Storage of strain-specific rat blood limits cerebral tissue oxygen delivery during acute fluid resuscitation

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Background. The effect of blood storage on tissue oxygen delivery has not been clearly defined. Some studies demonstrate reduced microvascular oxygen delivery, whereas others do not. We hypothesize that storage of rat blood will limit its ability to deliver oxygen to cerebral tissue.

Methods. Anaesthetized rats underwent haemorrhage (18 ml kg⁻¹) and resuscitation with an equivalent amount of fresh or 7 day stored strain-specific whole blood. Arterial blood gases, co-oximetry, red cell counts and indices, and blood smears were performed. Hippocampal tissue oxygen tension (PBrO₂), regional cerebral blood flow (rCBF), and mean arterial pressure (MAP) were measured before and for 60 min after resuscitation (n=6). Data [mean (SD)] were analysed by ANOVA.

Results. After 7 days, there was a significant reduction in pH, PaO₂, an increase in PaCO₂, but no detectable plasma haemoglobin in stored rat blood. Stored red blood cell morphology demonstrated marked echinocytosis, but no haemolysis in vitro. MAP and PBrO₂ in both groups decreased after haemorrhage. Resuscitation with stored blood returned MAP [92 (± 16) mm Hg] and PBrO₂ [3.2 (0.7) kPa] to baseline, whereas rCBF remained stable [1.2 (0.1)]. Resuscitation with fresh blood returned MAP to baseline [105 (16) mm Hg] whereas both PBrO₂ [5.6 (1.5) kPa] and rCBF [1.9 (0.4)] increased significantly (P<0.05 for both, relative to baseline and stored blood group). There was no evidence of haemolysis in vivo.

Conclusions. Although resuscitation with stored blood restored cerebral oxygen delivery to baseline, fresh blood produced a greater increase in both PBrO₂ and rCBF. These data support the hypothesis that storage limits the ability of RBC to deliver oxygen to brain tissue.

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Red blood cell (RBC) transfusion is administered to treat symptoms of acute anaemia based on the assumption that enhanced blood oxygen-carrying capacity improves tissue oxygen delivery. Although some studies have demonstrated that RBC transfusion improves global oxygen delivery, other studies have failed to demonstrate this association. Furthermore, transfusion of stored blood may not improve patient outcomes and has been associated with increased morbidity and mortality.

Experimental studies have demonstrated that transfusion of stored RBCs reduced microvascular and tissue oxygen tension, despite augmented blood oxygen content. These results may be the consequence of the ‘storage lesion’ which is defined as a series of biomechanical and biochemical changes within the stored RBC, including distortion of RBC morphology and depletion of 2,3-DPG. Differences between measurements of global tissue oxygen utilization and local tissue oxygen tension may
explain why an effect of blood storage has not been consistently demonstrated.\textsuperscript{9, 10, 12–14}

Therefore, we designed a study to assess storage-related changes in RBC morphology, integrity, and oxygen affinity \textit{in vitro} and the ability of stored and fresh whole blood to deliver oxygen to the brain \textit{in vivo}. We hypothesized that storage of whole rat blood would limit cerebral oxygen delivery as a result of the biochemical storage lesions.\textsuperscript{15}

\section*{Methods}

\subsection*{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. Anaesthesia was induced in male Sprague–Dawley rats (Charles River, St Constant, PQ, Canada), with ketamine/xylazine 100/7.5 mg kg\textsuperscript{-1} intra-peritoneally (Parke-Davis/Bayer, Toronto, ON, Canada) and maintained with 1–2\% isoflurane (Abbott, St Laurent, PQ, Canada) delivered in 50\% oxygen after intubation. Ventilation was maintained with a pressure-controlled ventilator (Kent Scientific, Litchfield, CT, USA) and was adjusted to achieve normocapnia and normoxia as determined by blood gas analysis (Radiometer ABL 500; London Scientific, London, ON, Canada). Vascular access was achieved using 24-G catheters placed in the tail artery and vein for direct measurement of mean arterial pressure (MAP), assessment of blood parameters, and to perform haemorrhage and resuscitation. Resuscitation was performed using strain-specific whole blood harvested from isoflurane anaesthetized donor rats utilizing citrate phosphate dextrose adenosine (1.4 ml CPDA-1 per 10 ml of whole blood, Baxter, Deerfield, IL, USA) as an anticoagulant. Donor rat blood was re-infused into the recipient rats within 1 h (fresh blood) or after 7 days (stored blood) of storage at 4\degree C.

\subsection*{Blood storage and analysis \textit{in vitro} (Protocol 1)}

To assess the effect of storage \textit{in vitro}, strain-specific blood was collected from isoflurane anaesthetized rats into CPDA-1 (n=6). After completion of blood collection, the animals were killed by anaesthetic overdose (ketamine 100 mg i.v.; Parke-Davis). Whole blood was assessed using blood gas analysis, co-oximetry (ABL 500 and OSM 3, respectively, Radiometer, London, ON, Canada), haemoglobin content and red cell indices using impedance electronic counting (LH755, Beckman Coulter Inc., Miami, FL, USA), and blood smear analysis (Wright-Giemsa-stained), 1 h after collection (fresh blood) and after 7 days of storage (stored blood) in sterile conditions at 4\degree C, \textit{in vitro}. Plasma was obtained from each sample by centrifuging a separate aliquot of blood to determine the plasma haemoglobin concentration by co-oximetry. A 7-day storage period was chosen for rat blood to approximate the storage lesion acquired by human blood after 4 weeks of storage.\textsuperscript{16} When comparing stored human and rat blood characteristics, rat blood stored in CPDA-1 for 7 days has ATP and DPG levels and red cell integrity that are comparable with human blood stored for 4 weeks.

\subsection*{Assessment of re-infused rat blood \textit{in vivo} (Protocol 2)}

After assessing the effect of seven days of storage \textit{in vitro}, blood was harvested from an additional eight rats for assessment \textit{in vivo}. After 20 min of baseline measurements, anaesthetized recipient rats underwent 30\% blood volume haemorrhage by removing 18 ml kg\textsuperscript{-1} of blood from the tail vein at a constant rate over a period of 10 min. A post-haemorrhage sample was taken for arterial blood gas (ABG) and co-oximetry analysis. After an additional 20 min, an equal volume of either fresh autologous whole blood (\textless 1 h) or 7 day stored strain-specific whole blood was re-infused over 10 min to restore blood volume (n=4 rats per group). Three post-resuscitation blood samples were taken at 20 min intervals (60, 80, and 100 min) and assessed by ABG, co-oximetry, and impedance electronic counting. A blinded individual performed blood smear analysis (M.L.). Fresh and 7 day stored bloods were assessed before (\textit{in vitro}) and after (\textit{in vivo}) infusion into recipient rats.

The number of echinocytes per blood smear was rated on a scale from 0 to 4 and reported as an echinocyte count index (ECI).

\subsection*{Effect of haemorrhage and resuscitation on cerebral tissue oxygen tension and blood flow (Protocol 3)}

Isoflurane anaesthetized and ventilated rats were placed in a stereotaxic frame (ADI Instruments; Harvard Apparatus, St Laurent, PQ, Canada) and the skull exposed by a sagittal scalp incision (n=6 per group). A 5 mm diameter burr hole was trephined at the level of the bregma, 2–3 mm lateral to the sagittal sinus, exposing the intact dura. A combined oxygen sensing microelectrode, temperature probe, and laser Doppler flow probe (OxyLite and OxyFlow, Oxford Optronix, Oxford, UK) was inserted 3–4 mm past the dura into the region of the hippocampus in order to measure both cerebral tissue oxygen tension (PBr\textsubscript{a}) and regional cerebral blood flow (rCBF). A stable baseline was achieved and a heating pad and lamp were used to maintain the brain temperature near 35\degree C. This degree of systemic hypothermia was maintained to approximate operating room conditions associated with acute blood loss and crystalloid resuscitation. Brain temperature, PBr\textsubscript{a}, rCBF, and MAP were recorded with
a computerized data acquisition system (DASYLab 5.6; Kent Scientific).

After 20 min of baseline measurements, rats underwent a controlled haemorrhage of 30% of the estimated blood volume (18 ml kg$^{-1}$) at a constant rate for 10 min. After an additional 20 min of hypovolaemia, each animal was resuscitated with an equal volume of either fresh or 7 day stored blood infused over a period of 10 min. After completion of fluid resuscitation, all variables were recorded for an additional 60 min before animals were killed by anaesthetic overdose (ketamine 100 mg i.v.; Parke-Davis). For each group, arterial blood gas analysis and co-oximetry (Radiometer) were measured at baseline (10 min), after haemorrhage (40 min), and at 20 min intervals after fluid resuscitation (60, 80, and 100 min).

Data analysis
All data analysis was performed using SigmaStat version 3.5 software (Systat Software Inc.). All parametric data are presented as mean (SD) whereas non-parametric data are presented as the median (25th, 75th percentile). Comparison of fresh and stored blood in vitro was performed by t-test. Comparisons of blood smear echinocyte count indices were performed using a Mann–Whitney rank sum test. For in vivo studies, analysis of blood parameters and physiological data were performed using a two-way ANOVA. Post hoc analysis with Tukey’s test was performed when a significant time-group interaction was identified. Statistical significance was assigned at a value of $P<0.05$.

Results

Assessment of fresh and stored blood in vitro (Protocol 1)
After 7 days of storage in vitro, there was a significant reduction in pH, $P_{aco_2}$, and oxygen saturation and an increase in $P_{aco_2}$, relative to fresh blood (Table 1). Plasma haemoglobin was not detectable in either fresh blood or 7 day CPDA-1 stored blood (Table 1). There were no significant differences in red cell parameters when fresh and stored bloods were compared (Table 2).

Assessment of fresh and stored blood in vivo (Protocol 2)
Baseline haemorrhage and resuscitation co-oximetry and ABG data did not differ between fresh and stored blood (Table 3). After haemorrhage, there was a comparable drop in haemoglobin concentration and blood oxygen content in both experimental groups. However, resuscitation with both fresh and stored blood resulted in similar increases in these parameters back to baseline values (Fig. 1a). There were no differences in any red cell parameters between the groups, either before or after haemorrhage and resuscitation (Table 4, Fig. 1b). Plasma haemoglobin was not detected in any sample either before or after haemorrhage and resuscitation in either group.

Blood smear analysis demonstrated a significant increase in the number of echinocytes after 7 day storage in vitro, relative to fresh blood (Fig. 2a, $P<0.05$). After 30% resuscitation, fewer echinocytes were observed in vivo. However, blood smears obtained after re-infusion of stored blood demonstrated a significantly higher ECI relative to rats resuscitated with fresh blood (Fig. 2b, $P<0.05$).

Effect of haemorrhage and resuscitation on cerebral tissue oxygen tension and blood flow (Protocol 3)
There were no differences in any measured parameter between the groups at baseline (Table 4, Fig. 3). After haemorrhage, there was a comparable drop in MAP and $P_{br_c}$ in both groups (Fig. 3, $P<0.05$ for both) whereas $rCBF$ remained stable. Resuscitation with stored blood returned MAP [92 (16) mm Hg] and $P_{br_c}$ [3.2 (0.7) kPa] to baseline values, whereas $rCBF$ remained stable [1.2 (0.1)]. Resuscitation with fresh blood returned MAP to baseline [105 (16) mm Hg] whereas both $P_{br_c}$ [5.6 (1.5) kPa] and $rCBF$ [1.9 (0.4)] increased significantly (Fig. 3, $P<0.05$). Sixty minutes after resuscitation with fresh blood, the $P_{br_c}$

### Table 1
Arterial blood gas and co-oximetry data from fresh and 7 day stored rat blood in vitro. Values are mean (sd). Hb, haemoglobin; ND, non-detectable. $^*P<0.05$ vs fresh blood (n=6)

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>$P_{aco_2}$ (kPa)</th>
<th>$P_{a_cO_2}$ (kPa)</th>
<th>Hb (g litre$^{-1}$)</th>
<th>$O_2$ sat. (%)</th>
<th>CarboxyHb (%)</th>
<th>MetHb (%)</th>
<th>$O_2$ Content (mmol litre$^{-1}$)</th>
<th>Plasma Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh blood</td>
<td>7.26 (0.06)</td>
<td>5.6 (1.8)</td>
<td>27.7 (3.7)</td>
<td>116.0 (9.6)</td>
<td>99.3 (1.8)</td>
<td>0.03 (0.01)</td>
<td>1.4 (0.1)</td>
<td>6.9 (0.5)</td>
<td>ND</td>
</tr>
<tr>
<td>Stored blood</td>
<td>7.05 (0.05)$^*$</td>
<td>8.5 (2.1)$^*$</td>
<td>16.3 (5.3)$^*$</td>
<td>121.6 (6.2)</td>
<td>90.2 (6.6)</td>
<td>0.01 (0.01)</td>
<td>0.8 (0.7)</td>
<td>6.8 (0.8)</td>
<td>ND</td>
</tr>
</tbody>
</table>

### Table 2
Impedance electronic counting data of fresh and 7 day stored rat blood in vitro. RBC, red blood cells; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; RDW, red cell distribution width. Values are mean (sd). $^*P<0.05$ vs fresh blood (n=6)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Haemoglobin (g litre$^{-1}$)</th>
<th>Haematocrit (%)</th>
<th>RBC count (10$^{12}$ litre$^{-1}$)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g litre$^{-1}$)</th>
<th>RDW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh blood</td>
<td>120.7 (3.8)</td>
<td>0.34 (0.01)</td>
<td>6.8 (0.34)</td>
<td>55.7 (1.7)</td>
<td>19.6 (1.0)</td>
<td>351.3 (8.5)</td>
<td>13.4 (0.8)</td>
</tr>
<tr>
<td>Stored blood</td>
<td>127.5 (11.3)</td>
<td>0.36 (0.03)</td>
<td>6.3 (0.62)</td>
<td>57.2 (1.9)</td>
<td>20.3 (0.7)</td>
<td>355.3 (3.3)</td>
<td>13.5 (0.9)</td>
</tr>
</tbody>
</table>
and rCBF values remained significantly higher than baseline and stored blood values (Fig. 3, \(P<0.05\) for all).

**Discussion**

Our findings demonstrate that storage of strain-specific whole rat blood diminished its ability to deliver oxygen to cerebral tissue. Resuscitation with stored blood returned MAP, \(PBrO_{2}\), and rCBF back to baseline. Stored blood underwent the typical morphological storage changes but did not undergo haemolysis. Conversely, resuscitation with fresh blood returned MAP to baseline but significantly increased the \(PBrO_{2}\) and rCBF relative to baseline and the stored blood group.

The effect of RBC storage on tissue oxygen delivery remains controversial. In a recent experimental study,
Critical oxygen delivery (DO₂crit) was not affected by blood storage, when systemic (whole-body) DO₂crit was utilized as a measurement of inadequate oxygen delivery. Although systemic DO₂crit is an important marker of the transition to anaerobic metabolism, it may not reflect regional tissue oxygen extraction in organs with high metabolic requirements, such as the brain. In another study, similar results were observed with respect to DO₂crit, despite a significantly lower p50 [partial pressure of O₂ at 50% oxygenated Hb (oxy-Hb) saturation] and PᵥO₂ (partial pressure of venous O₂) in the stored blood group. The inability of these studies to demonstrate an effect of RBC storage may be related to the methodology utilized and the absence of specific tissue oxygen tension measurements.

Studies which have measured microcirculatory oxygen tension have shown worsened oxygenation with stored when compared with fresh blood. In an exchange transfusion model, Tsai and colleagues showed that, at the skin microvascular level, stored RBCs reduced functional capillary density and blood flow, leading to a maldistribution of microvascular Pₒ₂ and to potential development of focal ischaemia. However, they did not find measurable changes in global oxygen delivery or consumption with stored blood, suggesting that the observed reduction in local tissue oxygen tension may not be detected by these methods.

Similarly, in other experimental models, stored blood was not as effective as fresh blood in restoring tissue oxygen tension in a number of resuscitation conditions. Although these studies utilized blood stored between 21 and 28 days, our study demonstrated reduced tissue oxygen delivery after 7 days of storage. This storage period was selected based on a previous study which demonstrated a significant storage lesion in rat blood by this time. Unlike previous studies, the current work focused on the brain, an organ with high oxygen requirement. The lower brain tissue oxygen tension observed with stored blood transfusion suggests that cerebral tissue oxygen delivery was limited. The clinical importance of this finding may impact patients with neurological vulnerability, such as those with traumatic brain injury. Alternatively, the observed increase in brain oxygen tension and cerebral blood flow after fresh blood transfusion may represent a post-ischaemic–reperfusion event. This cerebral hyperaemia after fresh blood

### Table 4

<table>
<thead>
<tr>
<th>Time</th>
<th>Sample</th>
<th>Haemoglobin (g litre⁻¹)</th>
<th>Haematocrit (%)</th>
<th>RBC count (10¹² litre⁻¹)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g litre⁻¹)</th>
<th>RDW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>Baseline</td>
<td>130.4 (13.4)</td>
<td>0.37 (0.04)</td>
<td>6.58 (0.81)</td>
<td>56.6 (1.0)</td>
<td>19.9 (0.5)</td>
<td>351.2 (6.6)</td>
<td>13.2 (0.4)</td>
</tr>
<tr>
<td>40 min</td>
<td>Haemorrhage*</td>
<td>128.2 (4.8)</td>
<td>0.37 (0.02)</td>
<td>6.54 (0.26)</td>
<td>56.5 (2.5)</td>
<td>19.6 (0.5)</td>
<td>447.4 (8.5)</td>
<td>14.8 (2.1)</td>
</tr>
<tr>
<td>60 min</td>
<td>Resuscitation #1</td>
<td>128.9 (4.2)</td>
<td>0.37 (0.01)</td>
<td>6.60 (0.17)</td>
<td>55.6 (2.6)</td>
<td>19.5 (1.1)</td>
<td>351.4 (4.3)</td>
<td>15.1 (3.0)</td>
</tr>
<tr>
<td>80 min</td>
<td>Resuscitation #2</td>
<td>127.9 (7.6)</td>
<td>0.35 (0.03)</td>
<td>6.29 (0.45)</td>
<td>56.1 (2.7)</td>
<td>19.4 (0.6)</td>
<td>346.0 (8.5)</td>
<td>15.2 (2.6)</td>
</tr>
<tr>
<td>100 min</td>
<td>Resuscitation #3</td>
<td>131.4 (9.4)</td>
<td>0.39 (0.03)</td>
<td>6.93 (0.44)</td>
<td>55.7 (1.2)</td>
<td>19.3 (0.6)</td>
<td>345.3 (4.7)</td>
<td>14.1 (0.5)</td>
</tr>
</tbody>
</table>

Resuscitation with fresh blood

Resuscitation with stored blood

![Fig 2](https://academic.oup.com/bja/article-abstract/100/3/357/241236/1)

Fig 2  (a) Blood smear analysis pre- and post-transfusion of fresh and stored blood. Seven days of storage resulted in marked echinocytosis. (b) ECI pre- and post-transfusion of fresh and stored blood. Blood smears obtained after re-infusion of stored blood demonstrated a significantly higher ECI relative to rats resuscitated with fresh blood. *P<0.05 vs stored blood (n=4 per group).
Despite comparable MAP values, resuscitation with fresh blood resulted in a greater increase in brain tissue oxygen tension and CBF, relative to stored blood values. MAP, mean arterial pressure; \( P_{\text{BrO}_2} \), cerebral tissue oxygen tension; CBF, cerebral blood flow. \( *P<0.05 \) vs baseline; \( *P<0.05 \) vs stored blood (\( n=6 \) per group).
resuscitation could have deleterious effects in some clinical situations, such as those in which intracranial pressure is elevated. However, low haematocrit is also a risk for increased brain injury after neurotrauma. The balance of these risks must be weighed when considering transfusion of stored blood. In clinical settings, transfusion of autologous or allogeneic blood is often more than 2 weeks old, which would minimize the likelihood of the occurrence of the hyperaemic response.

The importance of defining the effect of blood storage is emphasized by a recent clinical study which demonstrated that almost 75% of stored RBCs are transfused in the third week of storage or later. Since de novo synthesis of 2,3 DPG takes up to 24 h to reach normal levels after transfusion of stored blood, the optimal timing for transfusion requires further definition.

There are some limitations to this study. First of all, we used strain-specific whole blood. Although, we have not studied the effect of allogeneic blood transfusion or component transfusion, this model simulates the transfusion of stored autologous blood in humans, which often occur in clinical practice. Secondly, our results can only be applied to normal brain tissue. We used a haemorrhagic model with healthy rats to assess the effect of fresh vs stored blood on un-injured cerebral tissue. Future studies will utilize this model to assess the effect of blood storage on the injured brain. We studied regional oxygen delivery because this methodology seems to be more sensitive than global measurements of oxygen delivery. Blood smears were assessed by a fully trained haematology technologist who was blinded to the experimental groups. Such blinding minimized any subjective bias in assessing the degree of the echinocytosis, which is an optimal methodology for assessing RBC morphology. Lastly, we did not measure cardiac output and its variation, which may partially account for the observed differences in CBF responses. Future studies will measure cardiac output to elucidate its potential role in increasing CBF in the fresh blood group.

In summary, our study supports the hypothesis that, in this rat model, storage of RBCs for 7 days limits its ability to deliver oxygen to brain tissue. A more thorough assessment and understanding of the storage lesion and its clinical implications may help to explain the morbidity and mortality associated with perioperative stored blood transfusion.

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