<table>
<thead>
<tr>
<th>UNCLASSIFIED</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD NUMBER</td>
</tr>
<tr>
<td>ADB252283</td>
</tr>
<tr>
<td>LIMITATION CHANGES</td>
</tr>
<tr>
<td>TO:</td>
</tr>
<tr>
<td>Approved for public release; distribution is unlimited.</td>
</tr>
<tr>
<td>FROM:</td>
</tr>
<tr>
<td>Distribution authorized to U.S. Gov't. agencies only; Proprietary Information; JAN 2000. Other requests shall be referred to Army Medical Research and Materiel Comand, Fort Detrick, MD 21702-5012.</td>
</tr>
<tr>
<td>AUTHORITY</td>
</tr>
<tr>
<td>USAMRMC ltr, 1 Jun 2001</td>
</tr>
</tbody>
</table>

THIS PAGE IS UNCLASSIFIED
Award Number: DAMD17-97-1-7009

TITLE: Emergency Interventions After Severe Traumatic Brain Injury in Rats: Effect on Neuropathology and Functional Outcome

PRINCIPAL INVESTIGATOR: Patrick M. Kochanek, M.D.

CONTRACTING ORGANIZATION: University of Pittsburgh
Pittsburgh, Pennsylvania 15260

REPORT DATE: January 2000

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Jan 00). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-97-1-7009
Organization: University of Pittsburgh
Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.
### Abstract

Traumatic brain injury (TBI) contributes to combat morbidity/mortality. Studies in models of TBI have focused on novel mediators and mechanisms. We used controlled cortical impact (CCI), a contemporary model of TBI in rats to study field-oriented treatments. The following technical objectives were addresses: 1) What is the optimal ventilation strategy? 2) Is hypothermia beneficial? and 3) what is the optimal sedative/analgesic? A fourth objective, combining hypothermia plus other therapies was abandoned due to the limited efficacy of hypothermia. The most important findings/publications include: Objective #1), in a report published in the Journal of Neurosurgery, we demonstrated that early aggressive hyperventilation worsened neuronal death, Objective #2), we published the first report showing that hypothermia was ineffective in the combat-relevant scenario of CCI followed by secondary hypoxemia. That work is in press in Critical Care Medicine, Objective #3), we reported remarkably poor outcome in rats treated with narcotics (fentanyl) versus general anesthesia (isoflurane) after CCI. That study is extremely relevant since narcotics are the current field treatment. That work was presented at the 1999 meeting of the National Neurotrauma Society by research trainee Dr. Kimberly Statler, who received the Women in Neurotrauma Award. The paper was submitted to Journal of Neurosurgery.

### Subject Terms

- Head and Trauma
Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

__Where copyrighted material is quoted, permission has been obtained to use such material.__

__Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.__

__Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.__

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature 1-13-2000  Date
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRONT COVER:</td>
<td></td>
</tr>
<tr>
<td>STANDARD FORM (SF), REPORT DOCUMENTATION PAGE:</td>
<td>2.</td>
</tr>
<tr>
<td>FOREWORD:</td>
<td>3.</td>
</tr>
<tr>
<td>TABLE OF CONTENTS:</td>
<td>4.</td>
</tr>
<tr>
<td>INTRODUCTION:</td>
<td>5.</td>
</tr>
<tr>
<td>BODY:</td>
<td>7.</td>
</tr>
<tr>
<td>KEY RESEARCH ACCOMPLISHMENTS:</td>
<td>14.</td>
</tr>
<tr>
<td>REPORTABLE OUTCOMES:</td>
<td>14.</td>
</tr>
<tr>
<td>CONCLUSIONS:</td>
<td>16.</td>
</tr>
<tr>
<td>REFERENCES:</td>
<td>17.</td>
</tr>
<tr>
<td>APPENDIX:</td>
<td>18.</td>
</tr>
</tbody>
</table>
(5) INTRODUCTION

Please note that although we have performed, presented and published a considerable number of studies, as outlined in this final report, we are still completing a few aspects of work on the third technical objective. There are also a number of manuscripts and abstracts that are either in press, in submission or in preparation. After discussion with our contracting officer, it was recommended that we submit a supplement to this report and its appendix. A supplement will be forwarded to your office on July 14, 1999. Additional supplements will follow for subsequent publications beyond that date.

Traumatic brain injury (TBI) is an important contributor to combat casualty morbidity and mortality. Although progress has been made in the development of rodent models of TBI (such as the controlled cortical impact (CCI) model), most of the studies with these models have focused on molecular mechanisms of damage. Because there has been a paucity of application of practical emergency interventions in TBI models, we felt that it was important to address this deficiency since this could have important implications for field and emergency management of both soldiers and civilians. Our overall hypothesis is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI (respiratory management, temperature control, and sedation) can reduce secondary brain injury in a rat model of brain contusion, and thereby improve functional and/or neuropathological outcome.

Funding year 1

In yr. 1 of funding, we addressed the first Technical Objective, namely, to investigate the effects of mechanical ventilation strategies (as applied by the first responder in the field) on functional and neuropathological outcome in our model. We found that aggressive, prophylactic hyperventilation (HV) applied for 4 h immediately after injury is detrimental (vs normal PaCO₂), and leads to increased neuronal death in selectively vulnerable brain regions. This study was published as a full manuscript in the Journal of Neurosurgery (1). The reviewers indicated that this was an important study that would be cited often. Dr. M. Forbes, a Critical Care Medicine fellow training in research with Dr. Kochanek authored the study.

To set the stage for the evaluation of therapies after injury (as proposed studies in technical objectives 2-4), it appeared that it would be optimal to have TBI models both with and without a secondary insult-- since such insults are common in the field. This was accomplished by two studies assessing our model (2,3), including, adding a 30-min of moderate hypoxemia to the CCI. Characterization of that model was described in the 1997 report and was presented in 1998 at the National Neurotrauma Society Meeting (3). During yrs. 2 and 3, we used both the CCI model and the CCI plus secondary hypoxemia model to test therapies.

Funding year 2

In yr. 2 we performed three studies addressing Technical Objective 2 and part of Objective 3. These studies included: 1) assessment of the effect of transient (4 h),
moderate hypothermia on outcome after TBI with a secondary insult, 2) assessment of the effect of prolonged (12-h) moderate hypothermia on outcome after TBI, and 3) assessment of the effect of the anti-excitotoxic therapy (the NMDA-receptor antagonist MK-801) early after TBI. The results of these studies showed that hypothermia (32 °C, for either 4 h or 12 h) reduced DNA damage early after injury but beneficial effects on long-term outcome could not be demonstrated in the model. It was particularly ineffective after the combined CCI plus hypoxemia. In contrast, we were surprised to find that MK-801 improved functional outcome. However, neither treatment improved brain histopathology after injury. Three research fellows (Drs. C. Robertson, M. Whalen, and R. Ruppel) worked on these projects with the PI (Dr. Kochanek) during yr-2. Dr. Robertson presented an abstract at the 1999 annual meeting of the Society of Critical Care Medicine (4). That work on hypothermia is in press as a full manuscript in the journal Critical Care Medicine (5). We also reported that 4 h of moderate hypothermia attenuates DNA damage after injury (6,7). That work was presented last year at the Society of Critical Care Medicine Meeting and the manuscript is in preparation. Our work on hypothermia in TBI was summarized in an invited review article published by the International Trauma, Anesthesia and Critical Care Medicine Society (ITACCS)(8). Dr. Randall Ruppel presented the work on MK-801 at the 1999 Meeting of the National Neurotrauma Society (9). It was one of 12 papers selected for oral presentation out of over 200 papers submitted. The manuscript is in preparation for submission to the Journal of Neurotrauma.

Funding year 3

During yr. 3, we carried out a comprehensive study of sedation/analgesia comparing a narcotic (fentanyl) to a conventional general anesthetic (isoflurane). Fentanyl or morphine are the most commonly used narcotics after human head injury while isoflurane is the most commonly used anesthetic in rat models. We reported remarkably poor outcome in rats treated with narcotics (fentanyl) versus general anesthesia (isoflurane) after CCI. That study is extremely relevant since narcotics are the current field treatment for combat casualties. That work was presented by Critical Care Medicine fellow research trainee Dr. Kimberly Statler at the 1999 meetings of the National Neurotrauma Society, the Society for Neuroscience, and the Society of Critical Care Medicine (10-12). Dr. Statler received the 1999 Women in Neurotrauma Award at the National Neurotrauma Meeting and an Educational Scholarship from the Society of Critical Care Medicine. The full paper was recently submitted to Journal of Neurosurgery (13). The lack of beneficial effect of hypothermia in our model coupled with the remarkably powerful effect of isoflurane suggested the elimination of technical objective 4 in favor of a more comprehensive study of sedatives/analgesics early after CCI (i.e., expansion of proposed technical objective 3). As this progress report is being prepared, we are completing work on the final study in this proposal, namely a comparison of 7 sedative/analgesic treatments applied in a field relevant paradigm.
(6) BODY

(a) Technical Objective 1: Mechanical ventilation strategies after severe TBI in rats (see summary for 1996-1997 [yr-1] and reference 1, both in appendix).

We showed that aggressive, early HV after TBI augments neuronal death in CA3 hippocampus in a rat model of cerebral contusion. The further reduction of CBF with HV during the low cerebral blood flow state immediately after injury coupled with alkalosis may increase the vulnerability of selected neurons to damage. The results of this study reinforce the meticulous attention necessary to prevent secondary injury after TBI. A risk in the use of HV is demonstrated.

Recommendation: Unless signs of impending herniation (unilateral pupillary dilation, hypertension, bradycardia) are present, this study supports the targeting of normocarbia for mechanical ventilation in the emergency stabilization of the brain trauma victim.

(b) Technical Objectives 2: Testing of field-relevant therapies (notably hypothermia) in experimental models of severe TBI (with and without a secondary hypoxemic insult) in rats.

(b1) Effect of transient (4 h), moderate (32°C) hypothermia on functional and histological outcome after experimental TBI with a superimposed secondary hypoxemic insult in rats. (see summary for 1998-1999 [yr-2] and reference 5, both in appendix).

We tested the effect of 4 h of hypothermia in our model of TBI with a 30-min secondary hypoxemic insult. Hypothermia is effective in a variety of experimental models with transient application (1-4 h) and in a single-center study in humans (32°C applied for 24 h). However, in neither experimental nor clinical TBI has hypothermia been tested when applied after the combination of TBI with a secondary insult. We found no benefit from hypothermia after this field-relevant combined insult. This suggests three possibilities. First, the combination of TBI plus a secondary hypoxemic insult may be so severe that no single treatment will be effective. Second, the insult is so severe that no therapies will be effective. Third, based on our work in technical objective 3, it is possible that a beneficial effect of hypothermia is being masked by using isoflurane anesthesia (vida infra). This work is in press as a full paper in the journal Critical Care Medicine (5).

Recommendation: Even in centers where hypothermia was shown to be effective after TBI, this has not been the case for severely injured patients GCS 3-4. It is likely that severe injuries, such as that modeled by CCI with a 30-min hypoxemic secondary insult, will require combination therapies or may be refractory to all therapy. Also, based on the work by our group on technical objective #3, it will be important in future studies to test hypothermia using a sedative/analgesic approach that is similar to that used in the field or clinic—i.e., with narcotics. To our knowledge, such a study has never been carried out in a rodent model of TBI.

Based on the aforementioned study, in the CCI model, we sought to test, to our knowledge for the first time in any laboratory, the prolonged application of hypothermia in a rodent model of TBI. This included over 13-h of controlled mechanical ventilation and physiological monitoring. To date, only brief 1-4 h applications have been tested. In this study, we examined TBI without a secondary insult. As described in last year’s progress report, we failed to observe important beneficial effects of 12-h of hypothermia on functional or histopathological outcome after CCI. This is a surprising finding which is discussed below.

Recommendation: Studies of 12 h of hypothermia in any experimental animal model are very labor intensive. The negative result of this study suggests one of two possibilities. First, there may be both beneficial and deleterious aspects to the use of hypothermia. Despite promising data from single clinical sites, recently, a randomized, controlled multi-center trial of hypothermia in human head injury failed to yield a positive result. Second, once again based on the work by our group on technical objective #3, it will be important in future studies to test hypothermia using a sedative/analgesic approach that is similar to that used in the field or clinic—i.e., with narcotics. It is our recommendation that this be tried first in a rodent model using either morphine or fentanyl anesthesia followed by either 1 or 4 h of hypothermia vs normothermia.

(b3) Effect of the anti-excitotoxic NMDA-receptor antagonist MK-801 on functional and histological outcome after experimental TBI in rats. (see summary for 1998-1999 [yr-2], in appendix, for detailed methods).

In the third treatment trial in year two, we sought to test the effect of the traditional NMDA-receptor antagonist MK-801 in our TBI model (without secondary insult). The NMDA antagonist MK-801 was effective in improving both motor function and some aspects of cognitive function after CCI. The motor effects were more dramatic than those seen with hypothermia. In a separate pilot study, MK-801 was not effective when tested in our TBI plus secondary hypoxemia model, again suggesting this insult may be too severe for any single therapy. Although this specific agent is not available for clinical use, it suggests that this category of agents –targeting excitotoxicity—represents a viable strategy.

Recommendation: This finding is particularly relevant since our studies addressing technical objective #3 suggested that an anti-excitotoxic general anesthesia strategy such as isoflurane produced a markedly better outcome than treatment with the narcotic analgesic fentanyl. Although there have been several negative clinical trials of anti-excitotoxic therapy, there is frequently a delay in administration of treatment for as much as 6 h in these trials. Several reports have suggested that important components of the excitotoxic response may occur in the initial 1-2 h after injury. Based on our findings,
anti-excitotoxic strategies should not be abandoned, rather consideration should be
given to the field application of these strategies. In addition, sedative/analgesics
with anti-excitotoxic properties must be extensively studied in experimental TBI, in
both small animal and large animal models.

(c) Technical Objective 3: Testing of the optimal field-relevant sedative/analgesic
therapy in an experimental model of severe TBI in rats (see reference 13 in
appendix).

(c1) Comparison of the effects of TBI on functional and histological outcome
after experimental TBI in rats anesthetized with fentanyl or isoflurane (described
below).

Currently, in clinical practice, fentanyl is the most commonly used emergency
sedative for the intubated patients with severe TBI. Fentanyl has little direct anti-
excitotoxic properties. Thus, to begin investigating this area, we tested how fentanyl
treatment compared to standard isoflurane anesthesia in our model.

Outcome protocol

Rats were initially anesthetized with N2O:O2 (2:1) and 4% isoflurane and then
endotracheally intubated and mechanically ventilated. Anesthesia was maintained for
the for surgery with 2 - 2.5% isoflurane and N2O:O2 (2:1). Pancuronium bromide (0.1
mg/kg/h) was given iv for muscle relaxation. Femoral venous and arterial vessels were
cannulated for continuous blood pressure measurement, blood sampling, and
administration of medications. A rectal probe was inserted to monitor core temperature.
The rat was then placed in a stereotaxic frame and a left parietal craniotomy was
performed. The dura and bone flap were left in place until immediately before CCI. A
burr hole was drilled into the left frontal bone for temperature probe placement into the
frontal lobe. Continuously monitored physiologic parameters included arterial blood
pressure and rectal and brain temperatures. Blood glucose, hematocrit, and arterial blood
gas samples were assessed every 15 min for the initial hour and every 30 min thereafter.
PaCO2 was controlled at 35 - 45 mm Hg. This protocol produced a PaO2 of greater than
70 mm Hg in all preparations. Both brain and rectal temperatures were maintained at
37.0 ± 0.5 °C.

Rats were allowed to stabilize for 5 min after completion of surgical preparation and
then randomized to receive either fentanyl or isoflurane anesthesia. In the fentanyl group
(n=9), isoflurane was discontinued and 10 μg/kg of fentanyl was administered iv,
followed by a continuous iv infusion at 50 μg/kg/h. In the isoflurane group (n=9),
inspired isoflurane concentration was reduced to 1%, and normal saline, the vehicle for
fentanyl-treated rats, was administered to match the volume received by fentanyl
infusion. Both anesthetic groups continue to receive N2O:O2 (2:1). After 30 min
equilibration, TBI was induced by CCI. In pilot studies comparing isoflurane and
fentanyl using our standard CCI model (6-mm tip, 4 m/s velocity, 50 msec duration of
deforation and 2.5-mm deformation depth), all of the fentanyl-treated rats developed
pulmonary edema and died early after injury. Thus, to compare the effect of isoflurane
vs fentanyl on long-term outcome in our model, our standard injury was reduced (2.0-mm deformation depth). After CCI, the bone flap was replaced and sealed with dental cement, and the scalp incision was closed. Anesthesia was continued for 4 h in the isoflurane group and 3.5 h in the fentanyl group to facilitate similar extubation times. At the end of the anesthetic period, rats received 100% oxygen, were allowed to awaken and resume spontaneous breathing, and were then extubated and returned to their cages. Sham rats underwent identical preparation/anesthesia, but no CCI (n=6 per group).

Motor function, including beam balance and beam walking tasks, was tested by an observer blinded to group assignment on days 1-5 after injury. Morris Water Maze (MWM) testing was performed using an acquisition paradigm on days 14-20 after injury. Lesion volume and hippocampal neuron survival were assessed on day 21.

**ICP Protocol**

Based both on results of the above protocol showing that fentanyl-treated rats had higher MAP throughout the experiment and on a recent report that increased MAP may exacerbate injury after TBI, ICP and percent brain water were monitored in a separate cohort of rats (n=9 per anesthetic group) subjected to either fentanyl or isoflurane anesthesia and CCI in an identical paradigm to that used to assess functional outcome.

Surgical preparation, randomization, anesthetic administration, and CCI were identical to the outcome protocol, with minor exceptions. Specifically, an intraparenchymal ICP monitor (Codman microtransducer) was inserted through a burr hole in the frontal bone into the contralateral (right) frontal cortex at the time of craniotomy. After CCI, anesthesia was continued and ICP was monitored for 4 h in both anesthetic groups. Cerebral perfusion pressure (CPP) was calculated as the difference between MAP and ICP. Rats were killed by decapitation at the end of the anesthetic period. Brains were immediately removed and a 3-mm coronal slice was made through the center of the contusion. Per cent brain water was determined in both the injured and the homologous region of the uninjured hemispheres.

As an added control, a separate cohort of rats (n=3) was subjected to CCI and allowed to
recover without anesthesia. These rats were prepared for CCI under isoflurane anesthesia as above, allowed to recover a tail-pinch response and then subjected to CCI. Arterial MAP was monitored via a femoral arterial catheter for 4 h during recovery without anesthesia.

Results

Time to extubation did not differ after injury between isoflurane and fentanyl treatment groups (269 ± 7 min vs 275 ± 15 min, $p = 0.29$). Physiologic values, including PaCO₂, PaO₂, blood glucose and Hct did not differ between anesthetic groups. In contrast, MAP was higher in injured rats treated with fentanyl compared to their isoflurane counterparts ($p < 0.05$) during the entire posttrauma period (Fig 1). Similarly, MAP was higher in shams treated with fentanyl vs isoflurane ($p < 0.05$) during the entire duration of anesthesia (Fig 1). Fentanyl-treated rats had a MAP of ~150 mm Hg compared to ~105 mm Hg in the isoflurane groups.

Rats anesthetized with isoflurane performed better on beam balance and beam walking tasks after TBI compared to their fentanyl counterparts ($p < 0.05$, Fig 2). Following injury, isoflurane-anesthetized rats also performed better than their fentanyl-treated counterparts during MWM testing with a hidden platform ($p < 0.05$, Fig 3). Motor and MWM performances did not differ between sham groups.

Lesion volume, expressed as mm$^3$ or as percent of uninjured hemisphere, at 21 d did not differ between treatment groups (see reference 13 in appendix for details). In contrast, neuron counts in the injured CA1 hippocampus were markedly greater in isoflurane-treated rats ($p < 0.05$, Fig 4). Neuron counts in the injured CA3 hippocampus, however, did not differ significantly between treatment groups.
In the ICP protocol, again physiologic values, including PaCO₂, PaO₂, glucose and Hct, did not differ between anesthetic groups. As in the outcome protocol, MAP was higher in rats treated with fentanyl compared to their isoflurane counterparts \( (p < 0.05) \). ICP was similar in both anesthetic groups; however, there was a trend toward higher ICP in rats anesthetized with isoflurane by 3-4 h after TBI (Fig 5). This strongly suggests that the higher MAP in fentanyl vs isoflurane treated rats did not exacerbate intracranial hypertension. As expected from the difference in MAP, CPP was higher in the fentanyl treatment group (Fig 6).

Brain water content, assessed at 4 h after injury, was higher in the injured vs uninjured hemisphere \( (p < 0.05) \) for both anesthetic groups (Fig 7). However, brain water in either the injured or uninjured hemisphere did not differ between isoflurane- and fentanyl-treated rats, indicating that edema was not exacerbated in the fentanyl group.

Average MAP during the 4 h post-injury observation period did not differ significantly between fentanyl-treated rats and those recovering from CCI without anesthesia \( (157 \pm 6.2 \text{ mm Hg vs } 147 \pm 7.1 \text{ mm Hg, NS}) \). In contrast, isoflurane-anesthetized rats had lower MAP \( (105 \pm 5.5 \text{ mm Hg}) \) vs both fentanyl-treated rats and rats recovering without anesthesia \( (p < 0.05 \text{ vs both groups}) \).

**Recommendation:** The theoretical advantages of isoflurane vs fentanyl are compelling; however, explanations for the observed improvement in neurologic outcome after TBI in isoflurane-anesthetized rats remain to be determined. What has become clearer is that anesthetic agents may have considerable impact upon outcome following TBI. The results of this study suggest two important potential ramifications. First, isoflurane may not represent the optimal anesthetic in experimental TBI since it may mask potential benefits of novel therapies. Second, despite common clinical and field use, narcotics such as morphine or fentanyl may not be the optimal sedative/analgesic agent to administer to patients in the acute phase after severe head injury. We feel this has considerable relevance since narcotics (either fentanyl or morphine) are first line agents in field or emergency
department. Consideration should also be given to the possibility that like isoflurane anesthesia could be provided in the field. However, additional studies in rodent and large animal models of TBI are indicated. Specifically, further study of the mechanistic differences between isoflurane and fentanyl anesthesia is warranted. Our suspicion is that narcotics are not deleterious, rather general anesthetics such as isoflurane are powerfully beneficial. Defining the factors responsible for improved outcome with isoflurane may help to direct the clinical application of more optimal sedative or analgesic agents and possibly to identify novel therapies. Finally, since the immediate post-trauma sedative/analgesic regimen has such a powerful effect on both functional and histopathological outcome, a comprehensive comparison of field-relevant sedative/analgesic agents is suggested by this study and is underway (as described below).

Fig 7: Percent brain Water 4 h after TBI. Percent brain water in the injured hemisphere was increased vs respective non-injured hemisphere in both isoflurane- and fentanyl-treated rats; however, brain water did not differ between anesthetic groups. * p < 0.05, isoflurane vs fentanyl.

(C2) Randomized, blinded study in the rat model of CCI of seven different sedative/analgesic strategies for field use in TBI.

We are currently in the midst of completing a nine group (seven anesthetic) study in our model. Rats are prepared for TBI exactly as described in our protocol comparing isoflurane and fentanyl above. Anesthesia for surgical preparations is 2% isoflurane in nitrous oxide/oxygen. After surgical preparation, anesthesia is discontinued until tail-pinch response is obtained and then CCI is delivered. Rats are then randomized to one of the 8 groups below (n = 8 per group, Table 1). There is also a sham group (thus, a total of 9 groups). The sedation or anesthesia is maintained for a period of 60 min and the rats are then weaned and extubated when recovered. Outcome parameters are identical to the isoflurane vs fentanyl outcome study (motor and MWM function; lesion volume, hippocampal CA1 and CA3 cell counts. To date we have completed 30 of the studies.

Table 1. Sedation/analgesia study posttrauma

<table>
<thead>
<tr>
<th>Anesthetic/Sedative</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>1% by inhalation for 1 h</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>50 mg/kg iv</td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>Fentanyl</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td></td>
</tr>
<tr>
<td>Propofol</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>Sham</td>
<td>NA</td>
</tr>
</tbody>
</table>

1Isoflurane anesthesia discontinued and TBI immediately on return of tail pinch reflex.

Comment: This study will complete the expanded technical objective #3 (in lieu of elimination of objective #4) and is obvious, particularly since our isoflurane vs fentanyl study showed such a
powerful difference in outcome. We anticipate completing this ambitious protocol by February 28, 2000. Delay in beginning this protocol related to the need to perform pilot studies with each anesthetic in our CCI model.

(7) KEY RESEARCH ACCOMPLISHMENTS

In order of importance

Narcotics
- Narcotics, the standard field-treatment of victims of severe head injury (after intubation) and a front line treatment in emergency departments in the civilian sector had not been compared head-to-head with general anesthesia in a contemporary rodent model of TBI. We found that after experimental TBI, rats anesthetized with isoflurane exhibited markedly better functional and histopathological outcomes versus those treated with a narcotic (fentanyl). Narcotics probably are not the optimal sedative/analgesic early after TBI. Consideration should also be given to the possibility that light isoflurane anesthesia could be provided in the field.

Hyperventilation
- Aggressive hyperventilation for 4-5 h early after TBI is associated with an exacerbation of hippocampal neuronal death in selectively vulnerable brain regions adjacent to the contusion site.

Hypothermia
- Transient, moderate hypothermia, effective after TBI alone in prior studies, was demonstrated to be ineffective after experimental TBI in rats subjected to TBI with a superimposed secondary insult (hypoxemia). This may be clinically important since hypoxic patients have not been randomized in current clinical trials of hypothermia after TBI.

Mechanisms
- Moderate hypothermia reduces markers of injury (such as DNA damage) early after experimental TBI. However, sustained (12 h) of hypothermia was also surprisingly ineffective (on long-term outcome) after experimental TBI in rats. This suggests that although there are beneficial effects of hypothermia, there are potential side effects.

(8) REPORTABLE OUTCOMES

Manuscripts


$^\text{Full manuscripts from abstracts (see below) Whalen et al., and Ruppel et al. are also in preparation.}$

Abstracts and Presentations


AWARDS


(2000) Educational Scholarship to Dr. Kimberly Statler from the Society of Critical Care Medicine for her abstract entitled, “Isoflurane improves long-term neurologic outcome compared to fentanyl after traumatic brain injury in rats.”

(9) CONCLUSIONS

1. Based on the important finding in this study where the use of fentanyl in our CCI model produced deleterious effects on outcome after TBI, animal models should utilize clinically relevant sedative/analgesic treatments. The beneficial mechanisms of isoflurane (possibly promotion of cerebral blood flow or reduction of excitotoxicity) should be investigated for the development of novel treatments. Narcotics may not be the optimal sedative/analgesic early after TBI. Better field therapy than narcotics must be developed. Finally, more powerful sedative agents already in clinical use may represent better alternatives (and are currently under investigation in studies completing technical objective 3).

2. Based on studies in rats using the CCI model, we have demonstrated tangible risk to aggressive, indiscriminate hyperventilation early after injury—specifically—augmentation of neuronal death in selectively vulnerable brain regions. This suggests that aggressive hyperventilation should not be indiscriminately used in the field treatment of TBI, rather it should be applied if there are signs and/or symptoms of herniation. Mild hyperventilation (used in our control group) or normocapnia may be preferable.

16
3. Based on our studies in rats, hypothermia, although showing some beneficial effects, particularly early after TBI (such as a reduction in DNA damage, etc), may have some deleterious effects which result in only modest overall beneficial effects on long-term outcome. This is particularly true in the setting of severe injury (such as TBI plus secondary hypoxemic insults) where it is possible that there is little to gain except side effects. Also based on our narcotic (fentanyl) vs isoflurane study, hypothermia should be re-examined in future studies with narcotic anesthesia, since beneficial effects of isoflurane may be masking any benefit from hypothermia.

(10) REFERENCES


(11) APPENDIX

1. Figure 1
   Figure 2
   Figure 3
   Figure 4
   Figure 5
   Figure 6
   Figure 7

2. 1997 Report

3. 1998 Report

4. Curriculum Vitae

5. Manuscripts:
   Forbes ML, Clark RSB, Dixon CE, Graham SH, Marion DW, DeKosky ST, Schiding JK, Kochanek PM: Augmented neuronal death in CA3 hippocampus following


6. Abstracts:


(12) **BINDING (N/A)**

(13) **FINAL REPORTS**
   a) Bibliography of all publications and abstracts (see appendix)
   b) List of personnel receiving pay from the research effort
      - Patrick M. Kocharnek, M.D.
      - Peter Safar, M.D.
      - Henry Alexander
      - Scott Heineman
      - Marci Provins
      - Linda Amick
FIGURE 1
FIGURE 2

Latency (seconds)

Time (days)

*
FIGURE 3
CA1 neurons per h.p.f.

injured hemisphere

uninjured hemisphere

FIGURE 4
FIGURE 5

Time (hours)

ICP (mm Hg)
FIGURE 7
Grant Number DAMD17-97-1-7009

TITLE: Emergency Interventions After Severe Traumatic Brain Injury in Rats: Effect on Neuropathology and Functional Outcome

PRINCIPAL INVESTIGATOR: Patrick M. Kochanek, M.D.

CONTRACTING ORGANIZATION: University of Pittsburgh
Pittsburgh, Pennsylvania 15260

REPORT DATE: January 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5400

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
Traumatic brain injury (TBI) contributes to combat morbidity/mortality. We hypothesized that optimal emergency treatment can reduce brain injury in a rat model. Our ultimate goal is translation to the human condition. In yr-1, we studied mechanical ventilation strategies. We found that aggressive hyperventilation early after TBI is detrimental. Also, we developed a model of TBI plus secondary hypoxemia to study therapies, since secondary insults are common. In yr-2, we performed 3 studies, and began a 4th addressing objectives 2-3. We found that TBI plus secondary hypoxemia was refractory to 4 h of hypothermia—suggesting the need for combination therapies. We also tested prolonged hypothermia (12 h) in our model. Hypothermia improved motor function early after injury. However, by 2 wks, rats treated with hypothermia deteriorated and were ultimately worse (vs normothermia). This suggests the need for studies of hypothermia plus other therapies. We found that the NMDA antagonist MK-801 improved outcome after TBI—suggesting excitotoxicity as a promising therapeutic target. Fentanyl is used in patients with TBI, but lacks anti-excitotoxic properties. We are evaluating fentanyl in our model. Two fellows worked with the PI, and presented 3 abstracts (2-4). We also published an invited review (6).
Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

13-Jan-99

PT - Signature Date

3
# U.S. Army Medical Research Acquisition Activity

## 1998 Annual Technical Report

### Table Of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF 298</td>
<td>2</td>
</tr>
<tr>
<td>FOREWORD</td>
<td>3</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>4</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>5</td>
</tr>
<tr>
<td>BODY</td>
<td>6</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>13</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>13</td>
</tr>
</tbody>
</table>
INTRODUCTION

In our application, we highlighted the fact that traumatic brain injury (TBI) is an important contributor to combat casualty morbidity and mortality. We also stated that although progress has been made in the development of rodent models of TBI (such as the controlled cortical impact (CCI) model), most of the studies with these models have focused on molecular mechanisms of damage. Because there has been a paucity of application of practical emergency interventions in TBI models, we felt that it was important to address this deficiency and that this approach could have important implications for field and emergency management of both soldiers and civilians with severe TBI.

Our overall hypothesis is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI (respiratory management, temperature control, and sedation) can reduce secondary brain injury in a rat model of brain contusion, and thereby improve functional and/or neuropathological outcome.

In the yr-1 of funding, we addressed the most important aspect of the first Technical Objective of our proposal -namely- to perform a comprehensive study of the effects of mechanical ventilation strategies (as applied by the first responder in the field) on both functional and neuropathological outcome in our model. We found that aggressive, prophylactic hyperventilation (HV) applied for 4 hours immediately after injury is detrimental (vs ventilation to a normal PaCO₂), and leads to an increase in the amount of neuronal death in selectively vulnerable brain regions. This study was published as a full manuscript in the Journal of Neurosurgery (1). We were pleased that the reviewers indicated that this was an important study that would be cited often.

Also, to set the stage for the evaluation of therapies targeting improvement in outcome after severe TBI (as proposed studies in technical objectives 2-4), it appeared that it would be optimal to have TBI models both with and without a secondary insult since such insults are common in the field. This was done by adding a 30 min period of moderate hypoxemia to the CCI insult. The characterization of that model was described in last year's report and presented this year at the National Neurotrauma Society Meeting (2). As evidenced below, during yr-2, we have used both the standard CCI model and the CCI plus secondary insult model to provide insight on important therapies.

This year we performed three comprehensive studies addressing Technical Objective III and part of Objective II. In addition, we have begun a fourth study. These studies included 1) assessment of the effect of transient (4 h), moderate hypothermia on outcome after TBI with a secondary insult, 2) assessment of the effect of prolonged (12 h) moderate hypothermia on outcome after TBI, 3) assessment of the effect of the application of anti-excitotoxic therapy (the NMDA-receptor antagonist MK-801) early after TBI in our model, and 4) comparison of injury using two different anesthetic regimens (isoflurane or fentanyl [the standard emergency department and ICU sedative]). The results of these studies are summarized below. Finally, two research fellows (Drs. C. Robertson and R. Ruppel) worked on these projects with the PI (Dr. Kochanek) during yr-2. Dr. Robertson presented two abstracts of this work-- at the 1999 annual meeting of the National Neurotrauma Society (2,3) --and will present another abstract at the
Annual Meeting of the Society of Critical Care Medicine (4). That work is currently being prepared in full manuscript form. Also, in related studies, we recently reported that 4 hours of moderate hypothermia attenuates DNA damage assessed at 4 hours after injury using the Klenow method (5). Finally, some of our work on hypothermia in TBI was summarized in an invited review article that we published in a monograph by the International Trauma, Anesthesia and Critical Care Medicine Society (ITACCS)(6).

(6) BODY

(a) Technical Objective 1: Mechanical ventilation strategies after severe TBI in rats (see summary for 1996-1997 [yr-1]). Also see reference 1.

Recommendation

We showed that aggressive, early HV after TBI augments neuronal death in CA3 hippocampus in a rat model of cerebral contusion. The further reduction of CBF with HV during the low cerebral blood flow state immediately after injury coupled with alkalosis may increase the vulnerability of selected neurons to damage. The results of this study reinforce the meticulous attention necessary to prevent secondary injury after TBI. A risk in the use of HV is demonstrated. Unless signs of impending herniation (unilateral pupillary dilation, hypertension, bradycardia) are present, this study supports the targeting of normocarbia for mechanical ventilation in the emergency stabilization of the brain trauma victim.

(b) Technical Objectives 2-4: Testing of field-relevant therapies in experimental models of severe TBI (with and without a secondary hypoxemic insult) in rats.

(b1) Effect of transient (4 h), moderate (32°C) hypothermia on functional and histological outcome after experimental TBI with a superimposed secondary hypoxemic insult in rats.

We tested the effect of 4 hours of hypothermia in our model of TBI with a 30-min secondary hypoxemic insult. Hypothermia has been shown to be effective in a variety of experimental models with transient application (1-4 h) and in humans (32°C applied for 24 h). However, in neither experimental nor clinical TBI has hypothermia been tested when applied after the combination of TBI with a secondary insult.

Method

All of the protocols for the studies outlined below were approved by the Animal Care and Use Committee of the University of Pittsburgh. Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 43) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) followed by a 30 min controlled hypoxic insult that reproducibly results in a PaO₂ of 40-45 mm Hg. Rats were treated with one of the following three regimens—1) Brain temperature maintained at 37°C applied throughout a 5 hours period (n = 19), 2) Brain temperature maintained at 32°C applied for 4 hours beginning after insult (beginning after both TBI and secondary hypoxemia) and then followed by re-warming over 1 hour (n = 14), and 3) Brain temperature maintained at 37°C applied immediately after TBI (before the secondary
hypoxemic insult) and continued for 4 hours and followed by re-warming over 1 hour (n = 10). After 5 h, rats were weaned from mechanical ventilation, extubated and returned to their cages. Beam balance/beam walking and Morris water maze (MWM) performance latencies were measured in eight rats from each group on days 1-5 and 14-20 post CCI, respectively. Rats were killed at 21 d. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

**Results**

There were no significant differences in recovery of motor function (beam balance, beam walking, Figure 1) tested on days 1-5 after injury or cognitive function (spatial memory acquisition paradigm on the Morris water maze [MWM], Figure 2) tested between days 14-20 after injury. There were also no significant differences in lesion volume or hippocampal neuron counts between groups at 21 days after injury (Table 1). There was a trend toward reduced contusion volume in the immediate post injury group, however, it did not reach statistical significance.
Table 1. Effect of transient moderate hypothermia on histological outcome at 21 days after experimental TBI with secondary hypoxemic insult in rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Rat survival rate</th>
<th>Contusion Volume</th>
<th>CA3 Survival, mean # neurons per hpf</th>
<th>CA1 Survival, mean # neurons per hpf</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>15/19 (78.95%)</td>
<td>mm² =65.34±6.94</td>
<td>19.8 ±4.6</td>
<td>19.4 ±4.2</td>
</tr>
<tr>
<td>32 °C, application delayed 30 min until after secondary hypoxemic insult</td>
<td>8/14 (57.14%)</td>
<td>mm² =53.69±7.93</td>
<td>18.5 ±7.3</td>
<td>13.7 ±5.8</td>
</tr>
<tr>
<td>32 °C, application begun immediately after TBI, before secondary hypoxemic insult</td>
<td>8/10 (80.00%)</td>
<td>mm² =50.17±8.23</td>
<td>15.6 ±7.3</td>
<td>13.2 ±8.7</td>
</tr>
</tbody>
</table>

All data are mean ± SEM

Discussion

Surprisingly, we found that the combined insult of TBI plus secondary hypoxemia was refractory to 4 hours of moderate hypothermia. This is an important finding that was presented in November, 1998 at the annual Meeting of the National Neurotrauma Society, and will be presented in January, 1999 Meeting of the Society of Critical Care Medicine. It suggests the need for combination therapies in this setting. Alternatively, it was possible that the combined TBI plus hypoxemia insult was too severe to favorably effect outcome with any therapy. To address that possibility, we proceeded to perform two studies. These are outlined below.

(b2) Effect of prolonged (12 h), moderate (32 °C) hypothermia on functional and histological outcome after experimental TBI in rats.

The need for combined therapies was suggested, again by the second trial of hypothermia we performed this year. In the second experimental paradigm, we sought to test, to our knowledge for the first time in any laboratory, the prolonged application of hypothermia in a rodent model of TBI. This included over 13 hours of controlled mechanical ventilation and physiological monitoring. To date, only brief 1-4 hours applications have been tested. In this study, we examined TBI without a secondary insult.

Method

Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 20) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) and treated with one of the following two regimens—1) Brain temperature maintained at 37°C applied throughout a 13 hours period (n = 10),

2) Brain temperature maintained at 32°C applied for 12 hours beginning after insult (beginning after TBI and followed by re-warming over 1 hour [n = 10]). Rats were then weaned from mechanical ventilation, extubated and returned to their cages. They tolerated the procedure remarkably well. Beam balance/walking and MWM performance latencies were measured in all
rats from each group on days 1-5 and 14-20 post CCI, respectively. Rats were killed at 21 days. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

Results

Motor function, as quantified by beam walking task score recovered more rapidly in rats treated with hypothermia. (beam walking p=0.06 vs normothermia, Figure 3A). In contrast, rats deteriorated between 5 and 14 days after injury as reflected by the fact that cognitive function (spatial memory acquisition paradigm on the MWM, Figure 4) tested between days 14-20 after injury was worse in the hypothermia treated group. Histology, from these rats is currently being processed.

![Figure 3. Effect of prolonged (12 h) of hypothermia on motor outcome after experimental TBI in rats. Mean beam walking score (mean ± SEM, in sec) in rats before and on days 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed a trend toward a significant difference in favor of hypothermia (p=0.06). Data are mean ± SEM.]

![Figure 4. Effect of prolonged (12 h) hypothermia on cognitive outcome after experimental TBI in rats. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on days 14-20 after CCI is depicted. There was a trend towards a worsening by hypothermia (p=0.082) when treatment groups were compared using ANOVA with repeated measures. Data are mean ± SEM.]

Discussion

In this demanding experimental paradigm, testing 12 hours of hypothermia, we found that there were beneficial effects of hypothermia on motor function during the initial 5 days after TBI. However, by 2-3 wks after injury, rats treated with hypothermia had deteriorated and their performance on cognitive outcome tasks (MWM) was worse than the group treated with normothermia. One possible explanation for this is the inhibition of nerve growth factor synthesis by hypothermia (previously shown by our co-investigator, S. DeKosky). Thus, acute benefits of hypothermia on mechanisms such as cerebral swelling may be counterbalanced by detrimental effects on “regeneration” or other mechanisms yet to be defined. It is our opinion that this may be an extremely important finding. These data also again strongly suggest the need for studies of hypothermia plus other therapies during and after re-warming. To further strengthen these data, in year 3 we will again compare 12 hours of hypothermia vs normothermia in a squadron of rats, examining its effect on brain edema, intracranial hypertension, and markers of neuronal death (DNA damage) early after insult (at the completion of the 12 hours period of temperature control). If these markers are favorably affected (as anticipated), it would mirror the clinical condition, and strengthen the relevance of our model for the proposed studies in year 3 (combination treatments). Recently, we demonstrated that 4 hours of hypothermia reduces DNA damage in our CCI model (Whalen et al, Soc for Neurosci Abstract,

(b3) Effect of the anti-excitotoxic NMDA-receptor antagonist MK-801 on functional and histological outcome after experimental TBI in rats.

In experimental cerebral ischemia, Dietrich et al (J Cereb Blood Flow Metab 15:960, 1995) demonstrated efficacy of transient hypothermia plus sustained treatment (for several days after insult) with the anti-excitotoxic agent MK-801. The delayed deterioration after 1 wk in our model seen with the application of hypothermia suggests the possible need for combined therapies. In the third experimental paradigm, we sought to test the effect of the traditional NMDA-receptor antagonist MK-801 in our TBI model (without secondary insult), to set the stage for combination therapies.

Method

Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 30) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) and treated with one of the following two regimens—1) MK-801 (a single 1 mg/kg IP dose immediately after injury) or vehicle. A separate sham group (all surgery including craniotomy, but no TBI was also studied. Brain temperature maintained at 37°C during TBI. Rats were then weaned from mechanical ventilation, extubated and returned to their cages. They tolerated the procedure remarkably well. Beam balance/walking and Morris water maze (MWM) performance latencies were measured in all rats from each group on days 1-5 and 14-20 post CCI, respectively. Rats were killed at 21 d. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.
Results

Motor function, as quantified by both beam balance and beam walking tasks recovered more rapidly in rats treated with MK-801 (Figure 5). MWM performance in MK-801-treated rats did not differ between treatment groups (Figure 6). However, a significantly improved performance in the probe trial (Figure 7) was seen in MK-801 vs vehicle groups. Lesion volume data did not differ between groups (Table 2). There was similar tissue loss in both MK-801 and vehicle treated groups in the injured hemisphere at 21 days after injury. Hippocampal cell counts are still being processed.

Figure 5A-B. Effect of MK-801 treatment on motor outcome after experimental TBI in rats. Mean beam balance (A) and beam walking (B) performance latencies (mean ± SEM, in sec) in rats before and on days 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed a significant group difference. For both tests, MK-801 treated groups recovered sooner than saline treated groups (*p<0.05 vs vehicle). Data are mean ± SEM.

Morris Water Maze Performance

Figure 6. Effect of MK-801 on cognitive outcome after experimental TBI in rats. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on days 14-20 after CCI is depicted. There was no significant effect of MK-801 treatment (vs vehicle). Data are mean ± SEM.
Discussion

Remarkably, the NMDA antagonist MK-801 was effective in improving both motor function and some aspects of cognitive function after CCI. The motor effects were as dramatic or more dramatic than those seen with 12 hours of hypothermia. In a separate pilot study, MK-801 was not effective when tested in our TBI plus secondary hypoxemia model, suggesting this insult may be too severe for any single therapy. Although this specific agent is not available for clinical use, it suggests that this category of agents—targeting excitotoxicity—is a viable strategy for application with hypothermia.

(b4) Comparison of the effects of TBI on functional and histological outcome after experimental TBI in rats anesthetized with isoflurane or fentanyl.

Many, but not all, sedatives (such as barbiturates and Ketamine) target excitotoxicity. Currently, in clinical practice, fentanyl is the most commonly used emergency sedative for the
intubated patients with severe TBI. Fentanyl, has little direct anti-excitotoxic properties. Thus, we have begun to investigate how fentanyl anesthesia compared to standard isoflurane anesthesia in our model. In pilot studies, we noted that rats became markedly hypertensive and died early after TBI when anesthetized with fentanyl (but not isoflurane) in our standard TBI model. Thus, we are currently testing the use of fentanyl vs isoflurane anesthesia in our CCI model, using a slightly lesser degree of injury (2.0 mm depth of penetration rather than 2.5 mm depth—an insult with a low mortality rate in both groups). Since fentanyl is the standard of care in management of patients with TBI (in both the emergency department and the ICU), these results could have important clinical implications if fentanyl is found to be deleterious in our model.

(7) CONCLUSION

In our work during the second year of funding addressing portions of Technical Objective #2 and 3, we demonstrated that hypothermia plus anti-excitotoxic therapies represent an excellent potential combination therapy to test in our model of experimental TBI. In addition, we demonstrated that the combination of TBI plus a secondary insult not only results in severe deficits and large lesions after injury, but is remarkably refractory to either hypothermia or anti-excitotoxic treatment. In addition, we have begun studies suggesting that the current agent used for sedation in emergency departments and ICUs (fentanyl) may not be an optimal sedative agent. In year three we are going to first define the optimal sedative approach for field use in our TBI model (Completing Objective 2 and 3). We will then combine that approach with hypothermia in an attempt to target Objective 4 and model the best possible clinically-relevant approach for field use, both in civilian and military settings.

(8) REFERENCES


Grant Number DAMD17-97-1-7009

TITLE: Emergency Interventions After Severe Traumatic Brain Injury in Rats: Effect on Neuropathology and Functional Outcome

PRINCIPAL INVESTIGATOR: Patrick M. Kochanek, M.D.

CONTRACTING ORGANIZATION: University of Pittsburgh
Pittsburgh, Pennsylvania 15260

REPORT DATE: January 1998

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
Emergency Interventions After Severe Traumatic Brain Injury in Rats: Effect on Neuropathology and Functional Outcome

Patrick M. Kochanek, M.D.

University of Pittsburgh
Pittsburgh, Pennsylvania 15260

U.S. Army Medical Research and Material Command
Fort Detrick, Maryland 21702-5012

Our hypothesis is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI (respiratory management, temperature control, and sedation) can reduce brain injury in a rat model of brain contusion, and thereby improve functional and neuropathological outcome. In the first year of funding, we addressed the first Technical Objective of our proposal — to perform a comprehensive study of the effects of mechanical ventilation strategies on outcome. We found that aggressive hyperventilation applied for 4 h immediately after injury is detrimental (vs normal ventilation) and increases neuronal death in vulnerable brain regions.

Also, to set the stage for the evaluation of therapies targeting improved outcome after TBI (proposed in Technical Objectives 2-4), the severity of the insult was increased in our model. This was done by more accurately simulating the field scenario (adding a secondary insult). Finally, another injury station was established and a technician was trained to perform the studies proposed in y 2-3. Dr. Michael Forbes, a fellow completed his training during this year and was the first Author of the manuscript described above.

Traumatic brain injury (TBI) contributes to combat casualty morbidity and mortality. Our hypothesis is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI (respiratory management, temperature control, and sedation) can reduce brain injury in a rat model of brain contusion, and thereby improve functional and neuropathological outcome. In the first year of funding, we addressed the first Technical Objective of our proposal — to perform a comprehensive study of the effects of mechanical ventilation strategies on outcome. We found that aggressive hyperventilation applied for 4 h immediately after injury is detrimental (vs normal ventilation), and increases neuronal death in vulnerable brain regions.

Also, to set the stage for the evaluation of therapies targeting improved outcome after TBI (proposed in Technical Objectives 2-4), the severity of the insult was increased in our model. This was done by more accurately simulating the field scenario (adding a secondary insult).

Finally, another injury station was established and a technician was trained to perform the studies proposed in y 2-3. Dr. Michael Forbes, a fellow completed his training during this year and was the first Author of the manuscript described above.
Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

✓ Where copyrighted material is quoted, permission has been obtained to use such material.

✓ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

✓ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

✓ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

✓ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

✓ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

✓ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

✓ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

[Signature]

[Date: 17, 1998]
<table>
<thead>
<tr>
<th>Paragraph</th>
</tr>
</thead>
<tbody>
<tr>
<td>5) INTRODUCTION</td>
</tr>
<tr>
<td>In our application, we highlighted the fact that traumatic brain injury (TBI) is an important contributor to combat casualty morbidity and mortality. We also stated that although progress has been made in the development of rodent models of TBI (such as the controlled cortical impact (CCI) model), most of the studies with these models have focused on molecular mechanisms of damage. Because there has been a paucity of application of practical emergency interventions in TBI models, we felt that it was essential to address this deficiency and that this strategy could have important implications for field and emergency management of both soldiers and civilians with severe TBI. Our overall hypothesis is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI (respiratory management, temperature control, and sedation) can reduce secondary brain injury in a rat model of brain contusion, and thereby improve functional and/or neuropathological outcome.</td>
</tr>
<tr>
<td>In the first year of funding, we addressed the most important aspect of the first Technical Objective of our proposal - namely-to perform a comprehensive study of the effects of mechanical ventilation strategies (as applied by the first responder in the field) on both functional and neuropathological outcome in our model. We found that aggressive, prophylactic hyperventilation (HV) applied for 4 h immediately after injury is detrimental (vs ventilation to a normal PaCO₂, normal ventilation [NV]), and leads to an increase in the amount of neuronal death in selectively vulnerable brain regions. This study, is now in press as a full manuscript in the Journal of Neurosurgery (1), (also see Appendix #1). We were pleased that the reviewers indicated that this was an important study which would be cited often.</td>
</tr>
<tr>
<td>In addition, to set the stage for the evaluation of therapies targeting improvement in outcome after severe TBI (as proposed studies in technical objectives 2-4), it appeared that it would be optimal to increase the severity of the insult in our model. This was done by attempting to more accurately simulate the field scenario — i.e., adding a 30 min period of moderate hypoxemia to the insult. The characterization of that model for our future studies will also be described below.</td>
</tr>
<tr>
<td>During the first year of funding, Henry Alexander, an experienced technician assumed the technical duties of the injury model and has successfully learned the model to perform all of the subsequent injury studies in years 2 and 3. Also, a new injury device and station were purchased and is in operation for these studies. Finally, Dr. Michael Forbes, a fellow in Pediatric Critical Care Medicine completed his training during this first year of funding and was the team leader on our study assessing the effect of HV in our model. He was the first author of the manuscript describing that work. Dr. Forbes is now Associate Director of the Pediatric Intensive Care Unit at Allegheny General Hospital in Pittsburgh.</td>
</tr>
<tr>
<td>6) BODY</td>
</tr>
<tr>
<td>(a) Technical Objective 1: Mechanical ventilation strategies after severe TBI in rats</td>
</tr>
<tr>
<td>For over two decades, HV has been one of the most utilized strategies in the management of TBI. Laboratory and clinical studies, however, have verified that early after TBI, there is usually a state of reduced cerebral perfusion that may increase vulnerability to secondary injury. HV reduces intracranial hypertension by reducing cerebral blood volume; however, this generally is accompanied by a reduction in cerebral blood flow. A recent clinical study</td>
</tr>
</tbody>
</table>
suggested that HV may worsen outcome after TBI. However, in the field or during the initial stabilization, HV is often used (either planned or iatrogenically) and the first blood gas of patients in the emergency room can reveal significant hypocarbia. Using the CCI model in rats, we tested the effect of 4 h of aggressive HV (vs NV), beginning immediately after injury, on functional and neuropathological outcome.

**Methods:**

All of the protocols for the studies outlined below were approved by the Animal Care and Use Committee of the University of Pittsburgh. Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats \((n = 26)\) were subjected to CCI \((4 \text{ m/s}, 2.5 \text{ mm depth of deformation})\) and randomized after 10 min to either HV \([n = 13, P_aCO_2 = 20.3 \pm 0.7 \text{ mm Hg}]\) or NV \([n = 13, P_aCO_2 = 34.9 \pm 0.3 \text{ mm Hg}]\) for 5 h. Beam balance and Morris water maze (MWM) performance latencies were measured in eight rats from each group on d 1-5 and 7-11 post CCI, respectively. Rats were killed at 14 d. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

**Results:**

HV was readily achieved and could be sustained for 4 h in our model, and produced the anticipated systemic alkalosis (Figure 1). In addition, other variables could be tightly controlled for 4 h in our model (Figure 1).

![Figure 1](image-url)  
*Figure 1. Entire time course of (A) PaCO_2 (mm Hg), (B) arterial pH, (C) Mean arterial blood pressure (MABP, mm Hg), and (D) brain temperature (°C) in all rats treated with either NV (▲, \(n = 13\)) or HV (■, \(n = 13\)) after CCI. *p < 0.05 for NV vs HV. Data are mean ± SEM.
Mortality rates were similar in both groups (2/13 vs 3/13, NV vs HV, respectively, NS). There were no differences between groups in mean arterial blood pressure, brain temperature, and serum glucose concentration. There were no differences between groups in either performance latencies for both beam balance (Figure 2) and MWM (Figure 3) or contusion volume (Figure 4).

**Figure 2.** Mean beam balance performance latencies (mean ± SEM, in sec) in rats before and on d 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed no difference in duration of balance maintained between the two groups. (▲, NV, n = 8; ■, HV, n = 8). Data are mean ± SEM.
Figure 3. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on d 7-11 after CCI. There was no between group difference (▲, NV, n = 8; ■, HV, n = 8) when performances were compared using ANOVA with repeated measures. Data are mean ± SEM.
Figure 4. Graph depicting mean lesion area (left y-axis, mm$^2$) vs distance from occiput (mm) measured 14 d after CCI (NV, open bars, n = 11, HV, closed bars, n = 10). Contusion volume (mm$^3$) was calculated as the sum of these areas in each group and is depicted as cumulative volume (right y-axis) in the NV, △, and HV, ■, groups. There was no difference between groups in contusion volume (27.8 ± 5.1 vs 27.8 ± 3.1 mm$^3$ NV vs HV, mean ± SEM).

However, in brain sections through the center of the contusion, hippocampal neuronal survival in HV reduced the number of surviving hippocampal CA3 neurons (29.7 [24.2 - 31.7] vs 19.9 [17.0 - 23.7] cells/high power field (NV vs HV, median [25$^{th}$ -75$^{th}$ percentiles] *p < 0.05, Mann-Whitney Rank Sum Test, Figure 5). In contrast to the detrimental effect on CA3 neurons, CA1 neuronal death was not increased by aggressive HV.
Figure 5. Box plots representing the number of surviving CA1 and CA3 hippocampal neurons in coronal brain sections through the center of the lesion in the hemisphere ipsilateral to the contusion. Cells were counted 14 d after injury. The median line is placed within the shaded 25th - 75th range. There was a reduction in the number of surviving CA3 hippocampal neurons after injury comparing NV and HV groups (29.7 [24.2 - 31.7] vs 19.9 [17.0 - 23.7], cells/high power field (hpf), *p < 0.05, Mann-Whitney rank sum test).

Conclusion:
Aggressive HV early after TBI augments CA3 hippocampal neuronal death; however, it did not impair functional outcome or expand the contusion. These data suggest that CA3 hippocampal neurons are selectively vulnerable to the effects of HV early after TBI. The injudicious application of HV early after TBI, such as in the field, may contribute to secondary neuronal injury.

Comment:
Hippocampal CA3 neurons are selectively vulnerable to delayed neuronal death after TBI. The mechanisms underlying this process remain speculative. Potential mechanisms include ischemia, TBI-induced excitotoxicity, apoptosis, and inflammation. We previously demonstrated that the hippocampus and cortex ipsilateral to the impact have marked flow reduction (at least 60%) at 2 h after TBI in the CCI model (2). CBF approaches ischemic levels in the core of the contusion at 2 h after injury. Although we have not evaluated the status of reactivity of the cerebral circulation to changes in PaCO2 at 2 h after TBI in this model, we have reported that CO2 reactivity is impaired, although still present (62-71% of baseline) in and around the contusion at 24 h after CCI in rats (3).
HV produces cerebral vasoconstriction and alkalosis. Alkalosis exacerbates N-methyl-D-aspartate (NMDA)-receptor mediated neurotoxicity. As a result of aggressive HV, the rats in our study were quite alkalotic as indicated by arterial pH measurements. Alkalosis appears to have deleterious effects on neurons. It could also be that the combined effect of alkalosis and further flow reduction by HV is deleterious in regions vulnerable to excitotoxicity such as CA3. Early, aggressive or prophylactic HV, therefore, in the context of reduced CBF, may exacerbate excitotoxic mechanisms and augment neuronal death.

Aggressive HV in the early low flow period did not worsen functional outcome or expand the contusion. The cognitive deficits in this model are modest. Indeed, to test therapies targeting an improvement in outcome, we may need a more severe injury (see below). Additional unilateral or bilateral hippocampal damage may be necessary to create more marked functional deficits. CA3 damage alone may not mediate post-TBI MWM deficits. However, hippocampal damage and memory deficits are common after TBI in humans, and exacerbation of neuronal death in any brain region would be highly undesirable.

This study does not completely address the uncommon situation where early after severe head injury marked intracranial hypertension is observed. HV may, in fact, be life saving in the setting of impeding herniation. Similarly, we did not measure ICP or titrate ventilation to control cerebral perfusion pressure, and we evaluated only one level of HV and injury severity. We did not attempt to model the clinical scenario of optimal titration of ventilation when ICP is increased. Rather, we chose to evaluate the field setting and apply the worst case scenario, aggressive HV during the early post-trauma period when flow is already low and excitotoxicity is peaking. Our study does, however, show that HV is associated with a tangible risk to vulnerable neurons. To our knowledge, this is the first in vivo study demonstrating that HV can augment neuronal injury after TBI. This suggests that there is indeed a trade-off associated with this intervention.

**Recommendation**

We have shown that aggressive, early HV after TBI augments neuronal death in CA3 hippocampus in a rat model of cerebral contusion. The further reduction of CBF with HV during the low cerebral blood flow state immediately after severe TBI coupled with alkalosis may increase the vulnerability of selected neurons to damage. The results of this study reinforce the meticulous attention necessary to prevent secondary injury after TBI. A risk in the use of HV is demonstrated. Unless signs of impending herniation (unilateral pupillary dilation, hypertension, bradycardia) are present, this study supports the targeting of normocarbia for mechanical ventilation in the emergency stabilization of the brain trauma victim.

(b) A field scenario of severe TBI for the evaluation of therapies proposed in Technical Objectives 2-4.

Using our standard CCI model, neuronal death in selectively vulnerable regions and MWM deficits are present but modest. In that model, we are able to nicely demonstrate exacerbation of damage with a deleterious strategy, namely HV. However, in technical objectives 2-4, our goal is to define strategies (hypothermia, anesthetics, anti-excitotoxic therapies) that will mitigate damage. Thus, the severity of damage must be increased, both from the standpoint of both hippocampal neuronal death and MWM deficit, to achieve this goal. Recently, in studies separate from this application, we published a variation of our CCI model that was designed to
increase the amount of hippocampal damage without totally destroying the hippocampus (and making it impossible to resuscitate)(4). This was achieved by adding a 30 min period of moderate hypoxemia (FiO₂ = 0.11) which also results in accompanying mild hypotension. This mimics the secondary insults in head injury victims so commonly seen in the field. In addition, in studies by our group separate from this application (5), we reported that both necrotic and apoptotic neuronal death is seen in this new variant of the CCI model. To be certain that this model would be suitable for technical objectives 2-4, it was essential to determine if the insult was accompanied by a significant MWM deficit.

**Methods:**

All of the protocols for the studies outlined below were approved by the Animal Care and Use Committee of the University of Pittsburgh. Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 20) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) to the left parietal cortex using either a vertical or angled impact. Immediately after injury, the FiO₂ was reduced to 0.11 (inhalational anesthesia maintained constant by the addition of N2 to the ventilator circuit). At 5 min after reducing the FiO₂ and at the completion of the 30 min secondary hypoxic insult, a blood gas is obtained to document the level of hypoxemia achieved, and then the FiO₂ is increased. Shams were subjected to all surgical procedures, but no insult (i.e., neither CCI nor hypoxemia). After the recovery periods, catheters were removed and anesthesia was discontinued. Rats were weaned from mechanical ventilation, extubated, and returned to their cages until further study. Motor and cognitive outcome were assessed as previously described.

**Results:**

Both vertical and angled impacts resulted in significant motor and cognitive deficits as assessed by beam balance, and MWM paradigms (Figures 6, and 7, respectively).

![Figure 6](image_url)

*Figure 6. Mean beam balance performance latencies (mean ± SEM, in sec) in rats before and on d 1-5 after either vertical or angled CCI with secondary hypoxic insult. Analysis of variance with repeated measures revealed no difference in duration of balance maintained between the two insults. Both insults were significantly different from sham.*
Conclusion:

Combined with our prior publications showing both a well defined contusion and neuronal death by both apoptosis and necrosis in this model (4,5), the functional deficits produced with either a vertical or angled insult set the stage for studies proposed in Technical Objectives 2-4. We have chosen to use the vertical insult with hypoxemia, since all of our initial studies with HV used a vertical impact. These will be addressed in years 2-3 of the funding period. In accordance with this plan, we are currently evaluating the effect of hypothermia in this model of CCI with a secondary insult (as outlined in Technical Objective #2). We plan to address Technical Objective 2 and part of Technical Objective 3 in funding year 2.

(7) CONCLUSION

In our work during the first year of funding addressing Technical Objective #1, we demonstrated that aggressive HV early after TBI augments CA3 hippocampal neuronal death. These data suggest that CA3 hippocampal neurons are selectively vulnerable to the effects of HV early after TBI. The injudicious application of HV early after TBI, such as in the field, may contribute to secondary neuronal injury. As previously discussed, the results of this study reinforce the meticulous attention necessary to prevent secondary injury after TBI. A risk in the use of HV is demonstrated. Unless signs of impending herniation (unilateral pupillary dilation, hypertension, bradycardia) are present, this study supports the targeting of normocarbia for mechanical ventilation in the emergency stabilization of the brain trauma victim. Finally, by adding a secondary insult to our injury model, we have set the stage to address the optimal
application of treatments to improve outcome as outlined in Technical Objectives 2-4, and those
studies are underway.

(8) REFERENCES


**FACULTY DATA SHEET**

<table>
<thead>
<tr>
<th>NAME:</th>
<th>PATRICK MICHAEL KOCHANEK, M.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAMPUS ADDRESS:</td>
<td>Safar Center for Resuscitation Research</td>
</tr>
<tr>
<td>CURRENT TITLE &amp; EFFECTIVE DATE:</td>
<td>Associate Professor February 1, 1991</td>
</tr>
<tr>
<td>DATE APPOINTED TO FACULTY:</td>
<td>July 1, 1986 LENGTH OF TERM:</td>
</tr>
<tr>
<td>DATE APPOINTMENT EXPIRES:</td>
<td>June 1999</td>
</tr>
<tr>
<td>DATES OF PROMOTIONS:</td>
<td>February 1991</td>
</tr>
<tr>
<td>CERTIFICATION:</td>
<td>ABA: American Board of Pediatrics Sub-Board of Pediatric Critical Care Medicine</td>
</tr>
<tr>
<td>TENURE:</td>
<td>Tenured - 1997</td>
</tr>
<tr>
<td>MEDICAL SCHOOL GRADUATE:</td>
<td>University of Chicago</td>
</tr>
<tr>
<td>HOSPITAL OF RESIDENCY:</td>
<td>University of California, San Diego</td>
</tr>
<tr>
<td>YEAR RESIDENCY COMPLETED:</td>
<td>1983</td>
</tr>
<tr>
<td>SOCIAL SECURITY NUMBER:</td>
<td></td>
</tr>
<tr>
<td>DATE OF BIRTH:</td>
<td></td>
</tr>
<tr>
<td>CITIZEN:</td>
<td>USA</td>
</tr>
<tr>
<td>NAME OF SPOUSE:</td>
<td>Denise</td>
</tr>
<tr>
<td>UPDATED:</td>
<td>January 10, 2000</td>
</tr>
<tr>
<td>NAME: Patrick M. Kochanek, M.D.</td>
<td>BIRTH DATE:</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>HOME ADDRESS:</td>
<td>BIRTH PLACE:</td>
</tr>
<tr>
<td>HOME PHONE:</td>
<td>CITIZENSHIP: USA</td>
</tr>
<tr>
<td>BUSINESS ADDRESS: Safar Center for Resuscitation Research 201 Hill Building 3434 Fifth Avenue Pittsburgh, PA 15260</td>
<td>SOCIAL SECURITY NO:</td>
</tr>
<tr>
<td>BUSINESS PHONE: (412) 383-1900</td>
<td></td>
</tr>
</tbody>
</table>

**EDUCATION AND TRAINING**

<table>
<thead>
<tr>
<th>Undergraduate</th>
<th>Graduate</th>
<th>Post Graduate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972 - 1976</td>
<td>University of Michigan</td>
<td>University of California</td>
</tr>
<tr>
<td>Ann Arbor, Michigan</td>
<td>San Diego, California</td>
<td>San Diego, California</td>
</tr>
<tr>
<td>B.S., 1976</td>
<td>M.D., 1980</td>
<td>Pediatric Internship</td>
</tr>
<tr>
<td>Zoology</td>
<td>William Nyhan, M.D.</td>
<td>William Nyhan, M.D.</td>
</tr>
</tbody>
</table>

**ACADEMIC**

| 1986-1991 | University of Pittsburgh School of Medicine Department of Anesthesiology/CCM Pittsburgh, PA |
| 1991 - present | University of Pittsburgh School of Medicine Department of Anesthesiology/CCM Pittsburgh, PA |
| Assistant Professor | Associate Professor |
Patrick M. Kochanek, MD

1986 - 1991
University of Pittsburgh School of Medicine
Department of Pediatrics
Pittsburgh, PA
Assistant Professor

1991 - present
University of Pittsburgh School of Medicine
Department of Pediatrics
Pittsburgh, PA
Associate Professor

NON ACADEMIC

1983 - 1986
Hyperbaric Medicine Program
Center/Naval Medical Research Institute
Washington, DC
Guest Scientist

1986 - present
Children's Hospital of Pittsburgh
One Children's Place
Pittsburgh, PA
Associate Director
Pediatric Intensive Care Unit

1992 - present
Children's Hospital of Pittsburgh
One Children's Place
Pittsburgh, PA
Director
Pediatric Critical Care Research

1994 - present
Safar Center for Resuscitation Research
3434 Fifth Avenue
Pittsburgh, PA 15260
Director

CERTIFICATION AND LICENSURE

SPECIALTY CERTIFICATION

1985
Diplomate, American Board of Pediatrics, #32806

1987
American Board of Pediatrics Sub-board of Pediatric Critical Care Medicine, #0081

1995
American Board of Pediatrics Sub-board of Pediatric Critical Care Medicine, Recertification

MEDICAL LICENSURE

1981
California, #G46392

1985
Maryland, #D32599

1986
District of Columbia, #15785

1986 - present
Pennsylvania, MD# 035634-E
MEMBERSHIPS IN PROFESSIONAL AND SCIENTIFIC SOCIETIES

1985
American Association for the Advancement of Science
1987
Society of Critical Care Medicine
1988
Pennsylvania Society of Critical Care Medicine
1988
International Society of Cerebral Blood Flow and Metabolism
1988
New York Academy of Sciences
1989
Stroke Council, American Heart Association
1989
Neurotrauma Society
1990
Society for Neuroscience
1990
Society for Pediatric Research
1994
Council on Critical Care, American Heart Association
1996
International Neurotrauma Society
1997
The American Association of Neurological Surgeons, Associate Member

HONORS

1976
Phi Beta Kappa
1980
Alpha Omega Alpha
1977 - 1980
Joseph Collins Foundation Scholar
1984 - 1985
Fellow of the Year
Department of Critical Care Medicine
Children's Hospital National Medical Center
1985 - 1986
Fellow of the Year
Department of Critical Care Medicine
Children's Hospital National Medical Center
1991
Fellow, American College of Critical Care Medicine
1992
Cited in "Best Doctors in America", Woodward/White, Inc.
1992
Named one of "Pittsburgh's Best Doctors", Pittsburgh Magazine
1993
Society of Critical Care Medicine Pediatric Award
1993 - 1995
Society of Critical Care Medicine Established Investigator Award
1993
Cited in "Best Doctors in America", 2nd Edition
1993
Neurotrauma Society Poster Award
(Research Mentor to Dr. Susan Kaczorowski)
Patrick M. Kochanek, MD

1993  Society of Critical Care Medicine  
      Educational Scholarship  
      (Research Mentor to Dr. Susan Kaczorowski)

1994  Outstanding Faculty  
      University of Pittsburgh Honors Convocation

1995  Society of Critical Care Medicine  
      (Scientific Award)  
      (Research Mentor to Dr. Robert Clark)

1996  Presidential Citation  
      Society of Critical Care Medicine

1996  Poster Award Finalists  
      Neurotrauma Society of Medicine  
      (Research mentor to Dr. Michael Bell and Dr. Michael Forbes)

1996 – 1997  Who’s Who in America

1997  Educational Scholarship  
      Society of Critical Care Medicine  
      (Research mentor to Dr. Michael Bell)

1997  Young Investigator’s Award  
      Society for Neurosurgical Anesthesiology and Critical Care  
      (Research Mentor to Dr. Elizabeth Sinz)

1997  Poster Award Finalists  
      Neurotrauma Society of Medicine  
      (Research mentor to Dr. Michael Bell)

1998  The American Board of Pediatrics  
      Sub-Board in Pediatric Critical Care Medicine

1998  SCCM In-Training Fellow Award  
      Society of Critical Care Medicine  
      (Research mentor to Dr. Michael Bell)

1999  Women in Neurotrauma Award  
      National Neurotrauma Society  
      (Research Mentor to Dr. Kimberly Statler)

PUBLICATIONS

Refereed Articles.

1.  Weiss DS, Kochanek PM.  Photochemistry of 2-Methylcyclooctadecanone:  


3.  Kochanek PM, Zaritsky A:  Nifedipine in the Treatment of Pulmonary Hypertension in a Patient with  


Reviews, invited published papers, proceedings of conference and symposia, monographs, books and book chapters:


37. **Kochanek PM, Clark RSB, Carlos TM, Carcillo JA, Whalen MJ, Bell MJ, Adelson PD, Marion DW, DeKosky ST:** Role of Inflammation after Severe Head Injury In: *Critical Care State of the Art*, D Porembka (ed), Society of Critical Care Medicine, Anaheim, California, pp 119-134, 1997.


63. Kochanek PM: Toxic levels of neurochemical found in shaken baby syndrome In University of Pittsburgh University Times, p 4, February 4, 1999.


65. Kochanek PM, Sinz EH, Clark RSB, Dixon CE, Bell MJ, Marion DW: Inducible NOS and Other Novel Mediators of Inflammation in Brain Trauma. Presented at the Sixth Wiggers Bernard Conference on Nitric


Abstracts:


Patrick M. Kochanek, MD


PROFESSIONAL ACTIVITIES

TEACHING:

March 1987  
Grand Rounds  
Controversy Surrounding Corticosteroids Administration in Septic Shock  
Children's Hospital of Pittsburgh  
Pittsburgh, PA

October 1987  
Instructor - Pediatric Advanced Life Support  
Children's Hospital of Pittsburgh  
Pittsburgh, PA

March 1988  
Pediatric Grand Rounds  
The Pediatric Arrest  
Children's Hospital of Pittsburgh  
Pittsburgh, PA

April 1988  
University of Pittsburgh Anesthesiology/CCM Grand Rounds  
Reperfusion Injury after Cerebral Ischemia  
Pittsburgh, PA

October 1988  
University of Pittsburgh Anesthesiology/CCM Research Conference  
How Important is Inflammation to the Evolution of Brain Injury?  
Pittsburgh, PA

April 1989  
Research Conference  
Granulocytes and Cerebral Trauma  
International Resuscitation Research Center  
University of Pittsburgh, Pgh, PA

June 1989  
Instructor for Board Review Course - Pediatric Critical Care Medicine  
Section on Cerebral Resuscitation  
New Orleans, LA

November 1989  
Pediatric Grand Rounds  
Pathobiology of the Pediatric Arrest  
Children's Hospital of Pittsburgh  
Pittsburgh, PA

November 1989  
Research Conference  
Inflammation and Brain Injury: An Update  
International Resuscitation Research Center  
University of Pittsburgh  
Pittsburgh, PA

February 1990  
Pediatric Grand Rounds  
Pathobiology of the Pediatric Arrest  
Mercy Hospital  
Pittsburgh, PA
Patrick M. Kochanek, MD

March 1990
Neonatology Grand Rounds
Pathobiology of the Pediatric Arrest
Magee-Womens Hospital
Pittsburgh, PA

April 1990
Scientific Affairs Research Conference
University of Pittsburgh, Anesthesia Department
"Activation of Endogenous Neutrophils in the Cerebral Circulation"
Pittsburgh, PA

April 1991
Children's Hospital of Pittsburgh
Trauma Conference
"Age-Related Differences in the Cerebrovascular Response to Neurotrauma"
Pittsburgh, PA

April 1991
IRRC Hornbein Research Symposium
"Adult Brain Distress Syndrome"
International Resuscitation Research Center
Pittsburgh, PA

May 1991
University of Pittsburgh
Neurosurgery Department
Basic and Clinical Science Conference
"Biochemistry of Cellular Injury and Repair"
Mentor to Dr. Peter Miller
Children's Hospital of Pittsburgh
Pittsburgh, PA

May 1991
Neuroanesthesia and CCM Lecture Series
"Inflammation and Brain Injury"
Eye and Ear Hospital
Pittsburgh, PA

April 1992
IRRC Research Conference
"Trauma Studies at CHP"
International Resuscitation Research Center
Pittsburgh, PA

November 1992
IRRC Research Conference
Blood Flow after Brain Injury
International Resuscitation Research Conference
Pittsburgh, PA

November 1992
University of Pittsburgh Interdisciplinary Seminars in Cerebral Blood Flow and Metabolism
"Cerebral Blood Flow After Traumatic Brain Injury"
(with Dr. Walter Obrist)
Pittsburgh, PA

November 1993
Novel Therapeutic Approach to Traumatic Brain Injury
Pediatric Grand Rounds
Children's Hospital of Pittsburgh
Pittsburgh, PA
November 1993  Head Injury in Children
               Pulmonary Medicine Conference
               Children's Hospital of Pittsburgh
               Pittsburgh, PA

November 1993  Inflammatory Response to Traumatic Brain Injury
               Pediatric Clinical Pharmacology Conference
               Children's Hospital of Pittsburgh
               Pittsburgh, PA

January 1994  Update on Traumatic Brain Injury Studies
               International Resuscitation Research Center
               Pittsburgh, PA

June 1994    Plans for the Future
               International Resuscitation Research Center
               Pittsburgh, PA

November 1994  Acute Inflammatory Response to Traumatic Brain Injury
               Department of Neurology
               University of Pittsburgh Medical Center
               Pittsburgh, PA

February 1995  Inflammatory Response to Traumatic Brain Injury
               Neuroscience Seminar Program
               University of Pittsburgh Medical Center
               Pittsburgh, PA

April 1995    Mini Symposium
               Department of Anesthesiology and Critical Care Medicine
               Safar Center for Resuscitation Research
               Overview and Traumatic Brain Injury Program
               University of Pittsburgh Medical Center
               Pittsburgh, PA

April 1995    Traumatic Brain Injury
               Safar Center Bendixon Symposium
               University of Pittsburgh Medical Center
               Pittsburgh, PA

May 1995     Pathophysiological Mechanisms in Head Injury
               Center for Clinical Pharmacology
               University of Pittsburgh
               Pittsburgh, PA

March 1996   Inflammatory Response to Traumatic Brain Injury
               Research Minisymposium for Dr. Paul Knight
               Department of Anesthesiology/CCM
               Pittsburgh, PA

February 1997  MRI-assessment of Cerebrovascular Failure after Traumatic Brain Injury in Rats
               Pittsburgh NMR Center for Biomedical Research
               Carnegie Mellon University, February 12, 1997
               Pittsburgh, PA

Page 30
Patrick M. Kochanek, MD

February 1997  MRI-Applications to TBI in Rats
                 Safar Center Monthly Lecture Series
                 Department of Anesthesiology/CCM
                 University of Pittsburgh, February 12, 1997
                 Pittsburgh, PA

February 1997  Adhesion Molecules and Quinolinic Acid in CSF after Head Injury in Humans
                 University of Pittsburgh Brain Trauma Research Center
                 University of Pittsburgh, February 25, 1997
                 Pittsburgh, PA

March 1997  MRI-assessment of Head Injury in Rats
                 Pittsburgh NMR Center for Biomedical Research
                 Carnegie Mellon University, March 11, 1997
                 Pittsburgh, PA

August 1997  Traumatic Brain Injury in Children: From Bench to Bedside
                 Pediatric Grand Rounds
                 Children's Hospital of Pittsburgh, August 14, 1997
                 Pittsburgh, PA

December 1997  MRI-Facilitated Assessment of Outcome After Traumatic Brain Injury in Rats
                 Pittsburgh NMR Center NIH Site Visit
                 Carnegie Mellon University, December 3, 1997
                 Pittsburgh, PA

December 1997  Mechanisms and Pharmacology in Suspended Animation
                 Suspended Animation Investigators Meeting
                 University of Pittsburgh, December 6, 1997
                 Pittsburgh, PA

                 Safar Center Monthly Lecture Series
                 Department of Anesthesiology/CCM
                 University of Pittsburgh, March 11, 1998
                 Pittsburgh, PA

April 1998  Traumatic Brain Injury in Children: From Bench to Bedside
                 Trauma Conference
                 Children's Hospital of Pittsburgh, April 9, 1998
                 Pittsburgh, PA

May 1998  Update on Adenosine
                 University of Pittsburgh Brain Trauma Research Center
                 University of Pittsburgh, May 26, 1998
                 Pittsburgh, PA

July 1998  General Clinical Research Center
                 Children's Hospital of Pittsburgh, July 14, 1998
                 Pittsburgh, PA

October 1998  Special Investigator Research Update
                 Journal Club
                 Children's Hospital of Pittsburgh, October 23, 1998
                 Pittsburgh, PA
Patrick M. Kochanek, MD

June 1999
MRI-in the Assessment of Experimentally Induced Traumatic Brain Injury in Rats
Pittsburgh NMR Center NIH Site Visit
Carnegie Mellon University, June 11, 1999
Pittsburgh, PA

October 1999
CSF analysis of secondary mediators in neurotrauma
Brain Trauma Research Center
Department of Neurological Surgery, October 13, 1999
Pittsburgh, PA

RESEARCH:

Grants Received:

The role of granulocytes in reperfusion injury after brain ischemia.
Health Research Service Foundation (United Way)
$13,089 7/87 - 6/88 Principal Investigator

Cerebrovascular and cerebro metabolic effects of platelet-activating factor.
Western Pennsylvania Heart Association
$17,908 7/88 - 6/89 Principal Investigator

Polymorphonuclear leukocytes in the genesis of posttraumatic cerebral edema.
Children's Hospital of Pittsburgh Human Rights Committee Grant
$6,275 7/88 - 6/89 Principal Investigator

The effect of the PAF antagonist Ginkgo Biloba extract on posttraumatic cerebral edema in rats.
Willman Schwabe Pharmaceutical Corporation, Karlsruhe, West Germany
$3,000 11/88 Principal Investigator

The effect of platelet-activating factor-receptor antagonists on posttraumatic cerebral edema in rats.
University of Pittsburgh Internal Grants Program
$10,000 7/89 - 6/90 Principal Investigator

Effect of activated polymorphonuclear leukocytes on cerebral blood flow in rats.
Western Pennsylvania Heart Association
$47,838 7/90 - 6/92 Principal Investigator

Regional cerebral blood flow after concussive head injury in adult and immature rats.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
$6,715 7/90 - 6/91 Faculty Supervisor

Polymorphonuclear leukocytes in traumatic brain injury.
Sunny von Bulow Coma and Head Trauma Foundation
$35,000 9/90 - 8/91 Principal Investigator

Effect of hyponatremia on brain Ph function morphology (Sheldon Adler PI).
NIH-ROI $736,936 Consultant (5% commitment)

Age related differences in blood brain barrier permeability after cerebral trauma in rats.
Children's Hospital Human Rights Committee Grant
$7,251 3/91 - 2/92 Faculty Supervisor
Patrick M. Kochanek, MD

Effect of hypothermia on traumatic brain injury in immature rats.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
$6826
2/93 - 1/94 Co-Principal Investigator

Role of inflammation in cerebrovascular failure after head injury.
Society of Critical Care Medicine, Established Investigator Grant
$225,000
7/93-6/96 Principal Investigator

Effect of leukocyte adhesion cell molecule antagonist on the acute inflammation response after traumatic brain injury in rats.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
$7,998
1/94 - 12/94 Co-Principal Investigator

The effect of SCR-1 on posttraumatic neutrophil accumulation and markers of injury after cerebral trauma in rats.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
$4,420
1/94 - 12/94 Co-Principal Investigator

The role of inflammation in cerebrovascular failure after head injury.
American Heart Association Pennsylvania Affiliate Fellowship Grant
$22,000
7/94 - 6/95 Mentor (Salary support of Dr. Robert Clark)

Perfusion MRI Assessment of Cerebral Blood Flow After Head Injury.
Laerdal Foundation
$12,290
1/95 - 12/95 Principal Investigator

Exsanguination Cardiac Arrest, Hypothermic Preservation, and Delayed Resuscitation.
Laerdal Foundation (S. Tisherman, PI)
$10,000
1/95 - 12/95 Consultant

University of Pittsburgh Brain Trauma Research Center
NIH Program Project (Dr. Donald Marion-Principal Investigator)
$3,051,980
4/01/95 - 3/31/00

Project #4 Neutrophils and the Acute Inflammatory Response to Traumatic Brain Injury.
$389,567
Principal Investigator

Core C RAT/SURGERY/IMAGING
$332,240
Principal Investigator

The Role of Adenosine in the Development of Cerebrovascular Failure Following Severe Head Injury in Children.
Children’s Hospital of Pittsburgh, General Clinical Research Center Advisory Committee
$7,350
Co-Investigator and Mentor - (for Michael Bell, M.D.)

Application of Magnetic Resonance Imaging to Measure Cerebral Blood Flow, CO2 Responsivity and Blood-Brain Barrier Integrity After Traumatic Brain Injury in Immature Rat.
Children’s Hospital of Pittsburgh Research Advisory Committee Seed Grant
$9,940
7/1/95 - 6/30/96 Co-Investigator and Mentor - (Michael Forbes, M.D.)

Nitric Oxide-Mediated Cerebrovascular Failure After Brain Injury.
Schertz Fellowship Award, University of Pittsburgh, Dept. of Anesthesiology/CCM
$60,000
7/01/96 - 6/30/97 Mentor - (for Lisa Sinz, M.D.)
Patrick M. Kochanek, MD

CSF Indexes of Inflammation and Tissue Injury to Pediatric Head Injury: Prognostic Implications.
Laerdal Foundation (S. DeKosky, PI)
$15,000  1/01/96 - Co-Investigator
  12/31/96

Nitric Oxide-Mediated Cerebrovascular Failure After Brain Injury.
American Heart Association Pennsylvania Affiliate
$69,996  7/01/96 - Principal Investigator
  6/30/98

Increasing Survival of Uncontrolled Hemorrhagic Shock in Rats: Oxygen Breathing and Hypothermia.
Geo-Centers/U.S. Department of Navy Grant
$332,996  3/15/96 Co-Investigator (5% committment)

Hypothermia in the Treatment of Severe Head Injury in Children.
Children’s Hospital of Pittsburgh, General Clinical Research Center Advisory Committee
(P. David Adelson, M.D., PI)
$6,510  11/95 - Co-Investigator
  11/96

Emergency Interventions After Severe Traumatic Brain Injury in Rats: Effects of Neuropathology and
Functional Outcome.
U.S. Army Medical Research and Material Command
$792,237 12/96 - Principal Investigator
  11/99

Cerebrovascular Response Following Severe Traumatic Brain Injury in Children.
Laerdal Foundation for Acute Medicine
(P. David Adelson, M.D., PI)
$11,500  7/96 - Co-Investigator
  6/97

Adenosine’s Role in Experimental Traumatic Brain Injury in the Rat.
University Anesthesiology and Critical Care Medicine
Michael Bell, M.D., PI
$8051  7/96 - Faculty Sponsor
  6/97

The Role of Inducible Nitric Oxide Synthase in Delayed Neuronal Death After Traumatic Brain Injury.
Children’s Hospital of Pittsburgh, Research Advisory Committee for Faculty Start-Up Projects
Robert Clark, M.D., PI
$75,808  12/01/96 - Co-Investigator
  6/97

The Role of Neuroprotective Genes after Traumatic Brain Injury.
NINDS-MCSDA
Robert S.B. Clark, M.D., PI
$409,320 12/01/96 - Co-Sponsor
  12/31/01

Severe Diffuse Traumatic Brain Injury in Immature Rats.
MCSDA-NINDS/KO8
P. David Adelson, M.D., PI
$409,320 12/01/96 - Faculty Sponsor
  11/30/01

Page 34
Patrick M. Kochanek, MD

Pathogenesis of Osmotic Induced Demyelination.
NIH-RO1
Sheldon Adler, M.D., PI
$858,487 05/31/97 - 05/31/99 Consultant [5%]

The Effect of Hypothermia on the Acute Inflammatory Response to Brain Injury.
Schertz Fellowship Award, University of Pittsburgh, Dept. of Anesthesiology/CCM
Michael Whalen, M.D., PI
$60,000 07/01/97 - 06/30/98 Faculty Sponsor

Quinolinic Acid, A Novel Mediator of Neurotoxicity after Brain Injury.
Laerdal Foundation for Acute Medicine
$6120 07/01/97 - 06/30/98 Principal Investigator

Cell Trafficking and Functions in Two Models of Cold Induction: Ultraprofound Hypothermia and Hibernation.
Navy Medical Research Institute
Florence Rollwagen, Ph.D., PI
$400,574 Co-Investigator

Augmenting Adenosine to Improve Outcome after Severe Head Injury
Laerdal Foundation for Acute Medicine
C. Robertson, M.D., PI
$10,000 01/01/98 - 12/31/98 Co-Principal Investigator

Reduction of Neuronal DNA Damage in Rats by Hypothermia after Traumatic Brain Injury.
Laerdal Foundation for Acute Medicine
Michael Whalen, M.D., PI
$9,310 07/01/98 06/30/99 Co-Investigator

Production of a Novel Macrophage-Derived Neurotoxin, Quinolinic Acid, in Brain after Severe Head Injury in Adults and Children.
University of Pittsburgh’s Center for Injury Research and Control/CDC
$39,860 10/01/97 - 08/31/98 Principal Investigator

A Multidisciplinary NMR Center for Biomedical Research
Collaborative Research Project 3
Magnetic Resonance Imaging in the Assessment of Experimentally Induced Traumatic Brain Injury in Rats.
Project Leaders: Chien Ho, Ph.D., PI and Patrick M. Kochanek, M.D.
$5,945,043 07/01/98 - 06/30/03

Intercellular Adhesion Molecule-1 (ICAM-1) and Secondary Damage After Traumatic Brain Injury.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
Michael Whalen, M.D., PI
$7,690 Faculty Sponsor/Co-PI
Patrick M. Kochanek, MD

A PARS Inhibitor in Brain Trauma
Inotek Corporation's Small Business Innovative Research (SBIR) Grant
NIH
George Hasko, M.D., PI
$20,208 07/01/98 - 12/31/98 Collaborator

Effect of Novel AK Inhibitor on Outcome after CCI Plus Secondary Insult in Rats.
Metabasis, Inc.
$25,156 09/17/98 06/30/99 Principal Investigator

Caspase-Mediated Neuronal Death after Head Injury
NIH RO1
Robert S.B. Clark, M.D., PI
$917,678 04/01/99 - 03/21/03 Co-Investigator [5%]

Programmed-Cell Death after Human Head Injury
Competitive Medical Research Fund (CMRF)
University of Pittsburgh Medical Center
Robert Clark, M.D., PI
$24,170 07/01/97 06/30/99 Co-Investigator

Relationship of CBF to Function after Severe TBI in Immature Rats Using Perfusion MRI.
CHP Seed Grant
P. David Adelson, M.D., PI
$10,400 01/01/99 12/31/99 Consultant

Training in Trauma and Sepsis Research (T32-GM-08516-04)
NIH/NIGMS
Timothy Billiar, M.D., PI
$137,896 07/01/94 - 06/30/99 Co-Investigator

Quinolinic Acid in Cerebrospinal Fluid Early After Severe Head Injury in Victims of Child Abuse
University of Pittsburgh CIRCL CDC
Donald Marion, M.D., PI of CIRCL Center
$219,468 09/01/98 08/31/03 Principal Investigator

Cylooxygenase 2 and Ischemic Neuronal Injury
NIH-RO1
Steven H. Graham, M.D, Ph.D., PI
12/01/98 - 11/30/02 Consultant

Hypothermia for Severe TBI in Children
NIH-RO1
P. David Adelson, M.D., PI
$1,658,419 04/01/99 03/31/02 Consultant
Suppression of Traumatic Brain Edema with an Inhibitor of PARS.
Inotek/CDC
$33,020 07/01/99
11/30/99

Adenosine and Traumatic Brain Injury
NIH-RO1
$1,593,730 12/01/98 - 11/30/03 Principal Investigator

CPC-211 (Dichloracetate) in the Controlled Cortical Impact Model of Traumatic Brain Injury in Rats
Cypros Pharmaceutical Corporation
$46,053 Principal Investigator

General Clinical Research Center for Children's Hospital of Pittsburgh
NIH 5M01 RR0084-37
Donald Fischer, M.D., PI
$5,082,660 12/01/99 Associate Program Director (12.5% effort)
11/30/04 Effective 10/1/99

Grants Pending:

Stress Responses in Early Life Traumatic Brain Injury
MCSD-K08
Elizabeth Gilles, M.D., PI (Ohio State University
$239,760 12/01/99 - Consultant
11/30/01

University of Pittsburgh Brain Trauma Research Center
NIH Program Project
Donald Marion, M.D., PI
$5,453,880 03/01/00
02/28/05

Project #3 iNOS and Traumatic Brain Injury
$633,143 03/01/00 Principal Investigator
02/28/05

Core C Animal Modeling and Outcome
$745,636 03/01/00 Principal Investigator
02/28/05

Training in Pediatric Neurointensive Care and Resuscitation Research
NIH Training Grant
$1,217,405 07/01/00 Principal Investigator
06/30/05

2. Seminars and invited lectureships related to your research:


22. Inflammation Response to Traumatic Brain Injury, Ohio State University, Department of Immunology Seminars, March 27, 1994.

23. Inflammatory Response to Traumatic Brain Injury, Johns Hopkins University, Department of Anesthesiology and Critical Care Medicine, May 1994.


27. Severe Traumatic Brain Injury in Children. Critical Care Medicine Research-in-Progress Conference, Emory University School of Medicine, Department of Pediatrics, March 16, 1995.

28. Inflammatory Responses to Traumatic Brain Injury. Basic Science Fellow's Conference, Emory University School of Medicine, Department of Pediatrics, March 16, 1995.

29. Magnetic Resonance Imaging in Assessment of Experimentally Induced Head Trauma in Rats. Advisory Committee Meeting, Pittsburgh NMR Center for Biomedical Research, Pittsburgh, Pennsylvania, June 2, 1995.


32. Inflammatory Response to Traumatic Brain Injury. AI duPont Institute, Wilmington, Delaware, November 7-8, 1995.


37. Novel Therapeutic Approaches to CNS Injury. 34th Annual Symposium on Critical Care Trauma and Emergency Medicine, University of Southern California School of Medicine, Las Vegas Hilton Hotel, Las Vegas, Nevada, February 12-16, 1996.
38. Traumatic Brain Injury in Children. 34th Annual Symposium on Critical Care Trauma and Emergency Medicine, University of Southern California School of Medicine, Las Vegas Hilton Hotel, Las Vegas, Nevada, February 12-16, 1996.

39. Case Studies in Brain Injury. 34th Annual Symposium on Critical Care Trauma and Emergency Medicine, University of Southern California School of Medicine, Las Vegas Hilton Hotel, Las Vegas, Nevada, February 12-16, 1996.

40. Pediatric Neurointensive Care. 34th Annual Symposium on Critical Care Trauma and Emergency Medicine, University of Southern California School of Medicine, Las Vegas Hilton Hotel, Las Vegas, Nevada, February 12-16, 1996.

41. Acute Inflammatory Response to Traumatic Brain Injury. Pathophysiology of Secondary Brain Injury and Implications for Contemporary Treatment, University of Pittsburgh Medical Center, Sheraton Hotel at Station Square, Pittsburgh, Pennsylvania, May 17-18, 1996.


43. Magnetic Resonance Imaging in Assessment of Experimentally Induced Traumatic Brain Injury in Rats, Advisory Committee Meeting, Pittsburgh NMR Center for Biomedical Research, Pittsburgh, Pennsylvania, June 18, 1996.

44. Acute Inflammatory Response to Traumatic Brain Injury, FASEB Summer Research Conference, Copper Mountain, Colorado, August 11-16, 1996.


47. Established Investigator Grant Lecture: Role of Inflammation After Severe Head Injury, 26th International Symposium of the Society of Critical Care Medicine, San Diego, California, February 6-10, 1997.


50. Inflammatory Response to Traumatic Brain Injury. Anesthesia Grand Rounds, Harvard Medical School, Children’s Hospital, Boston, Massachusetts, March 26, 1997.

51. Role of Inflammation in Cerebrovascular Failure after Head Injury. TraumaCare ‘97, 10th Annual Trauma Anesthesia and Critical Care Symposium (ATACCS), Baltimore, Maryland, May 15-17, 1997.

52. Traumatic Brain Injury in Children - From Bench to Bedside. European Society for Pediatric Research, European Society for Paediatric Haematology and Immunology, European Society for Paediatric Infectious Diseases, Joint Meeting, Budapest, Hungary, August 31-September 3, 1997.


60. New Developments in Head Trauma, 18th International Symposium on Intensive Care & Emergency Medicine, Brussels, Belgium, March 17-20, 1998.


79. Novel Therapeutic Approaches to Brain Injury. Pediatric Research Conference, University of Texas Southwestern Medical Center, Dallas, Texas, March 9-10, 1999.


Patrick M. Kochanek, MD

3. Other research related activities:

Editorial Board

Critical Care Medicine - 1996 - present
Journal of Neurotrauma - 1996 - present
Pediatric Life Support International, Founding Editor - 1996
Critical Care Medicine, Scientific Editor - 1997 - present
New Horizons, Scientific Editor - 1998 - present

Ad Hoc Reviewer:

Journals -

Critical Care Medicine, 1988, 1993 - present
Journal of Neurosurgical Anesthesia, 1988
Pediatric Pulmonology, 1992
Journal of Cerebral Blood Flow and Metabolism, 1993 - present
Brain Research, 1994, 1999-present
Journal of Neurotrauma, 1994 - present
Anesthesia and Analgesia, 1994, 1995
Journal of Neuroscience, 1995, 1998-present
Journal of Intensive Care Medicine, 1995, 1997
Experimental Neurology, 1995
Anesthesiology, 1996
Brain Research Bulletin, 1997
American Journal of Physiology: Heart & Circulatory Physiology, 1999 - present
PNAS-Proceedings of the National Academy of Sciences, USA, 1999-present
American Journal of Pathology, 1999 - present

Ad Hoc Reviewer:

Grants -

NIH/ADAMHA Peer Review Consultant, 1992
Western Pennsylvania Psychiatric Institute and Clinic, 1992 - 1994
PSI Foundation, Ontario, Canada, 1994
Children's Hospital of Eastern Ontario Research Institute, Ontario Canada 1994, 1995
University of Pittsburgh ADRC Seed Grant Proposal, 1995
Department of Veterans Affairs Merit, 1996
NIH NSD-A Study Section, 1997
The Wellcome Trust, 1998
The Hospital for Sick Children Foundation, 1998

Committees

Local -

Research Advisory Committee - Department of Anesthesiology and Critical Care Medicine,
University of Pittsburgh, 1993 - present
Scientific Affairs Committee, Department of Anesthesiology and Critical Care Medicine,
University of Pittsburgh, 1989 - present
GCRC Advisory Committee, Children's Hospital of Pittsburgh, 1991- present
GCRC Advisory Committee, University of Pittsburgh Medical Center, 1996 - September 1999
23rd Annual Meeting of ISOTT, Pittsburgh, PA, August 23-27, 1995
Anesthesia & CCM Newsletter, 1995 - present
Patrick M. Kochanek, MD

Reappointment and Promotion Committee - Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh, 1995 – 1996, 1998-present
Research Advisory Committee - Children's Hospital of Pittsburgh, 1997 - present
Executive Steering Committee, Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh, 1997 - present
Chairman, Scientific Affairs Committee, Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh, 1998 - present
Health Sciences Animal Research Advisory Committee (HSARAC), 1999 - present
Strategic Planning Committee - Children's Hospital of Pittsburgh, 1999 - present
Associate Director, GCRC Advisory Committee, Children's Hospital of Pittsburgh, 1999- present

National -

Program Chairman - Society of Critical Care Medicine Meeting, 1994
Selection Committee - Young Investigator Award, SCCM, 1989, 1990, 1994
Abstract Reviewer - SPR (Section on Critical Care Medicine), 1993
Selection Committee - Laerdal Foundation Lecture, SCCM, 1994
Selection Committee - In Training Award SCCM, 1994
Continuing Education Committee - Society of Critical Care Medicine, 1994 - 1996
Critical Care Consultant - Tenth International Brain Edema Symposium, 1996
Program Committee - Neurotrauma Society Meeting, 1997, 2000
Program Committee - Sixth Vienna Shock Forum, 1997
Program Chairman - Sixth Wiggers Bernard Conference, 1997
American Board of Pediatrics - Sub-board in Pediatric Critical Care Medicine, 1998 – present
Chair, Credentials Committee – American Board of Pediatrics, Pediatric Critical Care Medicine Subboard - 2000

Other -

Multidisciplinary Critical Care Knowledge Assessment Program (MCCKAP) of the Society of Critical Care Medicine, Editorial Board Member, 1991, 1992, 1993, 1994
Field Tester for the American Board of Pediatrics Subspecialty Board Examination in Pediatric Critical Care Medicine, 1993
Consultant to the First International Conference on Pediatric Resuscitation, Washington, DC, June 1994
Consultant - Cypros Pharmaceutical Corporation, 1997

ARTICLES IN SUBMISSION


ARTICLES IN PREPARATION


Augmented neuronal death in CA3 hippocampus following hyperventilation early after controlled cortical impact

MICHAEL L. FORBES, M.D., ROBERT S. B. CLARK, M.D., C. EDWARD DIXON, PH.D., STEVEN H. GRAHAM, M.D., PH.D., DONALD W. MARION, M.D., STEVEN T. DEKOSKY, M.D., JOANNE K. SCHIDING, B.S., AND PATRICK M. KOCHANEK, M.D.

Safar Center for Resuscitation Research, The University of Pittsburgh Brain Trauma Research Center, and Departments of Anesthesiology/Critical Care Medicine, Pediatrics, Neurology, Neurosurgery, and Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania

Minimizing secondary injury after severe traumatic brain injury (TBI) is the primary goal of cerebral resuscitation. For more than two decades, hyperventilation has been one of the most often used strategies in the management of TBI. Laboratory and clinical studies, however, have verified a post-TBI state of reduced cerebral perfusion that may increase the brain’s vulnerability to secondary injury. In addition, it has been suggested in a clinical study that hyperventilation may worsen outcome after TBI.

Object. Using the controlled cortical impact model in rats, the authors tested the hypothesis that aggressive hyperventilation applied immediately after TBI would worsen functional outcome, expand the contusion, and promote neuronal death in selectively vulnerable hippocampal neurons.

Methods. Twenty-six intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats were subjected to controlled cortical impact (4 m/second, 2.5-mm depth of deformation) and randomized after 10 minutes to either hyperventilation (PaCO₂ = 20.3 ± 0.7 mm Hg) or normal ventilation groups (PaCO₂ = 34.9 ± 0.3 mm Hg) containing 13 rats apiece and were treated for 5 hours. Beam balance and Morris water maze (MWM) performance latencies were measured in eight rats from each group on Days 1 to 5 and 7 to 11, respectively, after controlled cortical impact. The rats were killed at 14 days postinjury, and serial coronal sections of their brains were studied for contusion volume and hippocampal neuron counting (CA1, CA3) by an observer who was blinded to their treatment group.

Mortality rates were similar in both groups (two of 13 in the normal ventilation compared with three of 13 in the hyperventilation group, not significant [NS]). There were no differences between the groups in mean arterial blood pressure, brain temperature, and serum glucose concentration. There were no differences between groups in performance latencies for both beam balance and MWM or contusion volume (27.8 ± 5.1 mm³ compared with 27.8 ± 3.3 mm³, NS) in the normal ventilation compared with the hyperventilation groups, respectively. In brain sections cut from the center of the contusion, hippocampal neuronal survival in the CA1 region was similar in both groups; however, hyperventilation reduced the number of surviving hippocampal CA3 neurons (29.7 cells/hpf, range 24.2-31.7 in the normal ventilation group compared with 19.9 cells/hpf, range 17-23.7 in the hyperventilation group [25th-75th percentiles]; *p < 0.05, Mann–Whitney rank-sum test).

Conclusions. Aggressive hyperventilation early after TBI augments CA3 hippocampal neuronal death; however, it did not impair functional outcome or expand the contusion. These data indicate that CA3 hippocampal neurons are selectively vulnerable to the effects of hyperventilation after TBI. Further studies delineating the mechanisms underlying these effects are needed, because the injudicious application of hyperventilation early after TBI may contribute to secondary neuronal injury.

KEY WORDS • head injury • hyperventilation • alkalosis • hippocampus • rat

Traumatic brain injury (TBI) is often complicated by malignant intracranial hypertension, which is associated with high mortality rates and has been managed using a combination of therapies including osmotherapy, diuretics, sedation, neuromuscular blockade, optimization of cerebral perfusion pressure, and hyperventilation. Hyperventilation therapy has been an integral part of the clinical armamentarium in the management of severe TBI for more than 20 years; this therapy rapidly reduces cerebral blood flow (CBF) and cerebral blood volume in areas of the brain with intact CO₂ autoregulation, providing one option in the management of TBI complicated by malignant intracranial hypertension.

In recent studies, however, researchers have defined a state of reduced CBF early after TBI in humans and animals, particularly in the first 8 hours after TBI. Some authors have hypothesized that the brain is more...
vulnerable to secondary injury during this period and that additional reduction of CBF by hyperventilation may attenuate the delivery of important energy substrates. Yoshida and Marmarou reported that hyperventilation produced relative ischemia in cat brain after fluid-perfusion injury and demonstrated an increase in brain lactate and inhibition of recovery of the ratio of phosphocreatine to inorganic phosphate. Muizelaar, et al., also demonstrated a loss of brain interstitial bicarbonate buffer after sustained prophylactic hyperventilation in rabbits. It has been reported that hyperventilation after TBI in animals and humans can reduce CBF to what traditionally have been considered ischemic levels. However, defining the ischemic threshold in injured tissue is problematic. Muizelaar, et al., reported that prolonged hyperventilation after TBI in humans may worsen functional outcome, raising questions regarding the appropriate indications and timing for the optimum application of hyperventilation after TBI. Recently published guidelines for the management of severe head injury state that "in the absence of intracranial hypertension, hyperventilation (PaCO₂ ≤ 35 mm Hg) therapy should be avoided during the first 24 hours after severe TBI." Although "hyperventilation therapy may be necessary for brief periods where there is acute neurologic deterioration...", consistent with these guidelines, in the setting of acute neurological deterioration, aggressive hyperventilation is used by both emergency and critical care personnel. In addition, in the initial stabilization of the brain-injured patient, aggressive hyperventilation (appropriate in the setting of impending herniation, or iatrogenic) occasionally occurs in both the prehospital and acute care settings. The specific impact of hyperventilation during this early low-flow period remains to be determined. Despite the availability of well-characterized rodent models of TBI, which reproduce the early posttraumatic reduction in CBF, the effect of aggressive hyperventilation on histopathological and functional outcome has not, to our knowledge, been investigated.

Using a rat model of focal percussive contusion, we hypothesized that aggressive hyperventilation, beginning immediately after TBI and continuing for 5 hours, would worsen functional outcome, expand the contusion, and promote neuronal death in selectively vulnerable hippocampal neurons.

Materials and Methods

Animals and Study Groups

All experimental protocols used in this report were approved by the Animal Care and Use Committee of the University of Pittsburgh. Twenty-six virus-free Sprague-Dawley rats weighing 346 ± 5 g were studied. Food and water were continuously available in their home cages. After TBI the rats were randomly assigned to one of two groups of 13 animals, one receiving normal ventilation (PaCO₂ = 30-40 mm Hg) and one receiving hyperventilation (PaCO₂ = 15-25 mm Hg).

Surgery and Brain Trauma Model

Anesthesia was induced using 4% isoflurane in N₂O/O₂ (2:1). The rats were endotracheally intubated and mechanically ventilated. The isoflurane concentration was reduced to 2% followed by sterile surgical placement of a femoral arterial catheter for continuous mean arterial blood pressure (MABP) and arterial blood gas monitoring.

Intramuscular injections of penicillin (100,000 U) and gentamicin (10 mg/kg) were given to minimize the risk of infection. Pancreatin bromide was administered at dosages of 0.1 mg/kg/hour via the arterial line to control ventilation. The rats' core temperature was monitored using a rectal probe.

A craniotomy was made using a high-speed dental drill aided by a binocular operating microscope. A burr hole was made 5 mm anterior and 2 mm lateral to the bregma in the left side of the skull and a temperature probe (0.009-in outer diameter) was inserted through the burr hole and 2 mm into the left parietal cortex. The bone flap was left in place and the isoflurane was reduced to 1% followed by a 30-minute equilibration period. The brain temperature was maintained at 37 ± 0.5°C. Normal arterial blood gas levels were achieved in all rats and PaO₂ was maintained at greater than 70 mm Hg.

The TBI's were produced using a controlled cortical impact device as recently described with minor modifications. Fifteen minutes before controlled cortical impact, an arterial blood sample was obtained for measurement of arterial blood gas levels, glucose concentration, and hemotocrit. The bone flap was then removed and a vertical controlled cortical impact (4 m/s impactor velocity, 2.5-mm deformation depth) was delivered onto the exposed dura overlaying the left parietal cortex. The bone flap was replaced and sealed with dental cement and the scalp was sutured.

Study Design

The study protocol was designed to mimic the aggressive use of hyperventilation (as opposed to normal ventilation) in the immediate posttrauma period in the prehospital as well as early hospital setting. Ten minutes after controlled cortical impact, rats were randomized to either the normal ventilation group (13 animals, PaCO₂ range 30-40 mm Hg) or the hyperventilation group (13 animals, PaCO₂ range 15-25 mm Hg). The ventilator was adjusted to maintain normocarbia or hypocarbia for 5 hours after controlled cortical impact. Arterial blood gas readings were obtained at 30 minutes post-controlled cortical impact, then hourly. The MAPB was recorded every 30 minutes after controlled cortical impact. Brain and rectal temperatures were recorded every 15 minutes.

At 5 hours after controlled cortical impact, anesthesia was discontinued. Temperature probes and the femoral artery catheter were removed and the rat was weaned from mechanical ventilation in the course of 1 hour and underwent extubation. The time to extubation was recorded. After extubation, supplemental O₂ was administered for 30 minutes. When it had fully recovered, the rat was returned to its cage with full access to food and water.

Functional Outcome and Behavior Assessment

Beam Balance. Vestibulomotor function was tested using the beam balance test in eight rats from each group. One hour before surgery, the rat was placed lengthwise on a 1.5-cm-wide beam suspended above the ground. The time the rat remained on the beam and the procedure was repeated. Rats were considered trained when they remained on the beam for three consecutive periods of 60 seconds. Beam balance tests were also performed daily on Days 1 to 5 postinjury. Three trials were recorded and averaged each day for each rat.

Morris Water Maze. Cognitive function was tested in the same eight rats from each group using a standard variation of the Morris water maze (MWM) paradigm. A pool 180 cm in diameter and 60 cm deep was painted black and filled with water to a depth of 28 cm. A clear Plexiglas platform 10 cm in diameter and 26 cm high (2 cm below the water surface) was used as the hidden goal platform. The pool was located in a 2.5 × 2.5-m room with numerous extra-maze cues (for example, posters, pipes, bookcases) that remained constant throughout the experiment. Testing started 7 days after controlled cortical impact to avoid confounding effects of motor deficits. The rats underwent four trials per day for 5 consecutive days to assess spatial memory performance. The rats started each trial once from each of the four possible start locations.
Augmented neuronal death following hyperventilation post-TBI

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Normal Ventilation</th>
<th>Hyperventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>Postrandomization</td>
<td>Postrandomization</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.01</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td>*PaCO₂ (mm Hg)</td>
<td>36.7 ± 1.1</td>
<td>34.9 ± 0.3</td>
</tr>
<tr>
<td>*PaO₂ (mm Hg)</td>
<td>165 ± 6</td>
<td>167 ± 4</td>
</tr>
<tr>
<td>base deficit (mmol/L)</td>
<td>2.7 ± 3.4</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>serum glucose (mg%)</td>
<td>189 ± 9</td>
<td>174 ± 6</td>
</tr>
<tr>
<td>hct (%)</td>
<td>36 ± 2.3</td>
<td>35 ± 0.6</td>
</tr>
<tr>
<td>time to extubate (min)</td>
<td>NA</td>
<td>28 ± 6</td>
</tr>
<tr>
<td>rectal temperature (°C)</td>
<td>36.7 ± 0.1</td>
<td>37 ± 0</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>129 ± 4</td>
<td>123 ± 4</td>
</tr>
</tbody>
</table>

* All values are expressed as mean ± SEM. Abbreviations: hct = hematocrit; NA = not applicable.
† p < 0.05 at 30 minutes postrandomization compared with baseline.

Hippocampal Cell Counting. Neuronal loss in hippocampal regions CA1 and CA3 pyramidal layers was quantified.3 A coronal section cut from the dorsal hippocampus underlying the area of contusion, approximately 2.6 mm posterior to the bregma, was used for analysis in each rat. The region was visualized at X 100 magnification, then localized and counted at X 400 by an observer (M.L.F.) who was blinded to the treatment group. Contusion volume in each rat was calculated as the sum of these sections.

Hypocampal Cell Counting. Neuronal loss in hippocampal regions CA1 and CA3 pyramidal layers was quantified.3 A coronal section cut from the dorsal hippocampus underlying the area of contusion, approximately 2.6 mm posterior to the bregma, was used for analysis in each rat. The region was visualized at X 100 magnification, then localized and counted at X 400 by an observer (R.S.B.C.) blinded to treatment group. Only complete cells with a clearly defined body and nucleus were counted. Surviving pyramidal CA1 and CA3 hippocampal neurons were counted in six separate X 400 fields for each region in both hemispheres. Sections were excluded if the boundary of the contusion extended into the pyramidal layers of the hippocampus or if fixation artifacts precluded accurate counting. Data are reported as the average number of surviving neurons per high-power field for the CA1 and CA3 hippocampal regions in both the ipsilateral and contralateral hemispheres.

Statistical Analysis

Survival was compared between groups using Fisher's exact test. Between group comparisons of physiological parameters, beam balance, and MWM latencies were made using one- or two-way analysis of variance (ANOVA) for repeated measures where appropriate and post-hoc tests with appropriate correction for multiple comparisons. Contusion volume was normally distributed and was compared between groups using Student's t-test. Hippocampal neuronal survival in CA1 and CA3 was not normally distributed and was compared between groups using the Mann–Whitney rank-sum test. Significance was defined at a probability level of less than 0.05.

Sources of Supplies and Equipment

Pancuronium bromide and gentamicin were purchased from Elkins-Sinn, Cherry Hill, NJ, and penicillin was acquired from Upjohn, Kalamazoo, MI. The stereotactic head positioning system was obtained from Barad, San Diego, CA. The temperature probe was purchased from Physitemp Corp., Clifton, NJ. The video tracking system (Poly-Trak) was acquired from San Diego Instrument, Inc., San Diego, CA, and the image analysis system (MCID) was from Imaging Research, St. Catharines, Ontario, Canada.

Results

Physiological Parameters

Baseline and 30-minute postrandomization physiological data are presented for all measured parameters in Table 1. After randomization, there was a marked increase in pH and decrease in PaCO₂ in the hyperventilation group (compared with baseline, p < 0.05). Hyperventilation was also associated with a small increase (12 mm Hg) in PaO₂ compared with baseline (p < 0.05). This difference was attributable to the increased minute ventilation and mean airway pressure in the hyperventilation group. At no time were any of the rats hypoxic (PaO₂ < 100 mm Hg). The entire time course of PaCO₂, arterial pH, MABP, and brain temperature after TBI is given for both groups in Fig. 1. The PaCO₂ and pH levels differed between groups at all time points after randomization (p < 0.05). The MABP and brain temperature were similar in both groups.

Five of 26 rats died during the 14-day study, with all deaths occurring on the day of injury. Two rats remained unresponsive postinjury and were unable to demonstrate any spontaneous respiratory effort for 1 hour after discontinuation of anesthesia and were therefore killed. Three rats developed pulmonary edema and/or respiratory distress and died soon after extubation. There were no differences in mortality between groups (two of 13 in the normal ventilation group compared with three of 13 in the hyperventilation group). There were no differences between groups in time to extubation (Table 1).
Functional Outcome Assessment

**Beam Balance.** There was no difference between groups in motor performance latencies over time (F<sub>1,12</sub> = 0.17, p < 0.69, Fig. 2). Maximum impairment of performance occurred on Days 1 or 2 in both groups, and eventually returned to baseline. Beam balance performance did not differ significantly between normal ventilation and hyperventilation groups.

**Morris Water Maze.** There was no difference between normal ventilation and hyperventilation groups in the time needed to find the hidden platform in the MWM test (F<sub>U5</sub> = 0.50, p < 0.50, Fig. 3). In addition, there was a statistically nonsignificant tendency (t<sub>13</sub> = 1.77, p < 0.065) for the rats in the hyperventilation group to swim slower than the rats in the normal ventilation group (30.8 ± 1.0 compared with 35.4 ± 2.1 cm/second).

**Histopathological Studies**

**Contusion Volume.** At the injury level selected for this study, the contusion was generally restricted to the parietal cortex beneath the impact site. Contusion volume in both groups is shown in Fig. 4. There was no difference between groups (27.8 ± 5.1 mm<sup>3</sup> in the normal ventilation group compared with 27.8 ± 3.3 mm<sup>3</sup> in the hyperventilation group) in this outcome parameter.

**Hippocampal Cell Counting.** Figure 5 shows the number of surviving neurons/hpf in the CA1 and CA3 regions of the dorsal hippocampus ipsilateral to the contusion. There were no differences in the number of surviving CA1 hippocampal neurons between groups after controlled cortical impact. There was, however, a further reduction in the number of surviving CA3 neurons in the hyperventilation group after controlled cortical impact compared with the normal ventilation group (normal ventilation 29.7, range 24.2-31.7 neurons/hpf, compared with hyperventilation 19.9, range 17-23.7 neurons/hpf; median [25th-75th percentiles], p < 0.05). Neuronal cell counts in the CA1 and CA3 regions of the hemisphere contralateral to the contusion did not differ in either the normal ventilation or hyperventilation groups (CA1 counts = 55.3, range 52.1-59

---

**FIG. 2.** Graph showing mean beam balance performance latencies (mean ± SEM, in seconds) in rats before and on Days 1 to 5 after controlled cortical impact (4 m/second, 2.5-mm cortical deformation depth). Repeated-measures ANOVA revealed no difference in duration of balance maintained between the two groups (triangles = normal ventilation [eight rats]; squares = hyperventilation [eight rats]).

---

**FIG. 3.** Graph showing mean beam balance performance latencies (mean ± SEM, in seconds) in rats before and on Days 1 to 5 after controlled cortical impact (4 m/second, 2.5-mm cortical deformation depth). Repeated-measures ANOVA revealed no difference in duration of balance maintained between the two groups (triangles = normal ventilation [eight rats]; squares = hyperventilation [eight rats]).
Augmented neuronal death following hyperventilation post-TBI

![Graph showing MWM performance latency to find a hidden platform](image)

**Fig. 3.** Graph showing MWM performance latency to find a hidden platform (mean ± SEM, in seconds) by rats on Days 7 to 11 after controlled cortical impact. There was no difference between groups (triangles = normal ventilation [eight animals]; squares = hyperventilation [eight animals]) when performances were compared using ANOVA with repeated measures.

![Bar graph depicting mean lesion area](image)

**Fig. 4.** Bar graph depicting mean lesion area (left y-axis, mm²) compared with distance from occiput (mm) measured 14 days after controlled cortical impact (open bars, normal ventilation [11 rats]; closed bars, hyperventilation [10 rats]). Contusion volume (mm³) was calculated as the sum of these areas in each group and is depicted as the cumulative volume (right y-axis) in the normal ventilation (triangles) and hyperventilation (squares) groups. There was no difference between groups in contusion volume (normal ventilation, 27.8 ± 5.1 mm³ compared with hyperventilation, 27.8 ± 3.1 mm³, mean ± SEM).

Discussion

In a model of controlled cortical impact–induced focal contusion in rats, aggressive hyperventilation for 5 hours after TBI augments neuronal death in the CA3 region of the hippocampus ipsilateral to the contusion. However, hyperventilation did not worsen motor function or cognitive outcome, as assessed using standard beam balance and MWM paradigms, respectively, and did not increase contusion volume.

Hippocampal CA3 neurons are selectively vulnerable to delayed neuronal death after TBI.23,49,52,55 Theories about the mechanisms underlying this process remain speculative. Potential mechanisms include ischemia, TBI-induced excitotoxicity, apoptosis, and inflammation.49,49

Yamakami and McIntosh56,57 reported reduced CBF as early as 15 and 30 minutes after TBI. Using a piglet model of TBI, Pfenninger, et al.,40 reported CBF reduction as early as 5 minutes post-TBI. Some flow levels were in the range consistent with ischemia. We have previously demonstrated that the hippocampus and cortex ipsilateral to the impact show marked flow reduction (at least 60%) at 2 hours after TBI in the controlled cortical impact model.25 Cerebral blood flow approaches ischemic levels in the core of the contusion at 2 hours postinjury. Although we have not evaluated the reactivity status of the cerebral circulation to changes in PaCO₂ at 2 hours after TBI in this model, we have reported that CO₂ reactivity is impaired, although still present (62-71% of baseline) in and around the contusion at 24 hours after controlled cortical impact in rats.16

Hyperventilation rapidly reduces cerebral blood volume and intracranial pressure (ICP).11 In some studies, this intervention has been associated with CBF values consistent with ischemia or brain tissue hypoxia.10,11,42,46 After global cerebral ischemia in dogs, hyperventilation did not increase neuronal death;52 however, the brains were assessed at 8 hours after reperfusion, and neuronal death may be delayed. Although ischemia may be considered a contributing mechanism in the observed augmented neuronal death, ischemia alone is an inadequate explanation for our findings in light of the preservation of CA1 neurons. Although CA1 neurons are known to be selectively vulnerable to ischemic injury,23 they were not affected by hyperventilation in this paradigm. Furthermore, in our model, CA1 neurons are more proximal to the point of impact in the cortex compared with CA3 neurons. The lack of CA1 neuronal death in light of ischemic and (presumed) anatomical vulnerability weighs against ischemia and primary injury as putative mechanisms of neuronal death in the hippocampus in this model. One limitation in this study is that neuronal counting using traditional histological methods may underestimate cell loss because of a loss of hippocampal volume.25 We did not use stereological methods in this study. However, CA1 neuronal counts did not differ between groups and were equivalent to those observed in sham-injured animals studied in our laboratory in prior published58 and unpublished work. In addition, comparisons were only made between injured groups within this study.

Hyperventilation produces cerebral vasoconstriction...
and alkalosis. Alkalosis exacerbates N-methyl-D-aspartate receptor-mediated neurotoxicity. As a result of aggressive hyperventilation, the rats in our study were quite alactic as indicated by arterial pH measurements. Although we did not measure brain pH, a decrease in PaCO₂ immediately reduces brain interstitial pH. Although alkalosis appears to have deleterious effects on neurons, acidosis has been shown to have both beneficial and detrimental effects. Giffard, et al., and Takadera, et al., reported a neuroprotective effect of acidosis via an attenuation of the N-methyl-D-aspartate receptor activation in vitro. Rosner and Becker reported a deleterious effect of tissue acidosis after experimental TBI in cats. The spatial distribution of brain pH around the contusion and in the hippocampus has not been determined for either normal ventilation or hyperventilation conditions in our model.

Finally, the potential effects of hyperventilation on other mechanisms such as posttraumatic seizures or axonal injury may contribute to the enhanced vulnerability of CA3 neurons. The lateralization of the deleterious effects also raises the possibility that spreading wave depression may be a component of the neurotoxic milieu after TBI in this model of focal contusion. It could also be the case that the combined effect of alkalosis and further flow reduction by hyperventilation is deleterious in regions vulnerable to excitotoxicity such as CA3. Early, aggressive, or prophylactic hyperventilation, therefore, in the context of reduced CBF, may potentiate excitotoxic mechanisms and augment neuronal death.

Aggressive hyperventilation in the early low-flow period did not worsen functional outcome or expand the contusion, failing to support a significant portion of our initial hypothesis. Ultimate contusion size, in controlled cortical impact or other models of focal contusion, is relatively refractory to manipulation by a variety of interventions, however, application of hypothermia, particularly prior to injury, reduces continent volume resulting from controlled cortical impact and lateral fluid-percussion injury. Although we chose rather aggressive hyperventilation in an attempt to produce a maximum effect, we did not test the effect of hyperventilation on a milder contusion, which may be more manipulable to secondary insults. The contusion penumbra has not been clearly defined in either of the standard rodent TBI models (controlled cortical impact or fluid-percussion) for any level of injury. It is possible that selectively vulnerable CA3 hippocampal neurons are the only potential target for a deleterious effect of hyperventilation in our model. However, the effect of hyperventilation on the survival of neurons in the dentate gyrus or hilus (all vulnerable to TBI) was not assessed.

Hippocampal damage and memory deficits are common after TBI in humans. This study did not reveal any added effect of hyperventilation on functional outcome deficits as measured by beam balance and MWM latencies. A number of factors may have contributed to this. Our sample size may have limited statistical power; however, this sample size was adequate to detect the exacerbation of functional deficits by the addition of 30 minutes of moderate hypoxemia (PaO₂, 40 mm Hg) in our model. Second, the cognitive deficits in this model are modest compared with those detailed in previous reports. Bilateral hippocampal damage may be necessary to create more marked functional deficits. In addition, CA3 damage may not mediate post-TBI memory deficits, as manifested in MWM test results. Finally, the specific functional outcome paradigm may not have the necessary sensitivity to detect subtle functional deficits. For example, more demanding MWM paradigms have been used by other investigators. However, in support of the testing strategy used, our hypothesis was that hyperventilation would worsen functional deficits.

This study does not completely address the uncommon situation in which, soon after severe head injury, marked intracranial hypertension is observed. Hyperventilation may in fact be life saving in its ability to impede herniation. Similarly, we did not measure ICP or titrate ventilation to control cerebral perfusion pressure, and we evaluated only one level of hyperventilation and injury severity. We did not attempt to model the clinical scenario of optimum titration of ventilation when ICP is increased. In the clinical setting, some investigators have demonstrated a wide variety of beneficial effects of hyperventilation under those conditions, such as homogenization of CBF, normalization of cerebral glucose uptake, and improvement in autoregulation. Rather, we chose the worst-case scenario, aggressive hyperventilation during the early posttrauma period when flow is already low and excitotoxicity is peaking. However, our study does show that hyperventilation is associated with a tangible risk to vulnerable neurons in the controlled cortical impact model. To our knowledge, this is the first in vivo study demonstrating that hyperventilation can augment neuronal injury after TBI, suggesting that there is indeed a tradeoff associated with this intervention.

**Conclusions**

We have demonstrated that aggressive, early hyperven-
Augmented neuronal death following hyperventilation post-TBI

tilation after TBI augments neuronal death in CA3 hippocampus. The further reduction of CBF with hyperventilation during the low CBF state immediately after severe TBI, coupled with alkalosis, may increase the vulnerability of selected neurons to traumatic injury. Further studies are needed to delineate the relative contributions of these mechanisms to the observed effects. The results of this study reinforce that meticulous attention is necessary to prevent secondary injury after TBI, and a risk in the use of hyperventilation is demonstrated.

Acknowledgments

The authors thank Drs. Peter Safar and Walter Ohrist for helpful comments. We also thank Raymond Griffith, Erica Baum, and Xiecheng Ma for technical assistance; Linda Amick and Marc Provis for preparing the manuscript; and Francie Siegfried for editorial assistance.

References

33. Meis G, Ishimaru S, Xie Y, et al: Ischemic thresholds of cerebral protein synthesis and energy state following middle cere-

Manuscript received June 9, 1997.
Accepted in final form October 23, 1997.
This study was funded by the following organizations: US Army Medical Research Acquisition Activity No. DAMD17–97–1–7009 (P.K.); the Laerdal Foundation (P.K.); National Institutes of Health Grants NS30318 (D.M.) and NS3198 (C.E.D.); Children's Hospital of Pittsburgh Seed Grant (M.F.); and Department of Veterans Medical Affairs Merit Review Program (S.G.).
Address reprint requests to: Patrick M. Kochanek, M.D., Safar Center for Resuscitation Research, 3434 Fifth Avenue, Pittsburgh, Pennsylvania 15260.
Therapeutic Hypothermia After Traumatic Brain Injury or Hemorrhagic Shock: From Mild Cooling to Suspended Animation

Objectives:
1. To familiarize the reader with contemporary studies on the application of resuscitative hypothermia in the treatment of traumatic brain injury and hemorrhagic shock.
2. To describe the potential mechanisms for the beneficial effects of hypothermia in these settings.
3. To present some recent findings from both laboratory and clinical studies of resuscitative hypothermia conducted at the University of Pittsburgh.
4. To discuss possible side effects and limitations of the application of therapeutic hypothermia.
5. To discuss future directions for novel applications of hypothermia in combination with pharmacologic interventions.

Historical Perspective

One of the earliest reports of the potential beneficial effects of hypothermia in the treatment of traumatic brain injury was described by Charles Phelps in 1897 in his classic textbook "Traumatic injuries of the brain and its membranes." It is fitting that this monograph was assembled on the 100th anniversary of this remarkable description.

"The shaving of the head, which had been advised as a means of facilitating diagnosis, is at the same time a measure of treatment... The essential advantage... to be derived from this procedure is that it permits the effective application of the ice-cap, which next to trephination, is most nearly a directly curative resource... It is contraindicated in hemorrhages and cerebral lacerations when uncomplicated by serious contusion; but, as those lesions are constantly thus complicated, it may be held a proper resort when such symptoms are manifest, without regard to exact diagnosis."

In the early 1940s, Fay and colleagues examined the deliberate application of hypothermia in traumatic brain injury, and this was followed by several additional series of case reports and uncontrolled trials between 1943 and 1979 by other pioneers in this field including Woringer et al., Sedzimir, Lazorhes and Campan, and Rosomoff in traumatic brain injury, Albin et al. in spinal cord injury, Bigelow et al. and Swan et al. in cardiothoracic surgery, Rosomoff et al. in focal cerebral ischemia, Siebke et al. and Conn et al. in near drowning. Wolfe, Benson et al., Ravitch and Safar in cardiopulmonary arrest, and Rush et al. in the application of deep hypothermia for total circulatory arrest. Although remarkable effects were suggested in many of these reports, they failed to demonstrate convincingly that hypothermia was beneficial and did not result in the widespread application of resuscitative hypothermia. These reports were complicated by a number of difficulties including variation in depth and duration of hypothermia, and failure to include concurrent normothermic controls. In addition, reports of potential infectious complications in patients treated with the sustained application of moderate hypothermia tempered enthusiasm for further studying resuscitative hypothermia in a controlled fashion.

Laboratory studies supporting the application of therapeutic hypothermia in traumatic brain injury and hemorrhagic shock

In the mid 1980s there was renewed interest in the laboratory investigation of the deliberate application of therapeutic hypothermia for protection (induced before the insult) or resuscitation (induced after the insult). This work was focused predominantly in models of global cerebral ischemia in rats and monkeys, cardiopulmonary arrest, and near drowning in dogs. Central to this resurgence in interest in hypothermia was the development of three novel concepts: 1) that remarkably mild hypothermia (a temperature reduction of between 3° and 5°C) was effective in reducing secondary brain damage, 2) that the duration of mild hypothermia necessary for a beneficial effect might be transient - as short as 1 or 2 hours, and 3) that brain temperature, not body temperature, was the critical therapeutic target.

The chance discovery of the efficacy of mild, transient hypothermia in these studies revived the importance of hypothermia research because mild and transient hypothermia are safer and easier to induce than the previously tried moderate, sustained hypothermia. It is important to define the approximate temperature ranges commonly used to describe specific depths of therapeutic hypothermia. Generally accepted definitions of these ranges are mild (34° to 36°C), moderate (28° to 32°C), and profound (< 15°C) hypothermia.
Specific investigation of the application of therapeutic hypothermia in the treatment of traumatic brain injury was renewed by the report of Clifton et al. who observed an inverse correlation between functional outcome and brain temperature (between 30° and 40°C). This was followed by a series of reports from several laboratories further defining the beneficial effect of hypothermia in a wide variety of models (both rodent and canine) of traumatic brain injury.\(^{12-20}\)

Recent controlled laboratory studies of the utility of resuscitative hypothermia in models of hemorrhagic shock developed from the initial work of Crippen et al. in our center\(^{21}\) and of Meyer and Horton.\(^{22}\) This resuscitative effect was demonstrated in models of both controlled\(^{23,24}\) and uncontrolled\(^{25}\) hemorrhagic shock (Figure 1), and with both mild and moderate hypothermia.\(^{26,27}\) In controlled laboratory studies addressing an additional hemorrhagic shock-related application of deliberate hypothermia, Tisherman et al.\(^{28}\) investigated the application of deep and profound hypothermic circulatory arrest to enable resuscitative surgery that would otherwise be impossible. Our series of studies into "suspended animation" has culminated so far in the study by Capone et al.\(^{29}\) who reported complete recovery of the brain in dogs after normothermic hemorrhagic shock of 1 hour followed by profound hypothermic circulatory arrest of 1 hour. This application of resuscitative hypothermia is being further developed as a possible novel therapeutic approach to the management of pulseless battlefield casualties, specifically, "suspended animation" for transport and repair of otherwise lethal extracranial wounds. "Suspended animation" could be induced and reversed by portable cardiopulmonary bypass\(^{30}\) and followed by subsequent delayed resuscitation.\(^{31}\)

**Why hypothermia? Proposed mechanisms for the beneficial effects of deliberate hypothermia in traumatic brain injury and hemorrhagic shock**

Laboratory and clinical trials in cerebral resuscitation from ischemic or traumatic brain injury have repeatedly highlighted the tremendous challenge involved in demonstrating reproducible efficacy, in a wide variety of injury models or injury types, when a single therapeutic agent is used.\(^{6,32}\) The complex, multifactorial nature of the cascades of secondary injury damage purported to occur in both ischemic and traumatic brain injury strongly suggests the need for multimodal therapies.\(^{33-36}\) A similar multifactorial pathogenesis is proposed in the evolution of visceral damage after hemorrhagic shock.\(^{37}\) A great deal of evidence suggests that hypothermia favorsably and simultaneously influences a large number of secondary injury mechanisms including: energy failure,\(^{38,39}\) oxidant injury,\(^{40-42}\) delayed neuronal death,\(^{43}\) excitotoxicity,\(^{44}\) intracranial hypertension\(^{45}\) edema formation,\(^{46,47}\) cytoskeletal protein degradation,\(^{48}\) blood-brain barrier permeability,\(^{49}\) IL-1β production\(^{50}\) (Figure 2), and neutrophil accumulation.\(^{51}\) It is very likely that some critical combination of beneficial effects on these mechanisms is responsible for the success of therapeutic hypothermia in experimental and clinical trials.

**Clinical investigation of therapeutic hypothermia in traumatic brain injury**

Although there is a much larger body of laboratory data supporting the use of mild, transient, resuscitative hypothermia in ischemic rather than traumatic brain injury, clinical application of deliberate hypothermia has been spearheaded in controlled trials after traumatic brain injury. Uncontrolled trials of moderate hypothermia in patients after traumatic brain injury looked promising\(^{52-54}\) but were abandoned because of management problems. Marion et al.\(^{55}\) reported a beneficial effect of moderate (32°C), transient (24 hours) hypothermia on intracranial hypertension in adults with severe closed head injury. A reduction in the need for other therapies for control of intracranial hypertension was observed. Clifton et al.\(^{56}\) reported a reduction in the incidence of posttraumatic seizures in adults treated with moderate hypothermia for 48 hours after severe head injury. A trend toward improved outcome was also observed. Similarly, Shiozaki et al.\(^{57}\) reported efficacy of mild hypothermia in controlling refractory intracranial hypertension in patients with severe traumatic brain injury. Most recently, Marion et al.\(^{58}\) demonstrated that moderate (32°C), transient (24 hours) hypothermia improved functional outcome as measured with the Glasgow outcome scale at 6 months after severe traumatic brain injury in 82 patients randomized to either hypothermia or normothermia. This beneficial effect extended to 12 months in the subgroup of patients with admission Glasgow coma score of 5 to 7 (Table 1). In addition, reductions in IL-1γ and glutamate concentrations were demonstrated in cerebrospinal fluid samples from hypothermic vs normothermic patients, suggesting the possibility of beneficial effects of hypothermia on posttraumatic inflammation and excitotoxicity, respectively. Remarkably, a significant reduction in cerebral metabolic rate for oxygen was not observed,\(^{59-61}\) suggesting that this beneficial effect was not due to a simple reduction in cerebral oxidative metabolic demands. A multicenter randomized controlled clinical trial of 48 hours of hypothermia vs normothermia in the treatment of human head injury is currently underway.

**Potential limitations and complications of the application of deliberate hypothermia**

Hypothermia is associated with potentially limiting side effects. Suppression of acute inflammation\(^{62}\) and an increased infection risk\(^{63-65}\) are concerns. These complications appear to be importantly related to the duration of hypothermia and the underlying condition that is being treated. In traumatic brain injury, Marion et al.\(^{66}\) and Clifton et al.\(^{67}\) did not observe increases in the incidence of infection with 24 hour and 48 hour...
applications of hypothermia, respectively. However, longer applications of hypothermia may have considerable risk.\textsuperscript{4} In addition, application of mild hypothermia in settings not associated with ischemia but associated with considerable infection risk (such as elective abdominal surgery in patients with malignancies) increases infection rates.\textsuperscript{56}

Coagulopathy is suggested as another potential complication of hypothermia. However, in the studies of severely head injured patients by Marion et al,\textsuperscript{64,66} platelet counts and prothrombin times did not differ significantly between groups, and no difference in posttrauma intracranial hematomas or other hemorrhagic complications were noted despite the fact that some of the patients had multiple trauma. Cardiac arrhythmias were also not observed. The threshold for these complications appears to be temperatures below 30°C.\textsuperscript{56,67} On the other hand, a recent report\textsuperscript{13} suggested that morbid cardiac events after non-cardiac surgery were more common in mildly hypothermic patients compared to those who remained normothermic. Although the systemic complications appear relatively minimal for the transient (24 hour) application of mild or moderate hypothermia, one area of investigation that deserves further study is that of the effect of hypothermia on regenerative and endogenous defense mechanisms in brain. Goss et al\textsuperscript{69} reported that 4 hours of moderate hypothermia resulted in a sustained inhibition of nerve growth factor production in brain after experimental contusion in rats. Nerve growth factor is an important homeostatic molecule in the central nervous system that upregulates antioxidant defenses and prevents apoptosis. The ramifications of this effect of hypothermia on brain parenchyma is currently under investigation.

Finally, another potential limitation of resuscitative hypothermia may be that it produces a temporary rather than sustained effect — i.e., delays rather than ameliorates damage. This possibility was first suggested in classic studies of the effect of hypothermia on acute inflammation,\textsuperscript{62,63} and was reintroduced in work by Dietrich et al\textsuperscript{72} in models of global cerebral ischemia, where brief episodes (1-3 hours) of hypothermia only delayed death of neurons in selectively vulnerable brain regions. Recent work by Colbourne et al,\textsuperscript{76} however, suggests that longer durations of hypothermia may produce permanent benefit.

### Future directions

Some of the most intriguing recent work in the therapeutic application of hypothermia in laboratory studies involves the combination of hypothermia with other therapies. Dietrich et al\textsuperscript{72} reported that combination of 3 hours of moderate hypothermia with sustained administration of the glutamate antagonist MK-801 produced a synergistic beneficial effect on neuronal survival in a model of global cerebral ischemia (Figure 3). Similar reports have been suggested for the combination of hypothermia and other therapies.\textsuperscript{77} Additional promising strategies that will require further study include the combination of hypothermia with either growth factors,\textsuperscript{38} anti-inflammatory agents or flow promoting treatments.\textsuperscript{78,79}
et al.: Improved survival of hemorrhagic shock with oxygen and hypothcr-
et al.: Mild posttraumatic hypothermia reduces mortality after severe
29. Tisherman S, Chahal C, Safar P, el al.: Resuscitation of dogs from cold-water submersion using car-
et al.: The influence of mild body and brain hypothermia
et al.: Extending the golden hour of volume controlled hemorrhagic shock
et al.: Prophylactic hypothermia (<15°C) compared with deep hypothermia (15°C) improves neurologic outcome in dogs after two hours' circulatory arrest induced to enable resuscitative surgery. J Trauma 31: 1001-1002, 1991.
et al.: Delayed postischemic hypothermia: A six-month survival study using lichav-
No Long Term Benefit from Hypothermia after Severe Traumatic Brain Injury with Secondary Insult in Rats

Courtney L. Robertson, MD; Robert S.B. Clark, MD; C. Edward Dixon, PhD; Henry L. Alexander, BS; Steven H. Graham, MD, PhD; Stephen R. Wisniewski, PhD; Donald W. Marion, MD; Peter J. Safar, MD; Patrick M. Kochanek, MD

Departments of Anesthesiology and Critical Care Medicine, Pediatrics, Neurological Surgery and Neurology, the School of Public Health, the Safar Center for Resuscitation Research, and the Brain Trauma Research Center, University of Pittsburgh, Pittsburgh, Pennsylvania, and Geriatric Research Educational and Clinical Center, Pittsburgh, VAHS.

Reprints will be ordered (see mailing address below)

Address reprint requests to:

Patrick M. Kochanek, M.D.
Safar Center for Resuscitation Research
3434 Fifth Avenue
Pittsburgh, PA 15260
Telephone: 412-383-1900
Fax: 412-624-0943
kochanek@smtp.anes.upmc.edu
ABSTRACT

Objectives: To evaluate the effect of application of transient, moderate hypothermia on outcome following experimental traumatic brain injury (TBI) with a secondary hypoxemic insult.

Design: Prospective, randomized study.

Setting: University-based animal research facility.

Subjects: Male Sprague-Dawley rats.

Interventions: All rats were subjected to severe traumatic brain injury (TBI) followed by 30 min of moderate hypoxemia, associated with mild hypotension. Rats were randomized to three groups: a) normothermia (37 ± 0.5 °C); b) immediate hypothermia (32 ± 0.5 °C initiated after trauma, before hypoxemia); and c) delayed hypothermia (32 ± 0.5 °C after hypoxemia). The brain temperature was controlled for 4 h after TBI and hypoxemia.

Measurements and Main Results: Animals were evaluated after TBI for motor and cognitive performance using beam balance (days 1-5 after TBI), beam walking (days 1-5 after TBI) and Morris Water Maze (days 14-18 after TBI) assessments. On day 21 after TBI, rats were perfused with paraformaldehyde and brains were histologically evaluated for lesion volume and hippocampal neuron counts. All three groups showed marked deficits in beam balance, beam walking and Morris Water Maze performance. However, these deficits did not differ between groups. There was no difference in lesion volume between groups. All animals had significant hippocampal neuronal loss on the side ipsilateral to injury, but this loss was similar between groups.

Conclusions: In this rat model of severe TBI with secondary insult, moderate hypothermia for 4 hours post-trauma failed to improve motor function, cognitive function,
lesion volume or hippocampal neuronal survival. Combination therapies may be necessary in this difficult setting.

Key Words: traumatic brain injury; hypothermia; hypoxemia
INTRODUCTION

Secondary insults after experimental traumatic brain injury (TBI) have been shown to exacerbate disturbances in key physiologic parameters, including hypoperfusion, energy failure, cerebral edema, and EEG suppression (1-3). In addition, animals subjected to a secondary hypoxemic insult after TBI have worse motor and histologic outcomes than those subjected to TBI alone (1, 3, 4). Following severe TBI, patients often experience a variety of secondary systemic insults related to extracerebral traumatic injury. As many as 20 - 50 % of patients presenting with severe TBI have experienced a period of hypoxemia (5-7). Autopsy findings of head injured patients (8) demonstrate evidence of ischemic neuronal death throughout the brain. Similarly, clinical studies have demonstrated higher morbidity and mortality among head injured patients who had experienced a secondary insult, specifically hypoxemia or hypotension (7). Often, the most severely devastated patients are those who experience the combination of TBI with hypoxemia and hypotension.

Hypothermia has been used as a successful treatment modality following brain injury in many experimental models and clinical settings. Neuroprotective effects of hypothermia in animal models include attenuation of release of excitatory amino acids (9, 10, 11), reduction in hydroxyl radicals (9) and inflammatory mediators (12, 13), and reduction in disruption of the blood-brain barrier (14). In the setting of experimental TBI, hypothermia improves outcome (15, 16). In models of fluid percussion injury (15) and controlled cortical impact (CCI) (16) reductions in both functional and motor deficits are observed in animals treated with moderate hypothermia after TBI when compared to normothermic animals. Transient, moderate hypothermia applied following global or
focal ischemic insult in animal models has improved histologic outcomes (17-19). Clinical studies have similarly demonstrated improvements in functional outcomes (20) and ICP (21, 22) in patients treated with moderate hypothermia.

Despite the importance of secondary insults to clinical outcome after TBI, the variety of experimental models of TBI and secondary insult that have been developed, and the success of hypothermia in both clinical and experimental TBI, the effect of the application of hypothermia in the setting of TBI with secondary insult has not been studied. We hypothesized that moderate hypothermia would improve outcome after CCI with secondary insult in rats.
MATERIALS AND METHODS

This study was approved by the University of Pittsburgh Animal Care and Use Committee. The care and handling of animals were in accord with National Institute of Health guidelines.

Experimental protocol

Virus-free male Sprague-Dawley rats (329-460 g) were studied. The animals were allowed free access to food and water before and after surgery. All surgical procedures were performed using aseptic technique.

Anesthesia was induced in a plastic jar with 4% isoflurane (Anaquest, Memphis, TN) in O₂. The trachea was intubated with a 14-gauge angiocatheter and the lungs were mechanically ventilated with 2% isoflurane/66% N₂O/balance O₂. A femoral arterial catheter (PE-50) was inserted for continuous monitoring of blood pressure and arterial blood sampling. Pancuronium bromide (0.1 mg/kg/h, Elkins-Sinn, Cherry Hill, NJ) was given for immobilization. A rectal probe was inserted to monitor core temperature.

Traumatic Brain Injury Model

The head was fixed in a stereotactic device (David Kopf, Tujunga, CA) and a midline scalp incision was made to expose the parietal bone. A craniotomy was made over the left parietal cortex with a dental drill, using the coronal and interparietal sutures as margins. The intact dura and bone flap were left in place until immediately before trauma. A temperature probe (0.009 inch outside diameter, Physiotemp Corp., Clifton, NJ) was inserted through a burr hole into the left parietal cortex 5 mm anterior to the bregma and 2 mm lateral to the sagittal suture. Rats were equilibrated under anesthesia (1.1% isoflurane/66 % N₂O/balance O₂) at a brain temperature of 37 ± 0.5°C for 30 min
before TBI. Fifteen minutes before trauma an arterial blood sample (0.5 ml) was obtained to verify normal arterial blood gas (ABG) tensions, serum glucose and hematocrit.

TBI was performed using the CCI device (23, 24), with minor modifications to the procedure previously described (25). Briefly, after removal of the bone flap, injury was produced using a device with a 6-mm metal impactor tip that is pneumatically driven in the vertical plane at a predetermined depth, velocity, and duration of brain deformation. For all studies, a depth of penetration of 2.5 mm, a velocity of 4.0 ± 0.2 m/sec, and a duration of deformation of 50 msec was used. Following trauma, the bone flap was replaced and sealed with dental cement (Koldmount, Vernon Banshoff Co., Albany, NY) and the scalp incision was closed.

Secondary Insult

Beginning 1 min after CCI, all rats underwent a 30 min period of hypoxemia as previously described (4). Air and oxygen were blended to achieve an FiO2 of 11% (1.1% isoflurane/74% N20/19% air/6% O2). This produced a PaO2 range of 46-51 and MAP range of 50-74. Arterial blood gas (ABG) samples were obtained in all rats at 10 and 25 min during the hypoxemic period.

Hypothermia

Rats were randomized into three groups: normothermia, immediate hypothermia and delayed hypothermia. Hypothermia (temp = 32°C) was achieved by the external application of ice packs to the head to lower brain temperature over a 15 min period. This temperature was maintained for 4 h and the brain was rewarmed over 1 h. ABG samples were obtained every hour during the hypothermia period. Physiologic parameters (MAP,
brain temperature, rectal temperature) were recorded every 30 min during hypothermia and during rewarming.

The immediate hypothermia group (n=10) had brain cooling initiated immediately after trauma, coincident with the onset of hypoxemia. The delayed hypothermia group (n=10) had cooling initiated upon the completion of hypoxemia, 30 min after TBI. The normothermia group (n=19) had brain temperature maintained at 37 ± 0.5°C throughout the experimental period. At the end of the experiment, after completion of rewarming, anesthesia was discontinued. Rats were extubated, placed in 100% oxygen for an additional 30 min, then returned to their cages, where they were allowed free access to food and water.

Motor Function Assessments

Gross vestibulomotor function was assessed using a beam-balance task (24, 26). The rats were trained by three trials prior to TBI to obtain a baseline measurement. Beam-balance latency (up to 60 sec) was measured on days 1-5 after TBI.

Fine vestibulomotor function and coordination were assessed using a beam-walking task (27). Performance was assessed by measuring the rat's latency to traverse the beam and enter a goal box. Beam-walking latency was measured on days 1-5 after TBI.

Cognitive Assessment (Morris Water Maze)

Water-maze testing started on day 14 postinjury. The hidden platform task assesses the rat's ability to learn spatial relations between distal cues and the escape platform. Performance is impaired by cortical and hippocampal lesions. We used a variant of the Morris water maze (28). Rats were given 120 sec to find the hidden platform. If the rat failed to find the platform within 120 sec, it was placed on the
platform by the experimenter. Rats were given four swimming trials per day for 5 consecutive days. Water maze tests were given on days 14-18 after TBI. The last 2 days of testing consisted of a visible platform task in which the platform was raised 2 cm above the water surface. This visible task controls for potential non-specific visual or motor deficits.

**Lesion Volume Analysis**

At 21 d after TBI, rats were anesthetized and perfused with 500 ml of 4% buffered formaldehyde. Brains were removed and post-fixed for a minimum of 1 wk at 4°C and cryoprotected in sucrose. Coronal sections (10-μm) were prepared through the entire brain at 1-mm intervals from the occiput. Sections were stained with cresyl violet. In the serial sections taken at 1-mm intervals, the margins of both the contusion and the total left hemisphere were outlined by a blinded observer using image analysis (Image Research). Contusion and hemispheric areas were measured. Contusion volume was calculated and expressed as mm³.

**Hippocampal Cell Counts**

Neuronal loss in hippocampal CA1 and CA3 pyramidal layers was quantified using a method previously described by Clark et al (4). A coronal section through the dorsal hippocampus underlying the area of contusion was used for analysis. This location was approximately 2.6 mm posterior to bregma. The regions were visualized at 400X magnification by a blinded observer. CA1 and CA3 hippocampal neurons were counted in six separate fields for each region in both injured and uninjured hemispheres. Only complete cells with a defined cell body and intact nucleus were counted. Hippocampal
neuron survival was reported as the average number of surviving neurons per high power field ipsilateral to injury.

Statistical Analysis

All data are presented as mean ± SEM. Because of the large number of physiologic variables recorded, comparisons of physiologic data were made using a multiple regression analysis, evaluating the effect of treatment and time for each variable. Beam balance, beam walking and Morris water maze data were analyzed using repeated-measures analysis of variance (ANOVA) using GB-STAT statistical software. Lesion volumes and hippocampal neuron counts were compared using The Kruskal-Wallis test and Dunn's test. A significance level of $p < 0.05$ was used for all tests.
RESULTS

Physiologic Variables

Physiologic data (including brain and rectal temperature, MAP, arterial pH and blood gasses, blood glucose and hematocrit) from all animals that survived the experimental protocol are presented in Table 1. There were no differences between groups in pH, PaCO₂, and hematocrit. As expected, the groups differed in brain and rectal temperatures ($p < 0.05$), and this difference was seen between groups when controlling for time. Brain temperature remained at $37 \pm 0.2 \, ^\circ{\text{C}}$ in the normothermia group. Brain temperature decreased from $37.2 \pm 0.1 \, ^\circ{\text{C}}$ before trauma to $32.0 \pm 0.1 \, ^\circ{\text{C}}$ by 25 min of cooling in the immediate hypothermia group. Brain temperature decreased from $37.1 \pm 0.1 \, ^\circ{\text{C}}$ before trauma to $32.1 \pm 0.1 \, ^\circ{\text{C}}$ by 25 min of cooling in the delayed hypothermia group.

Groups differed in MAP when controlling for time ($p < 0.05$). Initial MAP in the normothermia group was 91-92 and decreased to 62-63 mm Hg during hypoxemia, returning to a post-insult level of 84-85 mm Hg. The immediate hypothermia group started with a baseline MAP of 103-104, decreased to 68-74 during hypoxemia, and returned to 91-94 mm Hg post-insult. The delayed hypothermia group had the lowest overall MAP, starting with a baseline of 84-85, decreasing to 50-54 during hypoxemia and returning to 71-76 mm Hg post-insult.

Groups also differed in PaO₂ and glucose when controlling for time (both $p < 0.05$). The differences between groups appeared modest and unlikely to be clinically significant. Glucose levels were also different between groups over time. The normothermia group had initial glucose levels of 182 mg/dl and decreased to levels of 148 mg/dl at 3 h post-insult. The immediate and delayed hypothermia groups had more stable glucose levels.
Survival

Survival rate to 21 d for completion of motor and cognitive testing was 79 % for the normothermia group, 80 % for the immediate hypothermia group and 62 % for the delayed hypothermia group. Motor performance, cognitive performance, lesion volume analysis and hippocampal neuron counts are reported on all animals that survived to cognitive testing at 20 d and were able to swim for water maze testing.

Motor Performance

All three groups showed a decrease in beam balance performance and an increase in beam walking latency following trauma. However, there was no difference in beam balance duration (Figure 1) or beam walking latency (Figure 2) between normothermic and hypothermic groups.

Cognitive Performance (Morris Water Maze)

All three groups showed a marked latency in finding the submerged platform on days 14-18 following trauma. However, there was no difference between groups in performance in the water maze (Figure 3). The groups were similar in discovery of the visible platform on days 19-20 following trauma (Figure 3).

Lesion Volume Analysis

Lesion volumes (mm$^3$) measured at 21 d after TBI are shown in Table 2. There appeared to be a reduction in lesion volume in the hypothermia vs normothermic groups. However, this reduction in lesion volume was not statistically different from normothermia. Lesion area at various distances from the occiput is shown in Figure 4.

Hippocampal Neuron Counts
Surviving hippocampal neuron counts are shown in Table 3. Both normothermic and hypothermic animals had significant hippocampal neuronal loss on the side ipsilateral to injury. For comparison, average neuron counts in CA1 and CA3 hippocampus on the side contralateral to injury were 48-56 cells/hpf. There were no significant differences in the number of surviving CA1 or CA3 hippocampal neurons in the hypothermic versus normothermic groups.
DISCUSSION

To our knowledge, this is the first study to evaluate the effect of hypothermia on severe experimental TBI with secondary insult. Surprisingly, no difference in motor performance, cognitive performance, lesion volume or hippocampal neuronal survival was observed with the application of moderate hypothermia after severe TBI with secondary insult in a rat model. Also unexpectedly, both the immediate hypothermia group, with hypothermia initiated after trauma and before secondary hypoxemia, and the delayed hypothermia group, with hypothermia applied after both brain trauma and hypoxemia, demonstrated similar functional and histologic deficits when compared to each other and to the normothermia group.

Beneficial effects of hypothermia on histopathologic outcome following TBI have been demonstrated by several investigators. The timing of this histologic evaluation appears to be important. Dietrich et al (29) showed a reduction in cortical contusion volume and frequency of necrotic cortical neurons in rats that received 3 hours of immediate post-trauma hypothermia (30°C) following parasagittal fluid percussion injury. These animals were evaluated at 3 days post-trauma. However, in the same model, investigators found no difference in hippocampal CA1, CA3, CA4 or dentate neuronal survival in rats receiving post-trauma hypothermia compared to normothermic animals when brains were analyzed at 8 weeks following TBI (30). In models of ischemic brain injury, transient application of therapeutic hypothermia has also shown a temporary beneficial effect on hippocampal neuronal survival. Early evaluation revealed decreased hippocampal CA1 cell loss, but this protection by post-trauma hypothermia (30°C) was not seen in the animals evaluated at 2 months following ischemia insult (31). In our
model, severe TBI was followed by 30 min of hypoxemia. All animals showed a reduction in systemic blood pressure during the hypoxemic period, likely highlighting an ischemic component to the secondary insult.

The amount of tissue loss following experimental TBI varies greatly dependent on the model. This model of severe CCI followed by 30 minutes of hypoxemia produced lesion volumes of 50 to 65 mm$^3$. This is much larger than contusion volumes seen in other traumatic injury models, such as lateral fluid percussion (2.14 mm$^3$) (27), or in similar CCI models without hypoxemia (~30 mm$^3$), (5). The severe insult produced in this model might explain the failure of post-trauma hypothermia to show a significant reduction in lesion volume. However, other experimental TBI models applying hypothermia after injury have also failed to reduce necrotic volumes (30, 32). Cherian et al showed increasing sizes of contusion volume as the degree of secondary insult (bilateral carotid occlusion) increased following CCI in rats (33). In addition, it is likely that the lesion volume observed at 21 days post-injury is the result of damage by many different mechanisms operating in the early and late post-trauma phases. Clark et al (4) has demonstrated cells with either necrotic or apoptotic phenotypes in various brain regions following TBI in a similar model with hypoxemia. It is unclear if earlier assessment would have revealed more hippocampal protection with post-traumatic hypothermia in this model. However, our goal is to favorably influence long-term outcome. Hypothermia as a single treatment modality, and applied for only 4 hours following TBI, might be unable to reduce overall lesion volumes in such a model.

Previous experimental studies applying moderate hypothermia after TBI have demonstrated protection against motor and spatial memory deficits after both CCI (16)
and fluid percussion injury (30, 34). However these models did not include a period of hypoxemia or any other secondary insults after trauma. This secondary hypoxemic insult worsens histologic outcome and could, therefore, worsen behavioral outcome following TBI. In a similar model of CCI with secondary hypoxemia, rats demonstrated progressively worse motor function (beam-balance latency) with increasing amounts of post-trauma hypoxemia (4). This trend was seen even in rats who received mild hypoxemia (PaO₂ = 58-63 mm Hg) following CCI. In a fluid percussion injury model, Ishige et al (1) showed significantly worse neurological status scores in rats that underwent impact injury followed by a 30 minute period of hypoxemia (PaO₂ = 35-40 mm Hg) versus those injured without secondary hypoxemia. These neurologic deficits were also not observed in rats that received hypoxemia alone.

Clinical studies of patients with head injury have also reported marked worsening of outcome parameters in the setting of TBI with secondary insult such as hypoxemia or hypotension. In an analysis of 717 patients from the Traumatic Coma Data Bank, Chestnut et al (7) found that hypoxia and hypotension were independently associated with increases in morbidity and mortality from severe head injury. This study showed a marked shift towards vegetative/dead outcomes in patients who endured hypoxia (PaO₂ ≤ 60 mm Hg) or hypotension (systolic BP ≤ 90) during the pre-hospital or resuscitation period. This is especially relevant to our model of TBI because rats underwent a planned 30 min period of hypoxemia, which was also associated with a decrease in systemic blood pressure. The difference in MAP between groups may have caused additional experimental differences. The delayed hypothermia group had an overall lower MAP trend, and may have experienced more significant secondary ischemic flows.
Clinical studies applying hypothermia after TBI have yielded a variety of positive results. In a phase II study of moderate hypothermia, Clifton et al found a reduction in incidence of post-traumatic seizures (35). Shiozaki et al (22) documented improved control of intracranial pressure (ICP) with the application of mild (34°C) hypothermia after conventional therapies had failed to control ICP (22). Most recently, Marion et al (20) demonstrated faster recovery of functional outcome with the application of moderate hypothermia (32°C) after severe TBI in 82 patients randomized to either normothermia or hypothermia for 24 hours after injury. Relevant to our findings, these clinical trials showed important distinctions in the subset of very severely injured patients. Marion's study included all patients with initial GCS ≤ 8, but the beneficial effect of hypothermia only extended to 12 months in the subset of patients with initial GCS = 5-7 (20). In Shiozaki's study, the subset of patients admitted with GCS scores of 3-4 had a much lower incidence of favorable outcome at 6 months after injury (only 1 patient out of 22, 4.5%) versus the group with GCS scores of 5-7 (11 patients out of 40, 27.5%), despite the application of mild hypothermia (22). Importantly, Marion et al excluded patients with hypoxia or hypotension.

Our model of CCI with hypoxemia results in a very severe injury relative to other models. In addition, during the 30 min period of hypoxemia, the rats experience a significant drop in their mean arterial pressure. In a recent experimental TBI model (36), post-traumatic application of moderate hypothermia (30°C for 3h) resulted, after rewarming, in a lower cerebral perfusion without a corresponding decrease in cerebral glucose utilization, creating a state of metabolism to blood flow mismatch. This may be especially important in the clinical setting in which severely head-injured patients undergo
a secondary insult of hypoxia and/or hypotension prior to initiation of treatment for their head injury. Analysis of trauma patients has consistently revealed worse outcomes in patients who experienced sustained hypotension in the pre-hospital setting versus those who remained normotensive. Specifically, Chestnut et al (7) showed hypotension was associated with a 150% increase in mortality rate. Wald et al (37) also found that prehospital hypotension doubled the incidence of adverse outcome (37). In a review of pediatric trauma patients, Pigula et al (38) found that hypotension significantly increased the mortality rate. As a result, patients with significant secondary insult have been excluded from evaluation in clinical trials (20). This subset clearly represents a population in which currently available interventions may have limited efficacy --even those proven to be effective in the sets of TBI alone.

There are many mechanisms of cell injury and death after TBI. Investigators have shown additional pathophysiologic mechanisms operating when secondary insults were added to the already vulnerable, traumatically injured brain (1, 3, 33, 39). Recent experimental investigations have focused on the period following trauma, during which secondary insults may potentiate neuronal damage, utilizing a wide variety of therapies. However, many of these therapies have been tested in models of TBI where oxygenation and ventilation of animals following TBI are controlled. Given that many head-injured patients present with preceding hypoxemia, it may be important to reassess these therapies in models imitating this clinical setting. It is possible that proven treatments may be less effective in a model with applied hypoxemia and accompanying hypotension. Novel therapies targeting this complex clinical scenario have yet to be developed.
In conclusion, in this rat model of severe CCI with hypoxemia, moderate hypothermia for 4 hours post-trauma failed to improve hippocampal neuron survival, lesion volume, motor function or cognitive function. Combination therapies or development of novel therapies may be necessary to see significant improvement in outcome in this difficult setting.

Acknowledgment

This work was supported by U.S. Army #DAMD 17-97-1-7009. We thank Marci Provins for assistance with preparation of the manuscript.
REFERENCES


FIGURE LEGENDS

Figure 1. Beam balance latency in normothermia (closed circles), immediate hypothermia (open circles), and delayed hypothermia (triangles) on days 1-5 after CCI. Values are mean ± SEM.

Figure 2. Beam walking latency in normothermia (closed circles), immediate hypothermia (open circles), and delayed hypothermia (triangles) on days 1-5 after CCI. Values are mean ± SEM.

Figure 3. Morris Water Maze performance in normothermia (closed circles), immediate hypothermia (open circles), and delayed hypothermia (triangles) on days 14-18 after CCI. Visible platform latency in all groups on days 19-20 after CCI. Values are mean ± SEM.

Figure 4. Lesion area at various distances from the occiput (mm) in normothermia (closed circles), immediate hypothermia (open circles), and delayed hypothermia (triangles) groups. Values are mean ± SEM.
Table 1. Physiologic data

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain T (°C)</th>
<th>Rectal T (°C)</th>
<th>MAP (mm Hg)</th>
<th>pH</th>
<th>PaCO₂ (mm Hg)</th>
<th>PaO₂ (mm Hg)</th>
<th>Glucose (mg/dl)</th>
<th>HCT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C (n = 19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>37.1 ± 0.1*</td>
<td>37.3 ± 0.1</td>
<td>91 ± 3</td>
<td>7.44 ± 0.01</td>
<td>39 ± 1</td>
<td>169 ± 4</td>
<td>182 ± 10</td>
<td>40 ± 0</td>
</tr>
<tr>
<td>Insult</td>
<td>37.0 ± 0.1</td>
<td>37.3 ± 0.0</td>
<td>92 ± 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 min</td>
<td>37.1 ± 0.1</td>
<td>37.1 ± 0.1</td>
<td>63 ± 4</td>
<td>7.43 ± 0.01</td>
<td>40 ± 1</td>
<td>46 ± 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25 min</td>
<td>37.0 ± 0.0</td>
<td>37.1 ± 0.1</td>
<td>62 ± 3</td>
<td>7.42 ± 0.01</td>
<td>40 ± 1</td>
<td>47 ± 1</td>
<td>173 ± 13</td>
<td>39 ± 0</td>
</tr>
<tr>
<td>1 h</td>
<td>37.1 ± 0.0</td>
<td>37.2 ± 0.1</td>
<td>84 ± 2</td>
<td>7.43 ± 0.01</td>
<td>38 ± 1</td>
<td>160 ± 3</td>
<td>145 ± 4</td>
<td>39 ± 0</td>
</tr>
<tr>
<td>3 h</td>
<td>37.1 ± 0.1</td>
<td>37.2 ± 0.0</td>
<td>85 ± 3</td>
<td>7.42 ± 0.01</td>
<td>37 ± 1</td>
<td>172 ± 3</td>
<td>148 ± 4</td>
<td>38 ± 0</td>
</tr>
<tr>
<td>32°C - Immediate (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>37.2 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>103 ± 2</td>
<td>7.45 ± 0.01</td>
<td>40 ± 1</td>
<td>157 ± 3</td>
<td>171 ± 8</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>Insult</td>
<td>37.2 ± 0.0</td>
<td>37.3 ± 0.1</td>
<td>104 ± 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 min</td>
<td>32.5 ± 0.2</td>
<td>32.5 ± 0.2</td>
<td>74 ± 5</td>
<td>7.44 ± 0.01</td>
<td>37 ± 1</td>
<td>51 ± 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25 min</td>
<td>32.0 ± 0.1</td>
<td>32.1 ± 0.1</td>
<td>68 ± 3</td>
<td>7.45 ± 0.01</td>
<td>38 ± 1</td>
<td>50 ± 1</td>
<td>208 ± 22</td>
<td>39 ± 0</td>
</tr>
<tr>
<td>1 h</td>
<td>31.9 ± 0.1</td>
<td>32.0 ± 0.1</td>
<td>91 ± 4</td>
<td>7.45 ± 0.01</td>
<td>38 ± 1</td>
<td>201 ± 5</td>
<td>166 ± 11</td>
<td>39 ± 0</td>
</tr>
<tr>
<td>3 h</td>
<td>32.1 ± 0.1</td>
<td>32.0 ± 0.1</td>
<td>94 ± 3</td>
<td>7.42 ± 0.01</td>
<td>38 ± 0</td>
<td>193 ± 6</td>
<td>182 ± 15</td>
<td>39 ± 0</td>
</tr>
<tr>
<td>32° - Delayed (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>37.1 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>85 ± 2</td>
<td>7.43 ± 0.01</td>
<td>39 ± 1</td>
<td>176 ± 7</td>
<td>196 ± 14</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>Insult</td>
<td>37.1 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>84 ± 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 min</td>
<td>36.9 ± 0.1</td>
<td>37.1 ± 0.1</td>
<td>54 ± 3</td>
<td>7.42 ± 0.01</td>
<td>40 ± 1</td>
<td>47 ± 2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25 min</td>
<td>36.9 ± 0.1</td>
<td>37.1 ± 0.1</td>
<td>50 ± 2</td>
<td>7.41 ± 0.01</td>
<td>39 ± 1</td>
<td>47 ± 1</td>
<td>173 ± 10</td>
<td>39 ± 0</td>
</tr>
<tr>
<td>1 h</td>
<td>31.9 ± 0.1</td>
<td>31.9 ± 0.1</td>
<td>76 ± 2</td>
<td>7.43 ± 0.00</td>
<td>38 ± 1</td>
<td>205 ± 3</td>
<td>188 ± 11</td>
<td>39 ± 0</td>
</tr>
<tr>
<td>3 h</td>
<td>31.9 ± 0.1</td>
<td>32.0 ± 0.1</td>
<td>71 ± 5</td>
<td>7.41 ± 0.01</td>
<td>36 ± 0</td>
<td>215 ± 3</td>
<td>182 ± 9</td>
<td>37 ± 1</td>
</tr>
</tbody>
</table>

T = Temperature, MAP = Mean arterial pressure, HCT = Hematocrit

*Values are Mean ± SEM
Table 2: Lesion Volume

<table>
<thead>
<tr>
<th>Group</th>
<th>Lesion Volume&lt;sup&gt;a&lt;/sup&gt; (mm&lt;sup&gt;3&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermia</td>
<td>65.3 ± 6.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Immediate HT</td>
<td>50.2 ± 8.2</td>
</tr>
<tr>
<td>Delayed HT</td>
<td>53.7 ± 7.9</td>
</tr>
</tbody>
</table>

HT, hypothermia
<sup>a</sup> <i>p</i> = 0.32 by ANOVA
<sup>b</sup> Values are Mean ± SEM
Table 3. Hippocampal neuronal survival

<table>
<thead>
<tr>
<th>Group</th>
<th>CA1 Neurons(^a) (cells/hpf)</th>
<th>CA3 Neurons(^a) (cells/hpf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermia</td>
<td>19.4 ± 4.2(^b)</td>
<td>19.8 ± 4.6</td>
</tr>
<tr>
<td>Immediate HT</td>
<td>13.2 ± 8.7</td>
<td>15.6 ± 7.3</td>
</tr>
<tr>
<td>Delayed HT</td>
<td>13.7 ± 5.8</td>
<td>18.5 ± 7.3</td>
</tr>
</tbody>
</table>

HT = hypothermia, hpf = high-powered field
\(^a\) ipsilateral to injury
\(^b\) values are Mean ± SEM
January 7, 2000

Dr. John A. Jane, Sr.
Editor
Journal of Neurosurgery
1224 West Main Street, Suite 450
Charlotte, VA 22903

Dear Dr. Jane,

Enclosed please find our manuscript entitled "Isoflurane improves long-term neurologic outcome vs fentanyl after traumatic brain injury in rats" which we are respectfully submitting for publication in the Journal of Neurosurgery. This manuscript has not been submitted to any other journal. Please note that Dr. Kimberly Statler received the 1999 Women in Neurotrauma Research award for this work at the 1999 meeting of the National Neurotrauma Society.

We thank you in advance for consideration of our work.

Sincerely,

Patrick M. Kochanek, M.D.

cc: Kimberly Statler, M.D.
ISOFLURANE IMPROVES LONG-TERM NEUROLOGIC OUTCOME VS FENTANYL AFTER TRAUMATIC BRAIN INJURY IN RATS

Kimberly D. Statler, M.D.,  Patrick M. Kochanek, M.D., C. Edward Dixon, Ph.D., Henry L. Alexander, David S. Warner, M.D., Robert S.B. Clark, M.D., Stephen R. Wisniewski, Ph.D., Steven H. Graham, M.D., Ph.D., Larry W. Jenkins, Ph.D., Donald W. Marion, M.D., Peter J. Safar, M.D.

Departments of Anesthesiology and Critical Care Medicine, Pediatrics, Neurosurgery, Epidemiology and Public Health, and Neurology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
Safar Center for Resuscitation Research, Pittsburgh, Pennsylvania
Geriatric Research Educational and Clinical Center, Veterans Administration Pittsburgh Health Center, Department of Anesthesia, Duke University, Durham, North Carolina

Please direct correspondence to:
Patrick M. Kochanek, M.D.
Safar Center for Resuscitation Research
3434 Fifth Avenue
Pittsburgh, PA 15260
Phone: (412) 383-1900
Fax: (412) 624-0946
Email: kochanekpm@anes.upmc.edu

Running title: Isoflurane vs fentanyl after TBI in rats

Support: US Army DAMD Grant 17-97-1-7009 (P.M. Kochanek)
Abstract

Despite routine use of fentanyl in patients after traumatic brain injury (TBI), it is unclear if it is the optimal sedative/analgesic agent. Isoflurane anesthesia is commonly used in experimental TBI. We hypothesized that isoflurane would be neuroprotective vs fentanyl after TBI in rats. Rats underwent controlled cortical impact (CCI) and received 4 h of N₂O:O₂ (2:1) and either fentanyl (10 µg/kg iv bolus, 50 µg/kg/h infusion) or isoflurane (1% by inhalation) with controlled ventilation. Shams underwent identical preparation, but no CCI. Functional outcome (beam balance, beam walking, and Morris water maze [MWM] tasks) was assessed over 20 days. Lesion volume and hippocampal neuron survival were quantified on d 21. Additional rats underwent identical CCI and anesthesia with intracranial pressure (ICP) monitoring, and brain water content was assessed.

Motor and MWM performances were better in injured rats treated with isoflurane vs fentanyl (p < 0.05), but did not differ between shams. Damage to CA1 hippocampus was attenuated in isoflurane-treated rats (p < 0.05). Fentanyl-treated rats had higher mean arterial blood pressure (MAP) and cerebral perfusion pressure (CPP) after injury (p < 0.05); however, ICP and brain water were similar between treatment groups.

Ioflurane improved functional outcome and attenuated damage to CA1 hippocampus vs fentanyl in rats subjected to CCI. Isoflurane may be neuroprotective vs fentanyl by augmenting cerebral blood flow and/or reducing excitotoxicity, not by reducing ICP or brain water content. Alternatively, fentanyl may be detrimental. Isoflurane may mask beneficial effects of novel
agents tested in experimental TBI models. Additionally, fentanyl may not be the optimal sedative/analgesic agent early after TBI in humans.

Key words: sedation, analgesia, anesthesia, head injury, narcotics, opioids
Introduction

In current clinical practice, opioids are routinely administered after traumatic brain injury (TBI). Fentanyl is one of the first-line agents because of its short half-life and low incidence of hypotension. Despite standard clinical use, it remains unclear if fentanyl represents the optimal sedative/analgesic agent in the acute period following TBI. Unlike the clinical arena, opioids are rarely used in experimental TBI. In fact, most models of TBI use isoflurane or pentobarbital anesthesia.

Much of the study of opioids in TBI has focused on the actions of endogenous opiates, such as dynorphin, and specific opiate receptor effects.\textsuperscript{17,29,30,34,51} Although mu receptor agonists, such as morphine and fentanyl, have been shown to have some beneficial effects after central nervous system injury,\textsuperscript{29,30} recent studies in cerebral ischemia and focal cryogenic lesion suggest that isoflurane may be neuroprotective compared to fentanyl.\textsuperscript{37-39,48} In rats subjected to global cerebral ischemia, isoflurane reduced neuronal damage and improved motor function compared to fentanyl.\textsuperscript{37} Similarly, after focal ischemia, rats anesthetized with isoflurane had smaller infarct volumes than those receiving fentanyl. Lesion volumes in rats treated with fentanyl were similar to those in unanesthetized control rats.\textsuperscript{48} Isoflurane has been reported to enhance post-insult cerebral blood flow (CBF), produce widespread increases in brain surface $\text{PO}_2$ and reduce edema in a rabbit model of focal cryogenic lesion. Conversely, both CBF and regional $\text{PO}_2$ were decreased, and edema was increased, in rabbits anesthetized with fentanyl.\textsuperscript{38,39} Using a fluid-percussion model in cats, DeWitt et al, addressed the question of whether fentanyl was
detrimental in TBI and found that fentanyl produced no adverse effects compared to vehicle. However, in that study, fentanyl was administered to cats already anesthetized with isoflurane.³

To our knowledge, isoflurane has not been directly compared to fentanyl in a contemporary model of TBI with long-term functional outcome and histologic assessment. We hypothesized that isoflurane would be neuroprotective compared to fentanyl when administered early after TBI. To test our hypothesis, we directly compared fentanyl and isoflurane anesthesia in a controlled cortical impact (CCI) model of TBI.

Materials and Methods

Virus-free, mature male (280 - 400g) Sprague-Dawley rats were used in this study. The rats had free access to food and water before and after surgery. All studies were approved by the University of Pittsburgh Animal Care and Use Committee. All surgical procedures were performed using aseptic technique.

Outcome Protocol

Rats were initially anesthetized with N₂O:O₂ (2:1) and 4% isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL) via a nose cone and then endotracheally intubated with a 14-guage angiocatheter and mechanically ventilated. Anesthesia was maintained for the duration of surgical preparation with 2 - 2.5% isoflurane and N₂O:O₂ (2:1). Pancuronium bromide (0.1 mg/kg/h, Elkins-Sinn, Cherry Hill, NJ) was given intravenously for muscle relaxation. Femoral venous and arterial vessels were cannulated for continuous blood pressure measurement, blood
sampling, and administration of medications. A rectal probe was inserted to monitor core temperature. The rat was then placed in a stereotaxic frame (David Kopf, Tujunga, CA) and a left parietal craniotomy (7mm x 8mm) was performed using a high-speed dental drill. The dura and bone flap were left in place until immediately before CCI. A burr hole was drilled into the left frontal bone for temperature probe (2.28-mm outside diameter, Physiotemp Corp., Clifton, NJ) placement into the frontal lobe. Continuously monitored physiologic parameters included arterial blood pressure and rectal and brain temperatures. Parameters monitored intermittently included blood glucose, hematocrit, and arterial blood gas samples, which were assessed every 15 minutes for the initial hour and every 30 minutes thereafter. Throughout the experiment, PaCO$_2$ was controlled at 35 - 45 mm Hg. This protocol produced a PaO$_2$ of greater than 70 mm Hg in all preparations. Both brain and rectal temperatures were maintained at 37.0 ± 0.5 °C.

Rats were allowed to stabilize for 5 min after completion of surgical preparation and then randomized to receive either fentanyl or isoflurane anesthesia. In the fentanyl group (n = 9), isoflurane was discontinued and 10 μg/kg of fentanyl (50 μg/ml, Elkins-Sinn, Cherry Hill, NJ) was administered intravenously, followed by a continuous intravenous infusion at 50 μg/kg/h. In the isoflurane group (n = 9), inspired isoflurane concentration was reduced to 1%, and normal saline, the vehicle for fentanyl-treated rats, was administered to match the volume received by fentanyl infusion. Both anesthetic groups continue to receive N$_2$O:O$_2$ (2:1). After 30 min of equilibration, TBI was induced by CCI using a pneumatic-driven piston device that has been shown (with isoflurane anesthesia) to deliver a reliable and reproducible degree of injury with a mortality rate of less than 5%.$^{12,26}$ In pilot studies comparing isoflurane and fentanyl using our
standard CCI model (6-mm tip, 4 m/s velocity, 50 msec duration of deformation and 2.5-mm deformation depth), all of the fentanyl-treated rats developed pulmonary edema and died early after injury. Thus, to compare the effect of isoflurane vs fentanyl on long-term outcome in our model, our standard injury was reduced (6 mm tip, 4 m/s velocity, 50 msec duration of deformation and 2.0-mm deformation depth). After CCI, the bone flap was replaced and sealed with dental cement, and the scalp incision was closed. Anesthesia was continued for 4 h in the isoflurane group and 3.5 h in the fentanyl group to facilitate similar extubation times. At the end of the anesthetic period, rats received 100% oxygen, were allowed to awaken and resume spontaneous breathing, and were then extubated and returned to their cages. Sham rats underwent identical preparation and anesthesia, but no CCI (n = 6 per anesthetic group).

Motor function, including beam balance and beam walking tasks, was tested by an observer blinded to group assignment on days 1 - 5 after injury. Briefly, in the beam balance task, the rat was placed on a suspended, narrow wooden beam (1.5-cm wide) and the time that the rat remained on the beam was recorded (up to 60 sec). For beam walking, the rat was placed at one end of the beam and a dark, quiet chamber was located at the other end. An adverse stimulus of loud white noise was applied and the time for the rat to escape across the beam into the chamber was recorded (up to 60 sec). Rats were trained with three trials before CCI or sham injury, which also served as baseline values.

Morris Water Maze (MWM) testing was performed using an acquisition paradigm on days 14 - 20 after injury. Briefly, on post-injury days 14 - 18, the rat was placed into a pool (2-m diameter) and required to locate a hidden platform in order to escape the water. On post-injury days 19 and 20, the platform was raised so that the surface was visible 5-cm above the water
level. Latency to find the platform was used to compare performances. Swim speed was measured on post-injury day 20 to insure that rats in all experimental groups exhibited equal motivation and motor function.

Lesion volume and hippocampal neuron survival were assessed on day 21 after injury. Rats were re-anesthetized and then perfused with heparinized saline followed by 4% paraformaldehyde. Brains were removed, post-fixed and cryoprotected. Serial coronal sections (10-μm) were made at 1-mm intervals through the entire brain. Sections were mounted on slides and stained with cresyl violet. The areas of both tissue loss and the entire uninjured hemisphere were determined by an observer blinded to experimental group using an image analysis system (MCID, Imaging Research, St. Catherines, Ontario, Canada). Lesion volume was reported in cubic mm, as a percentage of uninjured hemisphere, and as area (mm$^2$) vs distance from the occiput (mm). Surviving hippocampal neurons were counted under 400X magnification in the entire anatomic CA1 and CA3 hippocampal regions in a coronal section taken 5-mm from the occiput by an observer blinded to experimental group (KS). Neuronal counts were reported as the mean number of surviving neurons per 400X field.

ICP Protocol

Based both on results of the outcome protocol showing that fentanyl-treated rats had higher MAP throughout the experiment and on a recent report that increased MAP may exacerbate injury after TBI, ICP and brain water were monitored in a separate cohort of rats (n
= 9 per anesthetic group) subjected to either fentanyl or isoflurane anesthesia and CCI in an identical paradigm to that used to assess functional outcome.

Surgical preparation, randomization, anesthetic administration, and CCI were identical to the outcome protocol, with minor exceptions. Specifically, an intraparenchymal ICP monitor (Codman microsensor transducer, outer diameter 1.0-mm, Johnson and Johnson, Raynham, MA) was inserted through a burr hole in the frontal bone into the contralateral (right) frontal cortex at the time of craniotomy. After CCI, anesthesia was continued and ICP was monitored for 4 h in both anesthetic groups. Cerebral perfusion pressure (CPP) was calculated as the difference between MAP and ICP. Rats were killed by decapitation at the end of the anesthetic period. Brains were immediately removed and a 3-mm coronal slice was made through the center of the contusion using a rat brain slicer. Per cent brain water was determined in the coronal slice using the wet-dry weight method.\(^{26}\) The section was weighed immediately, dried in an oven at 110° C for 48 hours and then reweighed. Brain water content was determined in both the injured and the homologous region of the uninjured hemispheres.

As an additional control, a separate cohort of rats \((n = 3)\) was subjected to CCI and allowed to recover without anesthesia. These rats were prepared for CCI under isoflurane anesthesia as described, allowed to recover a tail-pinch response and then subjected to CCI. Arterial MAP was monitored via an indwelling femoral arterial catheter for 4 h during recovery without anesthesia.
**Statistical Analysis**

Physiological parameters (PaCO$_2$, PaO$_2$, glucose, hematocrit, MAP, ICP, CPP) and beam balance, beam walking, and MWM latencies were assessed by two-way analysis of variance for repeated measures. Swim speed, brain water content, and hippocampal neuronal survival were compared by one-way analysis of variance. Appropriate post-hoc tests corrected for multiple comparisons were applied. Time to extubation and lesion volume were compared between treatment groups using unpaired student’s t-test. A $p$ value $< 0.05$ was considered statistically significant. Values are expressed as mean ± SEM.

**Results**

**Outcome Protocol**

Average time to extubation did not differ after injury between isoflurane and fentanyl treatment groups (269 ± 7 min vs 275 ± 15 min, $p = 0.29$) Physiologic values, including PaCO$_2$, PaO$_2$, blood glucose and hematocrit did not differ between anesthetic groups. In contrast, MAP was higher in injured rats treated with fentanyl compared to their isoflurane counterparts ($p < 0.05$) during the entire posttrauma period (Figure 1). Similarly, MAP was higher in shams treated with fentanyl vs isoflurane ($p < 0.05$) during the entire duration of anesthesia (Figure 1). Fentanyl-treated rats had a MAP of approximately 150 mm Hg compared to approximately 105 mm Hg in the isoflurane groups.

Rats anesthetized with isoflurane performed better on beam balance and beam walking tasks after TBI compared to their fentanyl counterparts ($p < 0.05$, Figure 2). Following injury,
isoflurane-anesthetized rats also performed better than their fentanyl-treated counterparts during MWM testing with a hidden platform (\( p < 0.05 \), Figure 3). Motor and MWM performances did not differ between sham groups. All experimental groups showed improved MWM performance during visible (vs hidden) platform testing (\( p < 0.05 \) for all groups) and had similar swim speeds (Figure 3), indicating that all rats had similar motivation and motor ability during MWM testing. However, performance on the visible platform paradigm of the MWM was better in isoflurane vs fentanyl treated rats after TBI (\( p < 0.05 \), Figure 3). This suggests that the difference in MWM performance may not be solely attributable to cognitive deficits.

Lesion volume, expressed as mm\(^3\) or as percent of uninjured hemisphere, at 21 days after TBI did not differ significantly between isoflurane and fentanyl treatment groups (Figure 4A,B). In contrast, neuron counts in the injured CA1 hippocampus were markedly greater in isoflurane-treated rats (\( p < 0.05 \), Figure 4C). Neuron counts in the injured CA3 hippocampus, however, did not differ significantly between treatment groups (Figure 4D). In the uninjured hemisphere, neuron counts in both CA1 and CA3 hippocampus did not differ between isoflurane- and fentanyl-treated rats, and were similar to shams (data not shown).

**ICP Protocol**

Physiologic values, including PaCO\(_2\), PaO\(_2\), glucose and hematocrit, did not differ between anesthetic groups. As in the outcome protocol, MAP was higher in rats treated with fentanyl compared to their isoflurane counterparts (\( p < 0.05 \), Figure 5A). ICP was similar in both anesthetic groups; however, there was a trend toward higher ICP in rats anesthetized with isoflurane by 3 - 4 h after TBI (Figure 5B). This strongly suggests that the higher MAP in
fentanyl vs isoflurane treated rats did not exacerbate intracranial hypertension. As expected from the difference in MAP, CPP was significantly higher in the fentanyl treatment group (Figure 5C). Brain water content, assessed at 4 h after injury, was higher in the injured vs uninjured hemisphere ($p < 0.05$) for both anesthetic groups (Figure 6). However, brain water in either the injured or uninjured hemisphere did not differ between isoflurane- and fentanyl-treated rats, indicating that edema was not exacerbated in the fentanyl group.

Average MAP during the 4 h post-injury observation period did not differ significantly between fentanyl-treated rats and those recovering from CCI without anesthesia (157 ± 6.2 mm Hg vs 147 ± 7.1 mm Hg, NS). In contrast, isoflurane-anesthetized rats had lower MAP (105 ± 5.5 mm Hg) compared to both fentanyl-treated rats and rats recovering without anesthesia ($p < 0.05$ vs both groups, one-way ANOVA).

**Discussion**

Isoflurane anesthesia administered to rats subjected to CCI improved performance on both motor and MWM tasks and attenuated damage to CA1 hippocampus after TBI. Although fentanyl-treated rats had higher MAP and CPP than their isoflurane counterparts, this was not accompanied by increased ICP or brain water during the first 4 h after TBI. This suggests that the improved functional outcome in rats anesthetized with isoflurane may be a direct result of either beneficial actions of isoflurane, and/or detrimental effects of fentanyl.

The pathophysiology of TBI includes a primary injury caused by the mechanical disruption of tissue and various secondary injuries mediated, at least in part, by post-insult
hypoperfusion, ischemia, and excitotoxicity.\textsuperscript{7,18,22,26,35} Isoflurane anesthesia may be neuroprotective vs fentanyl by decreasing excitotoxicity and/or augmenting cerebral blood flow.

Following TBI, interstitial levels of excitatory amino acids (EAAs), such as glutamate, are increased due to direct tissue injury and secondary ischemia. EAAs stimulate NMDA, AMPA/kainate, and metabotropic receptors, leading to neuronal membrane depolarization, cellular swelling, calcium influx, and ultimately, neuronal death.\textsuperscript{35} Although models of cerebral ischemia have shown conflicting effects of isoflurane on glutamate levels in blood and brain interstitial fluid,\textsuperscript{44,49} isoflurane has been shown to inhibit glutamate receptors, reduce NMDA-mediated calcium influx and delay neuronal injury induced by cerebral ischemia.\textsuperscript{4,45}

To attribute the potential neuromodulatory actions of isoflurane on neuronal injury to only glutamate toxicity\textsuperscript{4} and glutamate signaling transduction,\textsuperscript{11} however is an oversimplification since isoflurane has many neural actions that could contribute to neuroprotection. Some include the inhibition of some voltage sensitive potassium channels,\textsuperscript{23} the activation of specific receptor-coupled and voltage sensitive potassium channels,\textsuperscript{31,52,54} the uncoupling of muscarinic receptors,\textsuperscript{1,36,42} the enhancement of GABA A channels,\textsuperscript{28} and the reduction of intracellular calcium stores and inhibition of IP3 sensitive intracellular calcium release.\textsuperscript{21} Potential detrimental actions such as the enhancement of NMDA linked nNOS activation have also been documented;\textsuperscript{46} however, the potential beneficial actions of isoflurane on excitotoxic cascades far outweigh the potential detrimental actions.

Additionally, isoflurane is a potent cerebral vasodilator. Studies of CBF after experimental TBI have shown significant reductions in both local and global CBF early (0.5 – 4 h) after injury.\textsuperscript{7,18} Effects are greatest near the impact site, but global reductions in CBF are seen
as well.\textsuperscript{18} In clinical studies, early post-traumatic hypoperfusion has been strongly correlated with poor outcome.\textsuperscript{5,32} Although the effects of fentanyl on CBF have been subjected to limited study, Safo et al.\textsuperscript{47} have reported that CBF was markedly reduced in rats treated with fentanyl (100 $\mu$g/kg iv) vs control rats anesthetized with $\text{N}_2\text{O}$. In addition, using perfusion MRI in normal rats anesthetized with doses identical to those used in this study, we have shown that CBF is 2 - 3 times higher in rats treated with isoflurane vs fentanyl (unpublished data). By promoting CBF, isoflurane may help attenuate post-traumatic hypoperfusion, reducing secondary injury and improving recovery. The selective neuroprotection of CA1, but not CA3, hippocampus in isoflurane- vs fentanyl-treated rats is consistent with this concept. Additionally, another CBF promoting strategy, L-arginine, has recently been shown to improve outcome after TBI.\textsuperscript{8,10} Augmentation of CBF with an associated increase in cerebral blood volume therefore offers a potential explanation for both improved functional and histological outcome and the tendency toward higher ICP and brain water seen in isoflurane- vs fentanyl-treated rats in this study. Indeed, the combination of CBF promotion and reduced excitotoxicity may be particularly beneficial.

Alternatively, fentanyl may be detrimental after TBI. Opioids generally suppress neuronal excitability, however, mu receptor agonists, such as fentanyl, may contribute to hippocampal neuron excitation.\textsuperscript{5,40,41} In fact, high-dose fentanyl (25 - 100 $\mu$g/kg in humans and 400 $\mu$g/kg in rats) has been associated with subcortical seizures.\textsuperscript{14,24,33,43,47,50} Although fentanyl exhibits low affinity for kappa receptors,\textsuperscript{43} kappa receptor antagonists have been shown to
improve both neurological outcome after spinal cord injury and CBF after fluid-percussion injury in cats.\textsuperscript{14,33}

Other factors may contribute to improved outcome in isoflurane- vs fentanyl-treated rats. These include the disparity in both MAP and CPP and possible differences in the depth of anesthesia between treatment groups. Although increased MAP can have detrimental effects after TBI, in our study, ICP and brain water were not significantly different in isoflurane vs fentanyl treatment groups at the end of the 4 h treatment period. This suggests that the higher MAP associated with fentanyl vs isoflurane anesthesia had no acute detrimental effects on intracranial hypertension or brain edema. Additionally, MAP did not differ significantly between rats treated with fentanyl and those allowed to recover without anesthesia. The values for MAP observed in both isoflurane and fentanyl groups were within the reported range of cerebral autoregulation (50 - 170 mm Hg) for normotensive rats.\textsuperscript{19,20,53} The disparity in MAP is therefore unlikely to have contributed importantly to the observed difference in functional outcome. Although a recent study using the CCI model in rats suggests that increased MAP and CPP may exacerbate injury after TBI, in that study, systemic hypertension was induced by large doses of dopamine,\textsuperscript{27} that may have produced detrimental effects after injury which were independent of blood pressure.\textsuperscript{2} Additionally, our study does not address the important detrimental effect of hypotension following clinical TBI, particularly with multiple trauma. The blood pressure supporting effects of fentanyl (vs other sedative agents) may be beneficial in this section.

Although comparison of anesthetic depth between inhalation and intravenous agents is difficult, differences in anesthetic depth are unlikely to explain the observed difference in outcome seen between isoflurane- and fentanyl-treated rats. The dose of isoflurane used (1% by
inhalation), in combination with N\textsubscript{2}O:O\textsubscript{2} (2:1) represents approximately 1.2 minimal alveolar concentration.\textsuperscript{16} The dose of fentanyl falls in the range between the ED50 for purposeful movement and complete blockade of this response\textsuperscript{25} and is similar to standard doses used in rat models of central nervous system injury.\textsuperscript{3,37-39,48} Additionally, fentanyl-treated rats did not exhibit signs of increased stress vs isoflurane-treated rats, such as higher blood glucose, suggesting that anesthesia was adequate. Finally, rats in both treatment groups emerged from anesthesia similarly in our paradigm. After discontinuation of fentanyl, the rats were fully alert and exhibited similar activity to isoflurane-treated rats following extubation.

The theoretical advantages of isoflurane vs fentanyl are compelling; however, explanations for the observed improvement in neurologic outcome after TBI in isoflurane-anesthetized rats remain to be determined. What has become clearer is that anesthetic agents may have considerable impact upon outcome following TBI.

The results of this study suggest two important potential ramifications. First, isoflurane may not represent the optimal anesthetic in experimental TBI since it may mask potential benefits of novel therapies. Second, despite common use, fentanyl may not be the optimal sedative/analgesic agent to administer to patients in the acute phase after severe head injury. Although we do not suggest that isoflurane represents a clinically applicable therapy for the initial stabilization and treatment of patients after TBI, further study of the mechanistic differences between isoflurane and fentanyl anesthesia is warranted. Defining the factors responsible for improved outcome with isoflurane may help to direct the clinical application of more optimal sedative/analgesic agents and possibly to identify novel therapies. Finally, more
comprehensive comparisons of clinically relevant sedative/analgesic agents are needed in experimental TBI.

Acknowledgement: We thank the US Army DAMD 17-97-1-7009 for generous support of this project. We thank Marci Provins for assistance with preparation of the manuscript. This study was presented, in part, at the 1999 meeting of the National Neurotrauma Society. Dr. Statler received the 1999 Women in Neurotrauma Research Award for this work.
References


41. Neumaier JF, Mailheau S, Chavkin C: Opioid receptor-mediated responses in the dentate gyrus and CA1 region of the rat hippocampus. *J Pharmacol Exp Ther* 244:564-570, 1988


Figure 1: MAP vs time after injury, outcome protocol. MAP in both fentanyl-treated injured (open circles) and sham (open triangles) rats was approximately 50 mm Hg higher than in isoflurane-treated rats at all time points (injured shown by closed circles and shams by closed triangles). * $p < 0.05$, isoflurane vs fentanyl at each time point after injury, § $p < 0.05$, isoflurane vs fentanyl at all time points, including baseline, in shams.

Figure 2: A. Beam balance latency vs time in days after injury. Sham rats (triangles) had similar latencies throughout the 5-day testing period. After injury, beam balance latency was shorter for both isoflurane (closed circles) and fentanyl (open circles) treatment groups vs sham; however, isoflurane-anesthetized rats recovered more quickly (vs fentanyl). * $p < 0.05$, injured vs sham. B. Beam walking latency vs time in days after injury. Again, performance was impaired after injury in both anesthetic groups vs shams. Although isoflurane-treated rats recovered by post-injury day 3, fentanyl-treated rats failed to regain normal function by the end of the 5-day testing period. * $p < 0.05$, injured vs sham.

Figure 3: A. Latency to find a platform vs time after injury in an acquisition paradigm of the MWM. Sham rats anesthetized with isoflurane (closed triangles) or fentanyl (open triangles) had similar performances throughout the testing period. During the first few days of testing with a hidden platform, injured rats in both fentanyl (open circles) and isoflurane (closed circles) treatment groups had impaired performance vs sham. By the third day of testing, latencies to find the hidden platform were similar in injured isoflurane-anesthetized rats and shams. In contrast, longer latencies persisted in injured fentanyl-treated rats throughout the 5-day hidden
platform testing period. Latencies in all experimental groups improved during visible (vs hidden) platform testing; however, shams and injured isoflurane-anesthetized rats performed better than injured fentanyl-treated rats on both days of visible platform testing. * $p < 0.05$, injured vs sham; § $p < 0.05$, isoflurane vs fentanyl. B. Swim speed, tested on day 20 after injury, did not differ between experimental groups.

Figure 4: A. Lesion volumes, measured on post-injury day 21, did not differ significantly between isoflurane (solid) and fentanyl (open) treatment groups. B. Lesion volume, expressed as area (mm²) vs distance (mm) from occiput, did not differ significantly between treatment groups. C. Neuron counts in injured CA1 hippocampus were greater in isoflurane- vs fentanyl-treated rats. Neuron counts in uninjured CA1 hippocampus were similar in both treatment groups. * $p < 0.05$, injured vs uninjured. D. CA3 hippocampus neuron counts in either injured or uninjured hemisphere did not differ significantly between isoflurane- and fentanyl-treated rats.

Figure 5: A. MAP vs time after injury, ICP protocol. MAP was approximately 40 mm Hg higher in rats treated with isoflurane (closed squares) vs fentanyl (open squares) throughout the observation period. * $p < 0.05$, fentanyl vs isoflurane vs fentanyl. B. ICP vs time after injury. Initial ICP was approximately 4 mm Hg in both isoflurane (open squares) and fentanyl (closed squares) treatment groups. ICP progressively increased, reaching 10 - 18 mm Hg by 4 h after injury. Although ICP was similar between anesthetic groups, isoflurane-anesthetized rats exhibited a trend toward higher ICP after injury (vs fentanyl treated rats) that did not reach significance. C. CPP vs time after injury. CPP was increased in rats treated with fentanyl (open
squares) vs isoflurane (closed squares), * $p < 0.05$, isoflurane vs fentanyl at all time points except 3.5 and 4h.

**Figure 6:** Brain Water 4 h after TBI. Brain water in the injured hemisphere was increased compared to the respective non-injured hemisphere in both isoflurane- and fentanyl-anesthetized rats; however, brain water did not differ between anesthetic groups. * $p < 0.05$, isoflurane vs fentanyl.
Figure 1

MAP (mm Hg)

Time (hours)

impact 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0

* §
Figure 3A

Latency (seconds)

Time (days)

submerged platform

visible platform
Figure 4B

Area (mm$^3$) vs. Distance from Occiput (mm)
Figure 4C

CA1 neurons per h.p.f.

- injured hemisphere
- uninjured hemisphere
Figure 4D

CA3 neurons per h.p.f.

<table>
<thead>
<tr>
<th></th>
<th>injured hemisphere</th>
<th>uninjured hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>50</td>
</tr>
</tbody>
</table>
Figure 6

Brain Water (%)

- Isoflurane injured
- Fentanyl injured
- Isoflurane uninjured
- Fentanyl uninjured

* indicates a significant difference.
CHANGES IN MITOCHONDRIAL MEMBRANE POTENTIAL IN STRETCH-INJURED ASTROCYTES AND NEURONS. S.M. Ahmed*, B.A. Rzigalinski and E.F. Ellis, Dept. of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA.

The dynamics of energy failure in traumatically injured astrocytes and neurons are unclear. In order to better understand mitochondrial function and cell energetics following trauma we utilized the fluorescent dye Rhodamine 123, which is normally sequestered in mitochondria where its fluorescence is quenched. When mitochondrial membrane potential (MMP) decreases, such as with mitochondrial poisons, the dye moves to the cytoplasm and fluorescence is increased. Pure neuronal or astrocytic cultures were subjected to mild (5.7 mm), moderate (6.5 mm) or severe (7.5 mm) stretch-induced injury and the change in MMP measured. There were no significant changes in MMP in mildly to moderately injured neurons at 15 min, 24 or 48 hr post-injury. However, severely injured neurons displayed an immediate 33% decrease in MMP that persisted to 48 hr. In contrast, mild and moderate astrocyte injury caused a dramatic, 39-52% drop in MMP at 15 min, with MMP returning to normal by 24 hr. Our results indicate that direct trauma-induced alterations in cell energetics vary greatly in neurons and astrocytes. We suggest that in vivo the deficit induced in astrocytes may alter astrocyte function, which in turn may produce dramatic effects on neuronal function. Supported by NS-27214 and NS-07228.

EVIDENCE FOR APOPTOTIC CELL DEATH FOLLOWING SUBDURAL HEMATOMA IN RATS. R. Alessandrini†, X. Di; H. Chen, P. Pullela. Div. of Neurosurgery, Medical College of Virginia, Richmond, VA. 23298, USA.

Subdural hematoma (SDH) is a common and dangerous secondary event following traumatic brain injury. The mechanisms leading to neuronal death, even after SDH removal, are not fully understood. A mechanism which might contribute to cell death is apoptotic cell death (ACD), which has been shown to be involved in the development of traumatic and ischemic brain damage.

The hematoma was produced by subdural injection of 250μL of autologous venous blood in Halothane anesthetized rats. Animals were allowed to survive 1 (n=3), 2 (n=3), 4 (n=3) or 7 days (n=4) after injection of SDH. Brain sections were stained by a commercially available apoptosis detection kit (FragEL™) for apoptotic cells (visualized by diaminobenzidine, DAB; counterstained by hematoxylin). Brain sections were examined light microscopically and DAB-positive cells were counted in both hemispheres in cortical, subcortical and hippocampal areas.

The DBA-pos. cell counts were 2.3±2, 54.5±8, 13.7±8, and 12.8±8 at 1, 2, 4 and 7 days after SDH, respectively. All apoptotic cells were within the cortex, within and in the border zone of the SDH lesion. There were no DAB-pos. cells in the contralateral side. The number of DAB-pos. cells was highly correlated with the lesion area (r = 0.689, p < 0.001). The results indicate that ACD occurs following SDH, and is maximal at 2 days. DAB-pos. cells were only found within or in the border zone of the lesion. The correlation of ACD and lesion area underlines the importance of this type of cell death in SDH. The contribution of ACD to SDH-induced brain damage and its relevance for therapy needs further study.

HYPERTHERMIA ADVERSELY AFFECTS OUTCOME AFTER MODERATE HEAD INJURY. Philip R. Aldana†, J. Marquez*, D. S. Petrin†, D. Johns*, W. D. Dietrich*, P. A. Villanueva* Department of Neurological Surgery, Neurotrauma Research Center†, University of Miami School of Medicine, Miami, Florida, 33101, USA.

Hypothermia has been shown to have beneficial effects after traumatic brain injury (TBI) in both human and animal studies. Conversely, hyperthermia after TBI has been shown to have deleterious effects in animals. No studies have addressed the effects of hyperthermia after moderate head injury in humans.

104 patients admitted with a Glasgow Coma Score 9-12 due to blunt head trauma were studied. Demographics, comorbid factors and characteristics of the hyperthermic episodes (>38.6°C) were examined. The number of patients either dead, in a vegetative state or severely disabled during discharge was significantly larger for the hypothermic group vs. the normothermic group (42.4% vs. 17.5%, respectively). A significantly larger percentage of the normothermic group had a good outcome compared to the hyperthermic group (50% vs. 20.3%, respectively). Among the hyperthermic patients, those with associated infections had significantly worse outcomes and a higher frequency of hyperthermic episodes than those without infections. We conclude that hypothermia in the face of an associated infection may adversely affect the outcome of patients with moderate head injury. We advocate maintenance of at least normothermic conditions if moderate hypothermia cannot be achieved and treatment of any underlying infection after TBI.


Although a variety of modifications of the controlled cortical impact (CCI) model exist, a comparison between the two most common variants, vertical1 and angled impact2, has not been performed. Rats were subjected to vertical (n = 8), angled (n = 8) or sham (n = 8) insults (4 m/s, 2.5 mm) to the left parietal cortex, using a CCI model with hypoxemia.3 Motor (beam balance, dl-5), cognitive (Morris Water Maze, dl-21) and histologic (lesion volume, CA1 and CA3 neuron counts, dl2) outcomes were studied. Motor and MWM performance were impaired, but did not differ between injury groups. Lesion volumes also did not differ (vertical = 92.2±7.2 mm3, angled = 79.4±7.8, p = 0.25). CA1 neuron counts were decreased ipsilaterally to injury in both groups vs sham (vertical = 20.4±8.4 cells/hpf, angled = 32.7±15.8, sham = 55.5±4.9, p < 0.05). However, CA3 neuron counts were decreased ipsilaterally to injury in the vertical group vs sham (23.2±8.5 vs 52.1±6.6, respectively, p < 0.05), but the angled group (32.7±4.8) was not different from sham. We conclude that the vertical and angled variants of the CCI model produce similar functional deficits; however, the vertical impact appears to produce greater local damage, particularly in CA3 neurons.1, J Neurotrauma 13:1655 3, J Neurol Med. 39:255; 5 J Neurotrauma 14:179; Support: US Army DAMD17-95-1-7099.
45

CHRONIC OVEREXPRESSION OF AMYLOID PREROSOR PROTEIN (APP) AFTER TRAUMATIC BRAIN INJURY IN RATS. J. R. Ciallella1*, H. Q. Yan1*, X. Ma1, D. W. Marion1, S. T. DeKosky2, and C. E. Dixon1.

Departments of 1Neurosurgery and 2Psychiatry, University of Pittsburgh Medical Center, Pittsburgh, PA USA.

Traumatic brain injury (TBI) and Alzheimer’s disease (AD) produce cholinergic and metabolic deficits that may contribute to neurodegeneration. There is increasing evidence linking AD and TBI, including upregulation of APP in head injured patients (McKenzie et al. 1994 NeuroRep:6;161). To further investigate this linkage, we tested the hypothesis that controlled cortical impact (CCI) injury would produce chronic upregulation of APP protein levels at 4 weeks following injury. Our previous studies demonstrated significant changes in cholinergic proteins at this time point (Ciallella et al. 1998. Exp. Neurol. In Press). APP immunohistochemistry (n=3-5) and western blot (n=4) were performed on cortical and hippocampal regions from injured and sham animals. The same N-terminal antibody was used in all studies. A marked increase in cortical and hippocampal APP protein was demonstrated bilaterally by both immunohistochemistry and western blot in injured rats compared to sham controls. This demonstrates that a single TBI can lead to chronic upregulation of APP, concurrent with chronic alterations in cholinergic markers. Supported by AG05133, NINDS-T32NS07391, CDC-CCR312296, NIH-NS30313, and NIH-NS33150.

46


Many reports have shown benefit from hypothermia (HT) in traumatic brain injury (TBI); but, its effect on TBI with secondary insult remains undefined. We hypothesized that HT would improve outcome after controlled-cortical impact (CCI) with secondary hypoxemia. Rats received severe CCI injury followed by 30 min of hypoxemia, and randomized to normothermia (NT=37°C brain temp, n=19), immediate HT (IHT=32°C, after CCI, n=10), or delayed HT (DHT=32°C, after hypoxemia, n=14) for 4 h. Motor (beam balance/ walking, d=5), cognitive (Morris Water Maze [MWM], d14-21) and histologic outcomes (lesion volume, hippocampal neuron counts, d21) were evaluated. Motor and MWM performance were impaired but did not differ between groups. Lesion volumes (mm³) did not differ between groups (NT=65.3±6.9, IHT=50.2±8.2, DHT=53.7±7.9). Neuron counts (CA1, CA3) were decreased 60-70% ipsilateral to CCI, but did not differ between groups. Mortality doubled (43% vs 20-21%) in DHT vs NT or IHT (p=0.3). HT did not improve outcome after severe CCI with secondary insult. Clinical studies exclude patients with secondary insults, and suggest HT is not effective after severe injury (GCS 3-4). Novel therapies may be needed in this setting. *J Neurotrauma 14:179; *NEJM 336:540-6; Support: US Army DAMD17-97-1-7009.
EFFECT OF CALCIUM CHLORIDE ON REGIONAL CEREBRAL BLOOD FLOW DURING CARDIOPULMONARY RESUSCITATION IN PILOGETS

Melody Palmer Land, John Kuluz, Barry Gelm, Michael Naes, Ee Xu, and Charles Schleien. Pediatric Critical Care Medicine, University of Miami School of Medicine, Miami, FL 33101.

Introduction: The use of calcium chloride (CaCl$_2$) during CPR remains controversial. CaCl$_2$ may improve the effectiveness of CPR by increasing systemic vascular tone and vital organ perfusion. Alternatively, CaCl$_2$ may cause regional vasoconstriction in the brain and heart, resulting in secondary ischemic injury. We hypothesized that administration of the standard dose of CaCl$_2$ during CPR decreases rCBF.

Methods: Under pentobarbital anesthesia, 2-4 week old piglets underwent 6 min of cardiac arrest by ventricular fibrillation, and 30 min of standard CPR. rCBF was measured with microspheres at baseline and after 5, 15 and 30 min of CPR. CaCl$_2$ 20 mg/kg (n=5) or saline (n=5) was given after 1 and 15 min of CPR. Data (mean±SE) were analyzed by ANOVA and Student’s t-test (*p<0.05).

Results: Ionized Ca decreased from 1.46±0.1 at baseline to 1.16±0.05* at 15 min and 1.18±0.05* at 30 min CPR. After CaCl$_2$, ionCa increased to 2.5±1.6 at 5 min and 2.0±0.2 at 30 min, and was different from baseline at 15 min CPR. Calcium increased aortic pressure (44±2 vs 38±2*), and cerebral perfusion pressure at 5 min CPR. Total CBF was not different between groups at any time point; however, severe regional ischemia (CBF<15 mL/100g/min) was more common after 30 min CPR when CaCl$_2$ was given, particularly in subcortical regions (p<0.05).

Conclusion: These data show that CaCl$_2$ administration has adverse effects on rCBF during prolonged CPR and may worsen ischemic brain injury. Future studies will determine the effect of CaCl$_2$ on functional and neuroradiologic outcome.

50 NO LONG-TERM BENEFIT FROM HYPOTHERMIA AFTER SEVERE TRAUMATIC BRAIN INJURY WITH SECONDARY HYPOXIA IN RATS


Introduction: Many reports have shown benefit from hypothermia in traumatic brain injury (TBI), but its effect in the setting of TBI with secondary insult remains undefined. Clinical studies show an increase in morbidity and mortality after severe TBI with secondary brain insult. In experimental rat models, outcomes were worse in brain injury with secondary hypoxia.* Recently, we characterized a model of TBI with secondary hypoxemia and reported prominent neuronal apoptosis after injury.1,2

Conclusion: These data show that CaCl$_2$ administration has adverse effects on rCBF during prolonged CPR and may worsen ischemic brain injury. Future studies will determine the effect of CaCl$_2$ on functional and neuroradiologic outcome.

51 BRAIN NITRIC OXIDE CHANGES AFTER CONTROLLED CORTICAL IMPACT INJURY IN RATS

Lesia Charlet, J. Clay. Goodman, Claudia S.Robertson. Departments of Neurosurgery and Pathology, Baylor College of Medicine, Houston, TX-77030.

Introduction: The marked reduction in CBF that occurs after severe controlled cortical impact (CCI) injury in rats can be ameliorated by postinjury infusion of L-arginine. Since L-arginine is a substrate for nitric oxide synthase, these studies suggest that a reduced production of nitric oxide (NO) may play a role in the CBI reduction that occurs after brain trauma. The purpose of this study was so measure brain tissue concentrations of NO after severe CCI.

Methods: 12 Long Evans rats were anesthetized with isoflurane and subjected to impact injury with a fluidicimpulse coupled impact device, and subjected to severe CCI (5 m/sec, 3 mm depth). NO was directly measured using a NO electrode which was implanted near the site of the impact after calibration using L-arginine-N-acetyl-D-argininamide at 77°C. A microdialysis probe was inserted near the NO electrode and perfused with artificial CSF at 2 uL/min. The concentration of nitric oxide was measured using a chemiluminescent method in serial 20 minute collections of dialysates. These measurements were obtained prior to injury, and for 3 hours after injury. Values were expressed as % of the pre-injury baseline value.

Results: Injury impact caused a transient increase in brain tissue NO concentrations to 175 % of the baseline values in the CCI animals, compared to 90% in the sham injured animals (p=0.002). After the initial transient increase in NO, the concentrations of NO declined and remained significantly lower than in the sham animals throughout the 3 hr study period. The results are summarized below as mean ± SE of the values obtained prior to injury, and for 3 hours after injury. Values were expressed as % of the pre-injury baseline value.

<table>
<thead>
<tr>
<th>Time after Injury</th>
<th>CCI injury (n=6)</th>
<th>Sham injury (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>175 (158,197)</td>
<td>97.5 (96,102)</td>
<td>0.002</td>
</tr>
<tr>
<td>1 hr</td>
<td>75.5 (70,80)</td>
<td>98.5 (96,110)</td>
<td>0.004</td>
</tr>
<tr>
<td>2 hr</td>
<td>73 (69,79)</td>
<td>101 (95,114)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Conclusion: These studies show that NO is reduced immediately after severe brain injury and subsequently is found in depressed concentrations in the brain for at least 3 hours after injury. The reduction in CBF that occurs after severe CCI can be related to the reduced NO levels.

Supported by NIH grant #PO1-HD27616

Compared to ischemic stroke, the mechanisms that underlie neuronal damage following intracerebral hemorrhage remain relatively unexplored. Parenchymal ischemia accompanying hemorrhage is typically mild (CFI 50-75% of baseline); therefore, this may favor apoptotic pathways over necrotic cell death. The aim of the present study is to characterize the spatial and temporal profile of apoptosis after hemorrhage and evaluate the therapeutic efficacy of caspase inhibition. We performed histological and immunohistochemical analyses of the lesion and bordering the penumbra of the injury from 1 to 3 weeks post-injury using TUNEL, caspase-3, and caspase-9. We demonstrated that TUNEL-positive cells were distributed more in the periphery than in the center between 24 and 48 hrs, and then declined in number at 72 hrs. Pre-treatment with the caspase inhibitor, z-VADfmk (80ng, icv), significantly reduced the number of TUNEL-positive cells at 24 hrs.

These findings suggest that apoptosis is an important pathological mechanism following intracerebral hemorrhage and caspase inhibition may have a therapeutic effect. Supported by Uehara Memorial Foundation (KM), N15028 (MAM), N532506 (EHL), and N537074 (EHL).

101.6 Inhibition of interleukin 1β converting enzyme family proteases reduces cold injury-induced brain trauma and DNA fragmentation in mice. Y. Mori-Matsumura*, T. Taniyama*, K. Kawasaki*, K. Murakami*, I. Li*, and H. Chao*. Dept. of Anesthesiology, University of California, San Francisco. Dept. of Neurosurgery, Neurology and Neurological Sciences, Stanford University School of Medicine, Palo Alto, CA 94304.

The interleukin 1β converting enzyme (ICE) family, a protease family implicated in apoptosis, are also expressed in blood vessels such as ischemia and trauma, and its inhibition reduces ischemic brain infarction (Hara et al., 1997; Yakuvevich et al., 1997). We examined the effect of z-VAD,FMK, a recently nonselective inhibitor of ICE, on brain trauma-induced apoptosis and found that pre-treatment with the cold injury-induced brain trauma in which apoptosis appears to play a role (Tominaga et al., 1992). The vehicle alone or z-VAD,FMK was intracerebroventricularly administered to 15 min post-trauma and 48 hrs after cold injury. At 48 hrs after cold injury, infarction volumes in z-VAD,FMK-treated animals were significantly less than infarction volumes in vehicle-treated animals, which were further decreased at 24 and 72 hrs (p<0.05). There were no significant differences between the treatment groups before 24 hrs. These data suggest that ICE inhibitors might be of therapeutic benefit in brain trauma. The ICE family of proteases appears to contribute significantly to cold injury-induced brain trauma. Blocking ICE activity increases neuronal survival by reducing apoptosis. Supported by grants NS15453, NS52372, NS61477 and NS082866.


The protective effects of mild hypothermia following traumatic brain injury (TBI) have been demonstrated in multiple studies within the last decade. However, while this protection has been evaluated in relation to the preservation of neuronal survival and behavior, little information is available on any potential protective effect on TBI-induced axonal injury, a known feature of human injury. To this end, we evaluated the protective effects of mild hypothermia on axonal injury following TBI in rats. Male Sprague Dawley rats weighing 300-400 grams were subdural exposed to experimental TBI induced by impact acceleration. These rats were subjected to experimental TBI induced by impact acceleration. These rats were then allowed to survive for 15 min post-injury and continued over the entire 2 hrs observation period. We confirmed that the number of TUNEL positive cells at 24 hrs. Pre-treatment with the caspase inhibitor, z-VADfmk (80ng, icv), significantly reduced the number of TUNEL-positive cells at 24 hrs.

These findings suggest that apoptosis is an important pathological mechanism following intracerebral hemorrhage and caspase inhibition may have a therapeutic effect. Supported by Uehara Memorial Foundation (KM), N15028 (MAM), N532506 (EHL), and N537074 (EHL).


Hypothermia applied prior to or shortly after traumatic brain injury (TBI) reduces brain swelling and improves outcome. However, whether hypothermia will attenuate the expression of DNA damage is still unknown. TBI was induced by impact acceleration. These rats were subjected to experimental TBI induced by impact acceleration. These rats were then allowed to survive for 15 min post-injury and continued over the entire 2 hrs observation period. We confirmed that the number of TUNEL positive cells at 24 hrs. Pre-treatment with the caspase inhibitor, z-VADfmk (80ng, icv), significantly reduced the number of TUNEL-positive cells at 24 hrs.

These findings suggest that apoptosis is an important pathological mechanism following intracerebral hemorrhage and caspase inhibition may have a therapeutic effect. Supported by Uehara Memorial Foundation (KM), N15028 (MAM), N532506 (EHL), and N537074 (EHL).

101.9 Altered expression of phospho-1 and the endothelin 1 receptor subtype (ETB) after spinal cord injury. J. L. Ebsig, A. E. M. Mutesi, H. Meschter, R. Willette, and L. J. Noble*. Deps. of Neurosurgery, University of California at San Francisco and Saarland University Medical School, Homburg/Saar, Germany, and Dept. of Cardiovascular Pharmacology, SmithKline Beecham Pharmaceuticals.

Glia overexpression is a prominent feature of the injured spinal cord. There is increased expression of ET-1 in the spinal cord of a rat subjected to spinal cord injury. In this study, we begin to address this putative role of ET-1 in the contused spinal cord of the rat. At 3 hours to 3 weeks after a moderate spinal cord injury or after sham surgery, a 2 cm length of cord, centered over the impact or sham surgery, was removed and divided into proximal, injury, and distal segments. Sections were reacted for ET-1 antibody, and the intensity of staining was quantified by an observer blinded to the experimental protocol. The data were analyzed using Kruskal-Wallis, followed by Mann-Whitney U.

There is enhanced immunohistochemistry of ET-1 at all time points and a significant increase in expression in response to spinal cord injury. There was more intense staining of the injured cord at 1 to 3 weeks post-injury compared to sham surgery. ET-1 is localized in reactive glia, bordering central and dorsal column cavities, and macrophage-like cells. There is pronounced ETB mRNA in similar specimens. The lesion and bordering the penumbra of the injury from 1 to 3 weeks post-injury.

The enhanced expression of ET-1 and ETB mRNA in glial and macrophage-like cells suggest that local ET-1 may influence both glial reactivity and macrophage activity. Supported by NS23324.
POSTER SYMPOSIUM PRESENTATIONS: EXPERIMENTAL BRAIN INJURY

44

DNA DAMAGE IS TEMPERATURE DEPENDENT EARLY AFTER TRAUMATIC BRAIN INJURY IN RATS
Michael Waxler, Min-Hee Chen, Robert Clark, Kueilin He, Patrick Kochard, Donald Marion, and Steven Graham. University of Pittsburgh Dep. Anesthesiology/CVM, the Safar Center for Resuscitation Research and Brain Trauma Research Center, University of Pittsburgh, Pittsburgh, PA 15260

Introduction: Hypothermia applied before or shortly after traumatic brain injury (TBI) attenuates while hyperthermia exacerbates neurologic damage in experimental TBI (Clark et al., 1997). Damage is highest in neurons undergoing necrosis and apoptosis after TBI (Clark et al., 1997). One mechanism by which hypothermia might mitigate neurologic injury is upregulation of neuronal DNA damage. We hypothesized that neuronal DNA damage after TBI would be temperature-dependent within a clinically relevant range.

Methods: Anesthetized male Sprague-Dawley rats were subjected to controlled cortical impact and maintained at brain temperature 32, 37, or 39°C (± 0.5°C; n=8/group) for 4 h. Rat brains were perfused with ice-cold 4% paraformaldehyde. DNA damage was assessed using biotinylated DAPI and the Klenow fragment of DNA polymerase I. DNA damage was quantified by light microscopy as the number of positively-labeled cells/100x field in cortex and hippocampal regions. Data are expressed as mean ± SEM. Results were analyzed by ANOVA and Student-Newman-Keuls test.

Results: DNA damage in cells was evident in ipsilateral cortex, dentate, and CA3 hippocampus but was rarely detected in CA1 or in the contralateral hemisphere. DNA damage was temperature-dependent in the dentate gyrus (9.5 ± 0.5 vs. 31.0 ± 8.3 and 63.6 ± 18.1 cells/100x field X 32°C vs. 37°C and 39°C, respectively; p<.05). CA1 and CA3 (4 ± 0.2 vs. 10 ± 3.2 and 23 ± 10 cells/100x field X 32°C vs. 37°C vs. 0.2, p<.05), but not in CA1 or peritumoral regions of the cortex.

Conclusions: DNA damage in regions of hippocampus vulnerable to delayed neuronal death appears to be temperature-dependent at least early after TBI. One beneficial effect of hypothermia may be inhibition of DNA damage after TBI. Funding: Charles Scherzer Fellowship Grant from the Univ. of Pittsburgh Dep. Anesthesiology/CVM, Lavalier Ford, NS30118, and K08NS01946.

46

PENTOXIFYLLINE EFFECT ON FUNCTIONAL, BIOCHEMICAL AND HISTOLOGICAL RATINGS AFTER EXPERIMENTAL SPINAL CORD IMPACT
Vinay Nadkarni, Justin Fraser, Lisa Tice, Carol Barone, David Corddry. Jefferson Medical College, A.I. du Pont Hospital for Children, Wilmington, DE 19898.

Introduction: Pentoxifylline (PTX) may increase blood flow, cellular deformability, and decrease platelet aggregation, inflammation and secondary damage after acute spinal cord injury. This study examined the effects of two PTX treatment protocols on functional, biochemical, and histological recovery after acute spinal cord impact injury in an unanesthetized rat model.

Methods: 60 rats were randomized and assigned to 3 experimental groups. All groups were subjected to spinal cord impact using a drop weight 21 days prior to test day. Group I: Saline; Group II: PTX 90 mg/kg, 5 mg/kg, 5 mg/kg; and Group III: PTX 21 mg/kg, 15 mg/kg, 15 mg/kg. Blinded to group, 3 scales (BBB, Tach, Rivall-Tator Angleboard) were serially applied to assess motor function for 28 days. At autopsy, spinal cord sections were assessed, and below impact site were analyzed for biochemical markers of injury (nerve growth factor, DNA content, and hsp-72), immunohistochemistry was performed to determine if PTX altered the presence of the APOE4 allele following a TBI.

Results: At baseline, the 3 groups were equivalent. At 30 and 60 min after impact injury and blinded treatment, groups I and II showed significantly higher mean HR and BP than both PTX groups (p<.05). All groups showed improvement in motor function over 28 days (p<.01), with no significant differences among groups (p>0.05). Biochemical analysis showed the highest serotonin and metabolite levels at the impact site, with no significant differences in the ratio of serotonin-to-metabolite (p>0.05). Evaluation of the protein expression and cellular localization of the protein.

Conclusions: The results of this study suggest that the presence of the APOE4 allele is related to enhanced levels of EAA (glutamate and aspartate) which contribute to secondary damage following TBI. The mechanism of this relationship is not known and warrants further study.

47

PRELIMINARY RESULTS ON THE IMPACT OF APOE GENOTYPES ON CEREBROSPINAL FLUID (CSF) EXCITATORY AMINO ACIDS (EAA) AND METABOLITES IN TRAUMATIC BRAIN INJURED (TBI) ADULTS
Mary E. Kerr, Marilyn Kran, M. Ivy Kaslob, Ada Pacino, Steve T. Delcozky, Donald W. Marion. University of Pittsburgh, Schools of Nursing, Graduate School of Public Health; University of Pittsburgh Medical Center, Departments of Neurosurgery, and Psychiatry, Pittsburgh PA 15261.

Introduction: Apolipoprotein E (APOE) genotype has been linked to beta amyloid deposits and a microtubule-associated protein, tau, in an isoform-specific manner. APOE4 may impair neuronal growth potentially impacting recovery following injury. In a study by Wilson et al. reported that patients with APOE4 alleles had poorer verbal memory and psychomotor slowing following injury when compared to patients without APOE4 alleles. The purpose of this project was to determine whether the trajectory of EAA and lac-tate/pyruvate ratio was altered based on APOE-genotypes following a TBI.

Methods: The APOE genotypes were identified in 24 adults by genomic DNA amplification followed by digestion with Bsal restriction enzyme. Serial CSF samples from a ventriculostomy catheter placed within the right ventricle were removed by gravity drainage every 12 hours for the first 72 hours after injury. Samples were immediately placed into a freezer at -40 degrees C for storage. Aspartate and glutamate were measured using high-pressure liquid chromatography (HPLC) with fluorescence detection. Lactate and pyruvate were measured using ultraviolet detection.

Results: Of the 24 subjects, 6 had APOE 2/3 genotype, 12 had APOE 3/3 genotype and 6 had APOE 3/4 genotype. Acase range from .66 to 3.34 (M=1.34: SD=1.34). Differences existed across time in aspartate, glutamate and lactate/pyruvate ratio. Aspartate and lactate/pyruvate ratio were altered based on APOE-genotypes following a TBI.

Conclusions: The results of this study suggest that the presence of the APOE4 allele is related to enhanced levels of EAA (glutamate and aspartate) which contribute to secondary damage following TBI. The mechanism of this relationship is not known and warrants further study.


Excitotoxicity is implicated as a key mechanism of secondary neuronal damage after traumatic brain injury (TBI). The NMDA receptor antagonist MK-801 has been shown to attenuate cerebral injury in focal ischemia and some models of TBI, but it has not been tested in controlled cortical impact (CCI). We hypothesized that MK-801 would improve functional and histopathologic outcomes in rats following CCI. Anesthetized Sprague-Dawley rats (n=8/group) were subjected to CCI (4 m/s, 2.5 mm depth), then randomized to immediate treatment with either MK-801 (1 mg/kg IP) or vehicle. Rats treated with MK-801 recovered motor function significantly earlier than vehicle controls, as shown by beam balance/walking performance (d 1-5). MK-801 treated rats also showed improvement in the probe trial of the Morris water maze (d 14-20) vs vehicle (p<0.05), but no differences were seen in latencies to target. Contusion volume and hippocampal cell counts (d 21) did not differ between the groups. These data demonstrate an important role for excitotoxicity early after cerebral contusion and support continued evaluation of anti-excitotoxic therapies for use in TBI.

Funding: DAMD17-97-1-7009


Acute activation of Group 1 metabotropic glutamate receptors (mGluRs) contributes to traumatic brain injury (TBI) pathophysiology (J Neurosci 16:6012, 1996). We infused over 1 hr, the selective mGlu1 Group 1 antagonist, (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA) (0, 4, 2, 10 nmol) into the hippocampus (n=6/group) of adult male Sprague-Dawley rats. Control animals were injected with vehicle only. Animals were killed at 24 hrs after TBI. Cortical neurons were stained with Fluoro-Jade (FJ), a fluorescent marker for neuronal degeneration. Positive staining cells were counted in 4 sections/rat. Significantly fewer (p<0.05) FJ positive neurons were detected in the 10 nmol AIDA group (184 ±32) compared to the vehicle group (310 ±47). In a second experiment, rats were administered 10 nmol AIDA or vehicle (n=10/group) after TBI as above and tested in the Morris water maze for post-injury. The mean swim distance for the 10 nmol AIDA-treated group (193 ±142cm) was significantly (p<0.04) shorter than vehicle controls (1493 ±142cm). Post-injury blockade of Group 1 mGluRs appears to reduce neuronal degeneration and improve functional outcome after TBI. Supported by NIH NS29995.
16.3


A marker used to identify peroxynitrite activity following CNS injury is the 3-nitrotyrosine protein modifica tion. Recently, a number of studies have purported measurement of 3-nitrotyrosine (3-NT) in brain protein digest by HPLC. These assays vary substantially in processing. Halliwell and collaborators (J. Neurochem 70:2220-2222, 1998) reported measurement of an artificial substance in brain tissue which exhibited chromatographic, electrochemical and chemical properties nearly identical to 3-NT. It was suggested that this substance might confer the detection of 3-NT in brain tissue. We have developed an HPLC assay for the measurement of 3-NT that circumvents the problem of artificial detection. This was accomplished by on-pipette and multi-channel electrochemical detection. Using this technology, we were able to measure, in injured brain protein digests, 3-NT as a percentage of tyrosine (3-NT/TYR) at levels much lower (0.004%) than purported (J. Cereb Blood Flow Metab. 18:123-129, 1998). In fact, at 24 hrs, after impact-acceleration head injury in rat, hippocampal 3-NT/RYR was small did not differ from sham animals at levels much lower (0.004%) than purported (J. Cereb Blood Flow Metab. 18:123-129, 1998).

The induction of cytokines has been demonstrated after acute stroke and trauma. Temporal pattern of changes in energy balance has been closely linked to cytokine expression. To investigate a possible correlation between cytokine production and energy metabolism after closed head injury, CHI), the present study was designed in a mouse model of CHI. Injury was performed using a weight drop device (Chen et al, 1999) and brains were frozen 4-24h after sham surgery and at 5 min, 4, 12 and 24h (n=4/group) following CHI. ATP, glucose and lactate contents were determined by computer-aided bioluminescence imaging in serial tissue sections. Results: i) ATP content was significantly decreased in 3 days, 12 and 24h after injury compared to sham. Contralateral side ATP did not change in comparison to sham. ii) glucose content ipsilaterally significantly decreased on 3min, and remained lower up to 24h. Contralateral glucose content did not change significantly as compared to sham. iii) no change in lactate could be discerned. Conclusions: CHI in the mouse lead to ipsilateral cortical energy depression. This may be related to the early production of harmful modulators such as cytokines, reactive oxygen species or vasoactive neurotransmitters that locally impair the blood supply.
B6


Despite routine use of fentanyl in patients after traumatic brain injury (TBI), it is unclear if it is the optimal analgesic. Isoflurane is routinely used in TBI models. Studies in cold lesion and ischemia suggest isoflurane is neuroprotective vs. fentanyl. To our knowledge, fentanyl and isoflurane have not been compared in TBI. We hypothesize that isoflurane is neuroprotective vs. fentanyl early after TBI. Male Sprague-Dawley rats (n=18) underwent controlled cortical impact and received 4 h of fentanyl (10 mcg/kg bolus, 50 mcg/kg/h infusion) or isoflurane (1% inhalation). Functional outcome (beam balance, beam walking and Morris water maze [MWM] tasks) and lesion volumes were assessed. Motor and MWM performances were better in rats treated with isoflurane vs. fentanyl (p<0.05). Lesion volumes were not different between groups. We speculate that isoflurane may be neuroprotective after TBI by increasing CBF, suppressing metabolism, and/or modulating excitotoxicity. Isoflurane may mask beneficial effects of novel treatments in experimental TBI. Finally, fentanyl may not be the optimal analgesic agent early after TBI in humans. Support: US Army DAMD17-97-1-7009.

B7

INFLUENCE OF POST-TRAUMATIC HYPOXIA ON BEHAVIORAL AND HISTOPATHOLOGICAL OUTCOME FOLLOWING MODERATE SPINAL CORD INJURY IN RATS.

Y. Yamazawa*, A. Marcelli, R. Garcia, K. Loer, W.D. Dietrich. Department of Neurological Surgery and The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL.

Pulmonary dysfunction leading to secondary hypoxia is a common complication of spinal cord injury (SCI). The purpose of this study was to investigate the consequences of an induced post-traumatic hypoxic event following SCI. Forty-five female Sprague-Dawley rats were randomly assigned to 1 of 4 groups, including 1) sham surgery and normoxia, 2) sham surgery and hypoxia, 3) NYU weight-drop and normoxia, and 4) NYU weight-drop and hypoxia. For these studies, a moderate injury was induced by adjusting the height of the weight-drop (10 gm) to 12.5 mm above the exposed spinal cord (T10). Immediately after injury, PaO2 in hypoxic rats was kept between 30-35 mmHg for 30 min, with 56% nitrous oxide, 31% nitrogen, and 13% oxygen. PaO2 in the normoxic group was maintained over 100 mmHg, while PaCO2 in all rats was maintained at 35-40 mmHg. The behavior of the rats was checked every 7 days using the BBB locomotor rating scale. Rats were sacrificed at 8 wks after behavioral testing and perfusion fixed for quantitative histopathological analysis of lesion areas. Although post-traumatic hypoxia tended to improve BBB scores, no significant difference in locomotor performance was demonstrated between the traumatized groups. In contrast, the percent of gray matter sparing at the impact epicenter was significantly reduced in hypoxic vs. normoxic SCI rats (p<0.05). These studies demonstrate that although moderate hypoxia following SCI does not significantly affect locomotive recovery, this secondary insult worsen gray matter pathology.

B8


The objective of this study was to determine whether an episode of hypoxia 24 hr after brain trauma augments histologic injury. Male C57BL/6 mice (n=10) were subjected to controlled impact injury using a deformation depth of 1 mm and impact velocity of 5 m/sec. After recovery for 24 hr, hypoxia was produced by lowering the percentage of 9% and 7% for an additional 30 min. After an additional recovery period of 5 days, the animals were perfusion-fixed with FAM, and the brains were embedded in paraffin, sectioned, and stained with acid fuchsin/thionin. The stained sections were examined for histologic alteration and the volume of cortical infarction was measured. Histopathologic alteration was not detected in any region of the contralateral hemisphere. Hypoxia significantly increased the size of the cortical lesion: Sham-hypoxia = 1.95 ± 0.42 mm3 (mean ± SD, n=5) vs. Hypoxia = 3.15 ± 0.48 (p<0.01). The only other histologic alteration detected was in the dentate granule cell layer of the ipsilateral hippocampus. There was both loss of neurons and acidophilic transformation of neurons in this layer. However, the number of acidophilic dentate granule cells was not altered by hypoxia (Sham-hypoxia = 74 ± 4 vs. Hypoxia = 70 ± 19). These results indicate that the traumatized cortex remains vulnerable for 24 hr to a level of hypoxia which does not cause histologic injury in the contralateral hemisphere. Supported by NIH Grant NS-08803.

B9

HYPOXIA EXACERBATES CA3 HIPPOCAMPAL NEURONAL DAMAGE AFTER FLUID PERCUSSION BRAIN INJURY IN RATS. Namiko Nomura*, Kojiro Wada, Yoshitani Matsushita, Hiroshi Nawashiro, Katsuji Shima Dept. of Neurosurgery, National Defense Medical College, Tokorozawa, Saitama, Japan.

We have reported that increased vulnerability of hippocampal CA3 neurons to hypoxia after mild concussion. The present study was designed to determine if a model of moderate fluid-percussion (F-P) brain injury with hypoxia exacerbates hippocampal CA3 lesions, if those lesions are associated with apoptosis using the terminal deoxynucleotidyl transferase-mediated biotin-dUTP nick-end labeling method (TUNEL). Anesthetized Sprague-Dawley rats were injured with a moderate severity fluid percussion pulse (3.5-4.0 atmospheres) administered over the right parietal cortex. The experimental animals were divided into 2 groups, traumatic brain injury (TBI) group (n=6), which was subjected to TBI alone, and TBI + hypoxia group (n=6), which was subjected to TBI followed by 20 min of moderate hypoxia (FiO2: 10%). Three days following TBI, % neuronal density per 1-mm length of CA3 neurons in the ipsilateral hippocampus was significantly decreased in the TBI + hypoxia group (45.2 ± 29.6 %; p < 0.05) compared to the TBI alone group (90.8 ± 24.1 %). No significant difference in the number of TUNEL positive cells was observed at 6-h, 24-h and 3-day (n=2) in both groups. These results suggest that TBI with moderate hypoxia induced more hippocampal damage due to not only apoptosis but also necrosis.

60

SOLUBLE FAS IS INCREASED IN CSF FROM INFANTS AND CHILDREN AFTER HEAD INJURY

Neal A Seidberg, Acud Hosp of Pittsburgh, Pittsburgh, PA; Robert S B Clark, Univ Pittsburgh and Safar Ctr for Resuscitation Res, Pittsburgh, PA; Patrick M Kochanek, Safar Ctr for Resuscitation Res, Pittsburgh, PA; P David Adelson, Margaret A Satchell, Randall A Ruppell, Acud Hosp of Pittsburgh, Pittsburgh, PA; Patricia J Janesko, Safar Ctr for Resuscitation Res, Pittsburgh, PA; Steven H Graham, Univ of Pittsburgh, Pittsburgh, PA.

**Introduction:** For a member of the TNF receptor family, and its ligand FasL, provide a system for regulating intercellular programmed-cell death (PCD), where binding of FasL to Fas receptor triggers apoptosis. FasL has been identified on neurons and astrocytes, and FasL is present on microglia and inflammatory cells, thus, PCD in brain after injury may be regulated in part by FasL-Fas interactions. Accordingly, we examined CSF from infants and children after traumatic brain injury (TBI) for alterations in FasL and Fas in children. Pan-caspase inhibition using systemic treatment with BAY 12-3258 (BAF) would reduce hippocampal cell death after controlled cortical impact (CCI) vs vehicle (DMSO) tp. in a randomized fashion. In one squadron of 14 children, Fas and FasL were measured by ELISA. **Results:** TBI patients ranged in age from 1mo to 11 y, 16 survived and 2 died. CSF Fas was increased 2-fold in TBI patients vs control (see table). Post hoc analysis also revealed an association between Fas and age and suspected child abuse. Conclusions: These data suggest that TBI induces alterations in the Fas/FasL system. Additional patients and multivariate analysis are required to further define associations between Fas and age and suspected child abuse. Therapies targeting cell death receptors, such as Fas, may represent effective strategies aimed at reducing PCD after TBI. **Support:** RO1 NS38492, K08 NS31094, & P60 NS31816.

62

SYSTEMIC TREATMENT WITH A PAN-CASPASE INHIBITOR IMPROVES HIPPOCAMPAL NEURON SURVIVAL AFTER TRAUMATIC BRAIN INJURY IN MICE

Neal A Seidberg, Acud Hosp of Pittsburgh, Pittsburgh, PA; Steven H Graham, Univ of Pittsburgh, Pittsburgh, PA; Patrick M Kochanek, Safar Ctr for Resuscitation Res, Pittsburgh, PA; C. Edward Dixon, Univ Pittsburgh, Pittsburgh, PA; P David Adelson, Univ Pittsburgh and Safar Ctr for Resuscitation Res, Pittsburgh, PA; David G Watts-Warner, Univ of Pittsburgh, Pittsburgh, PA; P David Adelson, Margaret A Satchell, Pittsburgh, PA; Edward Dixon, Univ Pittsburgh and Safar Ctr for Resuscitation Res, Pittsburgh, PA; Donald W Marion, Univ of Pittsburgh, Pittsburgh, PA; Keri L Janesko, Safar Ctr for Resuscitation Res, Pittsburgh, PA; Donald W Marion, Univ of Pittsburgh, Pittsburgh, PA; Robert S B Clark, Univ Pittsburgh and Safar Ctr for Resuscitation Res, Pittsburgh, PA; Jr, senior author.

**Introduction:** Traumatic brain injury (TBI) in mice produces cell death both immediately after injury as a result of direct mechanical disruption, and in a delayed fashion as a result of secondary injury. Programmed cell death (PCD), or apoptosis, contributes to cell death in the context of TBI. We hypothesized that systemic administration of the pan-caspase inhibitor bensoic (OMe)N-(4-methylbenzyl)-4-fluorophenylalanine (BAF) would reduce hippocampal cell death after controlled cortical impact (CCI) in mice. **Methods:** Anesthetized mice were subjected to severe CCI to the left parietal cortex. Immediately after CCI mice were given 100 nmol BAF or vehicle (DMSO) by IP injection. Brain water was quantified on d21. Additional rats (n=14) underwent CCI and anesthesia as described above with intracranial pressure (ICP) monitoring (Codman intraparenchymal transducer) for 4b. Brain water was quantified on d21. In a randomized fashion, in one squadron of mice Caspase-3 activity was measured in injured brain at 24 h (n=3-4/group).

63

ISOFLURANE IMPROVES LONG-TERM NEUROLOGIC OUTCOME COMPARED TO FENTANYL AFTER TRAUMATIC BRAIN INJURY IN RATS

Kimberly D Statler, Acud Hosp of Pittsburgh, Pittsburgh, PA; Patrick M Kochanek, Safar Ctr for Resuscitation Res, Pittsburgh, PA; C. Edward Dixon, Univ Pittsburgh, Pittsburgh, PA; P David Adelson, Margaret A Satchell, Pittsburgh, PA; Peter J Safar, Safar Ctr for Resuscitation Res, Pittsburgh, PA; Donald W Marion, Univ of Pittsburgh, Pittsburgh, PA; Peter J Safar, Safar Ctr for Resuscitation Res, Pittsburgh, PA; Jr, senior author.

**Introduction:** Despite routine use of fentanyl in patients after traumatic brain injury (TBI), it is unclear if it is the optimal sedative/analgesic agent. Isoflurane is commonly used in veterinary anesthesia and is neuroprotective. The current study was designed to compare the effects of isoflurane and fentanyl on the neuroprotection after TBI. We hypothesized that isoflurane would be neuroprotective vs fentanyl when given early after TBI in rats. Methods: Adult rats (n=19) underwent controlled cortical impact (CCI) with physiologic monitoring and then received 4mg/kg CCl4 and either fentanyl (10 mg/kg bolus, 50 mg/kg infusion) or isoflurane (1% inhalation). **Results:** Shams (n=8) underwent identical preparation and anesthesia but no TBI. We hypothesized that isoflurane would be neuroprotective vs fentanyl when given early after TBI in rats. Methods: Adult rats (n=19) underwent controlled cortical impact (CCI) with physiologic monitoring and then received 4mg/kg CCl4 and either fentanyl (10 mg/kg bolus, 50 mg/kg infusion) or isoflurane (1% inhalation). Shams (n=8) underwent identical preparation and anesthesia but no CCI. Functional deficits (beam balance, beam walking, Moriss water maze (MW)) tasks were assessed over 20d in injured and sham rats. Lesion volume was quantified on d21. Additional rats (n=14) underwent CCI and anesthesia as described above with intracranial pressure (ICP) monitoring (Codman intraparenchymal transducer) for 4b. Brain water (wet-dry weight method) was assessed at the end of the anesthetic period. Result: After injury, motor and LWM performance were better in isoflurane vs fentanyl treated rats (p<0.05, ANOVA) but did not differ between shams. Lesion volumes were similar between groups. There was increased frequency of ICP>30 mmHg and higher brain water in rats treated with isoflurane vs fentanyl (p<0.05, ANOVA). Conclusion: Both treated with isoflurane had improved long-term functional outcome after CCI compared to those treated with fentanyl, despite increased brain water. We speculate that isoflurane may mediate improved long-term functional outcome after CCI in rats through promotion of cerebral blood flow, suppression of malformation, and/or modulation of excitotoxicity. Fentanyl should not be used as a sedative/analgesic agent early after TBI in humans. **Support:** USAArmy DAMD17-97-1-7009; 1. Mira, et al. Anesthesiology 1998; 89:400-427.
MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for reports on the enclosed list be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management