (severe disability)					•
OPC 2 (moderate disability)	••••				•
OPC 1 (normal)	••	•••••	•••	••••••	••
NDS (%)	13 (0 – 27)	0 (0 – 3)	All 0	All 0	10 (0 – 39)
HDS	28 (4 – 36)*	8 (0 – 48)	2, 16, 18	8 (0 – 37)†	17 (10 – 22)

Fig. 3


Regional HDS
20
16
12
8
4
0
frontal cortex
parietal cortex
occipital cortex
temporal cortex
insular cortex
hippocampus
dentate gyrus
caudate nucleus
putamen
globus pallidus
amygdala
thalamus
midbrain
substantia nigra
pons
medulla
dentate nucleus
granule cells
purkinje cells

52

DELAYED INTRA-ISCHEMIC AORTIC COLD FLUSH FOR PRESERVATION DURING PROLONGED CARDIAC ARREST IN DOGS

Wilhelm Behringer, Peter Safar, Xianren Wu, Kentner Rainer, Ann Radovsky, Samuel A Tisherman, SCRR, Univ. of Pittsburgh, Pittsburgh, PA

Introduction: In exsanguination cardiac arrest (CA), conventional resuscitation attempts are futile. Therefore we introduced the use of cold aortic saline flush at start of CA to rapidly induce preservative hypothermia during prolonged CA for "suspended animation for delayed resuscitation". This study was to determine in dogs, how long the initiation of cold flush after onset of CA no-flow can be delayed without loosing its protective effect on brain and heart. Methods: Male dogs (20–25 kg) were exsanguinated over 5 min to CA of 30 min no-flow. At CA 2 min (n=7), 5 min (n=5), and 8 min (n=5), the dogs received a saline flush (100 mL/kg at 2°C over 4 min) using a balloon-tipped catheter inserted via the femoral artery into the abdominal aorta. Resuscitation was by closed-chest cardiopulmonary bypass, followed by assisted circulation for 2 h, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated at 72 h in terms of overall performance category (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=brain death); neurologic deficit score (NDS 0–10%=normal,100%=brain death); and total brain histologic damage scores (HDS 0=normal, >40=severe damage, >100=extensive damage). Results: Lowest tympanic membrane temperature (mean, standard deviation) during CA achieved with flush at CA 2 min, 5 min, and 8 min was 25.7°C (SD 2.5), 24.7°C (SD 1.1), and 26.6°C (SD 2.3) (p=0.4). After flush at CA 2 min, 4 dogs achieved OPC 1, and 3 dogs OPC 2; after flush at CA 5 min, 2 dogs achieved OPC 1, and 3 dogs OPC 2. After flush at CA 8 min, 4 dogs achieved OPC 3, and 1 dog OPC 4 (p=0.004, delay 8 min differed significantly from 2 and 5 min). NDS (median, range) were 4% (0–24), 14% (0–24), and 49% (41–56) (p=0.006, delay 8 min differed significantly from 2 and 5 min). Total HDS were 18 (0–46), 58 (20–94), and 150 (92–184) (p=0.003, delay 8 min differed significantly from 2 and 5 min). Total HDS correlated with the duration of delaying the aortic flush (rs=0.9, p<0.001). Conclusions: In prolonged CA no-flow, preservative moderate cerebral hypothermia by aortic flush has to be induced as soon as possible after the onset of CA to achieve maximal preservation of brain and heart. The therapeutic window seems to be 2 to 5 min. [Supp. by the US Dept. of Defense].

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Resuscitation 54 (2002) 89 98

RESUSCITATION

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Veno-venous extracorporeal blood shunt cooling to induce mild hypothermia in dog experiments and review of cooling methods

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Received 17 September 2001; received in revised form 24 February 2002; accepted 24 February 2002

Abstract

Mild hypothermia (33–36 °C) might be beneficial when induced during or after insults to the brain (cardiac arrest, brain trauma, stroke), spinal cord (trauma), heart (acute myocardial infarction), or viscera (hemorrhagic shock). Reaching the target temperature rapidly in patients inside and outside hospitals remains a challenge. This study was to test the feasibility of veno-venous extracorporeal blood cooling for the rapid induction of mild hypothermia in dogs, using a simple pumping-cooling device. Ten custom-bred hunting dogs (21–28 kg) were lightly anesthetized and mechanically ventilated. In five dogs, two catheters were inserted through femoral veins, one peripheral and the other into the inferior vena cava. The catheters were connected via a coiled plastic tube as heat exchanger (15 m long, 3 mm inside diameter, 120 ml priming volume), which was immersed in an ice-water bath. A small roller-pump produced a veno-venous flow of 200 ml/min (about 10% of cardiac output). In five additional dogs (control group), a clinically practiced external cooling method was employed, using alcohol over the skin of the trunk and fanning plus ice-bags. During spontaneous normotension, veno-venous cooling delivered blood into the vena cava at 6.2 °C standard deviation (SD 1.4) and decreased tympanic membrane (Tty) temperature from 37.5 to 34.0 °C at 5.2 min (SD 0.7), and to 32.0 °C at 7.9 min (SD 1.3). Skin surface cooling decreased tympanic temperature from 37.5 to 34.0 °C at 19.9 min (SD 3.7), and to 32.0 °C at 29.9 (SD 5.1) ($P = 0.001$). Heart rates at Tty 34 and 32 °C were significantly lower than at baseline in both groups, but within physiological range, without difference between groups. There were no arrhythmias. We *conclude* that in large dogs the induction of mild systemic hypothermia with extracorporeal veno-venous blood shunt cooling is simple and four times more rapid than skin surface cooling.

Keywords: Hypothermia; Temperature; Circulation; Extracorporeal; Brain injury

1. Introduction

In recent years, growing evidence in animal and human studies has documented or suggested the beneficial outcome effects of mild hypothermia (33–36 °C) during or after insults to the brain, as for cardiac arrest [1–11], stroke [12–15], or brain trauma [16–20]; for spinal cord injury [21–23]; for acute myocardial infarction [24–28]; and for hemorrhagic shock [29–33]. Hypothermia has the most positive effect when applied very early after cerebral ischemia [2,5,34–36] and for 12–24 h [6,9,10,17,18,20]. The main challenge in the clinical scenario remains the immediate induction of mild hypothermia without producing shivering. With surface cooling of the trunk, skin temperature has to be reduced substantially first, in order to achieve mild hypothermic core temperature. With cold air or ice applied to the skin, it takes at least 1 h to achieve mild systemic hypothermia in adult patients

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0300-9572/02/$ - see front matter
PII: S0300-9572(02)00046-1

[9,10,14,15,20,37]. For use out-of-hospital, cooling blankets and ice-bags are impractical, because they require cold storage and an electrically powered refrigerator. Cardiopulmonary bypass, once vessel access is achieved, is an effective tool to rapidly decrease whole-body temperature in dogs [2,6,38], and patients [39,40], but its clinical use has been limited to special hospitals; and vessel access, priming time, and bulky equipment are limiting factors.

We are searching for a simple method by which even paramedics in the pre-hospital setting could rapidly induce mild hypothermia in patients with circulation, after various insults. This study was to test, in dogs, the feasibility of a simple extracorporeal blood cooling method with a veno-venous shunt, using an improvised pumping-cooling device.

2. Methods

For humane and economic reasons, these experiments were performed in dogs prior to euthanasia 3 days after another experiment. This study was approved by the Institutional Animal Care and Use Committee, and the Department of Defense and followed national guidelines for the treatment of animals.

2.1. Animal preparation and surgical instrumentation

Custom-bred hunting dogs (21–28 kg, 6–12 months old) were used. After premedication with ketamine (10 mg/kg i.m.) and orotracheal intubation, the dogs were mechanically ventilated under anesthesia with titrated halothane 0.5–1.5% and N_2O:O_2, 1:1. Temperature thermister probes (Cole-Parmer Instrument Company, Vernon Hills, IL) were inserted for measuring tympanic membrane (Tty), esophageal (Tes) and rectal temperatures (Tr). Via a peripheral i.v. cannula (18 g), dextrose 5% in 0.45% NaCl, 100 ml/h, was administered. An 18-gauge PVC catheter was inserted into the left femoral artery via sterile cutdown for monitoring of arterial pressure. A pulmonary artery catheter (7.5 Fr) was advanced via the right external jugular vein for temperature measurements in the right atrium (Tra) (Vigilance®, Baxter, Irvine, CA).

2.2. Veno-venous blood cooling ($n = 5$)

Via sterile cutdowns, two PE 90 catheters were inserted into the inferior vena cava via the femoral veins bilaterally, one to 5 cm for venous outflow, and the other to 25 cm for venous inflow. The catheters were connected via a coiled plastic tube (15 m long, 3 mm inside diameter, 120 ml priming volume), which was immersed in an ice-water bath. A small electric roller-pump (Masterflex® L/S™, Cole-Parmer Instrument Company, Vermon Hills, IL) produced a veno-venous flow of 200 ml/min (about 10% cardiac output). Heparin 5000 IU was administered intravenously to avoid clotting in the catheters.

2.3. Skin surface cooling ($n = 5$)

A method was applied as clinically practiced, namely alcohol on the skin plus fanning over the thorax and abdomen, and ice-bags over both groins, around the neck, and on the thorax.

Arterial and central venous pressures, and EKG were continuously recorded on a polygraph (Grass Model 7D Polygraph, Quincy, MA). End-tidal CO_2 (CO_2 SMO respiratory profile monitor, Novametrix Medical Systems Inc., Wallingford, CT) and temperatures were continuously displayed and recorded every minute. Just before start of cooling, Tty was controlled at 37.5° standard deviation (SD 0.1) by heating blanket and lamp. During cooling, halothane was titrated to keep MAP above 75 mmHg, and the ventilation rate was adjusted to keep end-tidal PCO_2 at 30–35 mmHg. The cooling experiments were stopped once Tty reached 32 °C or MAP decreased to less than 75 mmHg. After the cooling experiments, the dogs were euthanized.

2.4. Statistical analysis

Data are given as mean and SD. We used the Independent-Samples T test to test for differences in temperatures, heart rate, and blood pressure between the two groups; and the Paired-Samples T test to test for differences in temperatures, heart rate, and blood pressure within groups with Bonferroni correction for multiple comparison. All data were computed with SPSS for Windows, release 8.0 (Illinois, USA). A P-value < 0.05 was considered statistically significant.

3. Results

Extracorporeal veno-venous blood cooling or skin surface cooling was performed, in five dogs each. The veno-venous blood cooler received blood at 37.5 °C which then decreased to about 32 °C, and delivered blood into the right atrium at a temperature of 6.2 °C (SD 1.4) throughout the experiments. In one dog in the veno-venous cooling group, the experiment was stopped at 12 min at a Tty of 32.3 °C, because MAP decreased below 75 mmHg, without arrhythmias.

Baseline Tty was 37.4 °C (SD 0.2) in the veno-venous cooling group, and 37.5 °C (SD 0.1) in the external cooling group ($P = 0.2$). The time needed to achieve Tty 34.0 °C was 5.2 min (SD 0.7, range 4.3–6.0) with veno-venous cooling, and 19.9 min (SD 3.7, range 15.7–24.5) with skin surface cooling ($P = 0.001$) (Fig. 1). The time

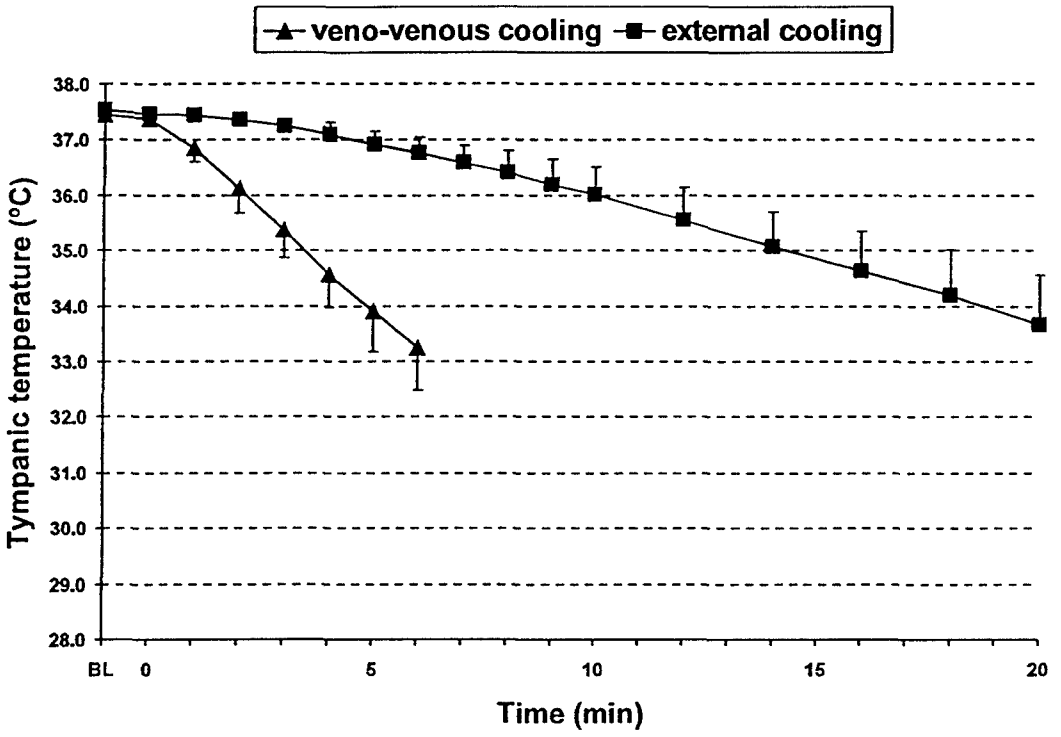

Fig. 1. Change in tympanic temperature from baseline to 34.0 °C with extracorporeal veno-venous blood cooling and with external cooling. Data are shown as mean and SD.

needed to achieve Tty 32 °C was 7.9 min (SD 1.3, range 6.0–8.8) with veno-venous cooling, and 29.9 min (SD 5.1, range 23.5–36.0) with skin surface cooling ($P < 0.001$). With veno-venous cooling, Tes followed Tty, Tra decreased faster than Tty, and Trec slower than Tty (Fig. 2). With external cooling, Tes and Tra followed Tty, and Trec decreased slightly slower than Tty (Fig. 3). The noninvasive equivalent to core temperature (Teso) and that to brain temperature (Tty) remained the same as they declined in parallel, while Tra declined more rapidly (without causing arrhythmias), and Tr declined more slowly (Fig. 2).

Heart rate (Fig. 4) and MAP (Fig. 5) changed moderately during cooling to Tty 34 °C. Heart rates at Tty 34 °C and Tty 32 °C were significantly lower than at baseline in both groups, without differences between veno-venous cooling and skin surface cooling (Table 1). None of the dogs developed arrhythmias during cooling to Tty 32 °C. MAP at Tty 34 °C and Tty 32 °C was only slightly lower than at baseline in the veno-venous cooling group, and did not change in the skin surface cooling group (Table 1).

4. Discussion and review

In this dog study, extracorporeal veno-venous blood shunt cooling proved to be a simple method to quickly induce mild hypothermia of brain and organism. To our knowledge, this exploration of extracorporeal veno-venous blood shunt cooling in dogs without the use of an oxygenator, is the first since exploratory experiments with a simple method in the 1950s [37,41,42]. In those experiments on normal dogs, core temperature decreased by 0.25 °C/min [41], compared to our method with almost 1 °C/min. In another study with a cold shunt flow of 300 ml/min in unanesthetized dogs, shivering delayed core cooling, Tr decreased to 28 °C over $2\frac{1}{2}$ h [42]; and three of the seven dogs developed ventricular fibrillation at Tr 26–27 °C.

The dogs in our study weighed between 21 and 28 kg, which makes a translation of our results to humans difficult. We do not know how the approximately 5 min to reduce Tty by 3.5 °C and the approximately 8 min to reduce Tty by 5.5 °C in our study (which was approximately four times faster than skin surface cooling) should be translated into the cooling of humans. We expect cooling times with extracorporeal veno-venous blood cooling to be considerably faster than skin surface cooling. For quick percutaneous insertion, the two separate venous catheters should be combined into one double-lumen catheter, to be advanced into the superior or inferior vena cava, with the outflow lumen more distally placed to minimize recirculation of cold blood. We used systemic heparinization, which should be avoided in trauma cases, where the entire extracorporeal veno-venous system should have non-clotting surfaces, e.g. be heparin-bonded.

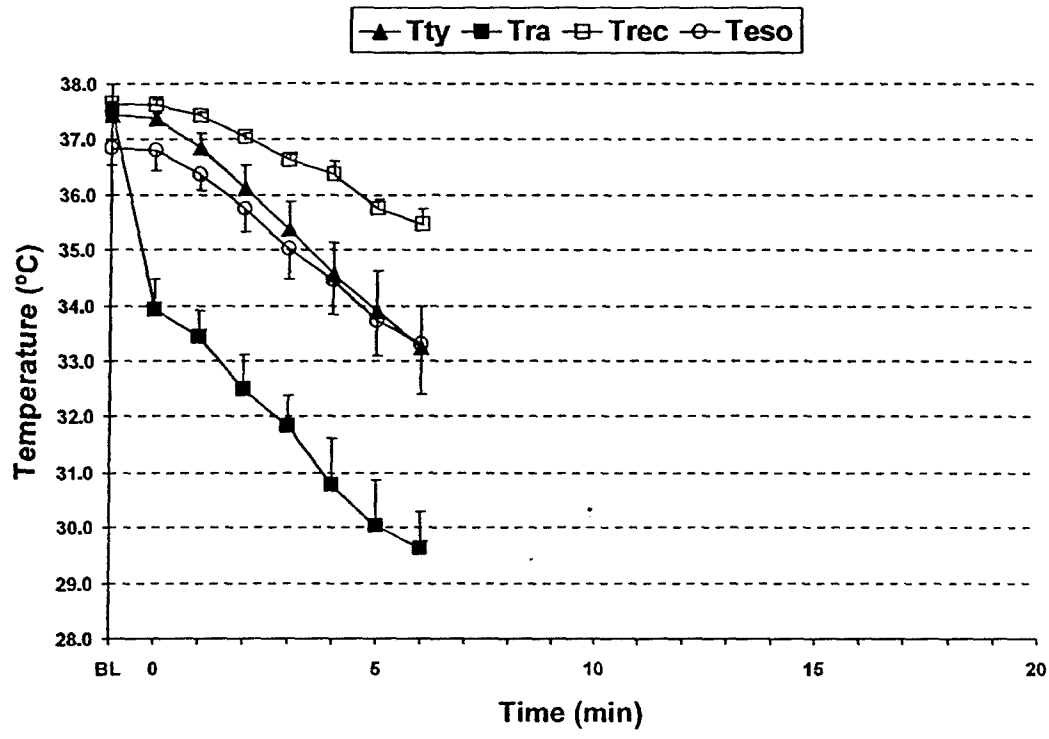

Fig. 2. Change in tympanic (ty), right atrial (ra), rectal (rec), and esophageal (eso) temperatures with extracorporeal veno-venous blood cooling during the first 6 min of cooling. Data are shown as mean and SD.

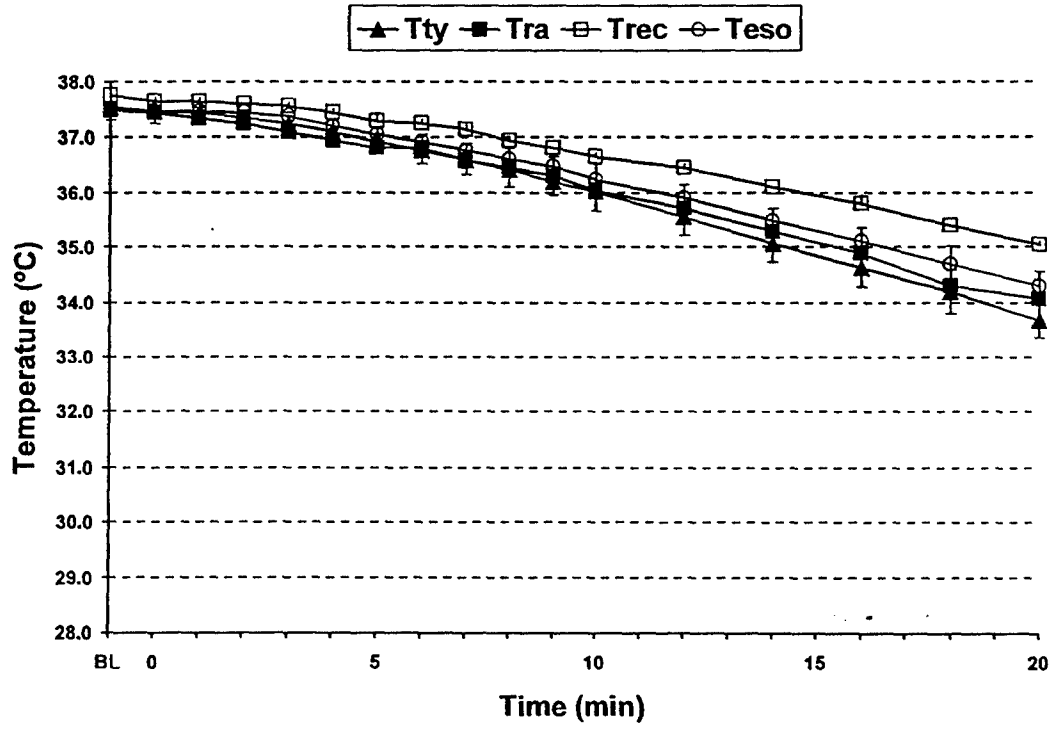

Fig. 3. Change in tympanic (ty), right atrial (ra), rectal (rec), and esophageal (eso) temperatures during the first 20 min of external cooling. Data are shown as mean and SD.

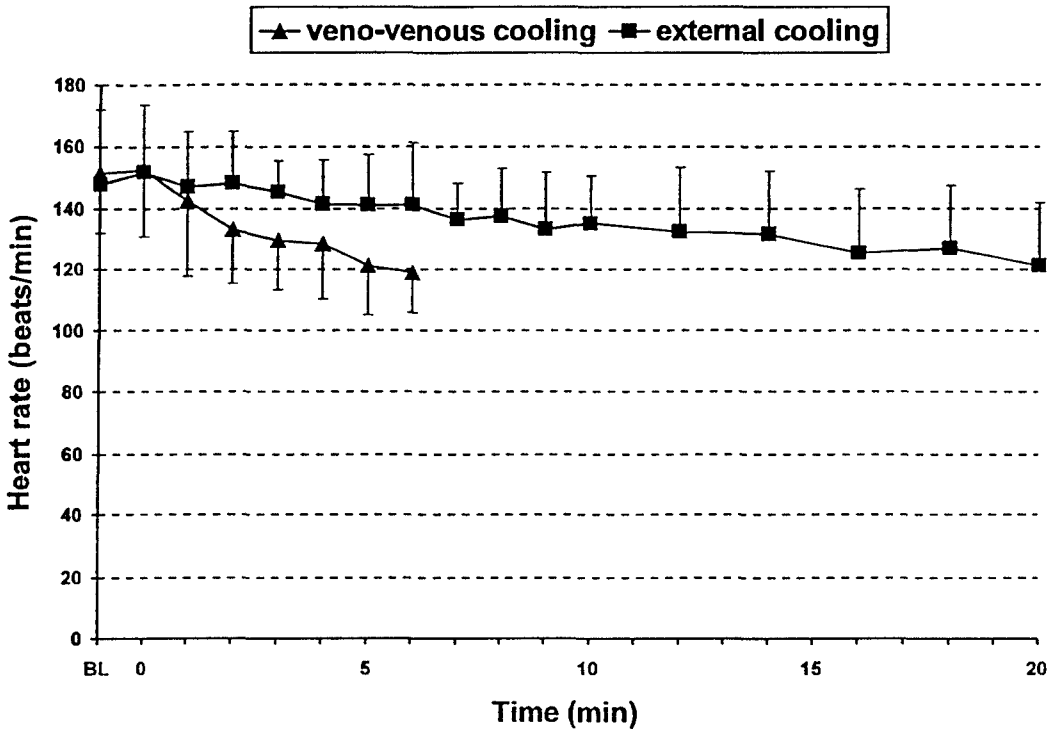

Fig. 4. Change in heart rate from baseline to 34.0 °C with extracorporeal veno-venous blood cooling and with external cooling. Data are shown as mean and SD.

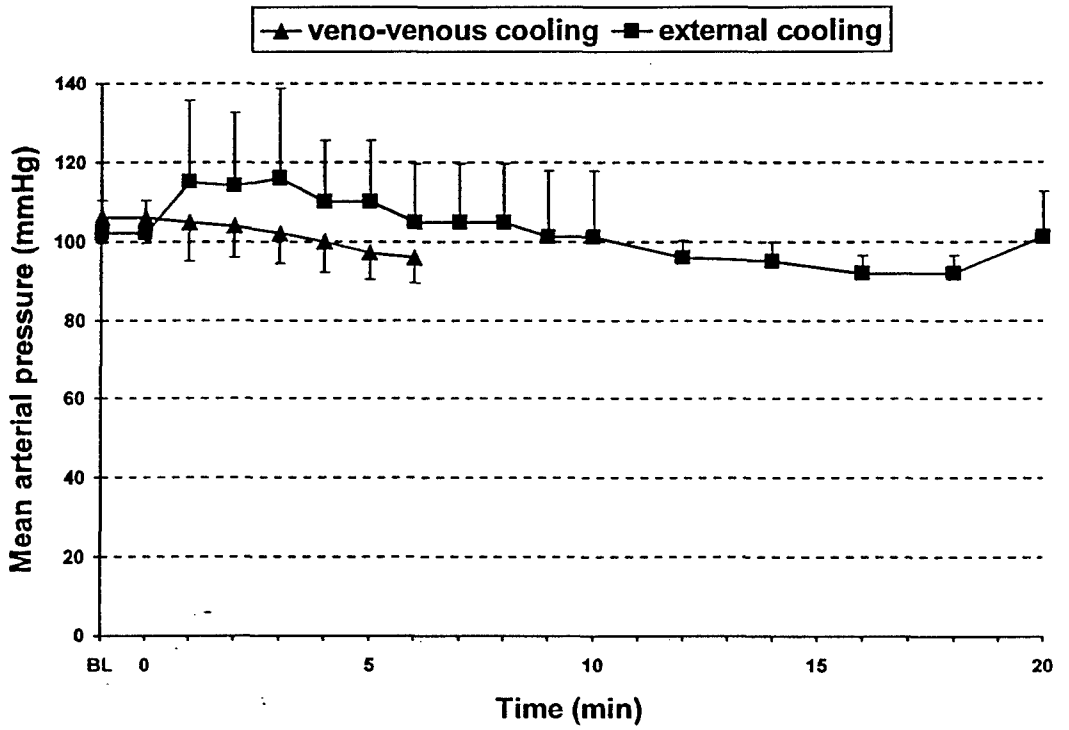

Fig. 5. Change in mean arterial blood pressure from baseline to 34.0 °C with extracorporeal veno-venous blood cooling and with external cooling. Data are shown as mean and SD.

Table 1
Heart rates and mean arterial pressures at baseline, and when tympanic temperature reached 34 and 32 °C for veno-venous cooling ($n = 5$) and external cooling ($n = 5$)

	Veno-venous cooling	External cooling
Heart rate (per min) at baseline	151 (SD 20)	148 (SD 24)
Heart rate (per min) at Tty 34 °C	120 (SD 13)[a]	127 (SD 25)[a]
Heart rate (per min) at Tty 32 °C	110 (SD 13)[b,c]	112 (SD 21)[d]
MAP (mmHg) at baseline	106 (SD 7)	102 (SD 8)
MAP (mmHg) at Tty 34 °C	96 (SD 6)	101 (SD 11)
MAP (mmHg) at Tty 32 °C	89 (SD 14)[b]	103 (SD 16)

Tty, tympanic temperature; MAP, mean arterial pressure.
[a] $P = 0.02$ compared to baseline.
[b] $n = 4$.
[c] $P = 0.04$ compared to baseline.
[d] $P = 0.006$ compared to baseline.

In our study, the vessels were already cannulated before the cooling circuit was connected. Vessel access time under clinical conditions would vary greatly with the skill of the operator. In the future, physicians would probably prefer to use the needle-wire-catheter method of Seldinger for inserting a double-lumen catheter into the vena cava. Paramedics would more likely cannulate two large peripheral veins (e.g. the v. basilica and external jugular veins). We suspect that vessel cannulation might be faster than obtaining and applying external skin-cooling devices.

Extracorporeal veno-venous pump cooling can deliver a steady state-flow rate until the target temperature is reached without fluid overload of the patient. In our study we arbitrarily used a flow rate of about 10% of the dogs' cardiac output, which is 200 ml/min. The delivery of blood at approximately 6 °C into the inferior vena cava at a flow rate of 200 ml/min did not cause any arrhythmias to Tty 32 °C. In a pilot experiment, delivering blood at 14 °C at a higher flow rate, 400 ml/min, resulted in superventricular extrasystoles at Tty 35.3 °C (Tes 33.7 °C). For use in humans, the safe flow rate and blood temperature would have to be titrated during monitoring of the electrocardiogram, which, by the way, can also be used to detect shivering.

Optimal temperature monitoring sites during veno-venous cooling remain to be determined. In our study we observed markedly different temperatures between the right atrium, the Tty, the esophagus, and the rectum. Tes remained close to Tty (Fig. 2). Myocardial and cerebral temperatures were not measured. The temperature probe in the right atrium reflects the temperature of the cold saline delivered from the vena cava catheter mixed with the warm blood coming from the superior vena cava, rather than the myocardial temperature. During normothermia, brain tissue temperature is well reflected in Tty [43]; but deep hypothermia induced rapidly during cardiopulmonary bypass causes large temperature gradients between brain and Tty [44]. Veno-venous cooling to mild hypothermia probably does not produce the large temperature gradients between brain and Tty seen with cardiopulmonary bypass and deep hypothermia. Once mild hypothermia is achieved, Tty and brain temperature equilibrate.

In recent years, therapeutic mild hypothermia was explored in humans for various conditions of brain injury [8–11,14,15,20,45,46]. A group in Japan [8] used cooling blankets and alcohol on the skin of trunk and extremities in 11 patients after cardiac arrest; the target core temperature of 33–34 °C was reached within 336 min (SD 180) of commencement of cooling. A group in Australia [9,11] used extensive surface cooling with ice packs in one-half of 77 randomized patients after cardiac arrest; core temperature decreased from 34.9 °C at 30 min after restoration of spontaneous circulation by 0.9 °C/h. A group in Europe [10,11] used external head and body cooling with cold blankets and a special air mattress with constant cold air flow in one half of 273 randomized patients after cardiac arrest. Core temperature was decreased from minimal spontaneous hypothermia after restoration of spontaneous circulation to the target core temperature of 33 ± 1 °C over a median of 8 h (1 QR 4 and 16). In stroke patients, Schwab et al. [14] used cooling blankets and cool-air fanning of the body surface in 25 patients; the time required for cooling to 33 °C bladder temperature ranged between 3.5 and 6.2 h. Krieger et al. [15] used cooling blankets, ice-water and whole-body alcohol rubs in 10 patients; the time required for cooling to 32 °C core temperature averaged 3.5 h (range 2–6.5 h). In traumatic brain injury, Marion et al. [20] used cooling blankets placed above and below the patient and nasogastric lavage with iced saline; a Tr of 33 °C was reached after a mean duration of 10 h after the injury. It is surprising that, in spite of such long delays, all of these human studies showed a higher proportion of patients with good cerebral outcome in the hypothermia groups. Animal data and mechanism studies suggest that faster cooling would have resulted in even better outcomes. Animal studies showed consistently that the earlier hypothermia is induced after an insult, the more effective it is [2,5,34,47,48]. In a recent multi-center study in patients with traumatic brain injury, hypothermia failed to result overall in beneficial effects on outcome [45]. We believe that the study results were negative in part because the target core temperature of 33 °C was reached at a mean time of 8.4 h (SD 3.0) after injury. Not surprisingly, in the same study, an apparent benefit was found in patients with spontaneous hypothermia on admission and continuation of hypothermia.

When external cooling was applied in the human studies mentioned above, neuromuscular blockade was used to avoid shivering during hypothermia. We are seeking a cooling method that can be applied to patients without the need for neuromuscular blockade, which requires intubation and prolonged intensive care. Skin cooling contributes a shivering stimulus to the thermoregulatory system [37,49]. The shivering threshold in healthy, unsedated volunteers is lower when the skin temperature is not altered [50]. Shivering thresholds can be decreased further with pethidine (meperidine, Demerol) [51], best administered i.v. in titrated doses. In contrast to the skin, large veins and the right heart are lacking temperature sensors of importance [52,53]. Since the dogs in our study had to be anesthetized, we could not assess the effects of extracorporeal veno-venous blood cooling on shivering without anesthesia. Though in both groups the concentration of halothane was reduced during cooling, titrated to keep the MAP above 75 mmHg, the dogs remained under anesthesia until the end of the experiments.

Methods other than external cooling have also been investigated. Plattner et al. [54] investigated five different cooling methods in anesthetized volunteers: gastric lavage caused abdominal cramping and diarrhea in the first volunteer and was not further investigated; bladder lavage provided only minimal cooling; forced air and cold mattress were equally slow with a cooling rate of 1.7 °C (SD 0.5) per h, and 1.6 °C (SD 1.1) per h; ice-water immersion is the most rapid external cooling method, as it reduced core temperature from 36.2 °C (SD 0.3) to 34 °C in only 20 min [50]. Ice-water immersion, however, is usually not readily available in out-of-hospital resuscitation attempts, and proved difficult and clumsy in the hospital. Baumgardner et al. [55] first cooled neurosurgical patients with skin surface cooling, and then, through a large-bore peripheral intravenous catheter, administered chilled (1–6 °C) 5% albumin solution, 5 ml/kg over 5 min or over 30 min; rapid infusion changed core temperature by only 0.6 °C (SD 0.1), and slow infusion by 0.4 °C SD (0.1). Rajek et al. [56] administered in anesthetized and intubated volunteers saline at a rate of 40 ml/kg over 30 min through a central venous catheter; with saline at 4 °C core temperature decreased by 2.5 °C (SD 0.4), and with saline at 20 °C, core temperature decreased by only 1.4 °C (SD 0.2) over 30 min. In this study [56], the infused volume of saline was 2.9 (SD 0.2) and the infusion rate was 96 ml/min (SD 13). In clinical use, volume overload might be problematic, and can lead to pulmonary problems, especially in patients with reduced myocardial contractility, as seen after cardiac arrest [57].

The veno-venous cooling of this study induces cerebral and overall mild hypothermia rapidly under normal circulation. The cooling rate is slower when blood flow is low, as during cardiac arrest and CPR steps A-B-C [3; Nozari et al., unpublished]. The rate of reduction in Tty was increased by initiating the veno–venous shunt flow with an intravenous cold flush of 25 ml/kg saline at 2 °C. Once no-flow occurs, cooling of the brain must be achieved within the first 5 min of arrest. That is easy when cardiopulmonary bypass via heat exchanger is already connected, as after heart surgery. For emergency conditions, without cardiopulmonary bypass we introduced aortic cold flush in dogs, either into the cerebral and heart arteries first via aortic arch balloon catheter [58,59], or into the abdominal aorta or femoral artery [60,61], using large volumes of saline at 2 °C; that reduced Tty by 2–3 °C/min, achieving protective profound hypothermia (Tty 10 °C) within 10 min of flush.

Finally, there are methods for selectively lowering brain temperature below heart temperature and maintaining cerebral differential hypothermia [62–74]. These methods are difficult, promising, and beyond the scope of this discussion. Recently, moderately rapid mild cooling and prevention of hyperthermia have been achieved in pigs and patients with use of a special vena cava heat exchange catheter that is perfused with externally cooled liquid [15,28,75]. The physiologically most rational approach for conscious or stuporous patients immediately after the onset of severe acute stroke symptoms would be pumping blood via a heparin-bonded system with heat exchanger from any peripheral artery into the carotid artery. This could quickly add carotid cold flow to normal carotid flow, and thereby lower temperature of the entire brain while preventing heart temperature from decreasing below 30 °C [63–67]. The veno-venous system of this study might also be used for carotid cooling. Many physicians, however, fear the risk of dislodging carotid plaques.

We *conclude* that in large dogs, induction of mild systemic hypothermia with extracorporeal veno-venous blood shunt cooling, using an improvised, inexpensive pumping-cooling system, is simple and rapid, four times faster than skin surface cooling. We *recommend* that industry develop a simple, portable unit, consisting of a non clotting, double-lumen venous catheter and a pump, a heat exchanger, and a miniaturized cooler, for exploration and use in humans, both inside and outside of hospitals.

Acknowledgements

Mirolsav Klain, M.D., Ph.D. made valuable suggestions. Sherman Culver, Jeremy Henchir, Yuichi Sakai, and Murugan Subramanian helped with preparation of the dogs. Fran Mistrick helped with preparation of the manuscript and Patricia Boyle helped with editing. Supported by US Department of Defense, the US Office of Naval Research, grant #N00014-97-1-1064 and US Army MRMC grant #DAMD17-01-2-0038.

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Portuguese Abstract and Keywords

A hipotermia ligeira (33–36 °C) pode ser benéfica quando induzida durante ou após agressão ao cérebro (paragem

cardíaca, trauma cerebral, acidente vascular), medula espinal (trauma), coração (enfarte agudo do miocárdio) ou visceras (choque hemorrágico). A capacidade para atingir rapidamente a temperatura alvo em doentes fora do hospital ou já internados permanece um desafio. Este estudo procurou testar a exequibilidade do arrefecimento sanguíneo veno-venoso extra-corporal para a indução rápida de hipotermia ligeira em cães, utilizando um sistema simples de bombagem-arrefecimento. Dez cães de caça de criação específica (21–28 kg) foram superficialmente anestesiados e ventilados mecanicamente. Em cinco cães foram colocados dois catéteres nas veias femorais, um periférico e outro na veia cava inferior. Os catéteres foram conectados através de um tubo plástico helicoidal de troca de calor (15 m de comprimento, 3 mm de diâmetro interno, 120 ml de volume de preenchimento), que foi mergulhado num banho de água gelada. Uma pequena bomba de rotação produziu um fluxo veno-venoso de 200 ml/min (cerca de 10% do débito cardíaco). Noutros cinco cães (grupo de controlo), utilizou-se um método de arrefecimento externo comum na prática clínica, com aplicação de álcool na pele, ventoinha dirigida ao tronco e sacos de gelo. Durante a normotensão espontânea, o arrefecimento veno-venoso fornecia sangue á veia cava a uma temperatura de 6.2 °C, desvio padrão (SD 1.4) e diminuiu a temperatura da membrana timpânica (Tty) de 37.5 para 34.0°C aos 5 min (SD 0.7) e para os 32 °C aos 7.9 min (SD 1.3). O arrefecimento da superfície corporal desceu a temperatura timpânica dos 37.5 para os 34.0 °C em 19.9 min (SD 3.7) e para os 32 °C em 29.9 min (SD 5.1) ($P = 0.001$). As frequências cardíacas com Tty 34 e 32 °C eram significativamente mais baixas que as de base em ambos os grupos, mas ainda dentro dos limites fisiológicos e sem diferenças entre os grupos. Não houve arritmias. Concluímos que em cães grandes a indução de hipotermia sistémica ligeira com arrefecimento por shunt sanguíneo veno-venoso extracorporal é simples e quatro vezes mais rápido que o arrefecimento cutâneo superficial.

Palavras chave: Hipotermia; Temperatura; Circulação; Extracorporal; Lesão cerebral

Spanish Abstract and Keywords

La hipotermia leve (33–36 °C) podría ser beneficiosa cuando es inducida durante o después de agresiones al cerebro (paro cardíaco, trauma cerebral, accidente cerebro vascular), a la médula espinal (trauma), al corazón (infarto agudo de miocardio), o a visceras (shock hemorrágico). El reto sigue siendo alcanzar las temperaturas buscadas rápidamente, dentro o fuera del hospital. Este estudio se realizó para probar la factibilidad de usar enfríamiento con circulación extracorpórea veno- venosa para la inducción rápida de hipotermia leve en perros, usando un equipo simple de bomba enfríadora. Se anetesiaron superficialmente diez perros de caza (21–28 kg) y se pusieron en ventilación mecánica. En cinco perros, se insertaron dos catéteres en las venas femorales, uno periférico y el otro hacia la vena cava inferior. Los catéteres se conectaron con un tubo plástico en espiral sumergido en un baño de agua helada (15 m largo, 3 mm diámetro interior, 120 ml. de volumen como intercambiador de calor). Una pequeña bomba de rodillo produjo un flujo veno-venoso de 200 ml/min (cerca del 10% del gasto cardiaco). En los otros 5 perros (grupo control) se realizó un enfriamiento con método clínico externo, usando alcohol sobre la piel del tronco y abanicado mas bolsas de hielo. Durante la normotensión espontánea, el enfríamiento veno-venoso entregó sangre en la vena cava a 6.2 °C, desviación estánda (SD 1.4) y disminuyó la temperatura timpánica (Tty) de 37.5 a 34.0 °C en 5.2 min (SD 0.7), y a 32 °C en 7.9 min (SD1.3). El enfriamiento de superficie de piel disminuyó la temperatura timpánica de 37.5 a 34.0 °C en 19.9 min (SD 3.7), y a 32.0 °C en 29.9 (SD 5.1) ($P = 0.001$). Las frecuencias cardíacas a Tty 34 y 32 °C fueron significativamente mas bajas que las basales en ambos grupos, pero dentro de rangos fisiológicos, sin diferencia entre los dos grupos. No hubieron arritmias. *Concluimos* que en perros grandes la inducción de hipotermia sistémica con enfríamiento por shunt veno-venoso es simple y cuatro veces mas rápida que el enfríamiento superficial de piel.

Palabras clave: Hipotermia; Temperatura; Circulación; Extracorpórea; Lesión cerebral

Suspended animation for delayed resuscitation

Peter J. Safar and Samuel A. Tisherman

'Suspended animation for delayed resuscitation' is a new concept for attempting resuscitation from cardiac arrest of patients who currently (totally or temporarily) cannot be resuscitated, such as traumatic exsanguination cardiac arrest. Suspended animation means preservation of the viability of brain and organism during cardiac arrest, until restoration of stable spontaneous circulation or prolonged artificial circulation is possible. Suspended animation for exsanguination cardiac arrest of trauma victims would have to be induced within the critical first 5 min after the start of cardiac arrest no-flow, to buy time for transport and resuscitative surgery (hemostasis) performed during no-flow. Cardiac arrest is then reversed with all-out resuscitation, usually requiring cardiopulmonary bypass. Suspended animation has been explored and documented as effective in dogs in terms of long-term survival without brain damage after very prolonged cardiac arrest. In the 1990s, the Pittsburgh group achieved survival without brain damage in dogs after cardiac arrest of up to 90 min no-flow at brain (tympanic) temperature of 10°C, with functionally and histologically normal brains. These studies used emergency cardiopulmonary bypass with heat exchanger or a single hypothermic saline flush into the aorta, which proved superior to pharmacologic strategies. For the large number of normovolemic sudden cardiac death victims, which currently cannot be resuscitated, more research in large animals is needed. Curr Opin Anaesthesiol 15:203–210.

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Current Opinion in Anaesthesiology 2002, 15:203–210

Abbreviations

ALS	advanced life support
CPB	cardiopulmonary bypass
CPCR	cardiopulmonary–cerebral resuscitation
CPR	cardiopulmonary resuscitation
OPC	overall performance category
NDS	neurologic deficit score
ROSC	restoration of spontaneous circulation
Tty	tympanic membrane temperature

0952-7907

Introduction

Before the 1950s, there was no effective resuscitation method available for use by laypersons outside hospitals to reverse airway obstruction (e.g. as a result of coma), apnea, or pulselessness [1•]. In the late 1950s, the documentation of step A [2], step B [3,4], and step C [5], and their assembly into basic life support [6,7], enabled the reversal of cardiac arrest, with laypersons starting oxygen delivery within the critical first 5 min of normothermic no-flow [8]. In 1961, continuation by professionals with advanced life support (ALS) for restoration of spontaneous circulation (ROSC) with early defibrillation and drugs, and brain-oriented prolonged life support, led to cardiopulmonary–cerebral resuscitation (CPCR), which proved physiologically effective [7]. Resuscitation attempts made outside hospitals' special care units, however, have yielded suboptimal results because they usually came 'too little too late' [9]. For 30 years, agencies responsible for clinical guidelines have focused on the heart [10–13]. Few have made the brain the target organ of resuscitation [7].

For normovolemic cardiac arrest, the still weakest first link in the life support chain is immediate initiation of life-supporting first aid by lay bystanders [14]. The second link, ultra-ALS possibilities [15], have yet to be explored clinically for types of cardiac arrest which currently (totally or temporarily) cannot be resuscitated. These include about 50% of out-of-hospital CPR–ALS attempts, which fail to achieve ROSC, and are given up in the field [9–12,16–18]. Ultra-ALS methods considered for clinical trials include open-chest CPR, which is physiologically superior to closed-chest CPR [19,20]; emergency (portable, closed-chest) cardiopulmonary bypass (CPB) [21–23]; and suspended animation [24]. After prolonged normothermic cardiac arrest in dogs, venous–arterial pumping via oxygenator, which permits full control over the flow, pressure, temperature, and composition of blood, improved ROSC rates and cerebral recovery [21].

For exsanguination cardiac arrest, cardiovascular resuscitation is only possible if hemostasis can accompany fluid resuscitation and CPCR. Arterial pressure infusion with epinephrine proved to be effective even without the need for cardiac massage [25–28]. The majority of soldiers who are killed in action [29] and a rather small number of trauma victims in civilian emergency medical services [30], cannot be resuscitated. They die of penetrating truncal injuries leading to rapid internal

exsanguination to cardiac arrest. The latter group have rarely been saved in spite of emergency thoracotomy in the emergency department, because rapid attempts at fluid resuscitation and hemostasis lose the race against the complete ischemia tolerance limits of 5 min for the brain [7,31–33] and about 20 min for the heart [21,22,32,34].

Suspended animation, the topic of this paper, is preservation, defined as treatment to preserve the viability of organs and organism during a lethal insult, such as no flow (cardiac arrest), low flow (shock), trauma, or intoxication. Preservation must be differentiated from protection, which is meant to be initiated electively before and continued during cardiac arrest, as for elective open-heart surgery. Resuscitation encompasses all the strategies meant to reverse the insult and support recovery. In the mid-1980s, the discovery of mild cerebral hypothermia (33–36°C), for protection preservation during cardiac arrest [21,32] and even for resuscitation after normothermic cardiac arrest, in dogs [35,36], rats [37], and human patients [38–40] initiated a new era of research into therapeutic hypothermia. That research includes mild (33–36°C), moderate (28–32°C), deep (15–27°C), and profound hypothermia (5–14°C). Deep and profound hypothermia are incompatible with spontaneous circulation. Mild hypothermia is simple and safe compared with lower temperatures, which are more difficult to achieve rapidly and which are risky under spontaneous circulation. We can identify 10 indications for use or research of 'new' therapeutic hypothermia [31]: cerebral preservation during risky elective surgery; CPCR after cardiac arrest; suspended animation during cardiac arrest; stroke; brain trauma; spinal cord trauma; hemorrhagic shock; septic shock; myocardial infarction (without cardiac arrest); and intractable seizures. Reexplorations of the preservative and resuscitative mechanisms of hypothermia revealed more than the reduction in oxygen demand, namely synergism of beneficial depressant effects on numerous deleterious chemical cascades [31]. This summarizing paper on suspended animation includes emergency preservation and resuscitation potentials with hypothermia, drugs, and solutions.

Suspended animation animal outcome studies

In 1984, Bellamy, a United States Army surgeon, and Safar met and pondered over recent military casualty data [29]. The majority of soldiers killed in action without brain trauma had penetrating truncal injuries and exsanguinated internally over a few minutes to cardiac arrest. Such casualties are still considered not to be resuscitable in spite of the fact that many had repairable injuries upon autopsy. These are cases with no opportunity and time for resuscitative surgery for hemostasis in the field. Case examples might include Martin Luther King, Israel's past president Rabin, Princess Diana, and the United States' first (still only) combat casualty of the war against terrorism. A completely new approach is needed. If instantaneous preservation of the viability of brain and organism could be achieved, one could buy time for transport and major hemostasis during clinical death, to be followed by restoration of blood volume and resuscitation, using CPB.

Over 40 years ago, cardiovascular surgeons had reported that in dogs or monkeys, after slow, elective surface cooling followed by CPB cooling, under anesthesia, total circulatory arrest of over 2 h under profound hypothermia (10–15°C) could be reversed, with CPB, to recovery of grossly normal function [41–45]. For pharmacologic preservation, which could be induced more rapidly than profound hypothermia, no drug has appeared promising so far [24,31]. Phenomena not relevant for suspended animation in cardiac arrest are hibernation of mammals [46], which involves downregulation of oxygen supply parallel with metabolism, without tissue hypoxia, or reflex protection of diving seals. Hibernating turtles, however, which preserve themselves through winter with profound hypothermia under severe tissue hypoxia [47], which has not yet been explored, may teach some lessons relevant for suspended animation.

Since the late 1980s, the Pittsburgh group has been engaged in systematic pathophysiologic outcome studies in dogs for the development of the suspended animation concept [24]. First, Tisherman *et al.* [48–53] explored hypothermic preservation induced by closed-chest CPB and heat exchanger. Profound hypothermia induced at the beginning of exsanguination cardiac arrest proved to be more preservative than deep hypothermia [48,49]. The University of Wisconsin's organ preservation solution standing in the microcirculation did not add cerebral benefit over that achieved with the same temperature using standard plasma substitutes [50]. The lack of systemic heparinization, using a heparin-bonded CPB circuit, did not offset the beneficial effect of profound hypothermia [51]. Differences in hematocrit from below 5% to 20% during no flow made no significant outcome difference [52]. Most significant was the last study of this series [53]; 60 min normothermic hemorrhagic shock was followed by rapid cooling using CPB (with hemodilution by crystalloids) and 60 min cardiac arrest at tympanic membrane temperature (Tty) 10°C. Reperfusion and restoration of normal hematocrit was via CPB. Complete functional recovery was achieved. There was also, documented for the first time, a histologically normal brain with a reproducible method [33] for semiquantitative scoring of 19 brain regions.

The Pittsburgh group then decided that neither in the field nor in the hospital emergency department could

CPB be initiated within the critical 5 min of clinically recognizable cardiac arrest, even if there were available a miniaturized portable CPB apparatus [54] and aortic catheters with one or two occluding balloons [55]. The latter are meant to enable selective hemostasis, perfusion, and temperature control of different vascular beds. Brain and heart could be targeted first by placing one balloon for aortic arch perfusion.

Aortic cold flush strategies were then documented in a systematic manner, using large dogs with about 25 kg body weight [56,57,58••,59–66,67••]. The authors flushed the aorta, via a (prototype) single balloon catheter (Cardeon Company, Cupertino, CA), with isotonic saline at 0–4°C, at a rate of 1–2 l/min, starting at 2 min no-flow. This could lower Tty by 3°C per min. The outcome model used included, under minimal general anesthesia and spontaneous breathing of air, rapid controlled hemorrhage from aorta and vena cava over 5 min to cardiac arrest (which was assured by adding electric ventricular fibrillation); and aortic cold saline flush started at 2 min cardiac arrest no flow. Cardiac arrest was allowed to last for 15 min [56], 20 min [57], 30 min [58••], or 60, 90, or 120 min [59], under preservative Tty levels decreasing from 34°C to 6–10°C. Then, reperfusion by CPB included rapid restoration of normal circulating blood volume and hematocrit and slow controlled restoration of mild hypothermia. Tty of 34°C was maintained for 12 h, controlled ventilation to 24 h and intensive care to 72 h. Endpoints were outcome at 72 h in terms of overall performance categories (OPCs), where OPC 1 represents normal; OPC 2, moderate disability; OPC 3, severe disability; OPC 4, coma; and OPC 5, death, and neurologic deficit scores (NDSs): NDS 0%–10%, normal; NDS 100%, brain death. Standardized necropsy included perfusion fixation of the brain and histopathologic damage scoring of 19 brain regions [33]. This scoring system, initiated by J. Moossy, documented over many years by the Pittsburgh group, gave total histopathologic damage scores which correlated with NDSs [33]. Some dogs with OPC 1 and NDS 0% at 72 h had cognitive function evaluated 2–3 months later (CE Dixon, unpublished data).

With cardiac arrest of 15 min no flow, presumably portable saline flush volume of 25 ml/kg at 24°C (room temperature) achieved Tty 36°C and, at 72 h, functional normality with histologic damage, while the same protocol with saline at 0–4°C achieved Tty 34°C and two of six brains were histologically normal [56]. Catheter design influenced outcome; with the opening at the tip, the straight flush resulted in better outcome than using a catheter with the tip closed and the flush through multiple lateral openings. With cardiac arrest of 20 min no flow [57], aortic arch flush rapidly lowered Tty to 34°C, and achieved survival to 72 h with functional normality and histologically minimal damage. For cardiac arrests of 15 or 20 min, the catheter balloon was inflated high for aortic arch perfusion. When cardiac arrest was at 30 min no flow [58••], flush volume of saline at 0–4°C was increased to 100 ml/kg to achieve Tty 28°C; this achieved functionally normal brains (in some dogs even histologically normal brains); the aortic catheter had to be withdrawn into the abdominal aorta to also cold-flush the viscera and spinal cord. For flush preservation for cardiac arrest of 30 min in adult humans of 70 kg body weight, this would translate to 7 l of iced saline, which is feasible to be carried by ambulances, but not by medics in the field. Aortic flush to Ttys of 20°C, 15°C, or 10°C preserved the brain and organism long enough to achieve intact survival (OPC 1) after 60 min, 90 min, and in some dogs even 120 min no flow [59]. All six dogs with cardiac arrest of 90 min and Tty 10°C were functionally normal, with no or minimal histopathologic damage scoring. One dog after cardiac arrest of 90 min, one after cardiac arrest of 60 min, and one normal dog without cardiac arrest, received cognitive function tests 3 months later, which proved normal. Delaying start of flush to 8 min normothermic no flow, in the cardiac arrest of 30 min model, negated the preservation achieved with flush starting at 2 min or 5 min cardiac arrest no flow [61]. Normothermic flush (saline at 37.5°C) improved outcome only minimally over no flush (NS) [56,60]. In the OPC 1 survivors, in general, there was grossly normal function of extracerebral organs; liver enzyme levels were abnormal only transiently [62]. In order to be certain that the gut, liver, and spinal cord will not suffer damage, and to avoid hind-leg weakness (which might also result from muscle ischemia), the authors found that the most reliable flush method might be a return to the simplest: a large-bore cannula in the femoral or iliac artery to include the entire organism in the flush. This, for cardiac arrest longer than 30 min, required very large volumes of cold flush solution [59].

Pharmacologic strategies with novel drugs and solutions would be simpler [63–66,67••]. Even if the aorta could be accessed and cold flush initiated within the first 5 min of normothermic no flow, and a drainage catheter inserted into the vena cava, the 10–20 l/70 kg cold solution (0–4°C) estimated to be required for an adult human to lower Tty to 10°C (and core temperature to about 20°C) would be infeasible in the field. Although difficult in the mobile intensive care unit ambulance or hospital emergency department, such large amounts of solutions could be stored there in a refrigerator. Another approach would be to start with a single, small flush, to achieve mild cerebral hypothermia and then, to recirculate diluted venous drainage blood, with or without oxygenator, through a cooler-heat exchanger, to reduce Tty to profound hypothermia [24].

The same Pittsburgh team conducted for the first time an exploration of pharmacologic cerebral preservation potentials of 14 different drugs, in 84 dogs. They used the same model of exsanguination cardiac arrest of 20 min no flow, a portable volume of solution at ambient temperature, which achieved only mild cerebral hypothermia, and added cerebral preservation with pharmacologic means [63–67]. In controls, 25 ml/kg flush volume at 24°C, with saline flush started at 2 min cardiac arrest, achieved survival with brain damage [57,63–66,67••]. In groups of three to six pilot experiments per drug, various doses were flushed into the aortic arch via balloon catheter and, in some experiments, additional intravenous medication was given during reperfusion with CPB. The drugs were selected and grouped according to six mechanistic strategies [67••]: (1) delaying energy failure; (2) protecting membrane integrity; (3) preventing structural degradation; (4) regulating protein synthesis; (5) preventing reoxygenation injury; and (6) preserving mitochondria. The authors were seeking a suggestion of a breakthrough effect, namely for the majority of dogs in the mini-series to achieve OPC 1 at 72 h. The selection of drugs and doses was influenced by published beneficial results in rodents and guidance by expert consultants. None of the 14 drug treatments explored resulted in a breakthrough effect [63–66]. Only an occasional dog achieved OPC 1 (and that with some histologic damage) after thiopental with phenytoin or glucose with insulin. The antioxidant tempol, however, gave a suggestion of benefit [67••]. All eight dogs that received tempol 150–300 mg/kg in the aortic arch flush at the start of cardiac arrest achieved OPC 1 or 2 (good outcome), with none of the eight controls achieving a good outcome ($P=0.03$) [67••]. Interestingly, the histologic damage was not significantly mitigated by tempol. Various explanations for this have been discussed [67••]. The only negative side effect of tempol, minimal transient methemoglobinemia, is clinically not significant. One may criticize this exploratory approach since it is not possible to rule out some benefit possibly revealed by larger sample sizes and randomized concurrent controls. To conduct such studies, however, cost and time involvement would be prohibitive. Tempol is available and inexpensive and penetrates the blood-brain-barrier, but is not US Food and Drug Administration approved.

The empirical use in the Pittsburgh studies of isotonic saline solution for flush and dextran 40/Ringer's solution for reperfusion led to some trials with theoretically more-physiologic solutions [68,69]. Using the cardiac arrest of 30 min model with Tty 28°C [58••], polynitroxylated albumin with tempol gave slightly better NDSs and histopathologic damage scores than saline [68], while albumin 5% or 25% gave no improvement over saline [68]. Using the 120 min cardiac arrest model with Tty 10°C [59], normosol (a pH normalized Ringer's solution) was employed for cold flush and Unisol (Organ Recovery Systems Company, Charleston, SC) designed by Taylor *et al.* [70,71] was implemented. This combination provided an intracellular fluid composition for stasis, and an extracellular fluid composition (including anti-reperfusion injury drugs) for reperfusion [70]. With these 'optimized' solutions, OPC 1 and only minimal to moderate histologic damage was achieved in five of six dogs [63]. There would be, of course, many other theoretically even more physiologic solutions to be explored.

The Pittsburgh group, in recent still unpublished experiments, under Nozari *et al.*, explored the above suspended animation approach with liver trauma and thoracotomy added. The coagulopathy due to hemodilution, cold, and ischemia was greatly worsened by trauma. Nevertheless, cardiac arrest of 60 min with severe trauma and use of fresh donor blood could be reversed to intact survival (Ala Nozari, unpublished data).

In addition to the Pittsburgh group, two other groups have explored the concept of suspended animation from somewhat different perspectives. Taylor *et al.* [72,73] were interested in developing a method for protecting the brain during otherwise infeasible neurosurgical procedures. They showed that asanguinous low flow perfusion of the organism with CPB over 3 h, under ultraprofound hypothermia (<5°C), could be survived with normal neurological function. Long periods of total circulatory arrest were not explored, however. From a clinical perspective, intermittent low flow during SA may be helpful for finding bleeding sites.

Rhee *et al.* [71] have explored suspended animation in a clinically relevant exsanguination model in pigs (HB Alam, in preparation). Using readily-available equipment, they induced profound hypothermia by aortic flush via a thoracotomy and direct aortic cannulation. Repair of the aortotomy was accomplished during no flow. After total circulatory arrest of up to 40 min, normal neurologic recovery could be achieved [71]. The same group under Alam *et al.* (HB Alam, in preparation) found with a clinically realistic open-chest model in pigs, normal cognitive function after prolonged asanguinous low flow (by CPB) or shock at 10°C.

Attempts at further extending the so far maximal duration of reversible cardiac arrest of 90–120 min no flow, would get suspended animation research into cryobiology. Could one further extend the preservation time by going below 5–10°C? Profound hypothermia (5–15°C) has been shown in itself not to damage brain tissue [53,59,74,75] (HB Alam, in preparation), but going below 5°C can cause denaturation of proteins and

permanent cell damage, irrespective of the damage caused by ischemic anoxia [76,77] (MJ Taylor, unpublished data). Ultraprofound cerebral hypothermia (<5°C) with a special acellular synthetic solution as blood substitute, could preserve viability of rat hippocampus under ultraprofound hypothermia [75].

Potential clinical use of suspended animation

The recently achieved outcome results in dogs, with emergency preservation of the organism during prolonged cardiac arrest using aortic cold flush, and delayed resuscitation with CPB, obtained by the University of Pittsburgh group and subsequently by others, justify the following conclusions concerning the potential clinical use of suspended animation in trauma-induced exsanguination cardiac arrest:

(1) In large dogs, isotonic saline at 0–4°C, flushed into the aorta at a rate of 1–2 l/min, with drainage of the vena cava, can achieve deep to profound hypothermia of vital organs at a cooling rate of up to 3°C per min; this achieves preservation of viability of the organism during predictable durations of no flow.

(2) Considering the similarity between dogs and humans of time limits in cardiac arrest and resuscitation, with cooling begun before normothermic no flow of 5 min, one can count on achieving survival without functional or histologic brain damage, after cardiac arrest no flow of 15–20 min at Tty of about 30–35°C; after 30 min at Tty 25°C; after 60 min at Tty 15°C, and after cardiac arrest of 90 min at Tty 10°C.

(3) Considering that rapid percutaneous access to the aorta is not yet available, arterial flush and venous drainage will have to be accomplished via cutdown cannulation of the femoral artery and vein or via emergency thoracotomy for direct cannulation of the aorta and drainage from the right atrial appendage. In severe hemorrhage without anesthesia, the patient would become unconscious when mean perfusion pressure is decreased below 40 mmHg, which could be a signal for accessing the aorta via thoracotomy. When apnea then ensues and pulsations are no longer palpable in the aorta, one can assume cardiac arrest. This would be the signal for the cold flush.

(4) Pharmacologic preservation potentials pale *vis-à-vis* hypothermic strategies. Nevertheless, antioxidants that penetrate the blood-brain-barrier, such as tempol, and other novel medicated solutions for flush, preservation, and reperfusion might provide some minor additional benefit. They might be explored further.

(5) Suspended animation could be initiated by hypothermic aortic flush or with use of a portable CPB apparatus with heparin-bonded circuit (without systemic heparinization). Vessel access within 2–5 min, not yet available, is the key to both. With CPB, recirculation would be possible when major vessel leaks are closed.

Suggestions

For traumatic exsanguination cardiac arrest, clinical feasibility trials are indicated, at least for the initiation of suspended animation in trauma hospitals' emergency departments, where emergency thoracotomy is used quite routinely in such cases (Fig. 1). Later, when appropriate devices become available for initiation of suspended animation outside the hospital, such feasibility trials would become part of emergency medical service research. Randomized clinical outcome studies for resuscitation research have limitations because of numerous uncontrollable variables (31). Studying clinical feasibility and side-effects is more important than randomized clinical outcome studies. Randomized withholding of a life-saving method that is reproducibly effective in clinically relevant large-animal outcome studies of otherwise lethal insults, raises ethical dilemmas.

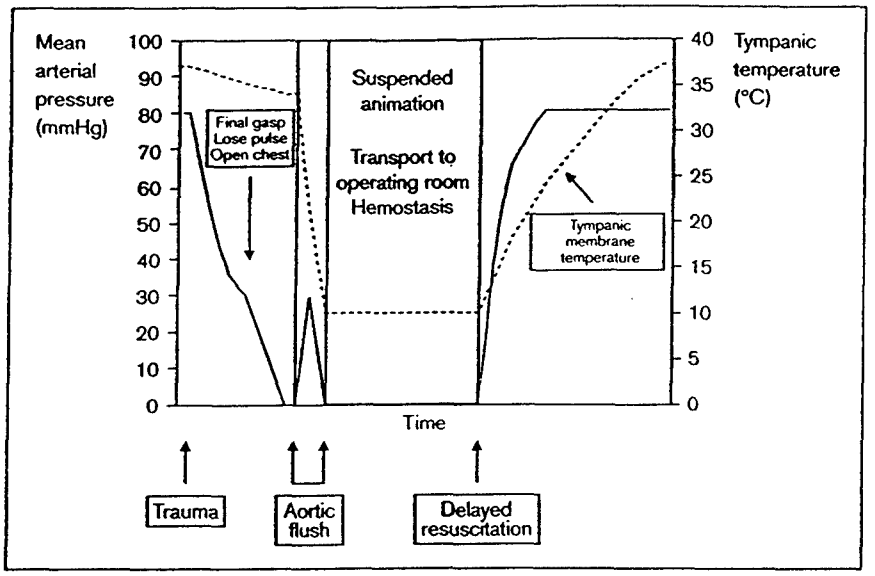
There is a need for conducting further basic research and achieving further bioengineering developments, simultaneously with clinical feasibility trials of suspended animation. Clinically-oriented suspended animation developments might include several topics: who may benefit from expensive and labor-intensive suspended animation? What logistic problems need to be overcome to initiate suspended animation? In field hospitals or trauma hospitals of poor countries without CPB available, could aortic flush preservation with profound hypothermic cardiac arrest be reversed with open-chest CPR and direct rewarming of the heart? Could large-volume flush be simplified by recirculating venous blood via a cooling device, without oxygenator?

Basic suspended animation research

There are a number of topics which might be investigated. (1) The multifactorial pathogenesis of the highly complex postischemic-anoxic derangements [31] calls for multifaceted, mechanism-tailored therapies. Search for and documentation of optimal drug combinations, dosing, and timing, in clinically relevant reproducible cardiac arrest outcome models in large animals would require several research teams' life-long commitments and funding. Therefore, combining serendipity with mechanism-specific selections of topics for evaluations may be more cost-effective. (2) The absolute limits to resuscitation attempts need clarification. What are patterns and rates of tissue death during total circulatory arrest (at different temperatures) without resuscitation? At which point do signs of irreversible cell death occur in

Figure 1. Possible clinical scenario for suspended animation in trauma victims with exsanguination cardiac arrest

As the patient becomes profoundly hypotensive, a last gasp and loss of pulse would be indications for rapid thoracotomy and cannulation of the aorta. This would be followed by aortic flush when cardiac arrest is confirmed. This would buy time for transporting to the operating room for control of major bleeders and delayed resuscitation using cardiopulmonary bypass.



different organs? We may hypothesize that chemical markers, for example, as now searched for in proteomics studies of the brain, precede irreversible morphologic changes, and that mitochondrial DNA damage precedes destruction of cell membranes and nucleus. (3) Is hypothermic preservation hampered by blood elements remaining in the microcirculation during prolonged stasis? How can integrity of the endothelium and of blood elements best be preserved or restored during and after prolonged stasis? (4) How do coagulation derangements influence the potentials of suspended animation, considering coagulopathies caused by hemodilution, ischemia, hypothermia, and tissue trauma? (5) Can suspended animation strategies offer new life-saving potentials in cases of normovolemic normothermic sudden cardiac death, in cases resistant to standard external CPR-ALS attempts at ROSC? Such cases include about 50% of out-of-hospital CPCR attempts. To give such patients a chance, responders must preserve viability of brain and heart until prolonged CPB (venous–arterial pumping via oxygenator) is started. CPB for several hours or even weeks, documented as feasible in patients, would enable the heart to recover from ischemic damage, be repaired (e.g. angioplasty), or replaced. Such bridging to CPB could be accomplished by CPCR steps A-B-C continued, with or without cooling, and compared with suspended animation. Other candidates for 'buying time for transport' might be normovolemic victims of terrorists, as of asphyxia from crush injuries, nerve gas poisoning, or smoke inhalation.

Devices development

Development of devices is needed primarily for initiation of suspended animation outside of hospitals. Life-supporting first aid by lay bystanders should include mild exposure hypothermia [14]. Mobile intensive care unit ambulances in civilian emergency medical services could bring to the scene electric power and could store kits, cooler, and large amounts of fluids. Military combat medics, however, would have none of this available. Everything suggested for their use would have to be ultra-miniaturized: (1) rapid percutaneous access to the aorta and vena cava, without thoracotomy, using a 'smart catheter' approach; (2) a miniaturized cooling-pumping device, considering that electric power for gas compression is not available. Evaporation of prepressurized gas, and thermoelectric approaches should be considered. Ideally, for portability in the field, the maximally miniaturized cooling source with pump could be developed for dual use. The two functions would be venous–venous shunt flow [78] or other method of rapid induction of mild systemic or cerebral hypothermia in conditions with circulation [79] (e.g. after cardiac arrest and ROSC; hemorrhagic shock, brain trauma, stroke), and profound hypothermic aortic flush in conditions without circulation: suspended animation for cardiac arrest [58••,67,69].

Conclusion

This article has summarized the initiation of new suspended animation strategies for attempts at saving some of the traumatologic and normovolemic cardiac arrest emergency patients who currently cannot be resuscitated outside of hospitals and operating rooms. We should also keep in mind, however, that suspended animation could also be useful when surgeons and anesthesiologists are unexpectedly losing ground with unmanageable hemorrhage during various surgical op-

erations and for performing otherwise infeasible cardiovascular or neurosurgical procedures.

Acknowledgements

Lyn Yaffe, MD of the US Navy Medical Research and Development Command (emeritus) initiated support of suspended animation research by the US Department of Defense. Suspended animation research has been supported by the A.S. Laerdal Foundation (1980s) and the US Department of Defense via the Office of Naval Research and the US Army MRDC and TATRC. Wilhelm Behringer, MD, Ala Nozari, MD, PhD, Miroslav Klain, MD, PhD, and Mr. William Stezoski provided input for this paper.

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Cerebral resuscitation potentials for cardiac arrest

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Permanent brain damage after cardiac arrest and resuscitation is determined by many factors, predominantly arrest (no-flow) time, cardiopulmonary resuscitation (low-flow) time, and temperature. Research since around 1970 into cardiopulmonary-cerebral resuscitation has attempted to mitigate the postischemic-anoxic encephalopathy. These efforts' results have recently shown outcome benefits as documented in clinically relevant outcome models in dogs and in clinical trials. Pharmacologic strategies have so far yielded relatively disappointing results. In a recent exploration of 14 drugs in dogs, only the antioxidant tempol administered at the start of prolonged cardiac arrest improved functional outcome in dogs. Cerebral blood flow promotion by hypertensive reperfusion and hemodilution has resulted in improved outcome in dogs, and brief hypertension after restoration of spontaneous circulation is associated with improved outcome in patients. Postarrest hypercoagulability of blood seems to yield to therapeutic thrombolysis, which is associated with improved cerebral outcome in animals and patients. In a clinically relevant dog outcome model, mild postarrest cerebral hypothermia (34°C), initiated with reperfusion and continued for 12 hrs, combined with cerebral blood-flow promotion increased from 5 to >10 mins the previously longest normothermic no-flow time that could be reversed to complete cerebral recovery. Mild hypothermia by surface cooling after prolonged cardiac arrest in patients has been found effective in recent clinical studies in Australia and Europe. Preliminary data on the recent randomized study in Europe have been reported. For presently unresuscitable cardiac arrests, research since the 1980s in dog outcome models of prolonged exsanguination cardiac arrest has culminated in brain and organism preservation during cardiac arrest (no-flow) durations of up to 90 mins, perhaps 120 mins, at a tympanic temperature of 10°C and complete recovery of function and normal histology. This "suspended animation for delayed resuscitation" strategy includes use of an aortic flush of cold saline (or preservation solution) within the first 5 mins of no flow. This strategy should also be explored for the larger number of patients with unresuscitable out-of-hospital cardiac arrests. Suspended animation for prolonged preservation of viability could buy time for transport and repair during hypothermic no flow followed by resuscitation, or it could serve as a bridge to prolonged cardiopulmonary bypass. (Crit Care Med 2002; 30[Suppl.]:S140–S144)

Key Words: cardiopulmonary bypass; cardiopulmonary-cerebral resuscitation; cerebral blood flow; cerebral ischemia; clinical trials; hemorrhage; hypothermia; reperfusion injury; suspended animation; thrombolysis

Current resuscitation attempts from cardiac arrest (CA) yield suboptimal results (1). Restoration of spontaneous circulation (ROSC) is not enough. Cardiopulmonary-cerebral resuscitation is needed to prevent brain damage. The goals are to minimize normothermic no-flow time with immediate bystander-initiated life-support (2) and earliest ROSC and to mitigate the secondary postischemic-anoxic encephalopathy (1, 3). Results with use of outcome models in monkeys and dogs (1) have been clinically important, whereas rodent models have been used to explore the complex mechanisms of the encephalopathy (1, 3). Beneficial results in rodents could not be consistently reproduced in large animals or human patients. Interventions that were effective for protection-preservation were not typically effective for resuscitation. Interventions that seemed effective after brain trauma or focal brain ischemia in rats were not consistently effective after global brain ischemia (CA) in monkeys, dogs, or human patients. Whereas pharmacologic strategies have been disappointing, hypothermic strategies have been promising.

PATHOPHYSIOLOGY

In sudden normothermic CA, brain oxygen stores and consciousness are lost within 20 secs, and glucose and adenosine triphosphate stores are lost within 5 mins. CA no-flow times of ≥5 mins and ROSC are followed by impaired cerebral blood flow (4–9). Transient cerebral hyperemia is followed by protracted global and multifocal hypoperfusion. Impaired reperfusion may be prevented with hypertensive reperfusion (4, 7, 9), which improved outcome in dogs (8) and is associated with good cerebral outcome in human patients (1, 7). Complex chemical derangements (1) account for the death of vulnerable neurons in selectively vulnerable regions (10). During no blood flow, there are membrane depolarization, calcium influx, glutamate release, acidosis, and activation of lipases, proteases, and nucleases, which set the stage for reoxygenation injury with cascades that involve iron, free radicals, nitric oxide, catecholamines, renewed excitatory amino acid release, and renewed calcium shifts, leading to mitochondrial damage, DNA fragmentation, and scattered cell

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Presented at the Wolf Creek VI Conference, Rancho Mirage, CA, June 2001.

Supported, in part, by the United States Navy and Army, the Deutsche Forschungsgemeinschaft of Germany, the European Union, and the A.S. Laerdal Foundation.

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death (1, 3). The post-ROSC encephalopathy matures over 3 days or longer (1, 10). For animal studies, a reproducible whole-brain histologic damage scoring mechanism was evolved in 1978 (1, 10). Post-CA coma in patients may be evaluated with the Glasgow Coma Score and Pittsburgh Brain Stem Score (11–14).

PHARMACOLOGIC CEREBRAL RESUSCITATION

After normothermic CA in animal models, barbiturates and calcium channel blockers improved cerebral outcomes (1, 3). Clinical trials, however, did not prove statistically significant better neurologic outcomes (11–14). Unfavorable cardiovascular side effects may have contributed to the overall negative results. Benefits might also come from inhibitors of neuronal apoptosis, excitatory amino acid receptor blockers, and free radical scavengers, together with improvements in the microcirculation, by inhibiting vasoconstrictive mediators, leukocytes, and coagulation (15). To date, no specific pharmacologic treatment option is available for clinical use.

Circulatory arrest induces selective and delayed neuronal cell damage, primarily in the CA1 sector of the hippocampus, in the thalamic reticular nucleus, and in specific areas of the neocortex (16, 17). In CA models in rats (16, 18), DNA fragmentation in neurons was seen in selectively vulnerable areas. Anti-apoptotic proteins like Bcl-2 and Bcl-X_L, and synthetic and viral caspase inhibitors, have shown positive effects in different models of global cerebral ischemia (19, 20). The blockade of the neurotoxic effects of glutamate with N-methyl-D-aspartate receptor antagonists was ineffective after global cerebral ischemia in rats. The use of AMPA receptor antagonists is being investigated (21). Experimental evidence suggests beneficial effects of transgenic expression of the free radical scavenger superoxide dismutase (22). Because the blood-brain barrier is preserved after cardiac resuscitation, antioxidant drugs may not be active. Tempol, an antioxidant that crosses into the brain tissue, is beneficial in conjunction with hypothermia.

When cerebral reperfusion is delayed or inadequate, blood cell sludging with increased blood viscosity is observed. Endothelial cell swelling; leukocyte-endothelial interactions; dysbalance among nitric oxide, adenosine, and endothelin; vasoactive mediators; and disseminated intravascular coagulation impede microcirculation (1, 4, 7, 15). Improvement in neurologic outcome was achieved in animals with heparin, dextran, and fluids containing fibrinolytic agents (1, 6–9, 23–25). In cats, the administration of hyperoncotic-hypertonic solutions improved cerebral microcirculation (23). The number of leukocytes is increased in the brain after CA (16), but reducing the number of polymorphonucleic cells failed to improve outcomes. The administration of an endothelin(A) receptor antagonist does improve cerebral blood flow, functional activity, and neurologic outcome after CA in rats (7, 24). Tissue-type plasminogen activator (rt-PA) combined with heparin significantly reduced the cerebral no-reflow phenomenon of the forebrain in cats (25). Microthrombi have been found in cerebral microvessels 5–10 mins after onset of CA (15, 25, 26). Fibrinolysis during CPR improved outcomes in patients (27).

HYPOTHERMIC CEREBRAL RESUSCITATION

Therapeutic hypothermia was introduced in the 1950s (28, 29). Benson et al. (30) reported then on moderate hypothermia (28–32°C) in patients after CA, yielding promising but inconclusive outcomes. Concerns about arrhythmias, coagulopathy, and pulmonary infection delayed further clinical use. In the early 1980s, the Pittsburgh group resumed research on resuscitative hypothermia (31). Until then, it was believed that moderate hypothermia levels, which are risky, are needed to be beneficial. In 1987 (32, 33), a brain-damage mitigating effect was discovered for mild hypothermia (tympanic membrane temperature [Tty] 33–36°C), which was accidentally present during CA in dogs. Mild hypothermia seems simple to induce and safe. This led to the first recommendation of resuscitative mild hypothermia after prolonged normothermic CA in clinically relevant dog outcome models (34–38). When temperature was reduced to 20°C, outcome was compromised (36). Delays of 15 mins after ROSC reduced the benefit of hypothermia (37). Induced hypertension and hemodilution enhanced the benefits of mild hypothermia and restored near normal function and brain histology in dogs after 11 mins of normothermic CA (38). In rats, mild hypothermia markedly reduced hippocampal injury after forebrain ischemia (1, 3, 39). Mild hypothermia is beneficial not primarily because of a reduction in oxygen demand but by mitigating excitotoxicity, free radical reactions, edema, intracranial pressure, cell destructive enzymes, and other deleterious cascades (1).

In patients, Bernard et al. (40) and Yanagawa et al. (41) may have improved outcomes in comatose survivors of out-of-hospital CA with surface cooling for 12 hrs (40) or 48 hrs (41). Studies in Japan (41–43) included cardiopulmonary bypass (CPB) in the emergency department (43) in patients who had out-of-hospital CA and did not respond to conventional cardiopulmonary-cerebral resuscitation, plus mild hypothermia for 2 days or more. The European study of 1996–2001 began with a feasibility trial in Vienna (44) and concluded with a multicenter international randomized clinical trial (45). The feasibility trial (44) showed that it was safe and feasible to mildly cool patients with ventricular fibrillation CA. Surface cooling with cold air was initiated within 62 mins (range, 41–75 mins) after ROSC. The target temperature (33 ± 1°C) was reached after 287 mins (range, 242–401 mins) and was maintained for an additional 24 hrs. Thereafter, patients passively rewarmed and reached >35°C after 7 hrs. After 6 months, good cerebral outcome (cerebral performance category 1 or 2) was achieved by 14 (52%) patients; two patients (7%) had poor recovery (cerebral performance category 3 or 4), and 11 (41%) died. Compared to historic controls in the same department, this represents a two-fold improvement of outcome. There were no major complications that could be directly related to hypothermia. Therefore, a definitive multicenter European trial was conducted (45) that ended in 2001. Preliminary results were presented at the Wolf Creek VI conference.

SUSPENDED ANIMATION

In trauma victims who rapidly exsanguinate to CA (no flow) as a result of uncontrollable intrathoracic or intraabdominal injury (e.g., combat casualties), conventional resuscitation attempts are futile, and mortality is near 100% at this time (46, 47). In searching for new approaches, Bellamy et al. (47) recommended research into "suspended animation for delayed resuscitation" to preserve the organism during CA of up to 2 hrs for

transport and surgical hemostasis and then to resuscitate to survival without brain damage. This would require emergency (portable) CPB (33). In dog outcome models, profound hypothermia (Tty, 5–10°C) induced and reversed with CPB, could fully preserve brain viability in CA to 60 mins of no-flow time (48, 49). In the field, CPB is not yet available, and in trauma victims who exsanguinate to CA, hypothermia must be induced before the brain loses its viability (i.e., within 5 mins of no flow). Therefore, an aortic cold flush was introduced to rapidly induce preservative hypothermia; CPB was used only for resuscitation and rewarming (50). Dogs were exsanguinated over 5 mins to a CA no flow of 15 to 120 mins (51–55). At 2 mins of CA, the dogs received the aortic flush via a balloon-tipped catheter, advanced via the femoral artery. CA of 15–120 mins was reversed with CPB, followed by ROSC, assisted circulation (with CPB) for 2 hrs, mild hypothermia for 12 hrs, controlled ventilation for 24 hrs, and intensive care to 72 hrs. Final outcome evaluation at 72 hrs was in terms of overall performance categories, neurologic deficit score, and total and regional histologic damage scores in 19 different brain regions (10). Results depended on flush volume and flush temperature. Results for CA of 15 mins (51), 20 mins (52), and 30 mins (53) have been reported. For CA of 30 mins or longer (54, 55), the flush had to include the spinal cord. For CA of 60 mins (54), aortic flush at the start of CA with around 3 L of saline at 2°C, to a Tty of 20°C, resulted in good cerebral outcome but with some disabilities in the hind legs. Aortic flush with around 6 L of saline at 2°C to a Tty of 15°C resulted in all dogs having normal outcome and only mild or zero histologic damage, as did a flush with around 2°C saline to a Tty of 10°C. For CA of 90 mins (55), aortic flush at the start of CA with around 10 L of saline at 2°C, decreasing Tty to 10°C, resulted in normal outcome and zero or minimal brain histologic damage. For CA of 120 mins (55), saline flush to a Tty of 10°C resulted in a mixed outcome. Delay of the aortic flush to 5 mins after the start of CA still resulted in a good neurologic outcome, whereas delays of 8 mins resulted in a poor neurologic outcome. Flush volumes required were large and impractical for field use. When using a small volume (25 mL/kg) for aortic arch saline flush at an ambient temperature (24°C) at the start of exsanguination CA of 20 mins of no flow, decreasing Tty to 36°C, 14 pharmacologic cerebral preservation potentials, according to six pharmacologic strategies, gave disappointing outcomes (56, 57). Only the antioxidant tempol, given in high doses by aortic flush at the start of CA of 20 or 40 mins, improved functional outcome, although histologic damage was the same as in controls (58).

Suspended animation for delayed resuscitation in patients with normovolemic sudden cardiac death who are resistant to CPR and advanced life support should be explored. About 50% of out-of-hospital CPR attempts fail to achieve ROSC. The decision would have to be made very early during ROSC attempts to preserve viability of vital organs with cold flush until long-term CPB can be initiated in the emergency department. This approach would have to be compared with continued conventional normothermic CPR low flow and with continued mild hypothermic CPR low flow, with external CPR steps A-B-C (airway control—breathing control—circulation support [chest compressions]) continued until start of prolonged CPB to let (or help) the heart recover, while evaluating the brain.

PERSPECTIVES

Preventing post-CA brain damage requires minimizing normothermic no-flow times (2) through early (automatic) defibrillation and early initiation of cooling. Rapid induction of mild cerebral hypothermia requires novel cooling methods for use by paramedics and emergency physicians. Surface cooling is too slow. Future pharmacologic combinations might reinforce the proven beneficial effects of hypothermia. Thrombolysis plus heparin and antiapoptotic strategies currently appear to be most promising. Portable CPB should become available for initiation outside hospitals. Suspended animation for delayed resuscitation should be tried on comatose trauma victims in trauma hospitals' emergency departments, using thoracotomy (on onset of pulselessness) and aortic cold flush (on apnea). Future breakthroughs might depend on a combination of: 1) basic science—elucidating why (and how), after CA and ROSC, only selectively vulnerable neurons die in a delayed manner; b) serendipity—having an open mind on potential breakthrough effects of new strategies; and c) implementation—minimizing arrest times and optimizing prevention of the cerebral postresuscitation disease (1, 59, 60).

Preventing postcardiac arrest brain damage requires minimizing normothermic no-flow times through early (automatic) defibrillation and early initiation of cooling.

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Editorial

Therapeutic Hypothermia after Cardiac Arrest

In this issue of the *Journal*, the reported results of two randomized clinical trials, one in Australia[1] and the other in Europe,[2] showed a neurologic benefit of mild therapeutic hypothermia (33°C in the first study and 32°C to 34°C in the second) in survivors of out-of-hospital cardiac arrest.[3] In the Australian study, which involved a total of 77 patients who remained comatose after the restoration of spontaneous circulation, 49 percent of those treated with hypothermia were discharged home or to a rehabilitation facility, as compared with 26 percent of those not treated with hypothermia ($P=0.046$).[1] There were no significant differences between the two groups with respect to the frequency of adverse events. The results of the European study, which involved nine centers in five countries and had a larger number of enrolled patients, were similar.[2] Taken together, the findings in these trials are important, because in the United States so far, permanent brain damage after cardiopulmonary–cerebral resuscitation[3] causes many delayed deaths and is seen in about 10 to 30 percent of survivors of out-of-hospital cardiac arrest.[4] The fact that the two studies yielded similar results makes the important conclusions even more compelling.

The rationale for the use of therapeutic hypothermia,[4-6] which was pioneered in the 1950s and 1960s, is complex. Spontaneous, uncontrolled hypothermia starts with potentially deleterious shivering, thermogenesis, catecholamine release, and vasoconstriction,[6,7] whereas therapeutic, controlled hypothermia is potentially beneficial.[1,2,4,8] Therapeutic hypothermia after cardiac arrest, as used in the two studies reported in this issue,[1,2] is directed at mitigating neurologic injury. Temperature levels are important; mild hypothermia (33°C to 36°C) may be most effective, and it is simple and safe. Moderate hypothermia (28°C to 32°C) can cause arrhythmias or even ventricular fibrillation and, if prolonged, can lead to coagulopathy and infection.[6,7] The timing and duration are important; mild hypothermia should be initiated as soon as possible after resuscitation,[4,8-11] but even when delayed for a few hours, mild hypothermia has been shown to have some benefit in animal models of global ischemia or cardiac arrest.[4,12] Mild hypothermia induced in patients for 12 hours, as in the Australian study,[1] or 24 hours, as in the European study,[2] does not appear to have the putative complications of moderate hypothermia (i.e., ventricular fibrillation, coagulopathy, and infection).[4-7]

Since the 1950s, moderate and deep hypothermia have been used for special surgical procedures,[6] but research on hypothermia to help reverse the neurologic insult after normothermic cardiac arrest lay dormant for over 20 years. In the early 1980s, our group rekindled research on therapeutic hypothermia after cardiac arrest, using clinically relevant models in dogs.[4,8,13] In 1987, mild hypothermia, accidentally present during prolonged cardiac arrest in dogs, was discovered to be beneficial.[13] This observation was followed by five studies in dogs showing positive outcomes with the use of mild hypothermia after cardiac arrest lasting 10 to 12 minutes without blood flow.[4,8] After resuscitation from ventricular fibrillation of 11 minutes' duration,[8] the use of mild hypothermia for 12 hours, combined with strategies to promote blood flow,[14] resulted in normal brain function and histologic findings.[8] At the same time, neuroscientists reported that mild changes in brain temperature can alter the degree of histologic damage to the hippocampus after incomplete forebrain ischemia in rats.[15]

In the 1950s, it was believed that the benefit of hypothermia was due to a reduction in oxygen requirements.[16] However, since even mild hypothermia, which does not lower oxygen uptake after cardiac arrest,[4] may be beneficial, it seems more likely that hypothermia provides protection against numerous deleterious biochemical mechanisms. Over a period of days after the restoration of spontaneous circulation, these mechanisms, which include calcium shifts, excitotoxicity, lipid peroxidation and other free-radical reactions, DNA damage, and inflammation, lead to the death of some neurons in vulnerable regions of the brain, such as the hippocampus and cerebellum.[4]

Surprisingly, the current trials[1,2] showed a benefit in spite of late and slow surface cooling. In the hypothermia group in the Australian study,[1] the core temperature decreased from 34.9°C at 30 minutes after the restoration of spontaneous circulation by 0.9°C per hour. In the hypothermia group in the European study,[2] cooling was initiated at a median of 105 minutes, and the target temperature of 32°C to 34°C was reached an average of eight hours after the restoration of spontaneous circulation. The majority of patients in the hypothermia and normothermia groups had mild hypothermia on arrival at the hospital. This suggests that early rewarming, as occurred in the control group, may be detrimental. Mild cerebral hyperthermia worsens brain injury.[9] The proportion of patients in whom cardiopulmonary resuscitation had been performed by a bystander was higher in the normothermia group than in the hypothermia group in the study by Bernard et al.[1] Had the proportions been equal and had the hypothermia group undergone immediate cooling and hypertensive reperfusion,[8,14] the beneficial effect of hypothermia

might have been even greater. The positive outcomes observed in the Australian and European studies had not been achieved with pharmacologic interventions in past clinical trials.[4] The fact that in the study by Bernard et al., the platelet count did not differ significantly between the hypothermia and normothermia groups suggests that mild hypothermia may also be safe in patients with trauma.[7]

Although brain and core temperatures equilibrate rapidly when the circulation is normal, brain temperature should be monitored during resuscitation. Monitoring can be performed noninvasively with the use of tympanic or nasopharyngeal temperature as a proxy measurement. Currently available cooling methods are not ideal for the induction of hypothermia. Immersion in ice water causes rapid cooling but is impractical. The removal of clothing and the application of ice packs to the head and torso, as in the study by Bernard et al.,[1] result in very slow cooling. The selective induction of cerebral hypothermia by surface cooling of the head and neck seems feasible only in infants. The method used in the European study[2] involves the circulation of cool air over the patient's body. Peritoneal cooling is rapid but is not generally used. Extracorporeal blood cooling is the most rapid method of reducing temperature, but it involves logistical difficulties. Although the use of cardiopulmonary bypass and a heat exchanger causes a rapid reduction in temperature, cooling is delayed because of the time required to obtain vascular access and to prepare the apparatus. In large animals, venovenous shunt cooling is rapid. Blood cooling through the lungs is currently under investigation.

The dismal outcomes after cardiac arrest call for novel therapeutic approaches. The investigators in the Australian and European studies[1,2] have successfully applied an old therapy — hypothermia — to a new clinical problem. We cannot rule out the possibility that despite the overall benefit, hypothermia has deleterious effects on regenerative or reparative mechanisms; such effects would warrant the use of titrated hypothermia in combination with other therapies. The need for additional laboratory studies, however, should not prevent clinical trials from proceeding. Clinical trials of mild hypothermia are also indicated for stroke,[17] traumatic brain injury, spinal cord injury, and hemorrhagic shock[7]; clinical trials of profound hypothermia (5°C to 15°C) induced at the start of cardiac arrest due to refractory, traumatic exsanguination are also indicated.[18] Additional experimental work is needed to determine whether hypothermia is beneficial for the treatment of septic shock and myocardial infarction. Although we await further studies with great interest, we recommend the use of mild hypothermia in survivors of cardiac arrest — as early as possible and for at least 12 hours.

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THERAPEUTIC HYPOTHERMIA IN TRAUMATOLOGY

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Hypothermia has been used off and on for clinical purposes since ancient times. The Edwin Smith papyrus (circa 3000 BC) describes the use of cold applications on wounds of the head and on ulcerated breast. Perhaps the first application in trauma was by Patroklos in Homer's *Iliad*, who dressed leg wounds with cold water packs after a javelin was removed. Hippocrates advocated packing patients in snow and ice to reduce hemorrhage.[2] Historical figures, such as Julius Caesar, Richard the Lion-hearted, and Mohandas Gandhi, were relieved of ailments by ice cold treatments.[151] Later, during the War of 1812, Napoleon's Surgeon General, Baron Larrey, noted that injured soldiers who were close to a fire died more rapidly than those who remained hypothermic.[84] The latter observations may have been related to the beneficial physiologic effects of hypothermia or to the detrimental effects of surface rewarming. Not long after this, cooling of the head was recommended to add benefit to trephination in patients with traumatic brain injury (TBI). More than 50 years ago, total body cooling was tried to reduce cancer pain[56] and was studied physiologically.[8, 51, 129]

When considering therapeutic hypothermia, one must differentiate[134] between uncontrolled (i.e., spontaneous, accidental) hypothermia—which can be deleterious because of initial shivering, sympathetic discharge, increased oxygen demand, and vasoconstriction[52, 54, 121, 153]—and controlled (i.e., therapeutic) hypothermia, with poikilothermia induced by insult or drugs, which can be beneficial.[51, 130, 131] One must also specify the temperature level in the brain, core, or elsewhere as mild (33°C–36°C), moderate (28°C–32°C), deep (10°C–20°C), profound (5°C–10°C), and ultraprofound hypothermia (0°C–5°C). As deep hypothermia stops the heart from pumping, temperatures below approximately 28°C require cardiopulmonary bypass (CPB) for induction and reversal.

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The application of moderate hypothermia for cerebral protection during complete circulatory arrest (i.e., treatment initiated before the insult) and preservation (i.e., treatment during the insult) has been used clinically since the 1950s when Bigelow et al[17, 18] documented and promoted its use to enable intracardiac operations in a bloodless field. From rudimentary machines to sophisticated cardioplegic solutions and heat exchangers, the application of cold temperatures has become a crucial component of modern cardiac surgery. In the 1950s, Rosomoff demonstrated benefit in dogs with moderate hypothermia during or after focal brain ischemia[123] or experimental TBI.[127] In the early 1960s, in Pittsburgh, Rosomoff and Safar[126] tried moderate hypothermia after TBI in patients. They stopped because of management problems, side effects, and uncertain benefit.[125]

Despite *the voices of the wild*, the discrepancy between animal studies and the scarcity or absence of prospective clinical human studies in trauma[61] has created a misleading environment of *false prophecies* in which hypothermia remains *the bad guy* in the multiple trauma patient.

Although induced hypothermia has become routine for cardiac surgery, the widespread use of therapeutic moderate hypothermia during and after states of hypoperfusion has not been embraced by the medical community, secondary to concerns regarding logistics of implementation, cardiac complications,[57–59] coagulopathies including transient peripheral sequestration of platelets,[68, 111, 115, 122] and infection.[54, 85, 123, 149] Core temperatures below 30°C, even without shivering, can cause these complications and life-threatening dysrhythmias,[54, 149] including ventricular fibrillation (VF).[106]

Recently, there has been renewed interest in therapeutic hypothermia for resuscitation (i.e., treatment after the insult), starting with cerebral resuscitation from cardiac arrest (i.e., temporary complete global brain ischemia).[134] Although, as early as 1961, Safar had recommended resuscitative hypothermia after cardiac arrest as cardiopulmonary cerebral resuscitation step H[131] and although there were clinical trials,[14, 117] this treatment potential lay dormant for 20 years. Pharmacologic cerebral resuscitation attempts were pursued, which have so far proved disappointing.[134, 172] In the early 1980s, Safar's group, including Brader et al,[21] Gisvold et al,[63] and Leonov et al,[90] resumed animal research into moderate resuscitative hypothermia after VF cardiac arrest. Outcome benefit was statistically significant but clinically not impressive. In 1987, Safar et al discovered in dogs the salutary effect of mild hypothermia when induced before[133] or after prolonged cardiac arrest.[88, 140] When used in a timely fashion, it prevents or decreases the ravages of the postresuscitation syndrome. Mild hypothermia, accidentally present during VF cardiac arrest of 10 to 15 minutes no-flow, was associated with unexpectedly good cerebral outcome.[133] Simultaneously, studies by others, with incomplete forebrain ischemia models in rats, revealed the benefit of accidental, mild hypothermia.[28, 26, 27, 35] In dogs, the Pittsburgh group conducted five systematic studies of normothermic cardiac arrest and resuscitation with mild hypothermia[82, 89, 140, 152, 174]; all achieved greater outcome benefit than with resuscitative moderate hypothermia,[90, 175] even when reperfusion was with clinically realistic external cardiopulmonary resuscitation and cooling.[152] Complete recovery after 11 minutes normothermic no-flow in dogs was achieved by combining cerebral blood flow promotion[139] with mild hypothermia from reperfusion to 12 hours postarrest.[140]

Mild hypothermia may be more effective than moderate hypothermia after the insult, because it reduces microcirculation less than with the high viscosity associated with lower temperatures.[141] Mild hypothermia is believed to be simple to induce without the previously mentioned risks observed with moderate hypothermia. The encouraging results in laboratories with mild hypothermia after cardiac arrest are also seen in preliminary data of patient trials.[16, 151, 184, 185] Preservative intra-arrest cooling is more effective than resuscitative postarrest cooling,[51, 163] but is usually not possible clinically. Brief postarrest mild hypothermia may give only transient benefit.[54] The neuron-saving effect, however, is permanent if mild hypothermia is induced as early after reperfusion as possible[82] and sustained for 12 to 24 hours.[32, 43, 140] Mild cooling, even if delayed by several hours, may save neurons,[40–43] whereas the slightest cerebral hyperthermia, which often occurs late after the insult, is deleterious.[40, 50] Revived research into hypothermia for focal[163] and global brain ischemia[130, 134] has been followed by re-exploration of the effect of hypothermia on outcome after TBI[37, 39, 55, 96–98, 117] or traumatic–hemorrhagic hypovolemic shock (HS).*

The mechanisms by which hypothermia can be beneficial, particularly mild hypothermia, must go beyond just decreasing metabolism. Hypothermia decreases blood flow and oxygen uptake by about 7% per °C in normal brain[124, 153] and in the whole organism.[12, 18] After cardiac arrest, mild hypothermia does not seem to decrease cerebral metabolism compared with normothermic controls.[81, 109] Hypothermia has been shown to decrease multiple mechanisms of secondary injury after ischemia and reperfusion or after TBI, including energy failure,[80, 166] oxidant injury,[5, 65, 67] delayed neuronal death,[95, 175, 176] excitotoxicity,[58, 64] cerebral edema, blood–brain barrier permeability, leukotriene production, intracranial hypertension,† cytoskeletal protein degradation,[155] interleukin-1β (IL-1β)production,[66] and inflammation with neutrophil accumulation.[79, 178, 177] Hypothermia tightens cell membranes. Hypothermia also has been shown to prevent the increase in capillary permeability that occurs in the gut after ischemia reperfusion.[75] In general, the protective, preservative, and resuscitative effects of hypothermia have been studied extensively in animal models and clinical settings of brain ischemia, but not in ischemia of the viscera. In traumatic HS, the brain and heart protect themselves by vasodilation, whereas the abdominal viscera suffer ischemia from compensatory vasoconstriction and blood sludging.

Drug treatments, including the use of inhalation anesthetics, have failed to mitigate brain damage, which can be explained, in part, by the fact that anesthetics depress active cerebral metabolism, whereas hypothermia also suppresses basal metabolism, preserving cell membrane integrity.[167] There are many ways to induce cerebral hypothermia,[134, 154, 169] ranging from external methods, which are slow in adults,[54, 129] to selective head-cooling feasible in infants[99, 136] or more rapid peritoneal cooling[182] and most rapid intra-arterial cooling.[12, 13, 116, 176, 180, 181]

Trauma patients are predisposed to hypothermia because of exposure by removal of clothing and opening of body cavities and administration of cold intravenous fluids and cold blood products. In addition, they have a decreased ability to maintain normothermia because of shock, anesthetic agents, or alcohol or drug intoxication. Consequently, they are often already cool in the emergency department.[62, 91] The degree of uncontrolled hypothermia in trauma patients has been correlated with injury severity score (ISS)[67] and trauma score.[91] Shivering, which increases oxygen demand and acidosis, may or may not be obvious. Controlled (i.e., therapeutic) hypothermia, which might also protect and preserve the abdominal viscera (i.e., the most vulnerable organs during and after HS) would have to be in the absence of shivering (i.e., in the presence of poikilothermia).[129, 134] The latter often occurs spontaneously because of ischemia; if not, it can be induced with sedatives or neuromuscular blockade.

*References 47, 76, 77, 88, 115, 156, and 158.
†References 36, 48, 55, 67, 73, 113, 125, 127, and 143–145.

The following brief reviews of the state of clinically relevant knowledge around the year 2000 concerning therapeutic hypothermia in trauma will be grouped into TBI, traumatic HS, and suspended animation for exsanguination cardiac arrest. Suspended animation is futuristic and is still in the experimental stage.

TRAUMATIC BRAIN INJURY

Severe TBI can kill within a few minutes by impact-induced apnea or coma-induced upper airway soft-tissue obstruction.[134] Delayed death can occur as a result of hematoma or brain swelling, resulting in brain herniation with vascular collapse and apnea. Hypothermia can at times prevent or mitigate post-TBI brain swelling and herniation.*

TBI can cripple survivors when contused areas' penumbra zones cause complex multifactorial vascular (i.e., hypoperfusion), neurogenic (i.e., excitotoxicity), and inflammatory damage to untraumatized remote brain tissue, such as the most vulnerable ipsilateral CA3 region of the hippocampus. In addition, TBI can cause axonal injury.[114] Hypothermia can protect against hypoperfusion, mitigate excitotoxicity,[64] protect the TBI-threatened DNA,[175] and mitigate inflammation.[79, 176, 177] Inflammation, however, has detrimental and beneficial components.

In the 1960s, mitigation of secondary brain damage by moderate therapeutic hypothermia in comatose patients after TBI or after intracranial operations seemed a possibility.[3, 54, 125–127, 178, 179] This treatment was not pursued in the 1970s and 1980s because of management problems and fear of arrhythmias, infection, and coagulopathy. Exceptions were sporadic attempts to reverse brain herniation in patients after TBI, using moderate hypothermia in intensive care units where it sometimes became the custom to cool victims of drowning or Reye's syndrome.[123, 134] Hypothermia was not included in the 1990s guidelines of steps to be taken for intracranial pressure (ICP) control after TBI.[25]

In 1991, Safar reinitiated research into resuscitative hypothermia after TBI in dogs; Pomeranz et al[113] developed an outcome model of temporary epidural brain compression, simulating epidural hematoma and drainage, followed by postinsult intensive care. Moderate hypothermia prevented or mitigated ICP rise to herniation, but when the temperature was changed to mild hypothermia (35°C) after 5 hours, ICP increased. Hypothermia diminished the size of the lesion, compared with controls, but after 62 hours of hypothermia, rewarming resulted in brain swelling and herniation in the same proportion of dogs as in normothermic controls. In a subsequent study, Ebmeyer et al,[55] using the same model, extended moderate hypothermia from 5 to 48 hours to prevent rebound swelling during rewarming. Despite slow rewarming from 48 to 72 hours, brain herniation again occurred in some dogs. In addition, this prolonged moderate hypothermia made some dogs develop coagulopathy and pulmonary infection.[55] Post-TBI, the cerebral hypoperfusion known to occur in rats[79] and human patients[108] also was found in this dog model.[55]

Also in 1991, Clifton et al[39] reported improved functional outcome in rats after experimental brain contusion, using moderate hypothermia. This beneficial effect on functional outcome was confirmed by Dixon et al.[53] Other animal experiments of TBI also suggested such benefit.[49, 99, 127, 146, 147] In contrast, even mild cerebral hyperthermia after TBI or ischemia can worsen the lesion.[50, 150]

*References 46, 55, 85, 86, 113, 114, 120, 125–127, and 144.

Patient trials of mild to moderate hypothermia after TBI, promising but not convincing in the early years,* lay dormant until, in 1993, feasibility trials of moderate hypothermia (32°C–33°C) in patients with TBI were published from three centers.[38, 97, 145] The first prospective, randomized clinical outcome study of moderate hypothermia after TBI was conducted in 82 patients by Marion et al[96] at the University of Pittsburgh. Moderate hypothermia for 24 hours did not cause coagulopathy or other complications. Although induction of hypothermia by cooling blankets was rather slow and late (within 10 hours of injury), the hypothermic patients had improved neurologic recovery, compared with normothermic controls in patients with Glasgow Coma Scale Score[139] of 5 to 7 on admission but not in more severely damaged patients.[96]

The previous patient study[95] and the extensive positive animal data led to a major multicenter, randomized, clinical outcome study coordinated by Clifton[37] of mild-to-moderate hypothermia (33°C) after TBI. The overall proportion of patients with good cerebral outcome was not different between treatment and control groups. In some centers, the hypothermia-treated patient group achieved better cerebral outcome than the control. In other centers, results seem the same or worse. Complications suspected of being related to hypothermia can depend on variable intensity and accuracy of post-TBI intensive care life-support, which is crucial for post-TBI recovery (even more crucial if therapeutic hypothermia is employed).

Attempts to prevent rebound swelling during rewarming[55, 72, 113] (i.e., to normalize continuously monitored ICP) may require titrated mild-to-moderate cerebral hypothermia in addition to osmotherapy, CSF drainage, transient hyperventilation, and barbiturate administration. This recommendation, based on clinical experiences in the early 1960s,[125, 126] may have to include frequent recooling.

For future laboratory and clinical studies of hypothermia after TBI, one of the authors (PS) recommends:

1. Earliest possible induction of cerebral hypothermia to prevent rather than treat noxious tissue reactions after impact, perhaps by intra-arterial cold flush in the field or on arrival in the emergency department
2. Preventing intracranial hypertension to herniation by titration of levels and timing of hypothermia and rewarming
3. Augmenting the beneficial effects of hypothermia with pharmacologic strategies
4. Exploring intraventricular-cisternal-lumbar irrigation for cooling, medicating, and removal of blood (i.e., toxic heme) and other noxious molecular species
5. Early surgical removal (débridement) of necrotic brain tissue under hypothermic protection

TRAUMATIC HEMORRHAGIC SHOCK

The relationship between hypothermia and outcome from traumatic HS is less clear than it is for TBI.[162] Mechanisms and outcome-oriented studies in animal models have documented benefits from controlled (i.e., therapeutic) mild-to-moderate hypothermia during HS and resuscitation.† In contrast, clinical

*References 56, 70, 86, 125, 126, 143, and 144.
†References 47, 76, 77, 88, 102–104, 115, 148, 156, and 158.

data suggest that uncontrolled (i.e., spontaneous or accidental) hypothermia is deleterious to trauma patients.[60, 63, 74, 94]

Laboratory Studies

Sori et al[148] studied the effect of hypothermia (29°C versus 34°C) on survival after prolonged, pressure-controlled HS in awake rats (mean arterial pressure [MAP] 30 mm Hg until 80% of the shed blood had been returned or a maximum of 7 hours). The hypothermic group required less return of shed blood to maintain MAP at 30 mm Hg and had greater 72-hour survival (50% versus 0%).

Meyer and Horton[120-122] examined the role of moderate hypothermia (33°C) in the treatment of HS in dogs. Dogs underwent pressure-controlled HS with a MAP 35 mm Hg for 2 hours. The dogs then were assigned to one of three groups: normothermia, hypothermia with correction of metabolic acidemia, and hypothermia without correction of acidemia. Hypothermia (33°C) was induced and reversed by peritoneal lavage. Hypothermic dogs had better preservation of cardiac function, less subendocardial ischemia, and better matching of systemic oxygen delivery and consumption. Long-term outcome was not assessed in these studies.

The Pittsburgh group initiated HS hypothermia studies in rats in 1988. Crippen et al[47] used a model of volume-controlled HS (3.25 mL/100 g blood withdrawn over 20 minutes) in awake rats without resuscitation. The effects on survival time of several treatments that could be initiated by medics in the field were examined. The effects of general anesthesia[1, 93] were avoided.[47] Hypothermia by surface cooling to 30°C and FiO_2 1.0 breathing prolonged survival time by life table analysis ($P < 0.05$). Both treatments kept the MAP higher longer than in the controls. Rectal fluids (5 mL/100 g LR) or external stimulation showed no significant benefit. In a follow-up study with fluid resuscitation in awake rats, Leonov et al[88] found that the combination of FiO_2 1.0 breathing and hypothermia allowed 100% long-term survival compared with 0% survival with room air breathing and normothermia.

The pressure- and volume-controlled HS models cited previously do not adequately duplicate the physiologic changes that occur in the trauma patient with ongoing bleeding. Consequently, Capone et al[30, 31] developed a three-phase model of uncontrolled HS (UHS) by tail cut in rats. The UHS phase I of 90 minutes consisted of initial volume-controlled hemorrhage (3 mL/100 g blood) over 15 minutes, followed by uncontrolled hemorrhage by a 75% tail amputation. The resuscitation phase II of 60 minutes consisted of hemostasis by tail ligation and infusion of shed blood and additional lactated Ringer's solution to achieve normotension. Phase I and II were done under light, standardized, general anesthetic with spontaneous breathing. The observation phase III was continued without anesthesia to 72 hours. Visceral dysoxia was monitored as a surface PCO_2 rise on the liver and gut.[126, 142] Survival was improved using limited fluid resuscitation to maintain a MAP of 40 mm Hg with lactated Ringer's solution during UHS, compared with no fluid resuscitation or aggressive fluid resuscitation attempting to normalize blood pressure.[30] Limited small volume fluid resuscitation is not new.[44] Using the same model, Kim et al[76] found that moderate hypothermia (30°C) and limited fluid resuscitation improved long-term survival. The best outcome was with the combination of limited fluid resuscitation and hypothermia. Kim et al[77] then examined the effects of increased oxygen breathing and moderate hypothermia (30°C) in a model of lethal UHS in rats, using only phase I of the three-phase model, simulating battlefield conditions with the casualty temporarily experiencing uncontrolled bleeding while waiting for evacuation. Previous studies had shown that hyperoxia can increase blood pressure during mild to moderate HS.[4, 19, 47] Survival times for the hypothermia groups were twice as long as the respective normothermia groups ($P < 0.001$). In contrast to previous studies with less severe HS,[4, 19] oxygen breathing had no effect on blood pressure or survival, perhaps because the insult was more severe.[158] Interestingly, a study by Brod et al[24] showed that hyperoxia could be detrimental if hemorrhage were uncontrolled.

Moderate hypothermia may cause several complications clinically.* Mild hypothermia, on the other hand, may have similar benefits on outcome with less risk of these complications. Takasu et al,[156] therefore, examined the effect of mild hypothermia, compared with normothermia and moderate hypothermia, using Kim et al's model of lethal, otherwise untreated UHS in rats.[77] The three temperature levels were also compared at three levels of inhaled oxygen (FiO_2 0.25, 0.5, and 1.0).[157] In this study, survival time was significantly improved with mild and moderate hypothermia. Oxygen inhalation had no effect on survival rates.

In a subsequent study, Takasu et al[159] examined the effect of the same temperature and oxygen inhalation levels on long-term outcome using the three-phase UHS model. In this study, mild and moderate hypothermia increased blood pressure, decreased visceral dysoxia, and improved long-term survival time and rate, compared with normothermia ($P < 0.05$). Oxygen inhalation again had no effect on survival rates.

In the studies by Takasu et al,[156, 158] there was no difference in the effects of mild versus moderate hypothermia on survival, which has clinical significance because mild hypothermia would be easier to induce and theoretically would be safer. These studies found that mild and moderate hypothermia increase blood pressure during HS. This finding was pursued further, because the beneficial effect of hypothermia during UHS, in part, may have been secondary to the blood pressure effect and not the metabolic effects of hypothermia. Using a pressure-controlled HS rat model, Prueckner et al[115] documented that hypothermia, not a blood pressure effect, increased HS survival time and rate.

Clinical Studies

During World War I, it was suggested that hypothermia had a detrimental effect on patients in traumatic shock. More recently, to help delineate the factors leading to hypothermia in trauma patients, Luna et al[94] retrospectively reviewed 94 trauma patients who were intubated and had an esophageal temperature probe inserted. Patients with mild (34°C–36°C) to severe hypothermia (< 34°C) had higher ISS[7] than those who remained normothermic. Survival correlated with temperature; 78% in the normothermia group survived, compared with 59% in the mild hypothermia group and 41% in the severe hypothermia group ($P < 0.05$, comparing hypothermia with normothermia).

In a similar retrospective review of 71 patients with truncal trauma and an ISS greater than or equal to 25, Jurkovich et al[74] attempted to analyze the relationships between body temperature, ISS, shock, and mortality. Overall, when stratified by ISS and by the presence or absence of shock, patients who became hypothermic had a higher mortality rate than those who remained normothermic. In addition, however, higher ISS, presence of shock, and large transfusion requirements were risk factors for becoming hypothermic. Stratifying by these factors, it seemed that the hypothermic patients had worse outcomes.

*References 52, 54, 57–59, 68, 83, 106, 111, 118, 122, 129, and 134

These studies[74, 94] were limited by the fact that a small number of patients were in each stratum and a multiple regression analysis was not done to separate the independent effects of hypothermia from those of other confounding factors statistically. In addition, the use of ISS for stratification in these studies could be questioned. The ISS is an anatomic, not physiologic, scoring system. Since the effects of hypothermia are on physiologic derangements, use of a physiologic scoring system for stratification (e.g., the Revised Trauma Score[33] or Acute Physiology and Chronic Health Evaluation [APACHE]-III system)[78] would seem more appropriate.

To test the hypothesis that stratification of patients by physiologic and anatomic measures of injury severity would more appropriately predict outcome in hypothermic trauma patients, Steinemann et al[150] retrospectively reviewed unanesthetized patients suffering blunt or penetrating injury with an ISS greater than 9 (n = 173) to compare outcomes of patients with a core temperature of less than 35°C (n = 37) with those of patients who remained normothermic. Hypothermia correlated with a higher ISS, lower (i.e., worse) trauma score and systolic blood pressure, and greater fluid requirements and base deficit. Hypothermic patients were less likely to survive when stratified by ISS. When the patients were stratified by their probability of survival using the TRISS methodology,[33] however, there was no significant difference in survival and length of stay in the intensive care unit between the hypothermic and normothermic groups—which suggests that hypothermia is not a primary factor in mortality; rather, it is the underlying shock processes that decreased survival and led to hypothermia.

In contrast to the previous retrospective, uncontrolled studies, clinical studies by Gentilello et al[60, 61] examined the role of rapid core rewarming versus slow standard external rewarming during resuscitation of the hypothermic critically ill patient. In these studies, continuous arteriovenous rewarming (CAVR) was achieved with a heparin-bonded, countercurrent heat exchanger (Level I Technologies, Rockland, MA), using large catheters placed into the femoral artery and vein. The first study included any hypothermic postoperative patient in the intensive care unit.[60] Use of CAVR dramatically decreased the time to resolution of hypothermia (i.e., temperature ≥ 35°C). Patients undergoing CAVR had lower crystalloid, blood product, and total fluid requirements than those undergoing standard rewarming (SR) alone. There was not, however, a statistically significant difference in overall survival.

In a more recent study by Gentilello et al,[61] trauma patients who were hypothermic (i.e., ≤ 34.5°C) and required a pulmonary artery catheter were prospectively randomized after admission to the intensive care unit to receive either SR (n = 28) or SR plus CAVR (n = 29). Total fluid requirement over the first 24 hours and time required to achieve normothermia were significantly lower in the CAVR group compared with the SR group. The frequency of organ system failures did not differ. There was no difference in survival to discharge from the hospital between the two groups (66% with CAVR; 50% with SR). Five complications occurred directly secondary to CAVR: clotting of the circuit, femoral artery pseudoaneurysm formation, arterial bleeding, and two patients requiring operative repairs of the femoral artery.

These results are difficult to interpret for several reasons. First, the two groups were not equivalent at the beginning of the study. For example, the CAVR group had twice as many patients who had undergone a laparotomy as the SR group. One would expect that the outcome of patients who become hypothermic secondary to exposure during a laparotomy might be different from that of patients who become hypothermic secondary to shock and fluid resuscitation. Second, the treatment (i.e., rewarming) was initiated late in the initial management of the patients (after resuscitation in the emergency department or operating room), and no information on treatment before arrival in the intensive care unit was included. Third, the choice of fluid requirement as the primary endpoint is concerning because of the potential for bias in this unblinded study and because it is a process, not an outcome, variable. Fourth, no data regarding causes of death were included. Although this study does compare outcome of two groups of trauma patients with significantly different temperatures, the hypothermic patients were not maintained intentionally at a certain temperature level, nor were any measures used to prevent shivering. In addition, other variables, such as blood pressure, ventilation, and metabolic acidosis, were not controlled. Clinical studies of therapeutic, resuscitative hypothermia during HS should include standardized fluid resuscitation algorithms, sedation and neuromuscular blockade to prevent shivering, and clearly defined outcome measures.

Finally, a beneficial effect of lowered core temperature on survival of combat casualties with severe HS has been suggested by observations in Indochina[34] and the Falkland Islands (M. Champion, MD, personal communication, 1997).

SUSPENDED ANIMATION

In 1984, Bellamy et al pondered over Bellamy's combat casualty data from the Vietnam War.[9-11] Most soldiers killed in action without severe brain trauma exsanguinated to cardiac arrest within 5 to 10 minutes. In some, external exsanguinating hemorrhage could have been stopped by life-supporting first aid. In many with penetrating truncal trauma, the internal exsanguinating injuries were found to be reparable. In such cases requiring surgical hemostasis for resuscitation, standard resuscitation methods before surgery have been useless. Bellamy and Safar decided that a totally new approach should be researched, which they called *suspended animation* (SA).[11, 13, 132] They defined SA as the induction of preservation of the whole organism immediately at the start of cardiac arrest, to maintain viability of vital organs during circulatory arrest of 1 to 2 hours, for transport and repair of the pulseless patient, to be followed by resuscitation to survival without brain damage. They believed that SA would not only help save victims of temporarily uncontrollable (i.e., currently hopeless) traumatic exsanguinations in war[9, 10] and peace,[6, 110] but also patients with nontraumatic exsanguination (e.g., ruptured aortic aneurysm); nontraumatic cases of sudden cardiac death that seem to be resistant to standard external cardiopulmonary resuscitation; and patients in need of selected elective surgical procedures that are only feasible during a state of no blood flow.[92]

In the laboratory, others have explored a technique to enable bloodless brain operations, without profound hypothermic circulatory arrest but with blood washout and continued asanguinous low-flow perfusion by CPB.[160] This approach uses special crystalloid and colloid solution mixtures of extracellular and intracellular compositions. At approximately 5°C to 10°C, 2 to 3 hours of asanguinous low-flow was tolerated in dogs.[161]

Between 1988 and 1994, the Pittsburgh team[23, 165-169] used hypothermic strategies in newly developed dog models to explore SA potentials in a series of six systematic outcome studies. These first SA dog studies relied on hypothermia induced and reversed with CPB.[45, 62, 71, 138, 137, 133] In the first study,[168] they found a 60-minute limit for the reversibility of deep hypothermic circulatory arrest (brain temperature: 15°C) after 30 minutes HS at a MAP 40 mm Hg. In the second

study,[165] they found that profound hypothermic circulatory arrest (brain temperature: 5°C–10°C) provided better outcome than deep hypothermic arrest, allowing survival after 2 hours of no-flow, but with some histologic brain damage. In the third study,[165] the University of Wisconsin organ preservation solution, used for blood washout before stasis during profound hypothermic circulatory arrest, provided no additional benefit. In the fourth study,[165] avoiding systemic anticoagulation with use of a heparin-bonded CPB circuit did not reduce the preservation effect. In the fifth study,[166] there was a hint that moderate hemodilution to hematocrit 20% may be better than total blood washout with hematocrit 5%, which had been used in the previous studies. The sixth study[29] is the most significant: Normothermic HS with a MAP 40 mm Hg for 60 minutes (not 90 minutes), followed by profound hypothermic circulatory arrest for 60 minutes, was reversed to complete functional recovery with histologically *clean* brains. In the year 2000, it is planned to pursue ultraprofound hypothermic circulatory arrest (brain temperature: 0°C–5°C) as a possibility to bring back normal brains after 2 hours of circulatory arrest.

In 1994, Bellamy et al invited 19 investigators to a discussion of SA research planning.[11] All agreed that investigation into this futuristic approach is indicated, is feasible, and is worthwhile for military and civilian objectives. SA, which means tolerance of prolonged tissue anoxia without blood flow, must be differentiated from hibernation, which is hypothermia without tissue anoxia and with sustained low blood flow and low metabolism.

Deep and profound hypothermia requires CPB, which is not available in the field but would be available in emergency departments of civilian emergency medical services (EMS) and in wartime, could be brought by helicopter by a far-forward resuscitative surgery team. Safar, Tisherman, and their colleagues,[12, 13, 156, 138, 182] in 1998 under US Navy funding, began explorations into methods for the instantaneous induction of SA in exsanguinating casualties by medics or emergency physicians in the field. It was believed that a rapid infusion of cold fluid into the aortic arch just before or during the first 5 minutes of pulselessness would be the best way to begin cooling the heart and brain for SA. In witnessed exsanguination cardiac arrest (which the medic could determine by the last breath, which coincides with a MAP 10 to 20 mm Hg), a technique for rapid access to the aorta is still to be developed. In dogs, after preinsertion of an aortic balloon catheter,[138] flushing of brain and heart by the aortic arch with about 25 mL/kg of normal saline solution (NSS) at ambient temperature (24°C) induced cerebral preservation instantaneously by reducing brain temperature from 37.5°C to approximately 35°C within 2 minutes.[116, 161] Exsanguination cardiac arrest of 15 minutes no-flow treated this way was followed by complete functional and histologic brain recovery.[116, 181] Preservation of normal brain during 20 minutes no-flow required flush of the aortic arch with NSS at 4°C.[13] Preservation during 30 minutes no-flow[12] for complete recovery of 72 hours required flushing the entire aorta, with the balloon inflated in the abdominal region using a large volume of NSS (100 mL/kg) at 4°C. For preservation during such a prolonged arrest, all vital organs, including spinal cord and abdominal viscera, need to be cold-flushed to achieve complete recovery. The heart cannot tolerate normothermic no-flow of 20 minutes or more.[32, 119] Because large volumes of NSS at 4°C are not available on the battlefield (although they would be available in civilian mobile intensive care unit ambulances or hospital emergency departments), a search for pharmacologic augmentation of the aortic arch flush approach with low volume (NSS 25 mL/kg) and ambient temperature (24°C) is under way.

The scenario envisioned for witnessed traumatic exsanguination cardiac arrest in the field or the emergency department would be as follows: Within the first 5 minutes of no-flow (i.e., apnea and pulselessness), rapid access to the aorta is gained by groin cut-down (or Seldinger technique or the *comb-needle* method),[161] by thoracotomy, or by a still-to-be-developed parasternal approach. The heart and brain are flushed with a cold medicated solution to gain preservation for at least 30 minutes. As soon as feasible, SA would be extended for another 1 to 2 hours after arrival of portable CPB and insertion of a venous drainage catheter (into the vena cava or right atrium). Brain temperature would be lowered as much as possible with CPB,[29, 166] recirculating the remaining blood and flush solution through a heat exchanger to allow 1 to 2 hours of SA with ultraprofound hypothermic circulatory arrest. Surgical hemostasis (i.e., damage control) by laparotomy or thoracotomy would be achieved during pulselessness as soon as possible, followed by very slow reperfusion and rewarming with CPB. The technology needed for this SA requires the development of Food and Drug Administration–approved devices for rapid vessel access[22, 100, 112, 161] and portable CPB.[135, 137, 138] The multifaceted research approach needed has to include optimizing pharmacologic composition of solutions for flushing, ultraprofound hypothermia (i.e., avoiding crystal formation and other deleterious effects of low temperature), new technology for rapid cooling of fluids, rapid vessel access procedures, heparin-bonded miniaturized CPB,[137] and ideal fluids for stasis and reperfusion. A multicenter, multidisciplinary, goal-oriented *mini–Manhattan project* for SA development was initiated in 1998 under the auspices of the US Navy.

DISCUSSION

Concerning TBI, the potential clinical benefit of early and prolonged, controlled mild-to-moderate hypothermia in patients with intracranial hypertension (i.e., brain swelling) after TBI—titrated to help normalize ICP—has been established. In the absence of threatened brain herniation, mild hypothermia may help preserve biochemical recovery of threatened cerebral neurons distant to the destroyed brain tissue. Details are under investigation. For discussion, the reader should refer to the appropriate articles previously quoted. An additional topic, beyond the scope of this article, that needs revival by laboratory and clinical studies is spinal cord trauma, the effects of which may be mitigated by hypothermia. This idea was conceived and documented 30 years ago by Albin et al[3] and by White.[183]

Concerning HS, a significant disparity exists between the results of laboratory and clinical studies with regard to the effects of hypothermia on outcome following HS. Laboratory studies of HS consistently show benefit of hypothermia, whereas clinical studies suggest that hypothermia is detrimental. Although Jurkovich et al[74] concluded that hypothermia is associated with increased mortality in trauma patients, they questioned "whether it is the hypothermia per se or the severity of the injury producing the hypothermia that is responsible for the subsequent mortality."

There are several differences between the laboratory and the clinical setting that need to be discussed to explain this disparity. Laboratory models do not mimic the clinical setting because of a lack of tissue trauma and coagulation abnormalities; the use of fresh, autologous (not banked donor) blood transfusions; and the need for anesthesia before and during HS. The need for anesthesia may affect physiologic responses to hemorrhage profoundly.[1, 95] Clinical studies are difficult to interpret because of a lack of a prospective, randomized trial of therapeutic, controlled hypothermia or a large, prospective, observational study; inadequate assessment of physiologic status; lack of temperature control; inabil-

ity to control for underlying disease states; and poorly defined outcome measures.

The effect of hypothermia on the coagulation system of trauma patients may be critical to the potential detrimental effect of hypothermia suspected clinically. Hypothermia has been shown to increase blood loss intraoperatively during trauma laparotomies.[15] Decreased platelet counts and platelet function seem to be the most consistent findings.[111, 118] The hypothermia-induced platelet trapping in capillaries seems reversible. Increased fibrinolytic activity during hypothermia also has been suggested.[111, 118] Clinical data on coagulation in patients with hypothermia may be confounded because standard measurements are performed with warming of the blood to 37°C. Gubler et al[85] cooled or diluted the blood of healthy volunteers and intensive care unit patients ex vivo to measure the effects of these two interventions on standard blood coagulation tests (e.g., prothrombin time, activated thromboplastin time, and platelet function). Cooling had similar effects on undiluted and diluted blood. Although there were statistically significant changes at 35°C, these changes did not reach clinical significance until the temperature was decreased to 33°C. Similarly, Reed et al[118] found that consistent and significant coagulation changes did not occur unless body temperature was 33°C or less. Gentilello et al[60, 61] did not find significant coagulation differences between their standard rewarming and CAVR groups. Coagulation factors in patients under mild hypothermia after cardiac events were found to be near normal.[185] No increased bleeding tendency was seen in patients after TBI under 33°C.[98, 120] These findings suggest that mild hypothermia (33°C–36°C) in patients may not have significant effects on the coagulation system. Species differences in coagulation physiology make animal studies of this potential risk of therapeutic hypothermia in patients less relevant.

Massive transfusions alone may have an impact on coagulation even if hypothermia does not. Thrombocytopenia is most common. Other deleterious effects of transfusions include acidosis, hyperkalemia, and hypocalcemia, which also can affect clotting.[64]

There has been concern about the effects of perioperative hypothermia on cardiac morbidity.[57, 58] Frank et al[59] have hypothesized that the cause of cardiovascular morbidity related to perioperative moderate hypothermia involves increases in catecholamines, arterial blood pressure, and peripheral vasoconstriction. Increase in blood pressure has been seen in recent animal studies of therapeutic mild hypothermia during HS.[156, 158] Because trauma usually involves young, previously healthy patients, it is not clear that mild hypothermia would have a significant impact on cardiac complications.

Retrospective studies and poorly controlled prospective studies cannot answer the question of whether or not therapeutic, controlled mild hypothermia has a beneficial effect on outcome after traumatic HS. A prospective, randomized, controlled trial is needed. Such a trial should be performed at multiple centers with well-integrated trauma systems. The patient population (i.e., trauma victims with evidence of HS) needs to be clearly defined. Mild cooling should be initiated as quickly as possible during resuscitation (i.e., in the field or emergency department), yet in a controlled manner, preventing overshoot. Because many trauma patients become hypothermic anyway, the hypothermic group may just need to be kept cool while the normothermic control group undergoes active restoration and maintenance of normothermia. To prevent shivering, patients in both groups need to be sedated, paralyzed, and intubated until all are rewarmed to normothermia. All physiologic parameters need to be controlled by protocol as well as possible. The primary outcome variable should be long-term survival. Secondary endpoints could include fluid requirements, clotting studies, morbidity (e.g., cardiac, infection), and development of organ system failure.

The appropriate level of hypothermia to be tested in clinical trials seems to be mild hypothermia (34°C) because this level has had as much benefit in animal models as moderate hypothermia[156, 158] and should have fewer complications. The question of the ideal duration of mild therapeutic hypothermia during HS and resuscitation remains unanswered. Most laboratory studies have examined cooling during HS only. Would more prolonged hypothermia during resuscitation improve outcome? Perhaps, yes, because post HS sepsis and the systemic inflammatory response syndrome may benefit from controlled hypothermia.[172, 173] Best timing and temperature levels for hypothermia in septic shock are not known. Future laboratory and clinical research also will need to focus on determining optimal methods of rapid, simple, and safe cooling.

Concerning SA, it is in an early stage of laboratory research.[11, 163, 170] Aortic cold flush induction of preservation of brain and heart, however, could be initiated clinically right now. The potentials for breakthroughs in traumatologic resuscitation with the further development of SA are considerable. Discussions of technology and methods of vessel access, cooling, and emergency CPB are beyond the scope of this article. Colleagues with novel ideas on SA should contact Drs. Safar or Tisherman.

SUMMARY

Despite its proven clinical application for protection–preservation of the brain and heart during cardiac surgery, hypothermia research has fallen in and out of favor many times since its inception. Since the 1980s, there has been renewed research and clinical interest in therapeutic hypothermia for resuscitation of the brain after cardiac arrest or TBI and for preservation–resuscitation of extracerebral organs, particularly the abdominal viscera in low-flow states such as HS. Although some of the fears regarding the side effects of hypothermia are warranted, others are not. Without further laboratory and clinical studies, the significance of these effects cannot be determined and ways to overcome these problems cannot be developed. Currently, at the turn of the century, there are significant data demonstrating the benefit of mild-to-moderate hypothermia in animals and humans after cardiac arrest or TBI and in animals during and after HS. The clinical implications of uncontrolled versus controlled hypothermia in trauma patients and the best way to assure poikilothermia for cooling without shivering are still unclear. It is time to consider a prospective trial of therapeutic, controlled hypothermia for patients during traumatic HS and resuscitation. The authors believe that the new millennium will witness remarkable advantages of the use of controlled hypothermia in trauma.[11, 23, 132, 134, 170] Starting in the prehospital phase, mild hypothermia will be induced in hypovolemic patients, which will not only decrease the immediate mortality rate but perhaps also will protect cells and reduce the likelihood of secondary inflammatory response syndrome, multiple organ failure, and late deaths. The most futuristic applications will be hypothermic strategies to achieve prolonged suspended animation for delayed resuscitation in traumatic exsanguination cardiac arrest.

ACKNOWLEDGMENTS

Patti Boyle, Donna Gaspich, and Valerie Sabo helped with the preparation of this article.

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THE SURGICAL CLINICS OF NORTH AMERICA

Trauma Care in the New Millennium

AURELIO RODRIGUEZ, MD,
KIMBALL I. MAULL, MD, AND
DAVID V. FELICIANO, MD
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VOLUME 79 • NUMBER 6 • DECEMBER 1999

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NOVEL SOLUTIONS FOR INTRA-ISCHEMIC AORTIC COLD FLUSH FOR PRESERVATION DURING 30 MIN CARDIAC ARREST IN DOGS

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Introduction: In exsanguination cardiac arrest (CA), conventional resuscitation attempts are futile. Therefore we introduced the use of cold aortic saline flush at start of CA to rapidly induce preservative hypothermia during prolonded CA for "suspended animation for delayed resuscitation". This study was to investigate, in dogs, three different solutions compared to saline for aortic cold flush. Methods: Dogs (20–25 kg) were exsanguinated over 5 min to CA of 30 min no-flow. At CA 2 min, the dogs received an aortic flush of 25 mL/kg at 2°C over 1 min, using saline (n=5), albumin 5% or 25% (n=6), Unisol-UHK (organ preservation solution) (n=5), or polynitroxylated albumin plus tempol (an antioxidant) (PNA-T) (n=5). The flush was through a balloon-tipped catheter inserted via the femoral artery into the abdominal aorta. Resuscitation was by closed-chest cardiopulmonary bypass, followed by assisted circulation for 2 h, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to 72 h, when outcome was evaluated as overall performance category (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=brain death); neurologic deficit score (NDS 0–10%=normal, 100%=brain death); and total brain histologic damage score (HDS 0=normal, >40=severe damage, >100=extensive damage). Results: Lowest tympanic temperature during CA was 32°C in all dogs. Outcome at 72h as OPC, NDS, and HDS, see table. Unisol resulted in pharmacologic defibrillation during CA, PNA-T in lowest NDS and HDS. Conclusions: Three aortic flush solutions, physiologically more rational than saline, may not give a breakthrough effect at moderate hypothermia, but Unisol might be beneficial for the heart and PNA-Tempol for the brain. [Supp. by US Dept. of Defense].

	Saline	Albumin	Unisol	PNA-T	p-value
OPC	3,3,3,3,3	3,3,3,3,4,4	3,3,3,4,5	3,3,3,3,3	0.4
NDS%	47 (38-58)	49 (38-90)	54 (48-97)	35 (31-44)	0.01*
HDS	88 (37-128)	132 (82-290)	132 (89-200)	68 (38-96)	0.02*

Data are median (range). *post hoc: $p<0.05$ Unisol vs PNA-T

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Objectives:

In exsanguination cardiac arrest (CA), conventional resuscitation attempts are futile. Therefore we introduced the use of cold aortic saline flush at start of CA to rapidly induce preservative hypothermia during prolonded CA for "suspended animation for delayed resuscitation". This study was to investigate, in dogs, three different solutions compared to saline for aortic cold flush.

Methods:

Dogs (20-25 kg) were exsanguinated over 5 min to CA of 30 min no-flow. At CA 2 min, the dogs received an aortic flush of 25 mL/kg at 2°C over 1 min, using saline (n=5), albumin 5% or 25% (n=6), Unisol-I (organ preservation solution with K^+) (n=5), or polynitroxylated albumin plus tempol (an antioxidant) (PNA-T) (n=5). The flush was through a balloon-tipped catheter inserted via the femoral artery into the abdominal aorta. Resuscitation was by closed-chest cardiopulmonary bypass, followed by assisted circulation for 2 h, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated as overall performance categories (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=death); neurologic deficit score (NDS 0-10%=normal, 100%=brain death); and brain histologic damage score (Total HDS 0=normal, >40=severe damage, >100=extensive damage).

Results:

Lowest tympanic temperature during CA was 32°C in all dogs. At 72h, all dogs achieved poor overall performance (OPC 3-5) (figure 1); Unisol resulted in pharmacologic defibrillation during CA; PNA-T in lowest NDS (figure 2) and HDS (figure 3).

	Control (n=5)	Albumin (n=6)	Unisol (n=5)	PNA-T (n=5)
OPC 5 (brain death)			●	
OPC 4 (coma)		●●	●	
OPC 3 (severe disability)	●●●●●	●●●●	●●●	●●●●●
OPC 2 (moderate disability)				
OPC 1 (normal)				

Figure 1. Overall performance categories (OPC) at 72 h after resuscitation, p=0.4.

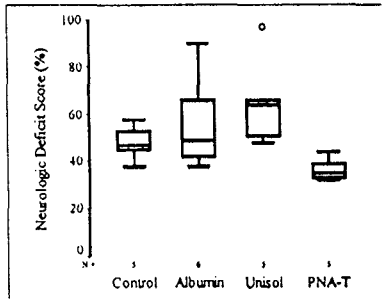

Figure 2. Neurologic Deficit Scores at 72 h after resuscitation, p=0.01 (post hoc: p<0.05 Unisol vs PNA-T)

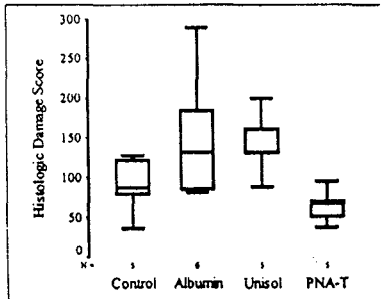

Figure 3. Total Brain Histologic Damage Scores at 72 h after resuscitation, p=0.02 (post hoc: p<0.05 Unisol vs PNA-T).

Conclusion:

Three aortic flush solutions, physiologically more rational than saline, do not give a breakthrough effect at moderate hypothermia, but Unisol might add benefit for the heart and PNA-Tempol for the brain.

(Supported by U.S. Department of Defense)

INTACT SURVIVAL OF 120 MIN CARDIAC ARREST AT 10°C IN DOGS. CEREBRAL PRESERVATION BY COLD AORTIC FLUSH

Wilhelm Behringer, Peter Safar, Ala Nozari, Xianren Wu, Rainer Kentner, Samuel A Tisherman, Ann Radovsky, SCRR, Univ. of Pittsburgh, Pittsburgh, PA; Michael Taylor, Organ Recovery Systems Inc, Charleston, NC

Introduction: In exsanguination cardiac arrest (CA), conventional resuscitation attempts are futile. Therefore we introduced the use of cold aortic saline flush at start of CA to rapidly induce preservative hypothermia during prolonged CA for "suspended animation for delayed resuscitation". In previous dog studies, saline flush to tympanic temperature (Tty) 10°C resulted in normal survival after CA 90 min, but not consistently after CA 120 min. This study was to find an optimized aortic flush, to consistently achieve normal outcome after CA 120 min. Methods: Male dogs (20–26 kg) were exsanguinated over 5 min to CA of 120 min no-flow. At CA 2 min, the dogs received an aortic cold flush, using a balloon-tipped catheter inserted via the femoral artery into the thoracic aorta, until Tty reached 10°C; then flush was continued from the femoral artery, until rectal temperature reached 20°C. Resuscitation was by closed-chest cardiopulmonary bypass (CPB), followed by assisted circulation for 2 h, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to 72 h, when outcome was evaluated as overall performance category (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=brain death); neurologic deficit score (NDS 0–10%=normal, 100%=brain death); and total brain histologic damage scores (HDS 0=normal, >40=severe damage, >100=extensive damage). The controls' flush was with saline at 2°C, 1 L/min; optimized flush was with Normosol at 2°C, 2 L/min from the femoral artery plus Unisol-UHK (organ-preservation solution) plus tempol (antioxidant) at the end of the flush; CPB was primed with Unisol-E (without K) instead of dextran/Ringers. Results: In the historic (plus concurrent) saline control group (n=6), OPC 1 was achieved in 2 dogs, OPC 2 in 1 dog, OPC 3 in 1 dog, and OPC 4 in 2 dogs; in the optimized flush group (n=6), OPC 1 was achieved in 5 dogs, and OPC 2 in 1 dog (p=0.06). Median (range) NDS was 26% (0–91) vs 1% (0–9) (p=0.09). HDS was 21 (10–172) vs 38 (12–98) (p=0.7). **Conclusion: An optimized single large volume cold aortic flush at start of CA can achieve normal survival with minor histologic damage after a no-flow time of 120 min. A field method for rapid vessel access and fluid cooling should be developed.** [Supp. by US Dept. of Defense].

INTACT SURVIVAL OF 120 MIN CARDIAC ARREST AT 10°C IN DOGS. CEREBRAL PRESERVATION BY COLD AORTIC FLUSH.

Wilhelm Behringer, Peter Safar, Ala Nozari, Xianren Wu, Rainer Kentner, Samuel A. Tisherman, Ann Radovsky, *Michael Taylor

Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA 15260, USA
*Organ Recovery Systems Inc

Objectives:

In exsanguination cardiac arrest (CA), conventional resuscitation attempts are futile. Therefore we introduced the use of cold aortic saline flush at start of CA to rapidly induce preservative hypothermia during prolonged CA, for "suspended animation" for hemostasis followed by resuscitation. In previous dog studies, saline flush to tympanic temperature (Tty) 10°C resulted in normal survival after CA 90 min, but not consistently after CA 120 min. This study was to explore an optimized aortic flush, to consistently achieve normal outcome after CA 120 min.

Methods:

Male dogs (20-26 kg) were exsanguinated over 5 min to CA of 120 min no-flow. At CA 2 min, the dogs received an aortic cold saline flush at 2-4°C, using a balloon-tipped catheter inserted via the femoral artery into the thoracic aorta, until Tty reached 10°C; then flush was continued from the femoral artery, until rectal temperature reached 20°C. The optimized flush was first with Normosol 650 mL/kg at 2-4°C, 2 L/min, to Tty 10°C , then at the end of the flush Unisol-I (with K^+) 50 mL/kg (organ-preservation solution) plus tempol 300 mg/kg (antioxidant). Resuscitation was by closed-chest cardiopulmonary bypass (CPB), primed with dextran/ringers in the saline flush group, and with Unisol-E (without K^+) in the optimized flush group. Then we used assisted circulation to 2 h, mild hypothermia to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated as overall performance category (OPC 1=normal, 2=moderate disability, 3=severe disability, 4=coma, 5=brain death); neurologic deficit score (NDS 0-10%=normal, 100%=death); and brain histologic damage scores (Total HDS 0=normal, >40=severe damage, >100=extensive damage).

Results:

Flush volume was 14.6 ± 1.7 L in the historic (plus concurrent) saline flush group, and 15.5 ± 4.0 L in the optimized flush group (p = 0.9). Lowest Tty during flush was 6.7 ± 0.6°C in the saline flush group vs 8.4 ± 0.5°C in the optimized flush group (p = 0.004) (figure 1). OPC ranged from normal to coma in the saline flush group, while in the optimized flush group 5/6 dogs achieved normality (OPC=1, figure 2). NDS and HDS varied greatly in the saline flush group compared to the optimized flush group, without a statistically significant difference (figures 3 and 4).

	Saline flush (n=6)	Optimized flush (n=6)
OPC 5 (brain death)		
OPC 4 (coma)	••	
OPC 3 (severe disability)	•	
OPC 2 (moderate disability)	•	•
OPC 1 (normal)	••	•••••

Figure 2. Overall performance categories (OPC) at 72 h after resuscitation, p=0.06.

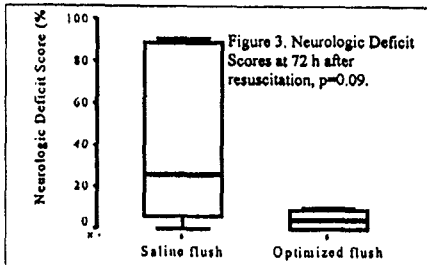

Figure 3. Neurologic Deficit Scores at 72 h after resuscitation, p=0.09.

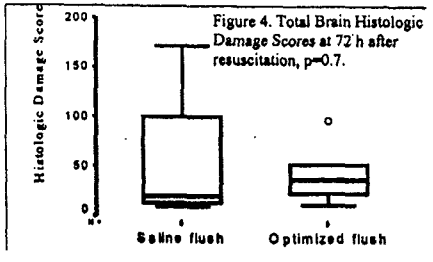

Figure 4. Total Brain Histologic Damage Scores at 72 h after resuscitation, p=0.7.

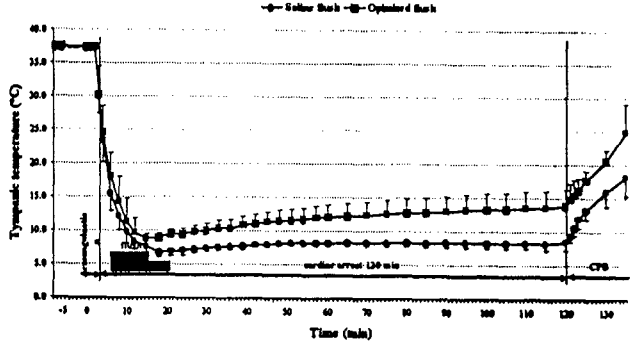

Figure 1. Tympanic membrane temperature (Tty) during exsanguination cardiac arrest (CA) of 120 min. Aortic flush was in the saline flush group via a balloon catheter with 1000 mL/min first into the thoracic aorta until Tty 10°C, continued into the femoral artery until Trectal 20°C and in the optimized flush group via cannula with 2000 mL/min into the femoral artery. The dog's head was put into ice-water during no-flow in the saline flush group starting 5 min after begin of CA. Area under the temperature curve during no-flow, p=0.002. Optimized flush gave better outcome although Tty was higher.

Conclusion:

An optimized single large volume cold aortic flush at start of CA can achieve normal survival with minor histologic damage after a no-flow time of 120 min. A field method for rapid vessel access and fluid cooling should be developed.

(Support by U.S. Department of Defense)

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ASA 2002 Annual Meeting

Filename: 651481
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Abstract Category: CRITICAL CARE - Life Support and Trauma
Journal Symposium: Yes
Disclosure: **A.** Agree **B.** Not Applicable **C.** Yes **D.** Agree **E.** Yes **F.** Yes
Study Supported By:
U.S. Department of Defence
G. No **H.** Yes

Title: Survival without brain damage with Suspended Animation after traumatic exsanguination cardiac arrest of 60 min in dogs.

Ala Nozari, M.D., Ph.D., Samuel Tisherman, M.D., Peter Safar, M.D., Xianren Wu, M.D. and S William Stezoski. [1]Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA.

Resuscitation attempts after traumatic exsanguination cardiac arrest (Exs-CA no-flow) rarely succeed. In dogs with non-traumatic Exs-CA of 90 min we achieved intact survival by aortic cold flush at CA 2 min to tympanic temperature (Tty) <10°C. In the present study, we explore the hypothesis that additional trauma would worsen the chance of intact survival.

Using 16 pilot experiments we defined the trauma, hemorrhage and flush. The definitive study was with traumatic Exs-CA 60 min. Fourteen male dogs were randomized into a control group without trauma (n=6) and a trauma group (n=8) which received at start of CA standardized laparotomy, spleen transection, and thoracotomy; and during CA splenectomy. In both groups, starting at CA 2 min, flush of saline at 2°C into the femoral artery was initiated and continued until Tty of 10°C. Restoration of spontaneous circulation and assisted circulation were with cardiopulmonary bypass (CPB) to 2 h (with heparin bonded system), and mild hypothermia (Tty 34°C) to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated as overall performance categories (OPC 1 = normal, 2 = moderate disability, 3 = severe disability, 4 = coma, 5 = death); neurologic deficit scores (NDS 0-10% = normal, 100% = brain death); and 72 h perfusion fixation, necropsy, and determination of total and regional brain histologic damage scores (HDS). Hematocrit was kept >25, if needed with donor blood.

All 14 dogs survived to 72 h. The 6 non-trauma control experiments resulted in prompt resuscitation and intact survival (OPC 1), NDS 1% (range 0-13%) and total HDS 11 (4-22). In 3/8 trauma dogs controlled ventilation was needed beyond 20 h because of airway edema, hypoventilation, cardiovascular complications, renal failure and neurologic deficit. 4/8 trauma dogs achieved final OPC 1, one OPC 2, one OPC 3, and two OPC 4; NDS was 13% (0-87). Blood loss in the trauma group ranged widely (up to 1300 mls) and was associated with poor outcome.

Coagulation studies revealed in both groups, after resuscitation, transient initial hypocoagulation with coagulation factors consumption, and fibrinolysis activation. This was followed by delayed hypercoagulation. There was no evidence of sustained DIC. Platelet count decreased to 50% baseline at 1 h after resuscitation, without normalization by 24 h. Plasma concentrations of plasminogen activator inhibitor peaked at 6-9 h after the insult. All changes occurred in both groups, but were numerically worse in the trauma group.

We conclude that rapid induction of profound hypothermia (Tty 10°C) (Suspended Animation) can enable survival without brain damage after Exs-CA of 60 min no flow even in the presence of trauma, although with worse exracerebral organ failure. Coagulopathy and possibly a thrombotic microangiopathy, as a result of ischemia, CPB, hemodilution and hypothermia, appear worsened by trauma.

Summary: In an experimental model of exsanguination cardiac arrest with or without tissue trauma dogs were resuscitated with suspended animation and survived 60 min of circulatory arrest without brain damage. Extracerebral organ complications and thrombotic microangiopathy were observed specially when trauma was added to this model.

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The work embodied in this abstract has not all ready been published, and it conforms to the ethical standards, duality of interest, and other requirements as listed in the associated instructions.

Signature of Contact Author:

Ala Nozari

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Submitted Abstract

on August 30, 01:55PM

for SCCM

CONTROL ID: 23256

CONTACT (NAME ONLY): Ala Nozari

Abstract Details

PRESENTATION TYPE: No preference

CATEGORY: Basic Science

SUBCATEGORY: CPR

KEYWORDS: coagulopathy; MODS; traumatic arrest;

AWARDS: Annual Scientific Awards, Specialty Section Awards, Educational Scholarships, Research Citations

Abstract

TITLE:

COAGULOPATHY AND MULTIPLE ORGAN FAILURE AFTER TRAUMATIC EXSANGUINATION CARDIAC ARREST (CA) OF 60 MIN IN DOGS

AUTHORS (ALL): Nozari, A [1]; Bontempo, F [1]; Safar, P [1]; Wu, X [1]; Stezoski, SW [1]; Tisherman, S [1]

INSTITUTIONS (ALL): 1. Safar Center for Resuscitation Research, Univ Pittsburgh, Pittsburgh, PA, USA;

ABSTRACT BODY:

Introduction: We could reverse CA of 120 min no flow at tympanic temperature (Tty) 10°C. Added tissue trauma caused coagulopathy and prevented survival.

Hypothesis / Methods: Fourteen dogs were exsanguinated over 5 min to CA and then randomized into a control group without trauma (n=6) and a trauma group (n=8) which received at start of CA standardized tissue trauma. In both groups, starting at CA 2 min, centripetal flush of isotonic saline at 2°C into the distal aorta was to Tty 10°C; after 60 min CA, reperfusion was with cardiopulmonary bypass with heparin-bonded system. Intensive care was to 72h. Hematocrit was normalized in both groups with initially shed blood. In the trauma group fresh donor blood was added.

Results: All non-trauma dogs survived without neurologic deficits or extracerebral organ complications. In 3 trauma dogs, cardiovascular- pulmonary complications and renal failure occurred. Blood loss in the trauma group was 0-1300 ml and associated with poor outcome. At 1h of recirculation, TEG indicated in both groups severe hypocoagulation with narrowed alpha angle (α), prolonged reaction time (r) and reduced maximum amplitude (MA). PT and PTT were prolonged and factors II, V, VIII and fibrinogen levels reduced. AT-III levels were reduced and remained so until 24 h in the control group and until 72h in the trauma group. Platelet levels were 50% baseline at 1 h and did not normalize. PAI increased 6x at 6-9 h, with higher levels in the trauma group. It gradually decreased thereafter and was followed by a delayed hypercoagulation toward 72h, with wide α, short r and high MA in the TEG curves. At 72h, PT, PTT and clotting factors had normalized, but plasmin, antiplasmin and fibrinogen were increased.

Conclusions: CA 60 min no flow at 10°C is resuscitable, but represents challenges with complex coagulopathy, which is worse with than without trauma. The derangements suggest a thrombotic microangiopathy.

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ASA 2002 Annual Meeting

Filename: 651557

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Member ID: Aff Member-pend

Abstract Category: CRITICAL CARE - Life Support and Trauma

Journal Symposium: Yes

Disclosure: **A.** Agree **B.** Not Applicable **C.** Yes **D.** Agree **E.** Yes **F.** Yes

Study Supported By:
U.S. Department of Defence

G. No **H.** Yes

Title: Hypothermia induced during cardiopulmonary resuscitation increases intact survival after prolonged normovolemic cardiac arrest in dogs.

Ala Nozari, M.D., Ph.D., Peter Safar, M.D., Samuel Tisherman, M.D., Xianren Wu, M.D. and S William Stezoski. [1]Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA, United States.

Studies by us and others have documented improved cerebral outcome with mild hypothermia (34°C) induced after cardiac arrest (CA) and restoration of spontaneous circulation (ROSC). We hypothesized that in a simulated unresuscitable CA dog model, intact survival can be achieved if hypothermia is induced during prolonged cardiopulmonary resuscitation (CPR) steps A-B-C.

Twelve dogs (20-25 kg) were subjected to 3 min of CA no-flow with ventricular fibrillation (VF), followed by 7 min CPR Basic Life Support and 30 min of unsuccessful CPR Advanced Life Support (ALS) with DC countershocks, FiO_2 1.0 and epinephrine boluses. Dogs were randomly allocated into two treatment groups: a control group with normothermic VF (n=6, tympanic temperature [Tty] 37.5°C throughout) and a hypothermia group (n=6) which received at VF 20 min a venous flush of 20 ml/kg normal saline at 2°C followed by veno-venous extra-corporeal blood cooling (cetheters in superior and inferior vena cava) until cardiopulmonary bypass (CPB) was initiated at VF 40 min. ROSC and assisted circulation were with CPB to 4 h and then mild hypothermia (Tty 34°C) to 12 h, controlled ventilation to 48 h, and intensive care to 96 h. Outcome was evaluated as overall performance categories (OPC 1 = normal, 2 = moderate disability, 3 = severe disability, 4 = coma, 5 = death); neurologic deficit scores (NDS 0-10% = normal, 100% = brain death); and 96 h perfusion fixation, necropsy, and determination of total and regional brain histologic damage scores (HDS).

Lowest Tty in the hypothermia group was 27°C (range 26-28°C). ROSC was achieved in all 12 dogs. In the control group, 1 dog survived to 96 h but remained comatose (OPC 4); and 5 dogs died during the intensive care period, the majority within 24 h, because of malignant arrhythmias and respiratory failure or vasopressor resistant shock; "best" NDS was 92% (range 92 - 98%). In the hypothermia group, 5 of the 6 dogs survived to 96 h with good neurologic outcome - OPC 1 (P=0.025) and NDS 0% (0-7%). HDS results are pending. In the control group there were renal failure and intestinal mucosal necrosis, severe subendocardial and epicardial hemorrhagic infarctions, and pulmonary infarctions. In the hypothermia group morphologic changes were absent or minimal (one with bilateral hemorrhagic pulmonary consolidations, 2 with mild subendocardial hemorrhage).

In conclusion, cooling during CPR attempts in prolonged normovolemic and presently unresuscitable cardiac arrest, as a bridge to prolonged CPB, results in survival with full neurologic recovery. A portable device for veno-venous cooling should be developed.

Summary: In a clinically relevant dog model of normovolemic ventricular fibrillation (VF) cardiac arrest, survival and outcome were evaluated after hypothermic vs. normothermic closed-chest cardiopulmonary resuscitation (CPR). Hypothermia was by cold saline flush and veno-venous blood cooling during CPR as a bridge to cardiopulmonary bypass. Survival with full neurologic recovery was achieved after hypothermic CPR of 20 min whereas normothermic CPR led to coma and deaths within 24 h.

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The work embodied in this abstract has not all ready been published, and it conforms to the ethical standards, duality of interest, and other requirements as listed in the associated instructions.

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Ala Nozari

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Submitted Abstract

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CONTROL ID: 23326

CONTACT (NAME ONLY): Ala Nozari

Abstract Details

PRESENTATION TYPE: No preference

CATEGORY: Basic Science

SUBCATEGORY: CPR

KEYWORDS: cardiopulmonary resuscitation; hypothermia; survival;

AWARDS: Research Citations, Annual Scientific Awards, Specialty Section Awards

Abstract

TITLE:
INTACT SURVIVAL IN DOGS AFTER CARDIAC ARREST (CA) OF 40 MIN WITH MILD HYPOTHERMIA (34C) DURING CLOSED CHEST CPR: MYOCARDIAL AND CEREBRAL PRESERVATION

AUTHORS (ALL): Nozari, A [1]; Safar, P [1]; Wu, X [1]; Stezoski, SW [1]; Tisherman, S [1]

INSTITUTIONS (ALL): 1. Safar Center for Resuscitation Research, Univ Pittsburgh, Pittsburgh, PA, USA;

ABSTRACT BODY:

Introduction: Mild hypothermia (34°C) *after* normothermic CA improves cerebral outcome. We hypothesized that mild or moderate hypothermia (30°C) *during* prolonged closed chest CPR steps A-B-C would further improve outcome.

Hypothesis / Methods: Twenty-four dogs were subjected to VF, normothermic no flow of 3 min, BLS of 7 min, and ALS for unsuccessful ROSC attempts of 10 min. They were then randomized to 4 groups: 1 (n=7) continued normothermic ALS; 2 (n=6) hypothermic flush (20 ml/kg normal saline IV at 2°C) and veno-venous extracorporeal shunt cooling to tympanic temperature (Tty) 26-28°C; 3 (n=6) same as group 2 but veno-venous shunt to Tty 34°C or 4 (n=5) normothermic flush and veno-venous shunt. After VF 40 min, reperfusion was with cardiopulmonary bypass. Intensive care was to 96 h. Outcome was evaluated as overall performance categories (OPC 1=normal, 5=death); neurologic deficit scores (NDS 0-10%=normal, 100%=brain death); and 96 h perfusion fixation, and determination of total and regional brain histologic damage scores (HDS). Groups 1 and 2 were presented before, group 3 and 4 are new.

Results: Of the normothermic CPR dogs, all in group 4 and all but one in group 1 (which remained comatose) died within 58 h, because of malignant arrhythmias and respiratory failure or vasopressor resistant shock. In groups 2 and 3 all survived to 96 h (Table); morphologic changes were absent or minimal.

Conclusions: Mild or moderate hypothermia *during* prolonged closed-chest CPR preserves viability of organs, without risk of complications, and improves outcome.

	Gr 1: 37.5°C	Gr 2: 27°C	Gr 3: 34°C	Gr 4: 37.5°C
OPC	6/7 died at 4-58 h 1/7 OPC 4	1,1,1,1,1,4	1,1,1,2,2,2	Died < 24h
NDS	6/7 died 1/7 NDS 92	1 (0-92)	1 (0-11)	Died < 24h
HDS	0, 26, 78	1 (0-66)	1 (0-4)	46

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