Diaspirin Cross-linked Hemoglobin Does Not Increase Brain Oxygen Consumption during Hypothermic Cardiopulmonary Bypass in Rabbits

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Background: Decreased erythrocyte deformability due to cardiopulmonary bypass (CPB) and/or hypothermia, may result in brain capillary beds that have decreased erythrocyte transit, resulting in a generalized impairment of brain oxygenation during CPB. Because brain capillary plasma flow continues even when erythrocyte flow is absent, the authors' hypothesized augmentation of plasma oxygen content with a non–erythrocyte-associated oxygen transport molecule would increase brain oxygen uptake during hypothermic CPB.

Methods: Anesthetized New Zealand white rabbits, maintained on CPB at 27°C, were randomized to one of three groups. In group 1 (n = 13), plasma oxygen content was increased by administration of α-α diaspirin cross-linked hemoglobin. In this group, pretreatment with 0.5 mg/kg verapamil was necessary to prevent hypertension. In group 2 (n = 13), α-α diaspirin cross-linked hemoglobin was not administered, but verapamil was given as before (control). In group 3 (n = 13), neither α-α diaspirin cross-linked hemoglobin nor verapamil was administered (control). At 60 min of CPB, cerebral blood flow (microspheres) and cerebral metabolic rate for oxygen (Fick) were determined.

Results: Systemic physiologic variables did not differ among groups. Although total arterial oxygen content was equivalent in all groups (~12.1 ml O2/dl), the α-α diaspirin cross-linked hemoglobin group had a much greater proportion of the total arterial oxygen content present in a non–erythrocyte-associated form, 29 ± 5% versus 6 ± 2% and 5 ± 3%, in groups 2 and 3, respectively. Nevertheless, neither cerebral blood flow (~34 ml·100g⁻¹·min⁻¹) nor cerebral metabolic rate for oxygen (~1.2 ml O2·100g⁻¹·min⁻¹) differed among groups.

Conclusions: Because oxygen was equally available to the brain in all groups, independent of whether oxygen was associated with erythrocytes or not, it was concluded that erythrocyte/capillary interactions do not limit oxygen transfer from blood to brain during moderately hypothermic CPB. The hypertensive response to α-α diaspirin cross-linked hemoglobin during CPB is probably a result of nitric oxide scavenging. (Key words: Brain; cerebral blood flow; hypothermia; metabolism. Blood substitutes: diaspirin cross-linked hemoglobin. Cardiopulmonary bypass: brain; hypothermia; nitric oxide.)

THIRTY to 60% of cardiac surgery patients have impaired cognitive and neuropsychological performance during the first week after surgery.1,2 Although neuropsychological impairment tends to improve with time, 10–30% of cardiac surgery patients have measurable deterioration in their cognitive status months to years after surgery.2–5 Because this degree of neuropsychological deterioration is unique to cardiac surgery patients,6 it seems likely that whatever causes this injury, it occurs during cardiopulmonary bypass (CPB). Microemboli, either gaseous7 or particulate,8,9 almost certainly contribute to post-CPB neurocognitive changes. Nevertheless, the diffuse, nonfocal, nature of neurocognitive changes suggest they might also be caused by a global impairment of cerebral oxygenation during CPB.

An impairment of cerebral oxygenation during CPB might, at first, seem unlikely, given that CPB is most often conducted with mild to moderate hypothermia, which decreases brain oxygen consumption,10,11 and is associated with increased cerebral venous oxygen saturation.11,12 Nevertheless, a number of non-CPB studies suggest that hypothermia can, in fact, impair oxygen transfer from blood to tissue.13–15 Because erythrocytes are larger than capillaries, erythrocytes must deform to enter capillary networks. Both hypothermia16–18 and CPB19–23 have been shown to decrease erythrocyte deformability. Decreased erythrocyte deformability, in concert with hypothermia-induced vasoconstriction, may result in capillaries becoming closed to erythrocyte transit, i.e., functionally "derecruited."15 Because of the low oxygen content
of plasma, derecruited capillary beds would necessarily have decreased tissue oxygen tensions and greater oxygen diffusion gradients, possibly resulting in a limitation of oxygen uptake. In support of this hypothesis, Hershenson et al. found, in hypothermic (30°C) dogs, that pentoxifylline (which reverses cold-induced erythrocyte deformability) significantly increased systemic oxygen consumption and maximal oxygen extraction as compared with nonpentoxifylline controls. The authors ascribed pentoxifylline-induced increases in oxygen consumption to increased erythrocyte deformability, with a resulting increase in the number of capillaries with erythrocyte transit. If the findings of Hershenson et al. are applicable to the brain, then brain oxygen uptake might indeed be limited during hypothermic CPB because of decreased erythrocyte deformability and decreased erythrocyte capillary transit.

Because plasma flow continues in brain capillaries even when erythrocyte flow is absent, cell-free hemoglobin solutions might provide a way by which physiologically meaningful quantities of oxygen could gain access to derecruited brain capillaries. We hypothesized that if brain oxygen uptake during hypothermic CPB is limited by the mechanisms discussed earlier, augmentation of plasma oxygen content by use of a cell-free hemoglobin solution would increase brain oxygen uptake as compared to conditions where no such augmentation was present. We tested this hypothesis at 27°C using our rabbit model of CPB. We chose α-α diaspirin cross-linked hemoglobin (DCLHb) to serve as our erythrocyte-free oxygen transport molecule.

Materials and Methods

Experimental protocols were approved by the Animal Care and Use Committee of the University of Iowa in accordance with the “Guide for the Care and Use of Laboratory Animals,” National Institutes of Health Publication No. 85-23, revised 1985. Forty New Zealand white rabbits (weight, 4–5 kg) were randomly preassigned to one of three groups, based on whether they would receive: (1) 10% (wt/vol) DCLHb (Baxter HealthCare Corporation, Round Lake, IL) and verapamil; (2) verapamil only (control); or (3) nothing (control), once CPB was established (see later).

Basic Preparation

Anesthesia was induced by inhalation of 3–5% isoflurane in oxygen. After local infiltration with 1% lidocaine, a tracheotomy was performed and the trachea was intubated with a 3.0 mm cuffed tracheal tube. Thereafter, the animals’ lungs were mechanically ventilated to achieve normocarbia, and anesthesia was maintained with 2% isoflurane in oxygen for the remainder of pre-CPB preparation, monitored by a calibrated agent analyzer (Datex, Puritan-Bennett, Helsinki, Finland). Animals were paralyzed with a 4 ml·kg⁻¹·h⁻¹ infusion of succinylcholine/lactated Ringer’s solution and placed prone. After a midline sagittal scalp incision, a 2-mm burr hole was drilled over the right fronto-parietal cortex and a 1-mm thermocouple (K-type, L-08419-02, Cole Parmer, Chicago, IL) was introduced under the cranium to rest on the dural surface. A posterior midline craniectomy was performed, exposing the confluens sinuum. Heparin was administered as a bolus (200 U/kg intravenous) and was added to the succinylcholine/lactated Ringer’s infusion to give a maintenance dose of 200 U·kg⁻¹·h⁻¹. The tip of a saline-filled polyethylene catheter (PE-90, Intramedic, Parsippany, NJ) was placed in the confluens sinuum, permitting collection of cerebral venous blood. Anatomic and physiologic studies in the rabbit have shown that venous blood at this site is derived almost entirely from the cerebral cortex, with no detectable extracerebral contamination (i.e., from emissary or diploic veins). The cortical thermocouple and cerebral venous catheter were secured with bone wax and fast-drying cyanoacrylate cement and the animals were placed supine.

The tip of a saline-filled catheter (PE-90), introduced via the right external jugular vein, was advanced to the superior vena cava to measure central venous pressure. Both brachial arteries were cannulated (saline-filled PE-160 tubing) for microsphere reference blood sampling. The left brachial arterial catheter was also used for arterial pressure monitoring and collection of arterial blood. A low midline abdominal incision was made. Viscera were packed away from the operative field with saline-soaked gauze and the distal abdominal aorta was isolated. The sternum was then divided at the midline, the thymus was retracted, and a polytetrafluoroethylene-pledgeted 4-O silk purse-string suture was placed in the right atrium. After systemic anticoagulation with 300 U/kg intravenous heparin, the distal aorta was ligated at the bifurcation and cannulated in retrograde fashion with a 10-French pediatric arterial perfusion cannula (Biomedicus, Eden Prairie, MN) 5–7 mm superior to the distal aortic bifurcation. A 21-French venous cannula (Polystan, Ballerup, Den-
mark) was then placed in the right atrium. The aortic and right atrial cannulas were connected to the perfusion circuit and CPB was initiated as described later. Approximately 30 min before CPB, maintenance fluids and the succinylcholine/heparin infusion were discontinued. Muscle relaxation was achieved with 0.2 mg/kg pancuronium.

**Cardiopulmonary Bypass**

The CPB circuit consisted of a venous reservoir, a membrane oxygenator/heat exchanger (Capiox 308, Terumo, Piscataway, N.), a variable-temperature water pump (VWR Scientific, San Francisco, CA), and a nonepulsatile centrifugal pump (Biomedicus, Model 540, BP-50 pump head). Circuit priming fluid consisted of 300 ml 6.5% (wt/vol) high-molecular-weight hydroxyethyl starch (McGaw Inc., Irvine, CA) in 0.72 N sodium chloride, 18 meq sodium bicarbonate, 250 mg CaCl₂, and 1000 U heparin. The priming fluid was circulated through a 40-μm filter for 15–20 min before the addition of ~150 ml fresh, filtered, packed rabbit erythrocytes, achieving a hemoglobin concentration of 6.6–9.7 g/dl (OSM3; rabbit absorption coefficients; Radiometer, Copenhagen, Denmark).

Cardiopulmonary bypass was initiated and maintained throughout the experiment at a systemic flow rate of 100 ml·kg⁻¹·min⁻¹, monitored with a calibrated inline electromagnetic flow meter (Biomedicus, TX-40P). The pulmonary artery was clamped to ensure complete venous outflow to the CPB circuit. To prevent left ventricular ejection and/or distortion, the tip of a 14-G catheter was placed transapically in the left ventricle to permit drainage to the venous reservoir. Systemic cooling began at onset of CPB with water to the heat exchanger being maintained at 27°C. The oxygenator was ventilated with a variable mixture of oxygen and nitrogen to maintain PaCO₂ near 40 mmHg and PaO₂ near 250 mmHg when measured at an electrode temperature of 37°C (α-stat acid-base management; IL1304, Instrumentation Laboratory, Lexington, MA). Isoflurane vapor was added to oxygenator inflow gases via a calibrated vaporizer. Inspired isoflurane concentration was kept at 2% at the onset of CPB, and was decreased to 1% once a brain temperature of 28°C was achieved. Isoflurane concentration in oxygenator exhaust gas was monitored by the agent analyzer. Blood from the surgical field was returned to the venous reservoir after passing through a 40-μm filter. Sodium bicarbonate was administered to maintain a base excess greater than −4 mEq/l, calculated at 37°C. No pharmacologic or mechanical means were used to control arterial pressure other than those described later.

**Protocol**

After CPB was established, animals entered the treatment phase of the experiment. Our goal was to establish equivalent total arterial oxygen contents in all groups but for approximately 30% of the arterial oxygen content to be in a non-erythrocyte-associated (“free”) form in animals receiving DCLHB versus approximately 5% of the arterial oxygen content to be in the free form in animals not receiving DCLHB.

In pilot studies, we found verapamil pretreatment was necessary to prevent a marked hypertensive response to DCLHB during CPB (see Discussion). Therefore, in animals receiving DCLHB (group 1, n = 14), 2.5 mg verapamil was administered into the venous reservoir 5 min after CPB was initiated. Thereafter, 120–170 ml of a 10% (wt/vol) DCLHB solution was administered into the venous reservoir over 5–10 min. Additional 10% DCLHB and/or rabbit erythrocytes were given to maintain a plasma DCLHB concentration of 2.5–3.5 g/dl and a native (erythrocyte-associated) hemoglobin concentration of ~6 g/dl (hematocrit [Hct] ~18%).

Two control groups were necessary to characterize the independent effect of verapamil on cerebral blood flow (CBF) and cerebral metabolic rate for oxygen (CMR氧气). In the first control group (group 2, n = 13), 2.5 mg verapamil was administered into the venous reservoir 5 min after the start of CPB, but no DCLHB was given. Rabbit erythrocytes were administered to maintain an erythrocyte-associated hemoglobin concentration of ~8 g/dl (Hct ~24%). In the second control group (group 3, n = 13), neither verapamil nor DCLHB were given, and only rabbit erythrocytes were administered during CPB to maintain an erythrocyte-associated hemoglobin concentration of ~8 g/dl (Hct ~24%).

After 60 min of CPB, the following were recorded: arterial pressure, central venous pressure, systemic flow rate, and brain (epidural) temperature. Arterial blood was collected for measurement of Hct (capillary microcentrifugation, 5 × 10³ RPM for 5 min), whole blood and plasma hemoglobin concentration (OSM3, rabbit coefficients), plasma sodium and potassium concentrations (Model 614, Ciba Corning, Medfield, MA), and oncotic pressure (Model 4400, Wescor, Logan, UT). Concurrent with these measurements, CBF was determined (see later) and arterial and cerebral

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venous blood was collected for blood gas analysis and measurement of total oxygen content \textit{via} the galvanic cell method (Lex-O₂-Con. Lexington Instruments Corporation, Waltham, MA).

\textbf{Cerebral Blood Flow and Cerebral Metabolic Rate for Oxygen Measurements}

Cerebral blood flow was measured by the radioactive microsphere technique. Isotopes used included $^{46}$Sc, $^{85}$Sr, $^{95}$Nb, and $^{141}$Ce (New England Nuclear, Boston, MA), although only one isotope was used in each experiment. Stock microspheres (200 µL, ~0.9 million microspheres), vigorously mixed for 5 min before withdrawal, were diluted in 1.5 ml suspending solution (10% dextran-40 in normal saline with 0.5% (vol/vol) polysorbate-80) and mixed an additional 60 s. Microspheres were injected over 30 s into the arterial perfusion line approximately 25 cm proximal to the distal tip of the aortic cannula. Starting 15 s before microsphere injection, and continuing 2 min thereafter, blood was simultaneously withdrawn from each branchial arterial catheter \textit{via} a calibrated withdrawal pump (1.96 ml/min). After the experiment, the brain was removed and dissected into the following regions: right and left cerebral hemispheres, cerebellum, midbrain, and medulla. Fresh tissue samples were weighed, placed in counting tubes and, with reference blood samples, each counted for 5 min in a sodium iodide well-type gamma counter (Minaxi $\gamma$ Auto-Gamma 5000, Packard Instruments, Meriden, CT). Isotope separation, background, and overlap corrections, and organ blood flow calculations (ml·100g$^{-1}$·min$^{-1}$) were performed by standard techniques.\textsuperscript{30–32} Weight-averaged values for right and left cerebral hemispheric blood flow were used to calculate mean hemispheric CBF.

$P_{O₂}$ was corrected to \textit{in vivo} (brain) temperature using equations derived by Severinghaus.\textsuperscript{35} Dissolved oxygen (ml O₂/dl) was calculated as the product of temperature-corrected $P_{O₂}$ and the temperature- and hemoglobin-corrected oxygen solubility coefficient (0.00337 ml O₂·dl$^{-1}$·mmHg$^{-1}$).\textsuperscript{34}

Data from groups 2 and 3 only (no DCLHb) were used to calculate erythrocyte-associated oxygen (ml O₂/dl) as a function of Hct as shown in equation (1).

\textbf{Statistics}

Right and left microsphere counts appeared to be normally distributed, permitting linear regression analysis to test adequacy of microsphere mixing and distribution. In contrast, box and whisker plots suggested that many physiologic variables were not normally distributed. Consequently, all physiologic variables were summarized using their median ± quartile deviation, the latter equaling half the difference between the first and third quartiles. Systemic physiologic variables were assessed qualitatively to preserve statistical power to detect differences in CBF and $CMRO₂$.

Analyses were performed using Systat statistical software.\textsuperscript{53} Cerebral blood flow appeared to follow a normal distribution. $CMRO₂$ appeared to follow a log-normal distribution. Thus, $CMRO₂$ was log-transformed before analysis. Analysis of variance on the logarithm of $CMRO₂$ yielded a large Studentized residual (3.183) for a rabbit with an extremely large $CMRO₂$. Data from this animal (from group 1) were excluded from further statistical analysis. Cerebral blood flow and log($CMRO₂$) were compared among groups by one-way analysis of variance. One-sided simultaneous confidence bands for differences between group 1 (DCLHb)
and groups 2 and 3 (controls) were performed for log(CMR$_{O_2}$) using Dunnett's test for multiple comparisons. Assumptions of the statistical tests were satisfied.

Results

**Microsphere Validation.** Paired right and left microsphere reference counts were well matched ($r^2 = 0.985$, slope = 1.09, intercept ($-75$ cpm) not significantly different than zero), indicating adequate microsphere mixing and uniform distribution. There were no right-left CBF asymmetries between the cerebral hemispheres.

**Systemic Variables.** Systemic physiologic variables at 60 min of CPB are summarized in table 1. There were no differences among groups with respect to the following: mean arterial pressure, central venous pressure, systemic flow, arterial pH, Pa$_{CO_2}$, or Pa$_{O_2}$, plasma sodium or potassium concentration, oncotic pressure, or dissolved oxygen content (0.7 ml O$_2$/dl). As intended, arterial oxygen content was equivalent among groups (~12.1 ml O$_2$/dl), but groups not receiving DCLHb (groups 2 and 3) had greater arterial Hct values (~24%) than did the DCLHb group (~18%). In group 1, DCLHb-associated oxygen was estimated as $2.8 \pm 0.4$ ml O$_2$/dl versus essentially zero in groups 2 and 3. Thus, the DCLHb group had a much greater proportion of the total arterial oxygen content which was in the free (non–erythrocyte-associated) form: 29 ± 5% in group 1, versus 6 ± 2% and 5 ± 3%, in groups 2 and 3, respectively.

**Cerebral Physiology.** Cerebral physiologic variables are summarized in table 2. There were no differences among groups with respect to brain temperature. Hemispheric CBF (~3.4 ml·100 g$^{-1}$·min$^{-1}$) did not differ among groups ($P = 0.55$). Similarly, CMR$_{O_2}$ (~1.2 ml O$_2$·100g$^{-1}$·min$^{-1}$) did not differ among groups ($P = 0.26$; fig. 1). Simultaneous 95% confidence bounds for CMR$_{O_2}$ indicate that mean CMR$_{O_2}$ in group 1 (DCLHb and verapamil) could be, at most, only 9% greater than mean CMR$_{O_2}$ in group 2 (verapamil, no DCLHb), and only 3% greater than group 3 (no DCLHb, no verapamil), respectively.

Discussion

Although DCLHb animals (group 1) had a much larger proportion of the total arterial oxygen content in the free (non–erythrocyte-associated) form, neither CBF, oxygen extraction, nor CMR$_{O_2}$ differed from groups having far less free oxygen (groups 2 and 3). We therefore conclude that it is very unlikely that brain oxygen consumption is limited during moderately hypothermic (27°C) CPB on the basis of impaired erythrocyte transit through brain capillary networks.

**Erythrocyte–Capillary Interactions and Oxygen Transfer**

It appears that erythrocyte deformability plays a role in determining the distribution of erythrocytes within capillary networks and the transfer of oxygen from hemoglobin to tissue. With decreasing erythrocyte deformability, flow impedance increases and should some capillary beds become functionally de-recruited (see earlier), regional tissue oxygenation may become impaired. For example, in clinical sepsis, Powell et al. noted decreases in erythrocyte deformability correlated with impaired systemic oxygen off-loading and worsened pulmonary ventilation/perfusion relationships. We wondered whether a similar process, resulting in impaired brain oxygenation, might occur during hypothermic CPB as a result of decreased erythrocyte deformability.

In hypothermic (30°C) dogs, pentoxifylline increased systemic oxygen consumption 20% over that observed in nonpentoxifylline controls, and increased maximal systemic oxygen extraction. Although the authors ascribed this increase to pentoxifylline’s effect on erythrocyte deformability, pentoxifylline also decreased systemic vascular resistance and low-shear blood viscosity, and increased cardiac output and systemic oxygen delivery. Hence, it is impossible to determine whether increased systemic oxygen consumption associated with pentoxifylline was caused by improvements in systemic hemodynamics or, instead, to improvements in erythrocyte/capillary interactions. In our experiment, we avoided the former problem by artificially supporting systemic hemodynamics. Systemic flow (i.e., cardiac output), systemic arterial pressure, arterial oxygen content, and, hence, net systemic oxygen delivery, were equivalent among groups.

Rather than attempting to alter erythrocyte deformability (which can be difficult to quantitate, and can differ significantly between methods), we chose to increase the oxygen content of plasma. Studies both in normal and ischemic brain indicate that even when capillary erythrocyte flow is absent, capillary plasma flow continues. We reasoned that if the oxygen
DCLHb AND BRAIN OXYGENATION DURING BYPASS

Table 1. Systemic Physiologic Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>1 DCLHb and Verapamil</th>
<th>2 Verapamil, No DCLHb</th>
<th>3 No Verapamil, No DCLHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>78 (6)</td>
<td>70 (5)</td>
<td>74 (7)</td>
</tr>
<tr>
<td>Central venous pressure (mmHg)</td>
<td>2 (1)</td>
<td>3 (2)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Systemic flow (ml·kg⁻¹·min⁻¹)</td>
<td>100 (1)</td>
<td>102 (3)</td>
<td>101 (2)</td>
</tr>
<tr>
<td>Exhaust isoflurane concentration (%)</td>
<td>0.8 (0.1)</td>
<td>0.8 (0.1)</td>
<td>0.8 (0.1)</td>
</tr>
<tr>
<td>pHᵣ [37°C]</td>
<td>7.41 (0.01)</td>
<td>7.41 (0.01)</td>
<td>7.39 (0.01)</td>
</tr>
<tr>
<td>PaCO₂ [37°C] (mmHg)</td>
<td>39 (1)</td>
<td>39 (1)</td>
<td>40 (1)</td>
</tr>
<tr>
<td>PaO₂ [37°C] (mmHg)</td>
<td>247 (12)</td>
<td>240 (8)</td>
<td>249 (7)</td>
</tr>
<tr>
<td>PaO₂ [temperature-corrected] (mmHg)</td>
<td>202 (12)</td>
<td>196 (6)</td>
<td>205 (7)</td>
</tr>
<tr>
<td>Plasma [Na⁺] (mEq/L)</td>
<td>149 (2)</td>
<td>147 (3)</td>
<td>147 (2)</td>
</tr>
<tr>
<td>Plasma [K⁺] (mEq/L)</td>
<td>3.0 (0.4)</td>
<td>2.5 (0.3)</td>
<td>2.5 (0.2)</td>
</tr>
<tr>
<td>Plasma oncotic pressure (mmHg)</td>
<td>27 (2)</td>
<td>21 (5)</td>
<td>24 (2)</td>
</tr>
<tr>
<td>Plasma hemoglobin concentration (g/dl)*</td>
<td>3.0 (0.4)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>18 (1)</td>
<td>24 (0)</td>
<td>24 (0)</td>
</tr>
<tr>
<td>Total arterial oxygen content (ml O₂/dl)</td>
<td>12.1 (0.4)</td>
<td>12.1 (0.2)</td>
<td>12.1 (0.3)</td>
</tr>
<tr>
<td>Dissolved oxygen (ml O₂/dl)</td>
<td>0.7 (0.0)</td>
<td>0.7 (0.0)</td>
<td>0.7 (0.0)</td>
</tr>
<tr>
<td>Erythrocyte-associated oxygen (ml O₂/dl)</td>
<td>8.7 (0.2)</td>
<td>11.5 (0.1)</td>
<td>11.5 (0)</td>
</tr>
<tr>
<td>DCLHb-associated oxygen (ml O₂/dl)†</td>
<td>2.8 (0.4)</td>
<td>0.1 (0.2)</td>
<td>0.2 (0.3)</td>
</tr>
</tbody>
</table>

Values are medians and quartile deviation (parentheses); n = 13 in all groups.

* Estimated spectrophotometrically with rabbit absorption coefficients. Reported value is total hemoglobin and does not account for met- and carboxy-forms of hemoglobin, which comprised about 15% of the total in the DCLHb group.
† DCLHb not present in groups 2 and 3. Reported values are calculated.

content of plasma could be substantially increased, limitations in brain oxygen uptake caused by unfavorable erythrocyte/capillary interactions (if present) could be circumvented. Recently, using a rat model of focal cerebral ischemia, and DCLHb as an oxygen carrier, Cole et al. have shown DCLHb increases oxygen delivery to low-flow regions and, as a probable consequence, reduces infarction volumes. It appears that DCLHb is able to enter capillary beds that erythrocytes cannot, providing physiologically meaningful amounts of oxygen. In our experiment, we achieved DCLHb concentrations (2.5–3.5 g/dl) equivalent to those shown by Cole et al. to result in the aforementioned effects. Despite a large increase in plasma oxygen content in the DCLHb group, there was no difference in CBF, cerebral oxygen extraction, or CMRO₂ between this group and controls. Verapamil, which was needed to control the pressor response to DCLHb (see later),

Table 2. Cerebral Physiologic Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>1 DCLHb and Verapamil</th>
<th>2 Verapamil, No DCLHb</th>
<th>3 No Verapamil, No DCLHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain temperature (°C)</td>
<td>26.6 (0.2)</td>
<td>26.8 (0.2)</td>
<td>26.7 (0.1)</td>
</tr>
<tr>
<td>Cerebral venous oxygen content (ml O₂/dl)</td>
<td>8.4 (0.6)</td>
<td>8.5 (0.7)</td>
<td>7.9 (0.9)</td>
</tr>
<tr>
<td>Cerebral arteriovenous oxygen content difference (ml O₂/dl)</td>
<td>3.5 (0.6)</td>
<td>3.9 (0.4)</td>
<td>4.7 (0.8)</td>
</tr>
<tr>
<td>Cerebral oxygen extraction ratio</td>
<td>0.30 (0.06)</td>
<td>0.30 (0.04)</td>
<td>0.39 (0.07)</td>
</tr>
<tr>
<td>Hemispheric cerebral blood flow (ml·100 g⁻¹·min⁻¹)</td>
<td>35 (5)</td>
<td>34 (3)</td>
<td>32 (5)</td>
</tr>
<tr>
<td>Cerebral metabolic rate for oxygen (ml O₂·100 g⁻¹·min⁻¹)</td>
<td>1.2 (0.2)</td>
<td>1.2 (0.2)</td>
<td>1.3 (0.3)</td>
</tr>
</tbody>
</table>

Values are median and quartile deviation (parentheses); n = 13 in each group.

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appeared to have only minimal independent effects on CBF and CMRO₂. Hence, it appears oxygen was equally available to the brain in all groups, independent of whether oxygen was associated with erythrocytes or not. Group sizes were sufficiently large to establish with 95% certainty that CMRO₂ in the DCLHB group could have been, at the most, only 3–9% greater than the control groups. We therefore conclude that unfavorable erythrocyte–capillary interactions do not limit oxygen transfer from blood to brain during moderately hypothermic CPB. Hence, there is no evidence that moderate hypothermia results in a global impairment of brain oxygenation during CPB. Our findings are consistent with those of Gutierrez et al.⁵⁵ and Wilford et al.⁴⁶ who observed no evidence of impaired systemic oxygenation in non-CBP dogs and pigs at 29–30°C.

**Diaspirin Cross-linked Hemoglobin as a Cell-free Oxygen Carrier**

Recent advances in hemoglobin purification and molecular stabilization have resulted in modified hemoglobin molecules that are essentially free of immunologic⁴⁷ and renal toxicities.⁴⁸⁴⁹ One such molecule is DCLHB, which we selected as our cell-free oxygen carrier. Purified from outdated human blood, individual hemoglobin molecules are internally cross-linked between the two alpha chains with bis(3,5-dibromosalicyl) fumarate.²⁸⁵⁰ The cross-linking process occurs over a 10 h period at 60°C, precipitating residual proteins and inactivating viruses.⁵⁰⁵¹ The resulting product is 99.8% cross-linked, has a Hill coefficient only slightly less than that of unmodified hemoglobin,⁷⁸ and has a P₅₀ of 32 mmHg⁴⁸⁵⁰ (i.e., slightly greater than that of erythrocyte-associated hemoglobin, 27 mmHg). Diaspirin cross-linked hemoglobin has an intravascular half-life of 5–21 h in rats, ~13 h in the rabbit, ~16 h in the monkey, and 7–22 h in pigs, with longer half-lives with larger doses.⁵² Currently, DCLHB is formulated as a 10% solution (10 g DCLHB per 100 ml isotonic electrolyte solution), with 5–7% of the DCLHB present in the met-DCLHB form. DCLHB has been studied as a blood substitute in animal models of hemorrhagic shock,⁵³⁵⁴ stroke,⁴⁴⁵⁵ and angioplasty,⁵⁶ and is currently in phase II clinical trials.

In addition to its oxygen-carrying properties, DCLHB has a pressor effect in several species,⁴⁸⁵³⁵⁴⁵⁵⁵⁷–⁵⁹ increasing mean arterial pressure 25–84% above baseline. The pressor response to DCLHB appears to be caused principally by nitric oxide scavenging⁶⁰⁶¹ although sensitization of peripheral α-adrenergic receptors⁵⁸ and/or endothelin receptors⁵⁰ may also contribute. In rats, the pressor response is blocked by α-adrenergic receptor antagonists,⁶² calcium channel blockers,⁶² and can be reversed by administration of L-arginine⁶⁰ or large doses of nitric oxide donors.⁶⁰⁶² In pilot experiments, we observed only modest (15–20%) increases in arterial pressure when 5–10 ml/kg 10% DCLHB was given to rabbits not on CPB. In stark contrast, when DCLHB was given either before or during CPB, profound hypertension (systemic arterial pressure greater than 300 mmHg) was uniformly observed within 5–10 min. It appears that CPB circumvents compensatory changes in cardiac output and/or systemic vascular tone, which, in the native circulation, serve to limit the hypertensive response to DCLHB in the rabbit. Although we could control DCLHB-induced hypertension during CPB with use of sodium nitroprusside or nitroglycerin, doses of 100–200 μg·kg⁻¹·min⁻¹ were necessary (i.e., doses 100–200 times that used clinically). We suspect that the hypertensive response to DCLHB, and the resistance to nitroglycerin and nitroprusside during CPB, were caused by DCLHB scavenging of both native and exogenous nitric oxide, respectively.⁶³ Consistent with the findings of Bilello et al. in rats,⁶² in our preparation, administration of a calcium channel antagonist (verapamil, 0.5 mg/kg) before DCLHB administration completely and uniformly prevented the hypertensive response. Although the pressor response...
to DCLHb during CPB is easily blocked, we believe a more complete characterization of this response is indicated before DCLHb is used as a blood substitute during human cardiac surgery and CPB. However, should this problem not exist in humans, or should it be avoided (as earlier), our data suggest that DCLHb is equivalent to native erythrocytes in support of cerebral blood flow and oxygen metabolism during moderately hypothermic CPB.

Overview of Studies with the Rabbit Model of Cardiopulmonary Bypass

We have serially investigated various processes postulated to impair or disorder cerebral blood flow and metabolism during CPB—mechanisms proposed to contribute to postoperative neurologic and neuropsychologic injury in adults undergoing cardiac surgery. We have found little or no evidence of an inherently damaging effect of steady-state CPB on the brain. For example, we have shown that arterial pulsation has no effect on brain blood flow or metabolism, and that at moderate hypothermia (27°C) acid–base management (a-stat vs. pH-stat) has no effect on brain oxygenation and only modest effects on blood flow. We have also shown there is very little spontaneous decrease in brain blood flow or metabolism during steady-state bypass, down-playing the concept of a major time-dependent deterioration in overall brain physiologic status during CPB. The experiment described here addresses yet another postulated mechanism of impaired brain perfusion/oxygenation during moderately hypothermic CPB. As before, we have found no evidence of impairment. Based on our collective work, we believe that generalized hypoperfusion and/or impairment of brain aerobic metabolism during well-conducted steady-state CPB should no longer be considered as a credible mechanism of postoperative neurologic and neuropsychologic dysfunction. We believe attention should now focus on how CPB modifies injuries that occur as a result of embolic events, and how the dynamics of CPB (e.g., cooling and warming) influence the brain and the brain’s response to injury.

In summary, in a rabbit model of moderately hypothermic CPB, a fivefold to sixfold increase the proportion of free (non-erythrocyte-associated) oxygen in arterial blood (using DCLHb) had no effect on CBF or CMRO₂. Because brain blood flow and oxidative metabolism were independent of the proportion of arterial oxygen content that was erythrocyte-associated, we conclude that hypothermia- and CPB-induced changes in erythrocyte–capillary interactions probably have little or no effect on brain oxygenation during moderately hypothermic CPB.

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