Severe hemodilutional anemia increases cerebral tissue injury following acute neurotrauma

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1Department of Anesthesia, Cara Phelan Centre for Trauma Research, Keenan Research Centre in the Li Ka Shing Knowledge Institute, University of Toronto, St. Michael’s Hospital, Toronto; 2Department of Physiology, University of Toronto, Toronto; 3Departments of Critical Care Medicine and Paediatrics and the Neuroscience and Mental Health Program, The Hospital for Sick Children, Toronto; 4Interdepartmental Division of Critical Care, Faculty of Medicine, University of Toronto, Toronto; and 5Division of Neuropathology, Department of Laboratory Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada

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Hare GM, Mazer CD, Hutchison JS, McLaren AT, Liu E, Rassouli A, Ai J, Shaye RE, Lockwood JA, Hawkins CE, Sikich N, To K, Baker AJ. Severe hemodilutional anemia increases cerebral tissue injury following acute neurotrauma. J Appl Physiol 103: 1021–1029, 2007. First published June 7, 2007; doi:10.1152/japplphysiol.01315.2006.—Anemia may worsen neurological outcomes following traumatic brain injury (TBI) by undefined mechanisms. We hypothesized that hemodilutional anemia accentuates hypoxic cerebral injury following TBI. Anesthetized rats underwent unilateral TBI or sham injury (n = 7). Target hemoglobin concentrations between 50 and 70 g/l were achieved by exchanging 40–50% of the blood volume (1:1) with pentastarch. The effect of TBI, anemia, and TBI-anemia was assessed by measuring brain tissue oxygen tension (PbrO2), regional cerebral blood flow (rCBF), jugular venous oxygen saturation (SjvO2), cerebral contusion area, and nuclear staining for programmed cell death. Baseline postinjury PbrO2 values in the TBI and TBI-anemia groups (9.3 ± 1.3 and 11.3 ± 4.1 Torr, respectively) were lower than the uninjured controls (18.2 ± 5.2 Torr, P < 0.05 for both). Hemodilution caused a further reduction in PbrO2, in the TBI-anemia group relative to the TBI group without anemia (7.8 ± 2.7 vs. 14.8 ± 3.9 Torr, P < 0.05). The rCBF remained stable after TBI and increased comparably after hemodilution in both anemia and TBI-anemia groups. The SjvO2 was elevated after TBI (87.4 ± 8.9%, P < 0.05) and increased further following hemodilution (95.0 ± 1.6%, P < 0.05). Cerebral contusion area and nuclear counts for programmed cell death were increased following TBI-anemia (4.1 ± 3.0 mm² and 686 ± 192, respectively) relative to TBI alone (1.3 ± 0.3 mm² and 404 ± 133, respectively, P < 0.05 for both). Hemodilutional anemia reduced cerebral PbrO2 and oxygen extraction and increased cell death following TBI. These results support our hypothesis that acute anemia accentuated hypoxic cerebral injury after neurotrauma.

MATERIALS AND METHODS

Effect of TBI and Anemia on Brain Tissue Oxygen Tension and Regional Cerebral Blood Flow

Animal model. All animal protocols were approved by the Animal Care and Use Committee at St. Michael’s Hospital in accordance with the requirements of the Canadian Council on Animal Care. Male Sprague-Dawley rats (350–400 g, 11–12 wk old, Charles River, St. Constant, Quebec, Canada) were assigned to one of three groups: 1) TBI only (TBI, n = 7); 2) hemodilutional anemia (anemia, n = 8),...
or 3) TBI and anemia (TBI-anemia, n = 10). Animals were initially anesthetized with ketamine-xylazine (100 and 7.5 mg/kg ip, Parke-Davis/Bayer, Toronto, Ontario, Canada), and anesthesia was maintained with 2–3% isoflurane in 50% oxygen (Abbott, St. Laurent, Quebec, Canada). Following tracheostomy, rats were ventilated with a pressure-controlled ventilator (Kent Scientific, Litchfield, CT) to achieve normocapnia as determined by blood gas analysis (Radiometer ALB 500, London Scientific, London, Ontario, Canada). The right jugular vein (polyethylene 90), tail vein, and the tail artery (polyethylene 50) were cannulated to measure mean arterial blood pressure (MAP) and central venous pressure (CVP) and to perform hemodilution. Co-oximetry (Radiometer, OSM 3) and blood gas analysis were performed on arterial and venous blood. Animals were then placed in a stereotaxic frame (ADI Instruments, Harvard Apparatus, St. Laurent, Quebec, Canada), and their skulls were incised sagittally.

**Fluid percussion injury.** A unilateral 5-mm-diameter burr hole was trephined over the left hemisphere at the level of bregma 0.0 to 5.0, 2 mm lateral to the sagittal sinus, to expose the intact dura. The pressure-conducting tube of the fluid percussion injury (FPI) device was then connected to the skull using acrylic cement. This provided a direct connection between the column of saline within the percussion device and the exposed dura. A calibrated 2-atmosphere (atm) impact was then performed as previously described (1). This degree of injury was utilized because it has been previously demonstrated to cause a unilateral region of brain injury (TBI) without causing evidence of histological damage within the contralateral cerebral hemisphere. The contralateral “uninjured” cerebral hemisphere could then serve as an internal control for experimental outcomes measured in the injured hemisphere. The 2-atm impact also correlates to clinical levels of injury associated with multiple trauma and systemic hemorrhage. Immediately postinjury, the cylinder was removed and a second burr hole was trephined over the right contralateral hemisphere at the corresponding position. The anemia group (sham TBI) was similarly attached to the FPI device, but no TBI was induced.

Subsequently, bilateral calibrated polarographic oxygen-sensing microelectrodes were inserted into the caudate nucleus using stereotaxic coordinates, as previously described (LICOX GMS, Harvard Apparatus) (15–17). Probes were inserted into the region of caudate nucleus approximately 4–6 mm past the dura, because this area is a large homogeneous region of gray matter with a high metabolic rate. Bilateral laser-Doppler flow probes (Oxyflo, Oxford Optronix, Oxford, UK) were positioned over the dura avoiding any visible large dural vessels. Measurements of regional cerebral blood flow (rCBF) were performed using probes at the surface of the cerebral cortex, remote from the oxygen electrode, to minimize local tissue trauma that might alter caudate tissue oxygen tension measurements. A period of 1 h was utilized to establish a steady baseline while a heating pad and heating lamp were used to maintain the brain temperature near 37°C. PaO₂, temperature, CBF, MAP, CVP, and heart rate (HR) were recorded digitally (DASYLab 5.6, Kent Scientific).

**Hemodilutional anemia.** After a stable baseline was established, physiological parameters were measured for a total of 60 min. Rats were assigned to one of three groups as outlined above: 1) TBI alone (sham hemodilution, n = 7); 2) anemia alone (sham TBI, n = 8); and 3) TBI plus anemia (TBI-anemia, n = 10). A sham-sham control group was not performed as previously published experimental studies have demonstrated stable PaO_2_ measurements near 18–20 Torr using these techniques (15, 17). In addition, the contralateral uninjured cerebral hemisphere served as an internal control to the effect of hemodilution in the TBI rats. In the two anemia groups, acute normovolemic hemodilutional anemia was induced over 10 min by simultaneous exchange of 30 ml/kg of arterial blood (50% estimated blood volume of 60 ml/kg), withdrawn from the tail artery, with an equivalent volume of pentastarch (Pentastan, Bristol-Myers Squibb Canada, St. Laurent, Quebec, Canada) infused via the jugular vein. Volume exchange was performed over 10 min using a programmable “push-pull” pump (PHD 2000, Harvard Apparatus). Both MAP and CVP measurements were interrupted during the volume-exchange period. Following completion of volume exchange, all parameters were recorded for an additional 30 min before killing the animal by anesthetic overdose (ketamine 100 mg iv, Parke-Davis, Toronto, Ontario, Canada). In each group, arterial blood gas samples were taken at baseline and after hemodilution at 15-min intervals.

**Effects of TBI and Anemia on Arterial and Jugular Venous Blood Gases**

Three different groups of rats were utilized for these studies. Rats were anesthetized and ventilated as above and assigned to one of three groups: control (sham-sham, n = 6), anemia without TBI (anemia, n = 8), and TBI plus anemia (TBI-anemia, n = 6). Animals were prepared as described above with the exception that cannulation of the right jugular vein (polyethylene 90) was performed in a retrograde

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**Table 1. Effect of hemodilution on co-oximetry and arterial blood gas measurements following TBI and anemia**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Sample</th>
<th>Hemoglobin Concentration, g/l</th>
<th>% Saturation</th>
<th>O₂ Content, mmol/l</th>
<th>pH</th>
<th>PaCO₂, Torr</th>
<th>PaO₂, Torr</th>
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<tbody>
<tr>
<td></td>
<td><strong>TBI (n = 7)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>15</td>
<td>Baseline</td>
<td>140±15</td>
<td>99.3±2.4</td>
<td>8.4±0.4</td>
<td>7.44±0.07</td>
<td>36.2±8.7</td>
<td>198.5±18.8</td>
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<td>45</td>
<td>Sham hemodilution</td>
<td>142±9</td>
<td>99.1±3.1</td>
<td>8.5±0.6</td>
<td>7.38±0.07</td>
<td>41.4±8.7</td>
<td>191.7±21.0</td>
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<td>60</td>
<td>Sham hemodilution</td>
<td>140±7</td>
<td>99.5±2.3</td>
<td>8.4±0.4</td>
<td>7.40±0.04</td>
<td>37.7±5.1</td>
<td>206.4±34.3</td>
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<td><strong>Anemia (n = 8)</strong></td>
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<td>15</td>
<td>Baseline</td>
<td>142±16</td>
<td>98.4±2.2</td>
<td>8.5±0.7</td>
<td>7.38±0.07</td>
<td>36.3±7.9</td>
<td>171.3±48.0</td>
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<td>30</td>
<td>Hemodilution</td>
<td>56±15*</td>
<td>99.1±3.3</td>
<td>3.2±2.9*</td>
<td>7.34±0.09</td>
<td>40.1±8.3</td>
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<td>Hemodilution</td>
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<td>99.3±2.9</td>
<td>3.5±2.8*</td>
<td>7.35±0.09</td>
<td>40.3±9.0</td>
<td>197.1±58.0</td>
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<tr>
<td>15</td>
<td>Baseline</td>
<td>144±11</td>
<td>97.5±3.9</td>
<td>8.7±0.8</td>
<td>7.33±0.07</td>
<td>37.2±10.0</td>
<td>152.8±34.4</td>
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<td>7.35±0.08</td>
<td>39.7±11.3</td>
<td>155.6±38.1</td>
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</table>

Values are means ± SD. TBI, traumatic brain injury; PaCO₂, arterial PCO₂; PaO₂, arterial PO₂. *P < 0.05 compared with corresponding baseline.
manner to allow for sampling of jugular venous blood. Venous blood samples were obtained before and after hemodilution by gravity-induced flow. The first 200 ml of each sample was discarded to minimize the effect of hemoconcentration secondary to venous occlusion between samples. Arterial and venous blood gas samples were taken simultaneously at similar time intervals as described above. The difference in arterial and venous oxygen content was calculated by subtracting corresponding venous and arterial values.

Effects of TBI and Anemia on Cerebral Contusion Area and TUNEL-Positive Cell Counts

Male Sprague-Dawley rats were anesthetized, intubated (14-gauge Angiocath), and ventilated before being assigned to one of three groups: control (sham-sham, n = 3), TBI alone (TBI, n = 7), and TBI plus anemia (TBI-anemia, n = 7). In this experiment, a less severe hemodilution protocol was utilized (40% estimated blood volume) by exchanging blood for pentastarch via the tail artery and vein to achieve a final target hemoglobin concentration near 70 g/l. This change allowed 100% survival to 5 days. In initial pilot experiments, hemodilution to a hemoglobin concentration of 50 g/l resulted in a survival rate near 70% with most deaths occurring within 12 h of surgery. Animals were then recovered and provided food and water ad libitum for 5 days. After 5 days, the rats were reanesthetized (2–3% isoflurane) and then perfused with 4% paraformaldehyde via a left ventricular catheter before whole brain extraction. After fixation, brain tissue was paraffin embedded. Whole mount coronal sections (5 μm) were prepared. Four coronal sections were obtained through the site of impact at the stereotaxic positions of bregma −2.3, −3.3 (anterior contusion), and bregma −5.3, −6.3 (posterior contusion). These sections were stained with cresyl violet and contusion areas were calculated for each section in a blinded manner, as previously described (36) [total magnification = 100×: eye piece (10×) × objective (10×), Leica DMLS Microscope, Wetzlar, Germany]. Microscopic images were captured and the area of contusion measured in the cortex of each rat ipsilateral to the site of injury using Image J image analysis software (version 1.37, National Institutes of Health, Bethesda, MD). The microscopists (JAL, RES) were blinded to the experimental group of the animals. The total cerebral contusion area was calculated by adding the contusion areas from each of the four histological sections for each rat. Two adjacent coronal sections (bregma −2.3 and −6.3) were utilized to assess the number of cells undergoing programmed cell death per coronal section in each rat as indicated by nuclear staining with terminal deoxynucleotidyl trans-
ferase biotin-dUTP nick-end labeling (TUNEL). TUNEL staining was performed utilizing a fluorescence in situ cell death detection kit as previously described (40) (Roche Applied Science, catalog no. 1684795, Laval, Quebec, Canada). TUNEL-positive immunofluorescent cells were counted in both injured and contralateral noninjured hemispheres of each coronal section at total magnification of 100× [eye piece (10×) × objective (10×), Olympus Provis Microscope]. Each cerebral hemisphere was divided into three sections, including 1) the contusion site, 2) the subcontusion cerebral cortex, and 3) the basal structures. Digital images were captured, and total TUNEL-positive cells were counted by blinded observers (ATM, RES). Patterns of TUNEL staining were compared, and the total number of TUNEL-positive nuclei were counted.

Statistical Analysis

Data are presented as means ± SD. Normal distribution of data was confirmed before statistical analysis was performed utilizing the SPSS software (SPSS, version 11.5.0). Physiological measurements (HR, MAP, CVP, PbrO2, and rCBF) were analyzed with a repeated-measures factorial ANOVA. The main effects of group, time, and hemisphere were assessed, and any interaction effects were defined. For cerebral tissue oxygen tension data, the baseline measurements for each cerebral hemisphere were held as covariates. Post hoc comparisons with Tukey’s test were performed if statistical differences were confirmed before statistical analysis was performed utilizing the SPSS software (SPSS, version 11.5.0). Physiological measurements (HR, MAP, CVP, PbrO2, and rCBF) were analyzed with a repeated-measures factorial ANOVA. The main effects of group, time, and hemisphere were assessed, and any interaction effects were defined. 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remained stable without any significant changes over time (data not shown). Hemodilution resulted in comparable reductions in arterial and venous hemoglobin concentrations and oxygen contents in both the anemia and TBI-anemia groups, relative to baseline values (Table 2, *P* < 0.05 for both). Arterial oxygen saturations remained between 97% and 100% in both groups. The baseline jugular venous oxygen saturation (SjvO₂) was increased in the TBI-anemia group (87.4 ± 8.9%, Table 2, Fig. 5, *P* < 0.05) relative to the anemia group without TBI (69.1 ± 6.4%). Following hemodilution there was a small transient rise in the SjvO₂ in the anemia group to 77.9 ± 2.6% and a larger increase in the TBI-anemia group (95.0 ± 1.6%) (Table 2, Fig. 5, *P* < 0.05, relative to baseline and between groups). The SjvO₂ remained increased for up to 45 min in the TBI-anemia group relative to the anemia group following hemodilution (Table 2, Fig. 5, *P* < 0.05 between groups) but was not different from the anemia group at 60 min. The arterial-venous oxygen content difference was significantly reduced in the TBI-anemia group relative to the anemia group (Fig. 5, *P* < 0.05).

### Effects of TBI and Anemia on Cerebral Contusion Area and TUNEL-Positive Cell Counts

Control (no TBI or hemodilution) rats did not exhibit any evidence of cerebral contusion (cresyl violet) and had less than 25 TUNEL-positive cells per cerebral hemisphere (data not shown). There was a significant reduction in the hemoglobin concentration in the TBI-anemia group relative to the TBI group (68 ± 5 vs. 140 ± 3 g/l, respectively, *P* < 0.05). In the TBI and TBI-anemia groups, two areas of cerebral contusion were identified within the cerebral cortex. One area was located immediately superficial to the impact site (a), and the second area was located deeper within the cerebral cortex (b) (Fig. 6). The contusion area was larger in the posterior sections at bregma −5.3 and −6.3 relative to the anterior sections at bregma −2.3 and −3.3 in both groups (Figs. 6 and 7). After 5 days recovery, the total contusion area in the TBI-anemia group was significantly larger than that measured in the TBI group that underwent sham hemodilution (4.1 ± 3.0 vs. 1.3 ± 0.3 mm², Fig. 7, *P* < 0.05). There was also a corresponding increase in the number of TUNEL-positive cells in the TBI-anemia group relative to TBI alone [686 ± 192 vs. 404 ± 133 nuclei per coronal section, respectively (Fig. 7, *P* < 0.05)]. The majority of TUNEL staining occurred in the cerebral cortex at the site of contusion or immediately inferior in the subcontusion region. Less staining was observed in the basal structures (Fig. 7). Very little TUNEL staining was observed in the contralateral uninjured cerebral hemisphere.

### DISCUSSION

We have demonstrated that acute anemia induced by normovolemic hemodilution led to cerebral hypoxia and accentuated cerebral injury in an experimental model of neurotrauma. Following unilateral traumatic brain injury, acute normovolemic hemodilution reduced regional cerebral tissue oxygen tension, while SjvO₂ and rCBF were elevated, suggesting that impairment of oxygen extraction had occurred. Acute normovolemic hemodilution also increased cerebral cortical tissue contusion area and cell death following TBI as measured in brain sections stained with cresyl violet and TUNEL. We also demonstrated that cerebral oxygen tension is maintained in the normal range and that no cerebral injury occurred following hemodilution without TBI. These data suggest that the brain is selectively vulnerable to anemia following neurotrauma.

The relevance of our experimental results is supported by a subanalysis of a clinical trial that suggests that patients suffering from acute neurotrauma may benefit from a higher transfusion threshold (18, 34). In addition, the “Lund therapy” management protocol, which contains higher transfusion thresholds, may lead to improved outcomes following acute neurotrauma (12). A recent clinical study in patients following cardiopulmonary bypass has demonstrated that relatively small changes in cerebral tissue oxygen tension may have an important impact on patient morbidity (38). Acute hemodilution is associated with increased neurological injury and higher mortality following cardiopulmonary bypass in animal models and in both adult and pediatric patients (14, 20, 25). The results of these studies, in combination with other studies showing that...
Table 2. Effect of hemodilution and TBI on arterial and venous co-oximetry and blood gas analysis

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Sample</th>
<th>Hemoglobin Concentration, g/l</th>
<th>%Saturation</th>
<th>O2 Content, mmol/l</th>
<th>pH</th>
<th>Pco2, Torr</th>
<th>P02, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anemia (n = 8)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Arterial blood sample</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Baseline</td>
<td>110±5</td>
<td>99.1±0.7</td>
<td>6.6±0.4</td>
<td>7.39±0.06</td>
<td>33.7±6.2</td>
<td>158.7±20.9</td>
</tr>
<tr>
<td>30</td>
<td>Hemodilution</td>
<td>44±5*</td>
<td>98.6±1.4</td>
<td>2.7±0.4*</td>
<td>7.36±0.04</td>
<td>37.9±4.9</td>
<td>184.8±43.6</td>
</tr>
<tr>
<td>45</td>
<td>Hemodilution</td>
<td>47±7*</td>
<td>98.1±1.8</td>
<td>2.8±0.4*</td>
<td>7.37±0.04</td>
<td>37.3±2.2</td>
<td>169.8±32.0</td>
</tr>
<tr>
<td>60</td>
<td>Hemodilution</td>
<td>47±7*</td>
<td>98.1±1.8</td>
<td>2.8±0.4*</td>
<td>7.37±0.04</td>
<td>37.3±2.2</td>
<td>169.8±32.0</td>
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<tr>
<td></td>
<td></td>
<td>Jugular venous blood sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Baseline</td>
<td>122±6</td>
<td>69.1±6.4</td>
<td>5.2±0.7</td>
<td>7.36±0.06</td>
<td>44.0±5.7</td>
<td>48.0±4.4</td>
</tr>
<tr>
<td>30</td>
<td>Hemodilution</td>
<td>45±5*</td>
<td>77.9±2.6*</td>
<td>2.2±0.4*</td>
<td>7.34±0.04</td>
<td>43.8±4.5</td>
<td>57.2±4.4</td>
</tr>
<tr>
<td>45</td>
<td>Hemodilution</td>
<td>47±8*</td>
<td>75.5±9.7</td>
<td>2.1±0.5*</td>
<td>7.35±0.03</td>
<td>44.2±5.1</td>
<td>55.2±5.3</td>
</tr>
<tr>
<td>60</td>
<td>Hemodilution</td>
<td>49±6*</td>
<td>69.0±7.9</td>
<td>2.1±0.3*</td>
<td>7.34±0.04</td>
<td>45.9±3.6</td>
<td>52.3±5.4</td>
</tr>
<tr>
<td></td>
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<td>TBI and anemia (n = 6)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Baseline</td>
<td>121±13</td>
<td>99.9±0.2</td>
<td>7.4±0.8</td>
<td>7.42±0.05</td>
<td>36.4±3.2</td>
<td>179.5±25.9</td>
</tr>
<tr>
<td>30</td>
<td>Hemodilution</td>
<td>42±4*</td>
<td>99.9±0.1</td>
<td>2.6±0.2*</td>
<td>7.41±0.06</td>
<td>37.9±3.3</td>
<td>206.3±10.0</td>
</tr>
<tr>
<td>45</td>
<td>Hemodilution</td>
<td>45±5*</td>
<td>98.9±0.2</td>
<td>2.8±0.3*</td>
<td>7.41±0.04</td>
<td>36.7±3.3</td>
<td>204.1±16.5</td>
</tr>
<tr>
<td>60</td>
<td>Hemodilution</td>
<td>45±4*</td>
<td>99.9±0.1</td>
<td>2.8±0.3*</td>
<td>7.41±0.05</td>
<td>35.7±3.8</td>
<td>202.6±10.6</td>
</tr>
<tr>
<td></td>
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<td>Jugular venous blood sample</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Baseline</td>
<td>119±16</td>
<td>87.4±8.9†</td>
<td>6.3±0.6</td>
<td>7.42±0.05</td>
<td>39.3±4.3</td>
<td>58.9±13.7</td>
</tr>
<tr>
<td>30</td>
<td>Hemodilution</td>
<td>48±6*</td>
<td>95.0±1.6†</td>
<td>2.7±0.4*</td>
<td>7.41±0.06</td>
<td>41.3±5.8</td>
<td>73.9±7.6†</td>
</tr>
<tr>
<td>45</td>
<td>Hemodilution</td>
<td>46±6*</td>
<td>93.3±3.9†</td>
<td>2.6±0.4*</td>
<td>7.40±0.04</td>
<td>41.5±5.9</td>
<td>68.4±12.8†</td>
</tr>
<tr>
<td>60</td>
<td>Hemodilution</td>
<td>50±5*</td>
<td>79.3±8.8</td>
<td>2.3±0.3*</td>
<td>7.40±0.04</td>
<td>43.5±3.8</td>
<td>54.0±6.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 compared with corresponding baseline values. †p < 0.05 compared with corresponding anemia values.

cerebral tissue oxygen tension is maintained during hemodilution in the absence of brain injury (11, 19, 31, 39, 44, 49), support our conclusion that the brain is selectively vulnerable to normovolemic hemodilution following TBI.

Reduced tissue oxygen tension may have a significant impact on neurological outcomes since relatively mild levels of systemic hypoxia have been shown to augment secondary cerebral injury following neurotrauma (2, 7, 8, 23, 36). Possible mechanisms of this selective vulnerability to hypoxia include impaired CBF responses and depletion of high-energy phosphates following TBI (10, 24). Additional experimental studies utilizing the current model have provided evidence that hemodilution triggers an increased expression of hypoxic molecules within the cerebral cortex, including hypoxia inducible factor-1α, neuronal nitric oxide synthase, and vascular endothelial growth factor and chemokine receptors (17, 35). These molecules may contribute to adaptive regulatory mechanisms following hemodilution. Alternately, they may contribute to secondary neuronal injury following TBI and hemodilution by mechanisms that include inflammation, oxidative stress, and apoptosis (26, 27, 32, 36, 41).

The increase in CBF associated with hemodilution is a well-characterized phenomenon caused by passive changes in rheology and active cerebral vasodilation (4, 22, 46). This CBF response may optimize cerebral oxygen delivery during anemia, thereby defending against cerebral tissue hypoxia (10). This response is observed in most areas of gray matter, including the caudate nucleus (42). CBF is initially reduced and autoregulation is impaired following TBI (10). In our study, the increase in CBF in response to hemodilution was relatively preserved in the injured and contralateral cerebral cortices. However, the associated increase in SjVO2 suggests that impairment of microcirculatory function may have led to increased shunting through larger conductance vessels and reduced cerebral oxygen extraction.

**Fig. 5.** Jugular venous saturation was abnormally high following hemodilution in rats with TBI. A: jugular venous oxygen saturation was higher in the TBI-anemia rats at baseline following TBI, and further increased following hemodilution, before returning to control values by 60 min. B: these changes were reflected in lower differences between arterial and jugular venous oxygen content observed in TBI-anemia rats following hemodilution, suggesting that cerebral oxygen extraction was reduced following TBI. *P < 0.05 vs. baseline, #P < 0.05 between groups.
Experimental studies suggest that the cerebral metabolic rate for oxygen (CMRO$_2$) is immediately increased following TBI (6, 30). However, this initial state of hypermetabolism rapidly evolves into a more sustained state of hypometabolism (10, 30), possibly secondary to impaired mitochondrial function (9, 21). Early cerebral hypermetabolism cannot fully explain the reduction in cerebral tissue oxygen tension observed following TBI because such hypermetabolism would be expected to reduce the SjvO$_2$. The existing literature suggests that there is a decrease in oxygen consumption after TBI. Nonetheless, reduction in blood oxygen-carrying capacity with severe hemodilution can lead to inadequate regional cerebral tissue oxygen delivery and increased tissue damage. After 60 min, the SjvO$_2$ returned to baseline, possibly because of compensatory mechanisms that restored oxygen extraction (49).

There are some limitations to this study. Only one level of trauma was evaluated as part of the experimental design. The level of TBI was chosen to approximate the level of clinical injury that is often associated with systemic hemorrhage. Although target hemoglobin values near 50 and 70 g/l were used, future studies will be required to establish the hemoglobin threshold at which secondary injury occurs following TBI and anemia. We did not measure intracranial pressure, which is important in the assessment of cerebral perfusion. We did measure rCBF, however, which may be a better index of cerebral perfusion. We used laser-Doppler flowmetry to measure red blood cell flux since it correlates strongly with CBF in previous studies in animal models (28, 33). Tissue oxygen tension and laser-Doppler flow probes were located in different regions of the brain to minimize the adverse effect of local tissue damage at the site of the oxygen electrode on rCBF measurements. Furthermore, comparable changes in cortical and caudate tissue blood flow have been demonstrated following hemodilution in another experimental study (43). Pentastarch was chosen for hemodilution because volume exchange with synthetic starch colloids has been previously
of increased CBF, suggesting that reduced cerebral oxygen extraction within the cerebral microcirculation likely contributed to the observed reduction in cerebral tissue oxygen tension. The increase in cerebral tissue contusion area and TUNEL-positive cell counts suggest that anemia leads to secondary cerebral injury following TBI. The evolving practice of restricting blood transfusion in critically ill patients might not be appropriate for patients with TBI. Optimal transfusion thresholds for patients with TBI may be different from other critically ill patients. Further research is required to determine if maintaining a higher hematocrit in patients suffering from TBI would improve clinical outcomes.

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GRANTS

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utilized to maintain adequate preload and prevent any significant reduction in blood pressure (37, 42, 46, 47). Maintenance of blood pressure was important because of the identified negative impact of systemic hypotension on cerebral tissue oxygen tension and neurological outcomes following TBI (19, 31, 37).

In summary, acute normovolemic hemodilutional anemia reduced cerebral tissue oxygen tension in the contused brain. This was associated with an increase in $S_{\text{JVO}_2}$, during a period


