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Award Number: W81XWH-12-1-0599

TITLE: Low Volume Resuscitation with Cell Impermeants

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REPORT DATE: October 2014

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE	*Form Approved* *OMB No. 0704-0188*

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 14	Annual Report	30 Sep 2013 - 29 Sep 2014

4. TITLE AND SUBTITLE

Low Volume Resuscitation with Cell Impermeants

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0599

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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Richmond, VA 23298

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. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

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

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Circulatory shock induces the loss of energy dependent volume control mechanisms, which cause lethal cell swelling, compression of capillaries by swollen tissues, and impairment of the microcirculation. The objective of the study was to increase the tolerance to the low flow state by passively moving water from the cell back into the microcirculation by loading patients with cell impermeant and oncotic agents, which osmotically pull water from the cell and interstitial space, respectively. Rats were hemorrhaged to a plasma lactate of 10 mM, given a low volume resuscitation (LVR) with saline (control) or various cell impermeants and colloids. When lactate again reached 10 mM following LVR, full resuscitation was started with crystalloid and red cells. Capillary blood flow was measured by the colored microsphere technique. Impermeants dramatically improved LVR outcomes in shocked rats. Small cell impermeants and PEG-20k in LVR solutions increased tolerance to the low flow state by 2 and 5 fold, respectively, normalized arterial pressure during LVR, and increased survival (100% Vs 0%) This was accompanied by higher capillary blood flow. Conclusions: Ischemia-induced lethal cell swelling during hemorrhagic shock is a key mediator of resuscitation injury, which can be prevented by cell impermeants in LVR solutions.

15. SUBJECT TERMS
Cell Impermeants, gluconate, ischemia, resuscitation, low volume resuscitation, PEG-20k

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	18	

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INTRODUCTION: The direct and indirect effects of severe and prolonged tissue hypoxia due to hemorrhagic shock are the leading cause of death for battlefield injuries (1,2). Resuscitation in the field is often seriously inadequate even if the patient makes it to the local field hospital for full resuscitation. The delay to evacuation in far forward units could take many hours to days so time becomes critical and maintaining patients in a low volume status for long periods is a real possibility (3). <u>Work done in this proposal will significantly increase this safe time period.</u> Past resuscitative attempts have focused on maintaining tissue perfusion and oxygen delivery to rebalance the oxygen supply-to-demand ratio by administration of large volumes of crystalloid. However, it is now recognized that controlled resuscitation using lower volumes is effective, more economical, and essential for battlefield operations (4). The objective should be to restore as much oxygen delivery in the lowest volume as possible only to patients that will benefit from the therapy. This approach can be improved by using agents in the low volume resuscitation solution that preserve tissue perfusion. One significant impediment to tissue perfusion during low pressure states is no reflow in the microcirculation caused by cell swelling induced by prolonged cellular ischemia. Endothelial cells in the capillaries swell during ischemia and shut off flow through already narrow capillary corridors (5,6). Parenchymal cell swelling further compromises the microcirculation by compressing the capillary from the outside. <u>The significance of this proposal is that it uses proven and tested technology and solutions developed for organ preservation for transplantation that resolve ischemia-induced cell swelling and applies it to shock and resuscitation injury</u>. Specifically, the use of simple cell impermeants in low volume resuscitation solutions will significantly attenuate cell swelling, capillary no reflow, and preserve DVO_2 to critical tissues both before and after full resuscitation. This lessens end organ failure and improves survival since distribution of the limited oxygen availability is enhanced. Additionally, cell swelling per se is lethal to tissues (7,8) so reversal with impermeants is salutary. A further enhancement of this approach is to combine the use of cell impermeants, which preferentially load into the interstitial space, with an oncotic agent, which stays in the capillary space. This creates a double osmotic gradient to first pull water from the cell (where it has the propensity to go during ischemia) with cell impermeants and then pull water from the interstitial space into the capillary where it belongs, with a colloid like PEG-20k. Thus, attacking one of the root causes of severe resuscitation injury (cell swelling) using proven methods should have a cascading effect that improves the survival of military personnel from severe hemorrhagic shock and resuscitation injury. These concepts and products will also be useful in civilian treatment of shock. <u>The significance of this approach, beyond the obvious medical benefits to humans, is the simplicity of the concept, its proven track record of use in organ transplantation, and the extreme stability of the active components (cell impermeants)</u>. The diagram below (**Figure 1**) summarizes the biological problem and the solution.

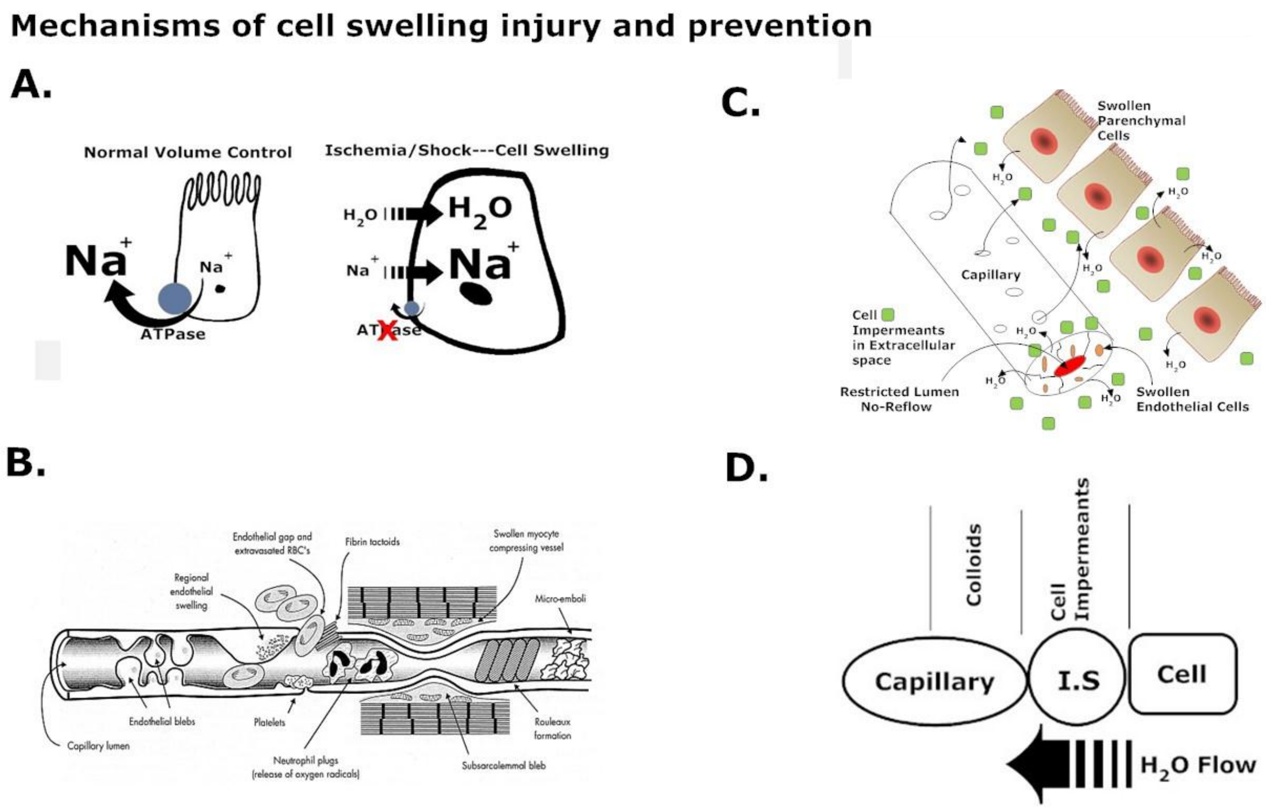


FIG 1. Cells swell as the sodium pump fails from lack of ATP during low flow states. Sodium enters the cell passively and water follows (A). Swollen parenchymal and endothelial cells than impair capillary flow by compression causing exacerbations in local perfusion during shock (B). Cell impermeants loaded into the interstitial space non-energetically and biophysically prevent water movement into the cell (C) and a dual osmotic gradient is created when cell impermeants are combined with oncotic agents to impel cell water movement out of the cell and into the capillary space where it belongs (D). This reduces lethal cell swelling and capillary compression AND increases capillary fluid volume, capillary pressure, and microcirculatory flow, which serves to restores microcirculatory oxygen delivery in the low flow state. This increases the tolerance to the low flow state when given in the low volume resuscitation solution and increases the “Golden Hour” on the battlefield. The time that a patient can safely stay in the low volume state until definitive medical care is needed is greatly expanded using this approach.

BODY:

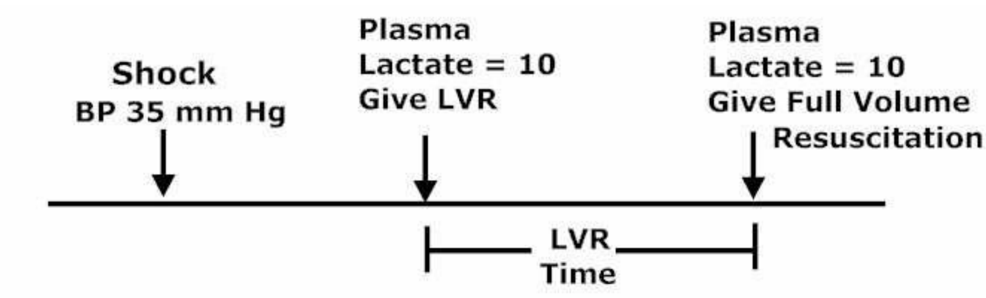
The objectives and specific tasks for the second year were to;

1. Identify the effects of adding novel oncotic agents WITH cell impermeants to further facilitate favorable movements of water during the low flow state in the LVR hemorrhage rodent model.
2. Define how this optimized LVR solution compares to current "State-of-Art" crystalloid solutions used for shock on the field and to define how these work (Mechanisms).

The following experiments and their results were used to address those objectives and tasks.

I. **Impermeant / Colloid Studies in Acute Rodent Shock Models**:

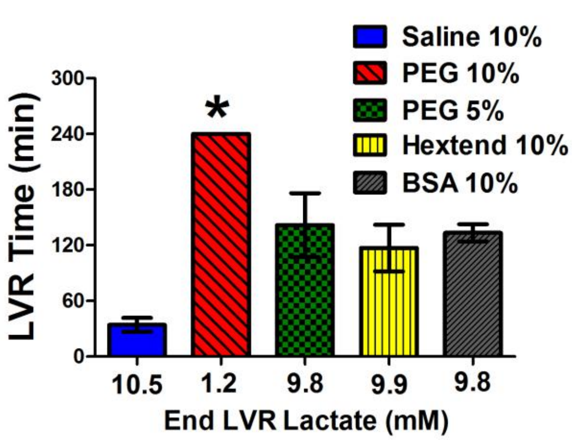
The response of impermeants in the rodent low volume resuscitation model has been well established in the prior year. Small molecule cell impermeants like gluconate, raffinose, and trehalose significantly increase the tolerance of the shocked patient to the low volume state as indexed by a doubling of the low volume resuscitation (LVR) time. The LVR time is the time from the start of the low volume resuscitation until the start of full resuscitation as shown in the **Figure 2**. This time represents the "golden Hour" or the time that a shocked victim can safely remain in the low volume state until full resuscitation and definitive medical care is necessary. While this time doubled with impermeants in the LVR solutions, it increased 6 fold when PEG-20k was added.



These data and studies are reported in extensive detail in the appended paper in Annals of Surgery (9).

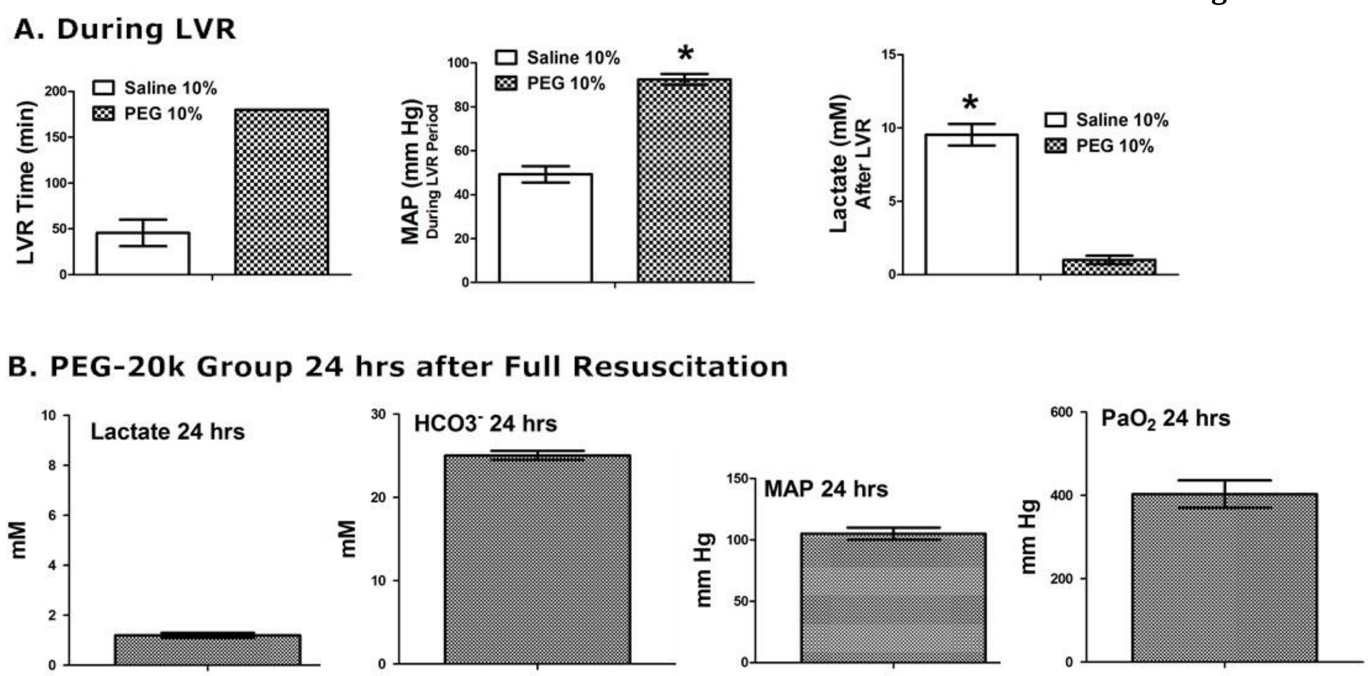
Parrish D, Lindell S, Reichstetter H, Aboutanos M, Mangino MJ. *Cell impermeant based low volume resuscitation in hemorrhagic shock: A biological basis for injury involving cell swelling*. **Ann.Surg. 2014 (in press).**

II. **Comparisons with "Other" Solutions**: The optimized solution developed for this project, which is based on an impermeant platform, has demonstrated that cell swelling is an important causal mechanism because using these agents that are known to reduce or prevent cell swelling greatly improves LVR times and outcomes in our shock models, compared to saline controls. However, since saline is not the treatment of choice anymore for shocked patients in the pre-hospital setting (10-12), we tested the impermeant based LVR solution against Hextend (13). In a further attempt to dissect out the pure colloid effects of PEG-20k in our studies from other effects, we also compared the PEG-20k effects with albumin, which is a pure oncotic agent. The responses of these solutions in our rodent LVR model are shown in **Figure 3**. Five groups of rats were shocked as in Figure 2 and the LVR time was assessed as the primary end point, which is an estimation of low volume tolerance or the "Golden Hour". In this particular trial, Polyethylene Glycol-20,000 (PEG-20k) used alone increased LVR time 8 fold more than the saline control and was at least twice as effective as Hextend or albumin, suggesting that PEG-20k probably is acting by mechanisms other than as an oncotic agent. In fact, the PEG 10% group LVR time of 240 minutes was arbitrarily determined and cut off before the lactate trigger



reached 10 mM. The plasma lactate in those animals that remained in the low volume state was only 1.2 mM after 240 min. In other words, had we waited until the lactate climbed to 10 mM like in the other groups, the LVR time would have been significantly longer than 240 minutes. So it is clear that PEG-20k alone is much more effective than any other solution available for low volume resuscitation in hemorrhagic shock states. It is also clear that PEG-20k, a putative oncotic agent (14,15), is as effective by itself as it is when combined with cell impermeants such as gluconate (9).

III. **Early Survival Data:** To accomplish scientific objectives with speed and efficiency, our models have used acute non-survival shock and low volume resuscitation as shown in figure 2. However, now that we have developed an optimized formulation of LVR solution using the impermeant platform, we need to determine if it works by increasing patient survival and preventing secondary illness after full resuscitation. In an early test group, three rats were given 10% PEG-20k at a volume of 10% of the calculated blood volume (equivalent to about 500 ml for an adult patient), and recovered over night after full resuscitation. The animals were allowed to stay in the low volume state for 180 minutes, which is longer than any subjects have survived using saline, albumin, or Hextend, and then recovered from anesthesia after removing the vascular catheters and closing all surgical wounds. The following day, all of the treated animals were alive, awake, ambulatory, and well. All of the saline control animals died in the low volume resuscitation period because they never made it to 180 minutes. Their lactates climbed well above 10 and they died of hypovolemic shock. In the survivors that received PEG-20k, we also measured hemodynamic and metabolic function 24 hours after recovering from a severe hemorrhage of 55% blood volume loss and low volume resuscitation. These data are summarized below in **Figure 4**.



The LVR period data are shown in panel A and the results from the following day (PEG-20k treated only) are shown in panel B. The LVR time for the control group only reached about 50 minutes and then the animals died while the PEG group easily went the full 180 minutes with an accumulated lactate of only 1.2 mM, indicating excellent microcirculatory function even in the low volume state. This is likely due to low resistance to flow in the peripheral capillaries due to prevention of cell swelling induced microcirculatory compression with PEG-20k. The blood pressure was normal in the PEG group during the LVR period where the animals had lost 55-60% of their total circulating blood volume. This compares to the severe hypotensive state of the controls where MAP was only around 50 mmHg during the LVR period. This is likely due to passive water movement from the interstitial space into the capillary space by the oncotic pressure produced by the PEG-20k molecule inside the capillary during hypovolemic shock. All animals treated with LVR solutions containing 10% PEG-20k survived the next day with normal lab values (Panel B). So, PEG-20k based low volume resuscitation solutions increase survival to 100% after very severe hemorrhagic shock. The LVR time chosen for this trial (180 min) is much lower than the capacity of the solution, which still remains unknown and untested. We have taken a few animals past 6 hours of LVR time with survival. These long times produce their own effects on the animals independent of shock state by virtue of prolonged time under general anesthesia.

IV. Mechanisms of PEG-20k: The Molecular Hybrid Model: The operational hypothesis of this last year's work was that the addition of an oncotic agent to LVR solutions already containing cell impermeants would produce additive effects on the movement of water. We discovered that the LVR times doubled with cell impermeants (like gluconate) but increased 6-8 fold when the oncotic agent PEG-20k was added. So, the effect was geometrically additive and not linearly. In testing just the PEG-20k component alone, we discovered that it had the same effect as both combined. Further experiments revealed that pure oncotic agents like albumin were not as protective as PEG-20k. So we concluded that the striking effects of PEG-20k are not attributable solely to an oncotic action alone and that there must be something else going on with this molecule. Another observation using PEG-20k was that it caused a diuresis after administration, even in shock!. These combined observations lead us to hypothesize that PEG-20-k, by nature of its unique molecular weight, may be acting as a hybrid molecule and assuming both oncotic and impermeant roles. Our data suggests that some of the PEG-20k may escape the capillary where it loads into the interstitial space (because it is impermeant to cells) and prevents cell swelling as an impermeant but some of the material stays in the capillary where it acts as an oncotic agent. This would explain our observations and suggests that impermeant solutions need only contain PEG-20k alone without specific cell impermeants like gluconate. To test this hypothesis, we measured the oncotic reflection coefficient (σ_d) for PEG-20k in normal rats. If a molecule has a reflection coefficient of 1.0, it is all reflected by the capillary wall and none escapes into the interstitial space or gets into the lymphatic system. If it has a reflection coefficient of 0, all of it escapes the capillary wall and gains access to the interstitial space and lymphatics. To measure σ_d for PEG-20k, we cannulated the thoracic duct to collect lymph flow and then volume loaded the rat with continuous infusions of saline to increase lymph flow rate. We then administered a bolus of FITC-labelled PEG-20k and tracked the accumulation of the fluorescent label in both the plasma and lymphatic compartments. The relationship of 1-[L]/[P] of the tracer at high lymphatic flow rates is assumed to estimate the oncotic reflection coefficient (16). A representative data set plotted is shown in **Figure 5** below. Not only does the PEG-20k appear quickly in the lymph, but it has a reflection coefficient of about 0.65, which means a large portion passes into the interstitium and a large portion stays in the capillary space where it acts oncotically. Therefore, these data support a compelling argument that PEG-20k is in fact a unique hybrid molecule, which explains its biological effects in shock.

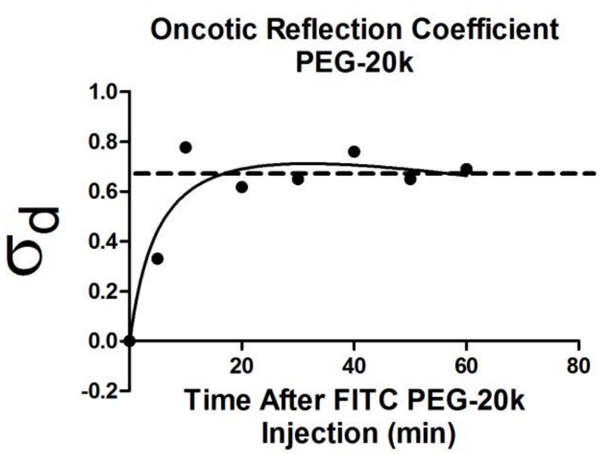


Figure 5. Oncotic reflection coefficients for PEG-20k in the rat microcirculation estimated by 1- lymph to plasma ration of FITC activity after I.V. injection of FITC-labelled PEG-20k tracer compound. The estimated σ_d is what would be expected for a molecule with hybrid oncotic-impermeant characteristics.

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KEY RESEARCH ACCOMPLISHEMENTS:

The following significant research accomplishments include:

1. Determine the effects of oncotic and impermeant agents together to increase the tolerance to the low volume state.
2. Detected a novel property of PEG-20k as a hybrid impermeant and oncotic agent with the ability to expand the golden hour up to 8 times the value observed with conventional low volume resuscitation crystalloids (saline).
3. Compared PEG-20k effects with conventional state-of-art crystalloids (Hextend and albumin).
4. Provided empirical evidence of the hybrid nature of PEG-20k in the microcirculation as indexed by the capillary oncotic reflection coefficient.
5. Demonstrated that the acute salutary effects of impermeant LVR solutions containing PEG-20k are also translatable to survival 24 hours after severe hemorrhagic shock and resuscitation.
6. By default, determined the preliminary safety profile of LVR solutions containing 10% PEG-20k.

REPORTABLE OUTCOMES:

1. Publication of the results in Annals of Surgery in 2014 (in press). This publication is included in the Appendix.
2. Presentation of results at the upcoming EAST conference (Eastern Association of the Surgery for Trauma) in Orlando FL.
3. Publication of the second round of findings in J. Trauma and Acuter Care Surgery, which will be published after the EAST meeting.
4. Formed a new company with a business partner in Washington DC to commercialize an impermeant based platform of low volume resuscitation solutions and similar solutions for other medical uses.
5. Informed FDA of a future Pre-IND meeting to discuss a regulatory pathway for the development of the new impermeant based LVR solution for military and civilian combat casualty care use in pre-hospital and hospital settings

CONCLUSIONS:

1. Cell swelling is a significant causal mediator of resuscitation injury after severe hemorrhagic shock.
2. Cell impermeants based LVR solutions containing PEG-20k increase tolerance to the low flow state (golden hour) 6-8 fold more than saline.
3. Cell impermeants based LVR solutions containing PEG-20k increase survival 100%, relative to saline.
4. Cell impermeants based LVR solutions containing PEG-20k increase the tolerance to the low flow state orders of magnitude more compared to commonly used crystalloid solutions such as albumin and Hextend.
5. PEG-20k can be effective alone in LVR solutions because it behaves as a hybrid molecule expressing both cell impermeant and colloid attributes together.
6. PEG-20k based cell impermeants appear safe as used in LVR solutions.
7. PEG-20k has an oncotic reflection coefficient of about 0.65 in the rodent systemic microcirculation.

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ORIGINAL ARTICLE

Cell Impermeant-Based Low-Volume Resuscitation in Hemorrhagic Shock

A Biological Basis for Injury Involving Cell Swelling

Dan Parrish, MD, Susanne L. Lindell, BSN,* Heather Reichstetter, LVT,* Michel Aboutanos, MD,* and Martin J. Mangino, PhD*†‡*

Objective: To determine the role of cell swelling in severe hemorrhagic shock and resuscitation injury.

Background: Circulatory shock induces the loss of energy-dependent volume control mechanisms. As water enters ischemic cells, they swell, die, and compress nearby vascular structures, which further aggravates ischemia by reducing local microcirculatory flow and oxygenation. Loading the interstitial space with cell impermeant molecules prevents water movement into the cell by passive biophysical osmotic effects, which prevents swelling injury and no-reflow.

Methods: Adult rats were hemorrhaged to a pressure of 30 to 35 mmHg, held there until the plasma lactate reached 10 mM, and given a low-volume resuscitation (LVR) (10%–20% blood volume) with saline or various cell impermeants (sorbitol, raffinose, trehalose, gluconate, and polyethylene glycol-20k (PEG-20k). When lactate again reached 10 mM after LVR, full resuscitation was started with crystalloid and red cells. One hour after full resuscitation, the rats were euthanized. Capillary blood flow was measured by the colored microsphere technique.

Results: Impermeants prevented ischemia-induced cell swelling in liver tissue and dramatically improved LVR outcomes in shocked rats. Small cell impermeants and PEG-20k in LVR solutions increased tolerance to the low flow state by two and fivefold, respectively, normalized arterial pressure during LVR, and lowered plasma lactate after full resuscitation, relative to saline. This was accompanied by higher capillary blood flow with cell impermeants.

Conclusions: Ischemia-induced lethal cell swelling during hemorrhagic shock is a key mediator of resuscitation injury, which can be prevented by cell impermeants in low-volume resuscitation solutions.

[AQ1]

Keywords: gluconate, osmotic effects, polyethylene glycol, resuscitation

(*Ann Surg* 2014;00:1–8)

Deaths due to injury in the United States reached more than 171,000 and costs more than $400 billion a year in health care costs and lost productivity in 2010.[1] Deaths from trauma are the number 1 cause of death for people younger than 44 years in the United States and the third leading cause of death overall for all age groups. Trauma accounts for about 30% of all life years lost in the United States, compared to cancer (16%), heart disease (12%), and human immunodeficiency virus (2%).[2] For all traumatic injuries, hemorrhagic shock is responsible for more than 35% of prehospital deaths and more than 40% of all deaths within the first 24 hours. This is second only to trauma deaths induced by severe CNS injury.[3] Finally, [AQ3] hemorrhagic hypotension exposes the patient to immediate complications of life-threatening infections, coagulopathies, and multiple organ failure.[4,5]

Early resuscitation strategies include the use of low volumes of intravenous blood products to increase oxygen delivery and to replace lost coagulation and clotting factors (coagulation proteins and platelets). Although this approach is fine for hospital emergency departments, it is not currently practical in prehospital settings where early intervention may be the key to preventing future complications following more definitive resuscitation. Crystalloids are available for prehospital use because they can be safely transported and stored but they are generally limited in their effectiveness. Attempts to modify basic intravenous crystalloids for prehospital resuscitation by adding hypertonic NaCl or starch (Hextend) as a volume expander have had disappointing results.[6,7] The future use of effective spray dried blood products will be a valuable tool in prehospital settings because they replace chemical coagulation precursors and factors. The use of fresh frozen plasma in the field, which is currently being tested at many centers, will also be useful but it too is limited by the need for refrigeration. There remains a need for a better crystalloid to resuscitate patients with severe hemorrhagic shock, especially in a prehospital setting. The successful design of such a solution is highly dependent on understanding the pathophysiological mechanisms that lead to injury during hemorrhagic hypotension and subsequent resuscitation. The optimal solution will likely be an effective new stable crystalloid that targets these mechanisms used together with reconstituted dried plasma products for the replacement and reconstitution of coagulation potential.

The predominant root mechanism of injury in hemorrhagic shock is energy failure. Although global ischemia and reperfusion injury are causally based at many levels, they all arise from changes that occur when the cell energetics drops because of a loss of adequate microvascular oxygen transport and subsequent loss of aerobically produced high-energy adenine nucleotides.[8–10] One mechanism of cell, tissue, and organ injury is cell swelling that occurs from the loss of ATP-dependent cell volume regulatory control mechanisms. In most cells, the single highest energy-consuming process is the running of the Na/K ATPase pumps in the cell membrane. These pumps actively transport sodium ions out of the cell to maintain membrane potentials and to run numerous Na^{+}-dependent facilitated membrane transport processes such as calcium, glucose, amino acids, and organic cation transporters. In the absence of ATP to run those pumps, as occurs in ischemia after hemorrhagic shock, the Na/K ATPase turns off and sodium enters the cell as it runs back down its electrochemical gradient. The elevated intracellular sodium futilely stimulates the sodium pump that cannot run because of loss of ATP.[11] Chloride then enters the cell down an electrical gradient and water

[AQ2]

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Disclosure: Supported by grants from the National Institutes of Health R01 DK087737 and the Department of Defense W81XWH-12-1-0599 to Dr Mangino. There were no conflicts of interest to declare.

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ISSN: 0003-4932/14/00000-0001
DOI: 10.1097/SLA.0000000000001049

follows the sodium chloride down a developing osmotic gradient, which causes the cell to swell. Hydropic degeneration from energy failure damages membrane and mitochondrial structures,[12] which may lead to cell death.

This basic mechanism of cell ischemic injury has been well described in organ preservation associated with transplantation.[13–15] Effective modern organ preservation solutions were developed around this concept and contain high concentrations of cell impermeants.[16] These are classes of nontoxic molecules, usually saccharides and small organic cations and anions, which are small enough to freely egress the capillary space in the microcirculation but are too large or too charged to cross the cell membrane. As such, they preferentially load into the interstitial space where they create an osmotic force that prevents the movement of water into the cell as the sodium concentrations rise during ischemia. They prevent lethal cell swelling. Cell impermeant, as a class of agents, are one of the most effective components of organ preservation solutions used today.[17] The University of Wisconsin solution contains high amounts of raffinose, lactobionic acid, sulfate, and phosphate, which all act as cell impermeants to prevent water movement. The Belzer-UW MPS solution uses gluconate and HTK solution uses both high concentrations of histidine and mannitol as impermeants. Histidine at physiological pH is charged and is an impermeant. Water movement in organ preservation is slower than ischemia at normal mammalian temperatures because hypothermia is used to preserve organs, which slows down the process. Because cell swelling during ischemia induced by hemorrhagic hypotension also occurs[18] and at a much faster rate than in organ preservation because of the warmer temperatures, it was hypothesized that loading the interstitial space with nontoxic cell impermeants during the low-volume period would prevent lethal cell swelling and increase the tolerance of the patient to the low-volume state and improve outcomes at resuscitation. Testing this was the objective of the study.

METHODS

All animal work was conducted under a protocol approved by the VCU Institutional Animal Care and Use Committee, which is governed by the rules and regulations set forth in the NIH guide and the USDA.

In Vitro Model

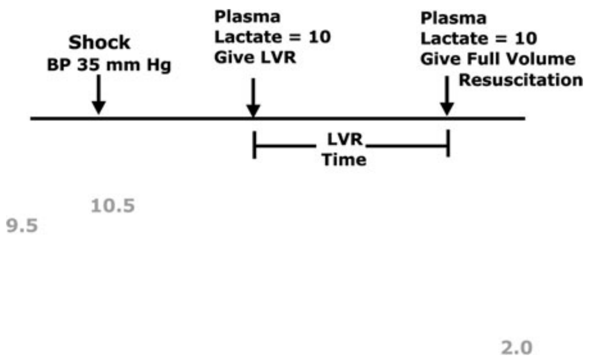
Warm ischemia-induced cell swelling and the effects of cell impermeants on this response was first characterized in mouse liver slices. Liver slices have been used before to characterize cell impermeants[19] because they are easy to prepare and there is abundant mass for many groups per liver. Adult mice (C57BL/6) were anesthetized with isoflurane and the liver was isolated, quickly removed, and immersed in cold saline on ice. Liver slices (3–4 per condition) were prepared with a Staddie-Riggs microtome to give a uniform thickness of less than 0.5 mm. About 150 mg of liver slices were incubated in 25-mL Erlenmeyer flasks in 1.5 mL Krebs buffer in a Dubanoff style metabolic shaking water bath under an atmosphere of oxygen or nitrogen always containing 5% CO_2. Tissue slices underwent ischemia by incubation under an atmosphere of 95% nitrogen and 5% CO_2 for 1 hour followed by reperfusion under an atmosphere of 95% oxygen and 5% CO_2 for an additional hour. Some tissue slice conditions contained impermeants in the Krebs buffer during ischemia and some did not (controls). Impermeants were used at 0, 25, 50, 100, and 150 mM final concentration. These impermeants consisted of sorbitol, gluconate, trehalose, raffinose, and an equal molar mixture of raffinose and trehalose. Tissue slices were sampled after preparation (Fresh), after ischemia, and after reperfusion in untreated and impermeant treated groups for analysis of total tissue water (TTW) content by calculating [wet-dry]/dry weight ratios. Dry weights were determined after drying the tissue slices in a 65°C oven for 48 hours.

Rodent Shock Model

A low-volume resuscitation (LVR) model was used in adult rats to test both the cell swelling hypothesis and to develop the impermeant-based LVR solution used for prehospital resuscitation of patients with severe hemorrhagic shock. Adult Sprague Dawley rats were anesthetized with isoflurane and maintained in a light surgical plane of anesthesia during the study. Polyethylene catheters were placed in both femoral arteries for blood pressure monitoring and blood sampling, and a catheter was placed in 1 femoral vein for administration of fluids. The animals were allowed to ventilate on their own to establish normal arterial blood gas (ABG) values. A 1-cm midline incision was created to induce soft tissue injury and for the placement of a temperature probe in the abdomen. The animals were kept at 38°C using a heating pad and an incandescent light source above them. Arterial blood pressure, heart rate, and temperature were continuously recorded using a PowerLab (ADInstruments, Boston, MA). After a 30-minute stabilization period, heparin was given (500 U/kg) and arterial blood was slowly removed at 1 mL/min into a syringe to maintain blood pressure at 30 to 35 mm Hg. This hypotension was maintained until the plasma lactate reached a value between 9 to 10 mM, as measured with both a handheld lactate analyzer (Lactate Plus, Nova Biomedical, Waltham, MA) and a blood gas analyzer (Radiometer 800). Once the target lactate was reached, an LVR equal to 10% to 20% of the calculated blood volume[20] of saline was administered intravenously over a 10-minute period using a syringe infusion pump. When the blood lactate again reached 9 to 10 mM, full resuscitation was started, which consisted of a volume of saline equal to the volume of the blood loss (about 55% to 60% of total blood volume) plus 30% of the removed red blood cells (washed) infused intravenously over 10 minutes (although this full resuscitation protocol using saline is now outdated, the study was started when it was acceptable to use saline so the authors finished the project using the same protocol). After 1 hour of full resuscitation, the animals were euthanized by an anesthetic overdose and terminal blood was removed for analysis. The time from the start of the LVR period until the start of full resuscitation is called the LVR time and it represents the tolerance of the animal to the low-volume state or the maximum amount of time that a shocked subject can safely remain in the low-volume state until more definitive resuscitation is required. This was a major outcome used in the study. The protocol is illustrated in Figure 1. [F1]

Regional Blood Flow

In another series of studies (n = 6 per group), the effects of hemorrhagic shock, LVR, and LVR with impermeants on local capillary blood flow were studied using the colored microsphere technique.[21,22] Animals were prepared as previously described but a catheter was also placed into the left ventricle by advancing the catheter through the right carotid artery using real time pressure and pressure waveforms as indicators of the catheter location. Once all catheters were in place, 0.2 mL colored microspheres (Triton Technologies, San Diego, CA) were rapidly injected into the left ventricle. A calibrated arterial reference blood sample was simultaneously removed from the femoral artery catheter by a withdrawal pump to calibrate the microsphere measurement. Three different microsphere colors were used at baseline, during LVR (immediately before full resuscitation), and 60 minutes after full resuscitation. After the study, tissue samples were removed from major organs and the microspheres in the tissues and in the reference arterial blood samples were recovered by alkaline digestion and repeated centrifugations. Dye coating the purified microspheres was extracted with dimethylformamide and



[AQ4] **FIGURE 1.** The LVR protocol that was used in these studies. Arterial hemorrhage is used to maintain a low-volume state (30–35 mm Hg) until the plasma lactate reaches 9 to 10 mM. At that time, an LVR solution is given, which temporarily reduces plasma lactate (due to dilution and increased perfusion) and increases arterial blood pressure. When lactate again reaches 10 mM, full resuscitation is started with 1 volume of saline containing 30% of the washed red cells that were hemorrhaged. The time from the start of the LVR solution until the start of the full resuscitation solution is called the LVR time and it represents the tolerance of the patient to the low-volume state or the maximum amount of time that a patient can safely remain in the low-volume state until more definitive medical care and full resuscitation can be started. The LVR time was a key outcome variable for these studies.

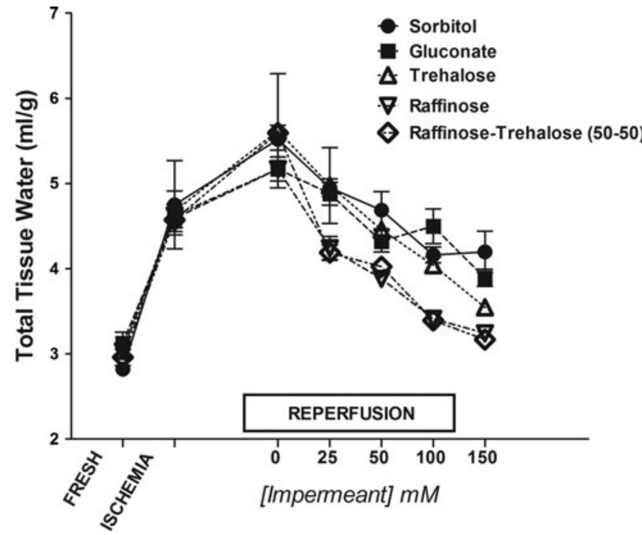


FIGURE 2. Cell swelling of liver tissue slices in vitro in response to hypoxic ischemia and the effects of various concentrations of cell impermeants on the cell swelling response. Cell swelling was indexed by measuring TTW of the liver slices an hour after ischemia and an hour after normoxic reperfusion or in fresh controls. Cell impermeants were in the Krebs buffer suffusing the slices during ischemia. In general, the impermeant effect is proportional to the molecular weight of the impermeant and its concentration in the extracellular space. n = 6 liver samples per group, values are mean ± SD. Each impermeant group also has a zero concentration control, which sees ischemia and reperfusion without any impermeants.

quantitiated using a UV-VIS spectrophotometer (Shimadzu). Individual colors were resolved using a matrix inversion algorithm from the composite spectra. Blood flow was calculated by the tissue dye content using the reference blood draw as a standard. [AQ5]

Experimental Design

Shocked animals were treated according to the following groups:

1. Saline Controls: Received saline as the LVR solution (n = 12).
2. Gluconate: Received an LVR solution of 15% gluconate in saline, a prototypical cell impermeant (n = 11).
3. Gluconate + PEG-20k: Received an LVR solution of 15% gluconate and 10% polyethylene glycol with a molecular weight of 20 kDa (PEG-20k). PEG-20k acts as an oncotic agent (n = 8).
4. PEG-20k: Received an LVR solution of 10% PEG-20k (n = 6).
5. BSA: Received an LVR solution of 10% Bovine Serum Albumin (BSA), a prototypical oncotic agent (n = 6).

The outcome variables for the study included LVR time, plasma lactate, mean arterial blood pressure, and regional tissue blood flow rates.

Statistical Analysis

Most data are expressed as the group mean ± the standard deviation. Each group consisted of 6 to 12 subjects per group, which was derived from power analysis and the known variance of the data in the studies. Data were analyzed by analysis of variance and Bonferroni's multiple comparison test. All data were first analyzed for normality of distribution. A $P < 0.05$ was considered statistically significant.

RESULTS

The impermeant effects of a variety of common cell impermeants in the in-vitro tissue slice model is shown in Figure 2. [F2] TTW measurements indicate that 60 minutes of hypoxic ischemia to murine liver slices caused tissue water accumulation to increase almost twofold after ischemia alone and after 1 hour of normoxic reperfusion. The addition of all of the molecular species of cell impermeants to the incubation media during ischemia prevented the ischemia-induced water accumulation after reperfusion. The magnitude of the response was generally directly proportional to both the molecular weight of the impermeant and the molar concentration in the media (25–150 mM). The optimal responses were observed with raffinose and mixtures of raffinose and trehalose used at about 60 to 100 mM.

The amount of time that a shocked subject can safely remain in the low-volume state is indexed by the LVR time in this experiment. These times are shown in Figure 3 for the various treated groups [F3] of shocked rats. The trigger to end the LVR period after the LVR solutions were given was the lactate climbing back up to 9 to 10 mM. Gluconate (15%) added to the saline-control LVR solution increased the LVR time by 100% from about 45 minutes for the saline control to more than 96 minutes for the gluconate solution. The addition of 10% PEG-20k to the gluconate LVR solution further increased the LVR time 5.3-fold over the saline control to 240 minutes. This LVR time was arbitrarily stopped because of anesthesia effects but it likely could have gone much longer because the target lactate of 10 mM was never reached even after 240 minutes after the start of the LVR solution. The lactate in the gluconate + PEG-20k group after 240 minutes was only 2.5 mM. Similarly, the LVR time in the group with only PEG-20k was also ended after 240 minutes with a lactate of only 2.2 mM. Because the time limit of these 2 groups was never met because the target lactates were never met, we do not know if

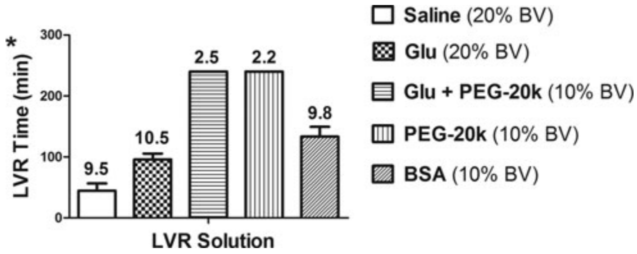


FIGURE 3. The LVR time measured in 5 groups of shocked rats. The LVR time is the time from the start of the LVR until the time of full resuscitation, based on the response of the plasma lactate levels. The values are mean ± SD with 6 to 12 animals per group. The numbers above the bars are the average plasma lactate value at the end of LVR. Because 10 was the cutoff to end LVR by definition, most of the lactate values are very close to 10. However, the 2 groups with PEG-20k in the LVR solution were arbitrarily ended at an LVR time of 240 minutes and the ending lactate values were still far below the target of 10 mM. The standard LVR volume was chosen to be 20% of the calculated blood volume but this had to be cut in half for the PEG-20k groups because the response and diuresis were too intense. $^{*}P < 0.05$ between all groups except between the 2 groups with PEG-20k, GLU = sodium gluconate.

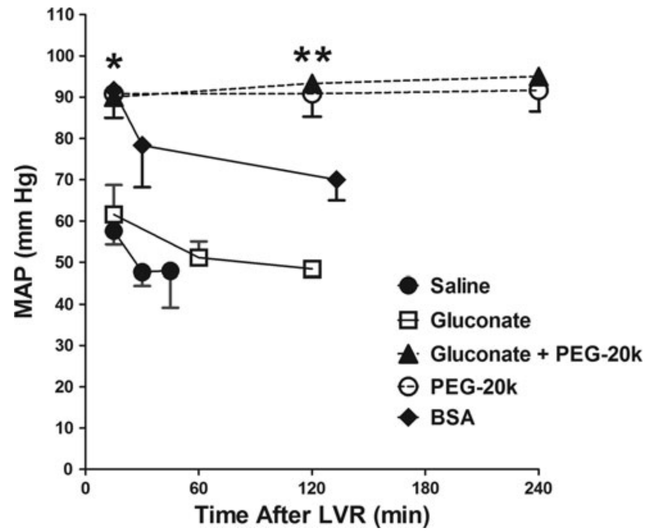


FIGURE 4. MAP in animals during the LVR time after hemorrhagic shock in the 5 groups of shocked rats. Values are mean ± SD, n = 6–12 per group, $^{*}P < 0.05$ relative to the gluconate and saline groups, $\dagger P < 0.05$, relative to the BSA and gluconate groups (at the same approximate time point).

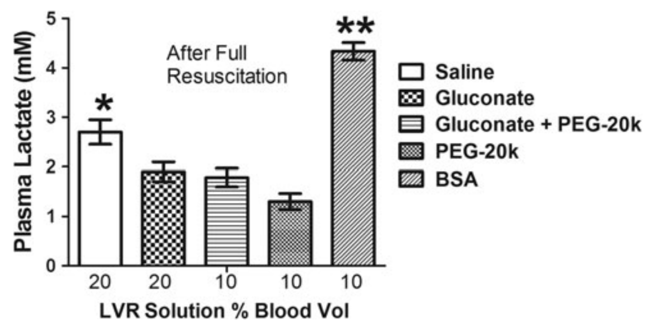


FIGURE 5. Plasma lactate values measured after 1 hour of full resuscitation in the 5 shocked rat groups. Values are mean ± SD, n = 6–12 animals per group, $^{*}\dagger P < 0.05$ relative to every other group.

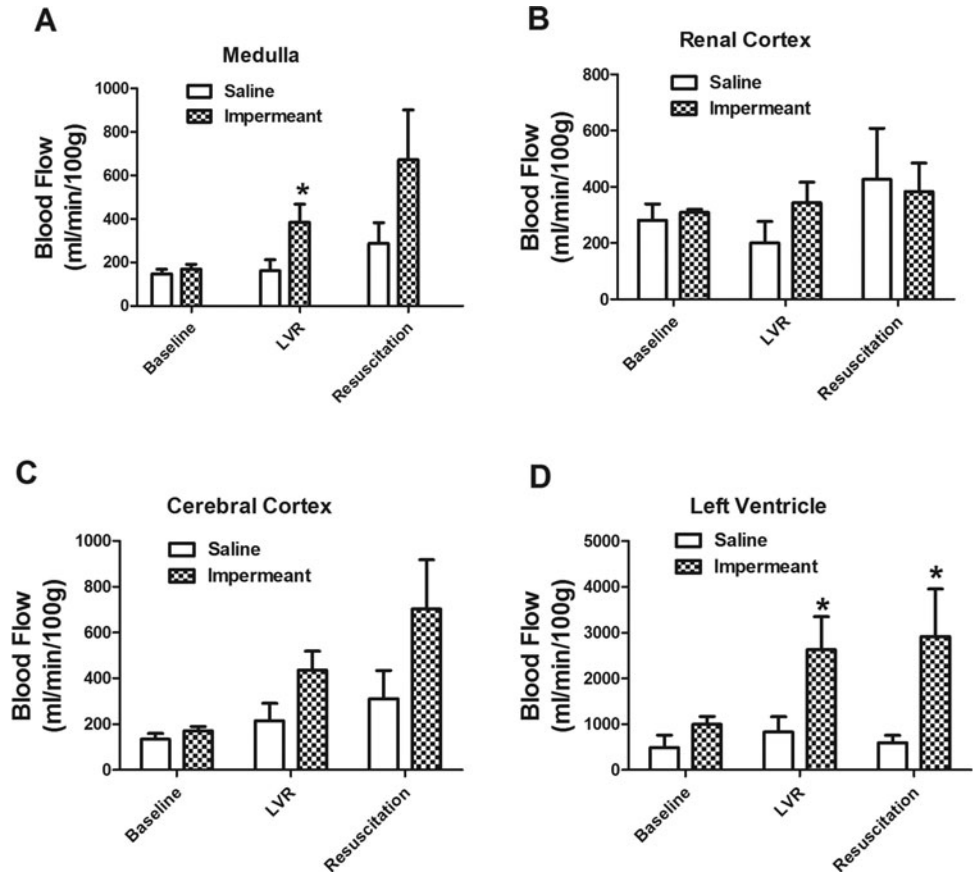
there is in fact a difference in the 2 groups with respect to LVR time. Finally, LVR solutions containing 10% BSA were also effective at increasing the LVR time (133 minutes) but not nearly as effective as LVR solutions containing PEG-20k. It is also important to note that the volume of LVR solution used that contained PEG-20k was half the volume (10%) used in the other groups (20%: saline control, gluconate, and BSA). Thus, PEG-20k based LVR solutions were more than 5 times more effective at expanding the LVR time compared to saline, at half the dose.

The mean arterial blood pressure in rats after shock and after administration of the LVR solution (for as long as the LVR period [F4] lasted) is shown in Figure 4. In the saline controls, the blood pressure after the shock period was 30 to 35 mm Hg, by definition of the model. After 10 minutes of saline LVR administration, the mean arterial pressure (MAP) rose initially to about 55 mm Hg but then rapidly fell back below 50 mm Hg as the LVR period ended after 45 minutes, because of the lactate reaching 10 mM. The gluconate group showed a similar pattern. Although the MAP did not get higher than the control group, it did last longer because gluconate doubled the LVR time. Groups resuscitated with PEG-20k in the LVR solution, however, had normal MAP throughout the 240 min LVR period and this was accomplished with only 50% of the LVR resuscitation volume of the controls. The BSA-treated oncotic controls started with a normal blood pressure immediately after LVR solution administration, which fell off to about 70 mm Hg at the end of the LVR period. This was significantly higher than the control MAP but significantly lower than the MAP for the groups resuscitated with LVR solutions containing PEG-20k.

[F5] Figure 5 shows the final plasma lactate levels in shocked rats after LVR and 1 hour after full resuscitation. The lactate levels were all significantly lower in animals given an LVR solution with an impermeant (gluconate, PEG-20k, or both) relative to the saline control group. Lactate in the BSA group after full resuscitation was significantly higher than all of the groups, including the saline controls.

Regional capillary blood flow in major organs and tissues in shocked rats treated with gluconate or with saline is shown in [F6] [F7] Figures 6 and 7. Local blood flow in the skeletal muscle, left ventricle, and brain (medulla) was significantly higher during the LVR period when an impermeant-based LVR solution was used, compared to saline. There were higher trends in other tissue beds too. After full resuscitation, regional blood flow was significantly higher in the left ventricle after impermeant-based resuscitation compared to saline. Again, there were strong trends in other beds.

ABG data are shown in the Table for rats given saline, saline [T1] with gluconate, or saline with gluconate + PEG-20k during the LVR period. ABG parameters are reported for each group after the baseline period before shock, after the hemorrhagic shock period, and after the LVR period (immediately before full resuscitation). In all groups, the changes in the ABG data from baseline to shock are predictable and not different between groups. Specifically, lactate rose to 10 mM in each group because the amount of shock that was induced was titrated and controlled to that level of oxygen debt (lactate). In addition, HCO_3^- and pCO_2 values fell as the pH remained unchanged. After LVR, however, some differences in the ABG data were apparent between the group receiving PEG-20k in the LVR solution and



[AQ6]

FIGURE 6. Capillary blood flow in medulla, cerebral cortex, renal cortex, and left ventricle at baseline, after the LVR period, and after the full-resuscitation period in 6 rats per group. The 2 groups compared were saline LVR solution (Saline) and a saline LVR solution with an impermeant mixture. $^{*}P < 0.05$ relative to the corresponding value for saline. Capillary blood flow was measured using the colored microsphere technique.

the other groups. Specifically, PEG-20k LVR prevented lactate levels from significantly rising above baseline (1.2 ± 0.4 mM during baseline vs 2.6 ± 1.1 mM after a 240-minute LVR period). A significant metabolic alkalosis with higher HCO_3^- and higher pH was also observed with PEG-20k resuscitation, relative to the other LVR groups. The pH of all of the LVR solutions was 7.2.

DISCUSSION

Severe hemorrhagic shock in the field can be life threatening because the blood pressure drops and the microcirculatory exchange capacity deteriorates, which cause the delivery of oxygen to tissues (DO_2) to fall. First responders are severely limited in what they can do to stabilize the DO_2. Recognizing now that high-volume crystalloid resuscitation that was once used to raise perfusion pressure is harmful, prehospital care now amounts to delivering LVR solutions. Given those constraints, LVR (<500 mL) should be looked upon as a vehicle to deliver agents that increase tolerance to the low-volume state rather than as a temporary volume expander to raise blood pressure per se. This is best accomplished by targeting significantly important causal mechanism and pathways of global ischemia and resuscitation injury. This study targeted cell swelling, which is a very specific and highly underestimated mechanism that contributes to the phenotypic changes associated with hemorrhagic shock and global ischemia.

Hemorrhagic shock is characterized by changes secondary to the loss or reduction in cellular energetics. As the cell ATP levels fall because of low oxygen delivery, the cell begins to lose ATP-dependent processes, including the active volume control mechanisms driven by the Na/K ATPase pump. Hydropic cellular degeneration then leads to cell and organelle membrane dysfunction, which can cause cell homeostasis abnormalities, lysis, and death. Furthermore, swollen parenchymal cells compress capillary exchange vessels to reduce capillary blood flow, which causes more ischemia and swelling in a vicious cycle. Although this mechanism is well appreciated in preservation injury of organs stored for transplantation, it is mysteriously unappreciated in global warm ischemia associated with shock, stroke, or infarction injury. The main objective of this study was to test this mechanism of shock by attempting to reverse it with cell impermeants that are known to prevent cell swelling but have few other biological effects. The results are clear, dramatic, and may represent a significant step forward in treating severe hemorrhagic shock with low-volume crystalloid-based resuscitation, especially in an austere prehospital environment.

Cell swelling plays a major role in organ preservation injury and may do the same in circulatory shock after trauma. Organ preservation causes cell swelling because depletion of ATP during cold ischemia and cold per se cause disruption of the normal ATP-dependent cell volume control mechanisms. Cell swelling is a major contributor to preservation injury in recovered donor organs because it can be largely mitigated by using cell impermeants in organ preservation solutions. In fact, cell impermeants are one of the most important and

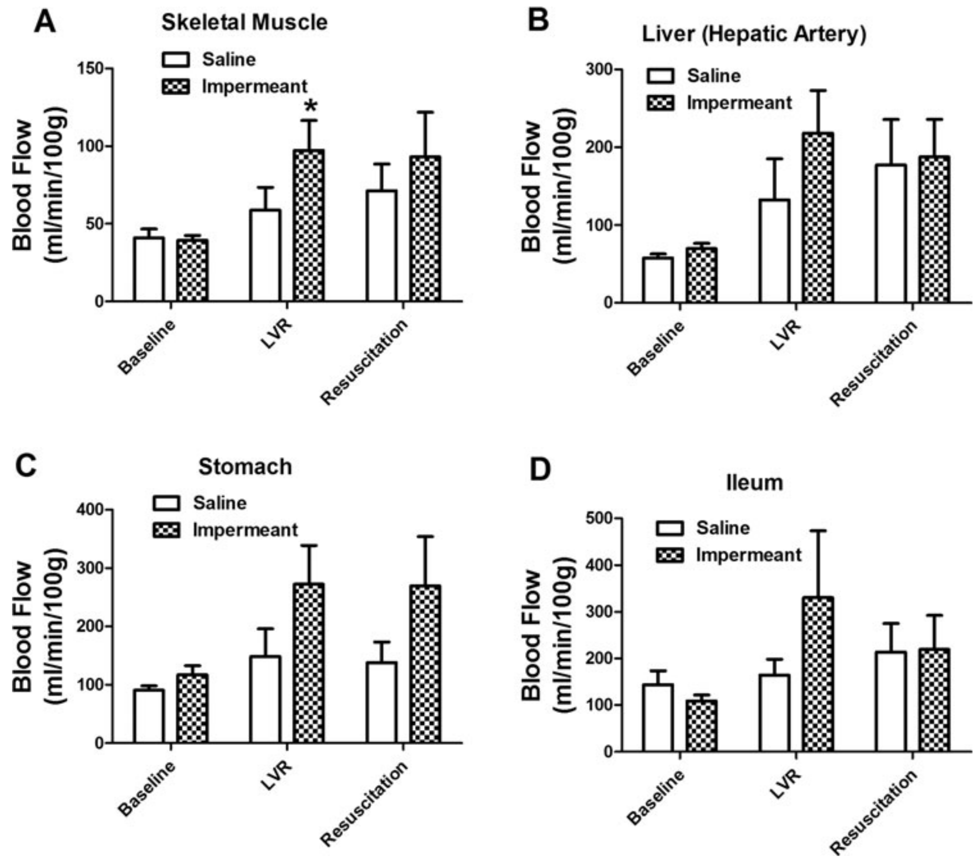


FIGURE 7. Capillary blood flow in skeletal muscle, stomach, liver (hepatic artery flow), and terminal ileum at baseline, after the LVR period, and after the full-resuscitation period in 6 rats per group. The 2 groups compared were saline LVR solution (Saline) and a saline LVR solution with an impermeant mixture. $*P < 0.05$ relative to the corresponding value for saline. Capillary blood flow was measured using the colored microsphere technique.

effective components of modern day organ preservation solutions.[17] The concept is simple, load the interstitial space with molecules that escape the capillary but are impermeant to the cell membrane. This preferentially increases the extracellular osmolarity and prevents water from moving into the cell, which is its natural propensity when the intracellular sodium concentration increases after ischemia-induced pump failure. A similar mechanism is proposed to exist in ischemic shock and a similar solution was tried because most cell impermeants are relatively nontoxic and can be administered in high enough concentrations to be theoretically effective at preventing ischemic cell swelling. The evidence supporting this parallel mechanism is seen in the impermeant effects on liver cell swelling after warm ischemia and the effects of impermeants on LVR times, blood lactate levels after shock, and capillary blood flow to major organs during shock. As cell swelling is prevented with gluconate in the LVR solutions, microcirculatory exchange improves, which lowers plasma lactate values in treated subjects. This is manifest as both increasing LVR times and higher capillary blood flow to vital organs with impermeant treatment during the low-volume period. This is consistent with reduced swelling compression on microcirculatory exchange vessels and reduced obstructive swelling of endothelial cells forming the capillary lumen,[23–25] which allows for better capillary perfusion and more efficient cellular metabolism (lower lactates). All these data are consistent with the hypothesis that circulatory shock states promote cell swelling, which is an important cause of tissue, organ, and systems injury, acting in part, through a microvascular mechanism.

In an attempt to further test the cell-swelling hypothesis and to make impermeant treatment more effective, a model of a microcirculatory osmotic gradient was developed and tested. In the osmotic gradient model, 3 microvascular compartments are identified in a shocked patient as intracellular, interstitial, and the capillary compartment. An osmotic gradient could be established both between the intracellular and extracellular space by the use of conventional cell impermeants (like gluconate), which occupy both capillary and interstitial spaces, and a gradient could be established between the interstitial and capillary spaces by the addition of an oncotic agent to the circulation, which only occupies the capillary space. The combination of impermeant and oncotic agent would create this double gradient, which may not only prevent cell swelling but also keep water flowing out of the interstitial space and into the capillary space where it belongs. There, the water that would otherwise have entered the ischemic cells in the tissue will expand the circulatory volume and promote capillary blood flow and oxygen exchange, which mitigates the shock state by increasing the efficiency of oxygen delivery during low flow. When this was tried by combining both gluconate (an impermeant) with PEG-20k, an oncotic agent, a *huge* potentiation effect was seen on LVR times. Furthermore, blood pressure during the LVR period was completely normalized with PEG-20k LVR solutions. Although gluconate doubled the LVR time, relative to the saline control,

TABLE. Blood Gas Data During the Shock and LVR Protocol in Rats Receiving Saline, Gluconate (Glu), or Gluconate + PEG-20k LVR Solutions

Group	ABG Parameter	Baseline	Hemorrhagic Shock	LVR
Saline (20% BW)	Lactate (mM)	1.35 (0.23)	9.72 (0.83)	9.49 (1.95)
	HCO_3^- (mM)	24.1 (2.6)	14.5 (3.17)	14.2 (1.63)
	pO_2 (mm Hg)	441 (32)	381 (63.8)	369 (36.3)
	PH	7.37 (0.04)	7.37 (0.04)	7.36 (0.05)
	pCO_2 (mm Hg)	39.8 (3.10)	26.4 (3.75)	22.9 (3.32)
Glu (20% BW)	Lactate (mM)	1.15 (0.33)	9.34 (0.37)	10.5 (1.67)
	HCO_3^- (mM)	26.2 (1.47)	16.3 (1.55)	13.0 (2.92)
	pO_2 (mm Hg)	406 (49.1)	398 (38.1)	379 (62.6)
	PH	7.39 (0.05)	7.39 (0.05)	7.30 (0.15)
	pCO_2 (mm Hg)	41.6 (3.11)	26.1 (2.59)	22.1 (2.76)
Glu + PEG-20k (10% BW)	Lactate (mM)	1.20 (0.37)	9.42 (0.21)	2.62 (1.06)*
	HCO_3^- (mM)	25.8 (1.28)	16.3 (0.99)	31.2 (2.54)*
	pO_2 (mm Hg)	448 (39.7)	417 (19.9)	419 (31.4)
	PH	7.40 (0.03)	7.34 (0.03)	7.48 (0.02)*
	pCO_2 (mm Hg)	36.1 (2.95)	25.2 (1.94)	39.9 (5.67)*

Values are mean ± SD, n = 6 per group.
*$P < 0.05$ relative to the corresponding value in the other LVR groups.
BW indicates volume based on calculated body weight.

gluconate and PEG-20k increased the LVR time five- to sixfold. It is not even known what the upper limits are to this effect since the PEG-Gluconate studies were cutoff by the experimenter 5 hours after the start of the LVR period. At that time, the lactate levels were still only at about 2.5 mM, far below the threshold of 10 mM needed to trigger full resuscitation in our model. Furthermore, the volume of PEG-20k or gluconate needed for this effect was half of the volume used for the saline control LVR group. This supports the concept that the addition of PEG-20k served to move water into the capillary space where it supports intravascular volume, blood pressure, and microcirculatory flow. The latter is supported by the low plasma lactate levels throughout the LVR period, which suggest good microcirculatory flow secondary to high capillary driving pressures (fluid expansion). What this means clinically is that a severely shocked patient (MAP in the 40s with 50% blood loss) can receive half of the volume of an impermeant-based LVR solution (intravenously) and safely remain in the low-volume state for at least 6 times longer than if conventional saline resuscitation were used, before definitive full resuscitation is needed.

LVR solutions containing PEG-20k largely prevent the accumulation of lactate in the blood even after 240 minutes after the initial 55% blood volume hemorrhage. Accompanying the low lactate was a slightly higher pH and a significantly higher bicarbonate concentration (double that of the other LVR groups). This metabolic alkalosis served to correct the lactacidosis of the low-volume state and is likely of renal origin since the pCO_2 remained normal and the pH of the LVR solutions were held at 7.2. After administration of the LVR solution in these studies, we always observed a temporary diuresis, which was attributable to the osmotic retention of water in the renal tubules secondary to PEG-20k filtration across Bowman's space and trapping in the tubular lumen. This diuresis (and maybe a concomitant naturesis) may result in the significant excretion of
[AQ7] hydrogen ions into the urine resulting in a normalization of pH and even a slight alkalosis. Metabolic studies are needed to define this possible mechanism. In any case, preserving proper pH during shock and LVR may help maintain the normal blood pressure observed in the PEG-20k LVR group.

To test the oncotic-impermeant model further, we conducted studies using albumin and PEG-20k alone in the LVR solution. Because high-molecular-weight PEG molecules are known to have other biological properties besides their oncotic ones, we used the physiological prototype oncotic agent albumin as an oncotic control. Albumin used alone to control for oncotic effects was not at all as effective as PEG-20k alone but it was better than saline. This suggests that there is something different about PEG-20k. The effects of PEG-20k in LVR shock models are attributable to more than just purely oncotic properties. There are 2 reasonable possibilities to consider: (1) The involvement of nononcotic PEG effects such as PEG's known effects on cell membranes, protein binding and hydration properties, or immunocamouflage effects or (2) Oncotic-impermeant hybrid effects, where, because of the unique molecular weight and attributes of PEG-20k, it is able to act both as a cell impermeant and as an oncotic agent.

There is evidence to support the idea that PEG-20k acts both as a cell impermeant by escaping the capillary space while remaining impermeable to the cell and as an oncotic agent whereby a large amount of the material remains trapped in the capillary space. This property may be caused by a slow equilibration time to cross the capillary barrier into the interstitial space, based on its size and other attributes. In fact, PEG-20k has been shown to effectively expand the vascular space and move water out of the interstitial space to stimulate thirst in rats and pigeons,[26] which demonstrates its oncotic effects. It has also been detected immunohistochemically in renal tubule epithelium and in monocytes in the liver and lung after intravenous administration, suggesting that it leaves glomerular capillaries and hepatic sinusoids,[27] which demonstrates its partial impermeant effects. Our own studies and observations indicate that it leaves Bowman's space because a significant but temporary diuresis is seen in rats after severe shock after receiving PEG-20k in the LVR solution. This diuresis may have been due to PEG-20k-induced restoration of the arterial pressure during the LVR period or it may have been due to an osmotic diuresis from PEG being filtered and trapped in the renal tubules, similar to mannitol. The latter is more plausible because we do not see a diuresis in shocked rats when their blood pressure is normalized using conventional resuscitation solutions but we do with PEG-20k solutions. Normalization of renal perfusion pressure and the institution of a mild filtration may be desirable during a shock state, as long as the diuresis does not jeopardize the newly normalized blood pressure. There is no evidence in our studies that this happens. The renal effects would tend to prevent the development of ATN after resuscitation. The diuresis observed in this study with PEG-20k is temporary and dose dependent because a 10% blood volume LVR dose of PEG-20k produces much milder diuresis compared to a 20% blood volume LVR dose. That's why we decreased the dose of the LVR solutions containing PEG-20k from 20% to 10%. The molecular weight of PEG-20k seems to be right on the size limit for partial capillary permeability because higher molecular weights approaching 30 kDa do not cross capillary spaces including Bowman's space.[27] A proposed hybrid oncotic-impermeant property of PEG-20k is also consistent with the observation from our study that PEG-20k was as effective alone as it was in combination with gluconate. In essence, partial capillary permeability characteristics of PEG-20k may allow enough osmotically active material to escape into the interstitial space to mimic the impermeant effect of gluconate while the majority of material stays behind in the capillary to act oncotically. Therefore, gluconate or other impermeants may not be necessary in LVR solutions using just PEG-20k alone, but further testing in survival studies is needed.

There are limitations to this study and to its projected clinical use. Our study used a controlled hemorrhagic shock model, which is highly relevant in limb or extremity injuries in the field or other compressible hemorrhagic injuries. In both cases, good hemorrhage control can be achieved with tourniquets and compression techniques. This stops the bleeding and allows the impermeant-based LVR solutions to expand the circulatory volume, drive up arterial pressure, and improve flow and oxygen exchange (in addition to protecting tissues from lethal cell swelling). In trauma cases, where bleeding remains uncontrolled or hard to control, the model is oversimplistic and overestimates its clinical utility because bleeding will continue. Bleeding will continue because (1) compression or hemostasis in the field is limited, (2) the increased arterial pressure (in the absence of hemostasis) from the osmotic volume shifts will exacerbate the pressure gradient for further hemorrhaging, and (3) the crystalloid solution does not provide any replacement of clotting factors and precursors, which could limit bleeding by active coagulation and platelet activation. These factors all limit the use of an impermeant-based LVR solution in many clinical settings. However, combining impermeant-based LVR solutions with plasma product replacements such as fresh frozen plasma or spray-dried plasma products may prove the most useful in many prehospital trauma settings involving uncontrolled hemorrhaging.

[AQ8]

CONCLUSIONS

Cell swelling due to global ischemia from severe hemorrhagic shock plays a significant role in the sensitivity of the victim to the low-volume state. This is attributable to effects on the microcirculation with improved efficiency of microvascular oxygenation and perfusion during low-volume states. The use of cell impermeants with oncotic agents or PEG-20k alone in LVR solutions dramatically increases the time that a patient can safely remain in the low-volume state until definitive medical care and full resuscitation are needed. The new LVR solution may be important in civilian prehospital resuscitation and for combat casualty care and resuscitation on the battlefield, especially when combined with plasma component replacement.

ACKNOWLEDGMENT

D.P. and S.L.L. contributed equally as first authors.

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Queries to Author

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