Building a Better Fluid for Emergency Resuscitation of Traumatic Brain Injury

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Hextend (HEX) is a colloid solution that is FDA-approved for volume expansion during surgery. ATL-146e is a novel adenosine A2A receptor agonist that has anti-inflammatory, neuroprotective, and coronary vasodilator properties. Three series of experiments were designed to evaluate the therapeutic potential of HEX±ATL-146e for emergency resuscitation from traumatic brain injury (TBI) + hemorrhagic hypotension.

Methods: In the first two studies in vivo, anesthetized, ventilated pigs (30–45 kg) received a fluid percussion TBI, 45% arterial hemorrhage, and 30 minutes shock period. In Series 1, resuscitation consisted of unlimited crystalloid (n = 8) or HEX (n = 8) to correct systolic arterial pressure >100 mm Hg and heart rate <100 bpm for the first 60 minutes (“emergency phase”), and then maintain cerebral perfusion pressure (CPP) > 70 mm Hg for 60–240 minutes. In Series 2 (n = 31), resuscitation consisted of a 1 L bolus of HEX + ATL-146e (10 ng/kg/min, n = 10) or HEX + placebo (n = 10) followed by crystalloid to the same endpoints. In Series 3 in vivo, the hemodynamic response evoked by 0, 10, 50, or 100 ng/kg/min ATL-146e was measured before or 60 minutes after HEX resuscitation from 45% hemorrhage.

Results: Following TBI+hemorrhage, there were 4/22 deaths in series 1 and 11/31 deaths in series 2. In those alive at 30 minutes, mean arterial pressure, cardiac index, mixed venous O2 saturation, and cerebral venous O2 saturation were all reduced by 40–60%, while heart rate and lactate were increased 2–5 fold. With no resuscitation (n = 2), there was minimal hemodynamic compensation and progressive acidosis. Upon resuscitation, these values corrected but intracranial pressure progressively rose from <5 mm Hg to 15–20 mm Hg. Series 1: With HEX (n = 8) versus crystalloid (n = 8), CPP was less labile, acid/base was maintained, and the fluid requirement was reduced by 60% (all p < 0.05). Series 2: With ATL-146e (n = 10) versus placebo (n = 10), stroke volume and cardiac output were improved by 40–60%, and the fluid requirement was reduced by 30% (all p < 0.05). Series 3: ATL-146e caused a dose-related increase (p < 0.05) in stroke volume after, but not before, hemorrhage. The effects on preload, afterload, and heart rate were similar before and after hemorrhage.

Conclusions: HEX alone is a safe and efficacious low volume alternative to initial crystalloid resuscitation after TBI. An adenosine A2A agonist combined with 1 L of HEX safely and effectively counteracted a decrease in cardiac performance noted after TBI+hemorrhage without causing hypotension or bradycardia.

Keywords: Hemorrhagic Shock, Adenosine, ATL-146e, A2A receptor, Hextend Swine


Each year in the United States, there are approximately 1.6 million cases of traumatic brain injury (TBI), which lead to 80,000 severe, permanent neurologic disabilities, and 52,000 deaths. The morbidity and mortality rates from TBI on the battlefield are even worse.

The fundamental goals of resuscitation of the TBI patient are the restoration of circulating volume, blood pressure, and oxygenation, but there are no evidence-based guidelines for the optimal fluid type and/or endpoint for resuscitation. We recently reported improved cardiopulmonary performance after low volume resuscitation from severe chest trauma with Hextend (HEX) supplemented with ATL-146e. HEX rapidly restored hemodynamics while ATL-146e improved early cardiac performance without causing hypotension or bradycardia. The overall aim of this study was to determine whether HEX with or without ATL-146e had salutary actions after severe TBI.

HEX is a high molecular weight hydroxyethyl starch in buffered electrolyte dextrose solution that is FDA-approved as a plasma volume expander for treatment of hypovolemia during elective surgery. Because of its favorable profile of...
side effects relative to other intravenous fluids. HEX has been selected for use as a field resuscitant by the U.S. military. ATL-146e is an adenosine A2A receptor-selective agonist that protects the spinal cord from reperfusion injury. It also has anti-inflammatory and coronary vasodilator properties and is in phase 2 clinical trials as a coronary dilator for pharmacological stress imaging.

A low volume resuscitation fluid supplemented with a compound that has well-described protective properties could offer logistical advantages for trauma patients in pre-hospital field conditions or whenever there is limited medical resources or prolonged transport times. These actions could be life-saving in the critical minutes after TBI, when the brain is especially vulnerable to secondary hypoxia or hypotension. Three series of experiments were designed to evaluate the therapeutic potential of HEX + ATL-146e for emergency resuscitation from TBI + hemorrhagic hypotension. The first series compared unlimited HEX alone to unlimited saline alone. The second series examined whether cardiac performance was enhanced when HEX was supplemented with ATL-146e. The third series tested the dose-related myocardial effects of ATL-146e before and after fluid resuscitation. This is the first study to examine the actions of HEX or ATL-146e after severe TBI.

METHODS

Materials

ATL146e (4-[3-[6-Amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl]-cyclohexanecarboxylic acid methyl ester) was provided by Dr. Jason Rieger of Adenosine Therapeutics, LLC, Charlottesville, VA. Hextend was provided by Dr. Paul Segall at BioTime, Inc, Berkeley, CA.

Study Population

Male and female, prepubertal, farm-raised cross-bred pigs (30–45 kg, n = 58) were housed in the university animal care facility, and maintained in exact accordance with all pertinent animal welfare regulations. All data were collected under general anesthesia with ketamine (Fort Dodge Animal Health, Fort Dodge, IA), xylazine (Fort Dodge Animal Health) and fentanyl citrate (Abbott Labs, North Chicago, IL). The Animal Care and Use Committee of the University of Miami approved all procedures.

Instrumentation

Three series of experiments were performed. The overall study design is outlined in Figure 1. In anesthetized swine, the trachea was intubated, and mechanical ventilation was initiated in the supine position. One liter of normal saline was administered and the ventilator was adjusted to maintain oxygen saturation (Sao2) of 100%, an inspired oxygen concentration (FiO2) of 40%, and an end-tidal CO2 (ETCO2) of 40 mm Hg. A left external jugular catheter was placed for administration of supplemental fluid. The right heart and pulmonary artery were catheterized via the right external jugular vein. The left femoral artery was catheterized for pressure measurements and for hemorrhage. Flow-through transducers were used to obviate the need for heparin. Following in vivo calibration (NOVA Ultra M, Waltham, MA), continuous measurements were made of all pressures, oxygen saturation, and cardiac output (Oximetrix3 SO2/CO com-

Fig. 1. Experimental design.
Additional Procedures

In Series 1 and 2 experiments only, animals were placed in the prone position. A 1 cm diameter craniotomy was made at the intersection of the transverse and sagittal sutures through which the superior sagittal sinus was cannulated. A fiberoptic probe (Integra Neurosciences, Camino, Plainsboro, NJ) was inserted into the subdural space through the midline craniotomy for measuring intracranial pressure (ICP). A second 1 cm craniotomy was made over the left fronto-parietal cortex, approximately 1 cm lateral and 2 cm anterior to bregma. A fluid percussion device was attached to a multiport, hollow bolt inserted into the second craniotomy. Finally, bispectral electroencephalogram (EEG) probes were attached and continuously monitored (Aspect BIS, Natick, MA). These procedures have been described in several previous publications from this lab.23–26

In Series 1 and 2 experiments only, after a 30–60 minutes stabilization period following instrumentation, a total of five separate measurements of the physiologic variables described below were made in pre-injury conditions at 10 minutes intervals. The first three measurements were made to verify baseline stability, then CO2 was infused into the ventilatory circuit, and titrated until the ETCO2 was 60. In Series 3, at 30 minutes after hemorrhage, resuscitation consisted of either HEX or normal saline was infused at 10 mL/hr starting at 25 minutes after TBI. At 30 minutes after TBI, the resuscitation consisted of 1 L of HEX followed by lactated Ringers that was titrated to SAP >100 mm Hg and HR <100 bpm for the “emergency phase.” After 60 minutes, lactated Ringers was titrated to maintain CPP > 70 mm Hg. An unlimited volume of lactated Ringers was administered to achieve the resuscitation endpoints. (see Fig. 1). In n = 2, no resuscitation fluid was administered.

In Series 2, either ATL-146e (10 ng/kg/min) or normal saline was infused at 10 mL/hr starting at 25 minutes after TBI. At 30 minutes after TBI, the resuscitation consisted of 1 L of HEX followed by lactated Ringers that was titrated to SAP >100 mm Hg and HR <100 bpm for the “emergency phase.” After 60 minutes, lactated Ringers was titrated to maintain CPP > 70 mm Hg. An unlimited volume of lactated Ringers was administered to achieve the resuscitation endpoints. (see Fig. 1)

In Series 3, at 30 minutes after hemorrhage, resuscitation consisted of 1 L of HEX followed by lactated Ringers titrated to SAP >100 mm Hg and HR <100 bpm. At 60 minutes, the dose-response curve for ATL-146e was repeated. (see Fig. 1)

Physiologic Variables

The following experimental data were collected: volume of intravenous fluid administered, blood temperature, HR, SAP, MAP, pulmonary artery pressure, pulmonary capillary wedge pressure, central venous pressure, cardiac output, mixed venous oxygen saturation (SvO2), SaO2, ETCO2, FiO2, bispectral EEG, EEG burst suppression ratio, sagittal sinus pressure, and intracranial pressure. Blood gases and electrolytes were drawn from the arterial line and from the sagittal sinus catheter at the 30 minutes intervals. A complete blood count was performed every 30 minutes (Abbott Cell-Dyne 1600).

Statistics

Data are expressed as mean ± SE. Differences were compared with two-way analysis of variance and non-parametric tests (Fisher PLSD and Scheffe F test) at the 95% confidence interval.

RESULTS

Series 1

TBI+40% hemorrhage produced 4/22 deaths within 30–40 minutes. Those that died (31.8 ± 3.5 kg) were hemorrhaged 958 ± 107 mL. In n = 2 survivors, no resuscitation fluid was administered. Both remained alive for the 240 minutes observation period but were moribund (e.g. MAP<40 mm Hg, HR >120 bpm, arterial lactate > 8
mmol/L, ischemic EEG changes, fixed-dilated pupils). The study population was comprised of 2 fluid resuscitated groups. The saline group (32.6 ± 1.8 kg, n = 8) was hemorrhaged 966 ± 44 mL. The HEX group (32.6 ± 2.1 kg, n = 8) was hemorrhaged 981 ± 64 mL. In the two groups, MAP and HR were indistinguishable in baseline and shock conditions, and were restored to similar values during the “emergency” phase, and throughout the “CPP management” phase (Fig. 2). By 240 minutes, 6/8 were alive with HEX compared with 5/8 with saline.

Cardiac output was 2.5–3.0 L/min before hemorrhage (70–90 mL/min/kg), and dropped to 1.0–1.5 L/min (30–40 mL/min/kg) post-hemorrhage, but then recovered to 3.5–4.5 L/min (80–120 mL/min/kg) with resuscitation. Arterial lactate increased from <2 mmol/L at baseline to >5.5 mmol/L after shock, but gradually corrected with fluid. SvO₂ decreased from 66–74% to 42–48% during shock, and then only partially corrected to 50–60%. In contrast, cerebral venous O₂ saturation fell less during shock (from 35–45% to 20–30%) and fully recovered to baseline with resuscitation. Intra-cranial pressures rose progressively during the resuscitation period, eventually reaching 15–20 mm Hg by the end of observation. However, there were no treatment-related differences between these values, so the data are not shown.

Cerebrovascular compliance (as reflected by the CO₂-evoked change in ICP) and cerebrovascular reactivity (as reflected by the CO₂-evoked change in cerebral venous O₂ saturation) were both significantly impaired after TBI, however, there were no treatment-related differences between these values, so the data are not shown.

![Fig. 2. Series 1 Experiments: For 30–90 minutes after TBI (“emergency phase”), an unlimited volume of either HEX or saline was administered to maintain SAP>100 mm Hg and HR<100 bpm. For the remainder of the 240 minutes post TBI observation period, CPP was maintained >70 mm Hg with as much of either fluid as necessary. These data show that MAP and HR were virtually identical between the groups at all time points.](image)

![Fig. 3. Series 1 Experiments: The total amount of HEX required to achieve the resuscitation endpoints was 60% less than the total amount of saline and the difference was significant (p < 0.05).](image)

![Fig. 4. Series 1 Experiments: CPP and Acid-base status were both better maintained with HEX versus saline. By 240 minutes post TBI, CPP was extremely labile and completely fluid dependent with saline, but was relatively stable at >70 mm Hg in the HEX group. This difference was significant (p < 0.05). With HEX, there was an arterial base excess at the end of the observation which was near the pre-injury baseline (3–6](image)
mEq/L). However, with saline, a progressive acidosis developed and by 240 minutes post TBI, there was a base deficit of 7–9 mEq/L. This difference was significant (p < 0.05). There was a similar trend with ICP, but that apparent difference was not statistically significant.

**Series 2**

In this series, TBI+45% hemorrhage produced 11/31 deaths within 30–40 minutes. Those that died (38.1 ± 1.6 kg) were hemorrhaged 988 ± 34 mL. In the placebo group (39.1 ± 1.3, n = 10), the hemorrhage volume was 1006 ± 32 mL. In the ATL-146e group (39.2 ± 1.9, n = 10), the hemorrhage volume was 1006 ± 50 mL. All that survived to point of resuscitation survived to the end of the 240 minutes observation period.

Figure 5 shows that throughout the observation period, MAP and HR were essentially superimposed with or without ATL-146e. At 60 minutes after the initiation of resuscitation, HR was 73 ± 6 bpm and 74 ± 4 bpm in the other. Systolic blood pressure, at the same time, was 118 ± 5 mm Hg in one and 120 ± 4 mm Hg in the other.

Several other variables, including arterial lactate, base deficit, SvO₂, and pH, showed the same basic pattern as Series 1 with shock and resuscitation. However, there were no treatment-related differences, so those data are not shown.

Figure 6 shows the total amount of fluid required to maintain systemic hemodynamics. The placebo + HEX + LR group required 6085 ± 582 mL (or about 150 mL/kg), while the ATL-146e + HEX+LR group received 4360 ± 513 mL (or about 115 mL/kg). This difference was significant (p < 0.05).

Figure 7 shows that, despite the relative volume deficit with HEX+ATL-146e versus HEX alone, cardiac output and stroke volume corrected faster and were maintained throughout the resuscitation period. Cardiac output, which was 3.0–3.4 L/min (75–80 mL/min/kg) before hemorrhage, dropped by half after hemorrhage. After initiation of resuscitation, stroke volume and cardiac index were increased by 20–40% with HEX+ATL-146e. Cardiac output after resuscitation rose from 1.23 ± 0.08 L/min (32 ± 2 mL/min/kg) post-hemorrhage to 3.50 ± 0.28 L/min (91 ± 9 mL/min/kg) with HEX alone at 60 minutes, and to 4.80 ± 0.85 L/min (122 ± 19 mL/min/kg) at the same time with the A2A agonist. The
elevation in cardiac output was sustained: the value at 240 minutes with HEX+A2A was 5.77 ± 0.81 L/min (148 ± 20 mL/min/kg) versus 3.70 ± 0.54 L/min (96 ± 15 mL/min/kg). These differences were significant (p < 0.05).

The first series of experiments basically showed that, when resuscitating to the systemic hemodynamic endpoints commonly used in patients (SAP>100 mm Hg, HR<100 bpm for first 60 minutes) with unrestricted volume, HEX was equivalent to crystalloid, except less volume was required (Fig 3; 1.9 ± 0.2 L or 56 ± 7 mL/kg versus 5.4 ± 0.5 L or 161 ± 17 mL/kg) and CPP and base deficit were better maintained (Fig 4).

This finding is important because the brain is particularly vulnerable to secondary hypotensive or hypoxic insults during transport and during the diagnostic work-up at the trauma center. The newly-arrived patient may be resuscitated for 1–2 hours before receiving a ventriculostomy (i.e. time to initial resuscitation, time obtaining and interpreting radiographic studies, and time to prepare and place a ventriculostomy catheter). Furthermore, decreasing the volume of resuscitation fluid could provide a logistical advantage in scenarios in which space and weight are at a premium, such as on the battlefield, during emergency transport, or during resuscitation in a rural setting. In addition, CPP resuscitative strategies, with their emphasis on maintaining intravascular volume, may lead to hypervolemia when crystalloids are utilized for resuscitation. This undesired consequence has been previously associated with an increased risk of pulmonary complications, including Acute Respiratory Distress Syndrome.1

Determining the exact mechanism for the protective effect of HEX after TBI is beyond the scope of this study, however it is probably not related to toxicity per se. The mOsm/L of NS, HEX, and LR are 308, 307, and 275 respectively. Furthermore, we measured blood osmolality and the values ranged from 280–290 and did not differ significantly between the HEX and NS groups. There are several studies which have clearly shown benefits of hypertonic saline after TBI in patients27–29 Whereas that mechanism is probably related to reduced endothelial swelling30 due to the hyperosmolarity, a similar physical effect probably could not explain the actions of HEX.

It is clear that in patients, HEX expands intravascular volume 2–3× more than crystalloid, for up to 48 hours. Even after as much as 8 L, HEX is apparently not associated with

**DISCUSSION**

The results from this experimental study on brain trauma confirm and extend our recent experimental study on severe chest trauma,3 which showed that low volume resuscitation with HEX + ATL-146e enhanced cardiopulmonary performance. In both injury models, for the sake of realism, soft tissue injury was superimposed on a life-threatening hemorrhage insult, and the control group received “standard of care” for 60 minutes, to simulate transport to the trauma center, initial evaluation, stabilization, radiographic studies, etc. In both studies, comparatively low volumes of HEX rapidly corrected the volume deficit, while the adenosine A2A agonist, ATL-146e, enhanced cardiac output and stroke volume without producing the well-described hypotension or bradycardia expected with its parent compound, adenosine.

**Table 1** Series 3 Experiments: Dose-Related Actions of A2A Agonist, ATL-146e, on Determinants of Myocardial Performance

<table>
<thead>
<tr>
<th>Absolute Values</th>
<th>A2A Dose ng/kg/min</th>
<th>Cardiac Output ml/min/kg</th>
<th>Heart Rate b/min</th>
<th>Stroke Volume ml/b/min</th>
<th>Mean Arterial Pressure mm Hg</th>
<th>Central Venous Pressure mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before hemorrhage</td>
<td>0</td>
<td>72 ± 5</td>
<td>49 ± 3</td>
<td>1.50 ± 0.10</td>
<td>99 ± 4</td>
<td>8 ± 1</td>
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<tr>
<td></td>
<td>10</td>
<td>79 ± 5</td>
<td>55 ± 4</td>
<td>1.49 ± 0.15</td>
<td>96 ± 4</td>
<td>8 ± 1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>85 ± 6</td>
<td>64 ± 8</td>
<td>1.44 ± 0.18</td>
<td>93 ± 4</td>
<td>7 ± 2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>95 ± 8*</td>
<td>81 ± 9*</td>
<td>1.28 ± 0.18</td>
<td>82 ± 4*</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>After hemorrhage + resuscitation</td>
<td>0</td>
<td>87 ± 13</td>
<td>58 ± 6</td>
<td>1.50 ± 0.17</td>
<td>105 ± 3</td>
<td>13 ± 2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>99 ± 13*</td>
<td>55 ± 6</td>
<td>1.79 ± 0.14*</td>
<td>103 ± 4</td>
<td>11 ± 1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>112 ± 14*</td>
<td>61 ± 6</td>
<td>1.85 ± 0.12*</td>
<td>97 ± 5*</td>
<td>11 ± 1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>133 ± 13*</td>
<td>70 ± 10*</td>
<td>1.97 ± 0.14*</td>
<td>91 ± 3*</td>
<td>9 ± 2*</td>
</tr>
</tbody>
</table>

* p < 0.05 change from baseline.
the coagulopathy often seen with other synthetic colloids.\textsuperscript{4–6} However, one problem with the design of series 1 is that in some field situations, or during emergency medical transport, unlimited volumes of fluid are not available.

The second series of experiments was intended to mimic a situation where resources are limited. In this design, a one liter bolus of HEX was administered after initial treatment with a placebo or ATL-146e (10 ng/kg/min). These data showed improved early myocardial performance with no undesired hemodynamic effects (Figs 5–7).

The third series of experiments suggests that cardiac output was increased because of an inotropic effect of ATL-146e. It is important to emphasize that in any given set of circumstances an increase in cardiac output could be attributed to an increase in pre-load (Frank-Starling effect), a decrease in afterload, an increase in heart rate, or an increase in contractility. Table 1 shows that ATL-146e had qualitatively similar, dose-related effects on pre-load, afterload, heart rate, and cardiac output before or after hemorrhage. However, there was a dose-related increase in stroke volume, after (but not before) hemorrhage, which is consistent with increased contractility. This increase is most likely due to a direct A2A mediated coronary vasodilatation\textsuperscript{15–16} or to an A2A effect to counteract the action of inflammatory mediators that inhibit myocardial contractility.\textsuperscript{7–14} In vitro, ATL-146e is a potent dilator (ED50 = 30 ± 4 nM) of pig coronary arteries, with a low affinity (EC50 = 440 ± 40 nM) at A1 receptors.\textsuperscript{33–34} An inotropic action could explain the further reduction in the amount of intravenous fluid required to resuscitate animals receiving ATL-146e (Fig 6). Further investigation is needed to determine whether this short-term action during the immediate resuscitation period has any practical significance for survival or long-term neurologic outcome.

Adenosine is a normal constituent of all body fluids that accumulates during inflammation, hypoxia, and ischemia and serves to modulate the pathologic responses associated with reperfusion. For almost 50 years, various investigators have reported benefits of fluids supplemented with ATP, adenosine or one of its related purine metabolites. Unfortunately, the putative benefits of most adenosine-related drugs\textsuperscript{31,32} are offset by short duration of action, and powerful vasodilator and bradycardic actions that are absolutely contraindicated during hypotensive shock states. The explanation is that, in addition to its metabolic actions inside the cell, the extracellular actions of adenosine are dependent upon on at least 4 sub-types of cell-surface receptors (A1, A2A, A2B, and A3), which vary widely in distribution between species and cell type.\textsuperscript{19} With the molecular cloning of these receptors, and the synthesis of several new agonists\textsuperscript{33,34} and antagonists,\textsuperscript{35} there has been a resurgence of interest in the theoretical potential of adenosine analogs to target individual receptors to protect against inflammation and reperfusion injury, particularly in the central nervous system.\textsuperscript{7–12,36} However, it should be emphasized that the beneficial effects in spinal cord reperfusion injury may not directly apply to TBI.

There are at least three elements of the experimental design that limit the practical relevance of these findings to the clinical situation. First, the observation period post TBI was relatively short; Second, there was no behavioral paradigm to test whether these short term physiologic changes improved neurologic outcome. Third, the pigs received fluid alone to address CPP during resuscitation from severe TBI + hemorrhage, whereas a TBI patient could receive mannitol, pressors, and/or transfusions. All of these issues have been addressed in a series of follow-up experiments.\textsuperscript{37}

In summary, these present observations substantiate our earlier work\textsuperscript{7} that HEX and ATL-146e have no obvious harmful effects after trauma. In addition, the use of HEX for TBI reduced the volume of intravenous fluid required to obtain standard, physiologic endpoints of resuscitation. Supplemental ATL-146e further reduced the volume required for resuscitation, by increasing myocardial contractility. Further studies are warranted to investigate the long-term effects of HEX and ATL-146e on intracranial pressure, cerebral inflammatory response to injury, and neurologic outcome.

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**REFERENCES**


