Regional cerebral blood flow in cats with cross-linked hemoglobin transfusion during focal cerebral ischemia

ANNETTE REBEL, JOHN A. ULATOWSKI, KARENA JOUNG, ENRICO BUCCI, RICHARD J. TRAYSTMAN, AND RAYMOND C. KOEHLER

1Department of Anesthesiology and Critical Care Medicine, Johns Hopkins Medical Institutions, Baltimore 21205; and 2Department of Biological Chemistry, University of Maryland, Baltimore, Maryland 21201

Received 10 October 2001; accepted in final form 5 November 2001

Rebel, Annette, John A. Ulatowski, Karena Joung, Enrico Bucci, Richard J. Traystman, and Raymond C. Koehler. Regional cerebral blood flow in cats with cross-linked hemoglobin transfusion during focal cerebral ischemia. Am J Physiol Heart Circ Physiol 282: H832–H841, 2002. First published November 8, 2001; 10.1152/ajpheart.00880.2001.—The beneficial effect of hemodilution on cerebral blood flow (CBF) during focal cerebral ischemia is mitigated by reduced arterial oxygen content (CaO₂). In anesthetized cats subjected to permanent middle cerebral artery occlusion, the time course of regional CBF was evaluated after isovolemic exchange transfusion with either albumin or a tetrameric hemoglobin-based oxygen carrier. The transfusion started 30 min after arterial occlusion. We tested the hypothesis that bulk oxygen transport (CBF × CaO₂) to ischemic tissue is increased by hemoglobin transfusion at a hematocrit of 18% compared with albumin-transfused cats at a hematocrit of 15% or control cats at a hematocrit of 30% and equivalent arterial pressure. In the nonischemic hemisphere, CBF increased selectively after albumin transfusion, and oxygen transport was similar among groups. In the ischemic cortex, albumin transfusion increased CBF, but oxygen transport was not increased above that of the control group. Hemoglobin transfusion increased both CBF and oxygen transport in the ischemic cortex above values in the control group, but the increase was delayed until 4 h of ischemia. Consequently, acute injury volume measured at 6 h of ischemia was not significantly attenuated. In contrast to the cortex, CBF in the ischemic caudate nucleus was not substantially increased by either albumin or hemoglobin transfusion. Therefore, in a large animal model of permanent focal ischemia in which transfusion starts 30 min after ischemia, tetrameric cross-linked hemoglobin transfusion can augment oxygen transport to the ischemic cortex, but the increase can be delayed and not necessarily provide protection. Moreover, an end-artery region such as the caudate nucleus is less likely to benefit from hemodilution.

blood substitute; hemodilution; oxygen transport; stroke

DURING FOCAL CEREBRAL ISCHEMIA, autoregulation is lost and cerebral blood flow (CBF) becomes passively dependent on perfusion pressure and blood viscosity. Some experimental models of middle cerebral artery (MCA) occlusion (MCAO) demonstrated that decreasing viscosity by hemodilution could promote intraischemic CBF and reduce injury as long as hypotension is prevented (9, 13, 44). However, several multicenter clinical trials of hemodilution in acute stroke failed to detect a beneficial effect (23, 28, 37), possibly because the degree of hemodilution and the increase in CBF was modest (49). In contrast, some improvement in neurological outcome has been reported in small trials using early, aggressive hypervolemic hemodilution (20, 29, 40).

Not all experimental studies showed an obvious improvement in outcome from hemodilution (1, 17). Decreases in arterial O₂ content (CaO₂) accompanying hemodilution may offset the effect of increased CBF on O₂ transport. Moreover, subcortical end-artery regions with few collateral vessels may not benefit from increased collateral flow and could be more vulnerable to reduced CaO₂ during hemodilution (26), as suggested by increased mortality in hemodiluted patients with deep brain structure lesions (37). Transfusions of cross-linked hemoglobin solutions have the ability to permit hematocrit to be decreased without a proportional decrease in CaO₂ and thereby augment O₂ transport. In addition, a plasma-based O₂ carrier has the potential to improve O₂ flux in individual red blood cell-poor capillaries compressed by swollen astrocytes. Indeed, several studies have shown increased CBF (10, 16) and decreased brain injury (1, 12, 15) after transient MCAO in rats transfused with diaspirin cross-linked hemoglobin (DCLHb), in which the tetramer is stabilized by a furmaryl crosslinker across the 99 lysines on the α-subunits. However, a clinical trial of DCLHb transfusion in acute stroke victims was halted because of worse outcome (35).

In studies with DCLHb transfusion in the rat, a hypervolemic exchange transfusion was performed either before or soon after the onset of MCAO. Studies of intraischemic CBF after cross-linked hemoglobin transfusion have not been performed over a prolonged period of MCAO or in a larger animal model of MCAO.
in which the time course of CBF could be followed. In the present study, we used a feline model of MCAO in which an isovolumetric exchange transfusion of a solution of albumin or cross-linked hemoglobin was started at 30 min of MCAO. Radiolabeled microspheres were used to make serial measurements of regional CBF in the cortex and caudate nucleus before and after transfusion. Arterial hypertension can act to decrease injury volume from MCAO (47). Because hemoglobin transfusion produces peripheral vasoconstriction in the cat (47), mean arterial blood pressure (MABP) in control and albumin-transfused cats was matched to that of hemoglobin-transfused cats by use of an aortic balloon catheter to determine if any benefit of hemoglobin transfusion was independent of the increase in MABP. Acute brain injury was measured at 6 h of permanent MCAO. Permanent MCAO rather than transient MCAO was used to determine if any changes in ischemic CBF after transfusion would be sustained. We tested the hypothesis that decreasing hematocrit equivalently by exchange transfusion of solutions of albumin or cross-linked hemoglobin after the onset of permanent MCAO will increase CBF in the ischemic cortex but that bulk O$_2$ transport will be augmented only with hemoglobin transfusion. We also postulated that the benefit of decreasing hematocrit on CBF in an end-artery region such as the caudate nucleus would be small.

**METHODS**

**Hemoglobin preparation.** A solution of intramolecularly cross-linked tetrameric hemoglobin was prepared by reacting deoxygenated human hemoglobin with bis(3,5-dibromosalicyl)-sebacate as previously described (8). This reagent produces intramolecular cross-links between the $\beta 82$ lysines and between the $\alpha 99$ lysines, respectively (7). The solution was purified from residual non-cross-linked material by affinity chromatography and therefore contained only cross-linked tetrameric nondissociable hemoglobin molecules. The partial pressure of O$_2$ at 50% saturation of hemoglobin (P$_{Sat}$) is near 34 mmHg at 37°C with a Hill parameter of $n_H = 2.2$. The highly purified, homogeneous solution was pasteurized and subjected to isovolémic dialysis with an isotonic solution containing 125 mM NaCl, 25 mM NaHCO$_3$, and 4 mM KCl. The solution was centrifuged, detoxified with Detoxygel (Pierce Chemical; Roxford IL) to remove endotoxin, and stored at $-70^\circ$C (46). Previous analysis indicated that endotoxin was not detectable in these solutions (8). On the day of the experiment, the nonpyrogenic solution was diluted with sterile lactated Ringer solution to adjust the hemoglobin concentration to 6 g/dl. The colloid osmotic pressure of the solution was 19 mmHg and was similar to a 5 g/dl solution of albumin (8).

**Surgical procedures.** All procedures were approved by the institutional animal care and use committee. Laboratory-bred male cats of mixed breed weighing 2.1–3.0 kg were anesthetized with halothane (2–3%) in order to perform the oral intubation and insert the femoral arterial and venous catheters. After the insertion of the first venous catheter, pentobarbital sodium (8 mg/kg) and fentanyl (40 $\mu$g/kg) were infused intravenously. The inspired halothane concentration was decreased to 1.5%. The inspired O$_2$ concentration was increased to 30% to maintain an oxyhemoglobin saturation $>97\%$ throughout the experiment. Mechanical ventilation was performed to maintain the arterial partial pressure of CO$_2$ (P$_{CO_2}$) near 35 mmHg. Muscle paralysis was achieved by periodic intravenous administration of pancuronium bromide (0.1 mg/kg) to facilitate electrophysiological monitoring and electrocauterization. Arterial pH was maintained near 7.4 with intravenous sodium bicarbonate (1 mmol/ml).

Two catheters were placed into the femoral veins and advanced into the inferior vena cava for administration of drugs and replacing fluid during the blood exchange. A catheter inserted into a femoral artery was advanced into the thoracic aorta to continuously monitor arterial blood pressure. A second arterial catheter, placed 5 cm distal to the first one, was used for withdrawal of blood during exchange transfusion and microsphere blood flow measurements. This catheter also had a balloon on its tip, which was used to regulate arterial blood pressure during the experiment (Edwards Swan-Ganz Monitoring Catheter Pediatric 4-Fr, Baxter). A left thoracotomy was performed, and a catheter was inserted into the left atrium for the injection of radiolabeled microspheres. After the chest wall incision was apposed, halothane was discontinued, and penobarbital sodium (6 mg/kg) and fentanyl (50 $\mu$g/kg) were injected intravenously. The cat was turned prone, and its head was positioned in a stereotaxic frame $\sim3$ cm higher than its heart. A thermistor was placed in the left temporal epidural space to estimate the brain temperature. Epidural temperature was maintained at 38 $\pm$ 0.5°C using a warmed water blanket and a heating lamp. The left MCA was exposed by a transorbital approach using microsurgical techniques (31). To produce focal ischemia, the left MCA was occluded near its origin from the intracranial carotid artery using microvascular clips (Roboz, Surgical Instruments; Rockville, MD).

The arterial pH, P$_{CO_2}$, and arterial partial pressure of O$_2$ (P$_{O_2}$) were measured with a self-calibrating electrode system (model 246, Chiron Diagnostics). Total hemoglobin concentration, arterial O$_2$ saturation, and CaO$_2$ were measured with a hemoximeter adjusted for cat blood (model OSM 3, Radiometer). Blood glucose was measured with a glucose analyzer (model 2300A, Yellow Springs Instruments). A multichannel signal averager (model CA-1000, Nicolet Biomedical Instruments) was used to measure bilateral somatosensory-evoked potentials (SEP) with stimulation of each foreleg, as previously described (31). The amplitude to the peak of the first major negative wave was measured from the peak of the proceeding positive wave. Cats that did not achieve at least a 90% reduction in SEP amplitude on the left side after MCAO were excluded.

Regional CBF was measured with radiolabeled microspheres ($15 \pm 0.5 \mu$m diameter, New England Life Sciences Products) by the reference method (32). Five of six radioactive isotopes ($^{57}$Co, $^{114}$In, $^{113}$Sn, $^{103}$Ru, $^{95}$Nb, and $^{46}$Sc) were injected in a random sequence into each cat. Approximately $1.5 \times 10^6$ microspheres were injected into the left atrium over a 20-s period, followed by a 5-ml saline flush. Reference blood samples were withdrawn from the aorta at 1.94 ml/min beginning 30 s before the injection and continuing for 90 s after the saline flush.

**Experimental protocol.** Thirty minutes after the end of the surgical preparation, baseline measurements of arterial blood gases, pH, hemoglobin concentration, CaO$_2$, glucose concentration, SEP, and regional blood flow were made. These measurements were repeated 20, 120, 240, and 360 min after the onset of MCAO. Plasma osmolality and colloid osmotic pressure were measured at 20, 120, and 360 min of MCAO. Cats were assigned to one of the following three groups: 1) control group, in which no exchange transfusion was performed (hematocrit 30%, n = 16); 2) albumin group,
in which an exchange transfusion was performed with a 5% human albumin solution to a hematocrit of 18% (n = 16); and 3) hemoglobin group, in which an exchange transfusion was performed with the cross-linked hemoglobin solution to a hematocrit of 18% (n = 15). The transfusion was started at 30 min of MCAO, thereby permitting measurements of CBF after the transfusion to be compared on a paired basis to both preischemic values and intraischemic values before the transfusion. The exchange transfusion was performed at a rate of 3.88 ml/min until the hematocrit was reduced to 18% (~10–15 min). The half-time of intravascular retention of this cross-linked hemoglobin is 6.5 h in the cat (8). To keep hematocrit near 18% for the remainder of the experiment, when protein is lost from the plasma space, additional amounts of the albumin or hemoglobin solution were slowly infused as needed without the withdrawal of additional blood. In the control and albumin groups, the aortic balloon was inflated at 40 min of MCAO to increase MABP by ~20 mmHg. This increase was equivalent to that seen 10 min after the hemoglobin transfusion was started. The aortic balloon remained inflated for the duration of MCAO. Sodium bicarbonate was administered to correct base deficit when arterial pH was <7.30.

At 6 h of MCAO, cats were killed with an intracardiac injection of potassium chloride. The brain was removed and immediately cut into 12 uniform coronal sections 2 mm thick to estimate brain injury with the 2,3,5-triphenyltetrazolium chloride (TTC) technique (4). Brain injury volume was corrected for swelling by subtracting the noninjured ipsilateral volume from the contralateral volume (41). Images of both the anterior and posterior surfaces were used to calculate injury volume (31).

The brain slices were placed in 10% buffered formalin before being sectioned into regions for blood flow analysis. For each hemisphere, the caudate nucleus, hippocampus, cortical gray matter, and subcortical white matter were dissected on each slice. Gray matter from the four anterior sections were pooled for anterior cortical blood flow determination. Gray matter from the four posterior sections were pooled for posterior cortical blood flow determination. For the four middle sections, gray matter was further subdivided into the superior parietal cortex, lateral temporal-parietal cortex, inferior temporal cortex, and medial inferior cortex. Tissue from the four middle sections were pooled to assure the presence of at least 400 microspheres. Samples of the small intestine and kidney were also analyzed to see if balloon inflation caused splanchnic and renal ischemia. Tissue and blood samples were analyzed for radioactivity spectra in an autogamma scintillation spectrometer (Minaxi model 5530, Packard Instruments). Blood flow was calculated as a product of the withdrawal rate (1.94 ml/min) times the counts in the tissue (corrected for the isotope overlap) divided by the counts in the arterial reference sample (46).

Statistical evaluation. Statistical differences among the three groups were evaluated by one-way analysis of variance. Post hoc analyses were performed with the Newman-Keuls multiple-range test. Significance was assumed when P < 0.05. Comparisons to baseline values and to 20-min MCAO values (before transfusion and aortic balloon inflation) were made within groups by paired t-tests. The false discovery rate procedure (18) was used to adjust for multiple t-test comparisons. The false discovery rate was set at 0.05 for the seven paired comparisons for each measurement. Values are expressed as means ± SD.

RESULTS

Physiological variables. After the exchange transfusion to a hematocrit of 18%, hemoglobin concentration and CaO₂ decreased in the albumin and hemoglobin groups compared with the control group (Table 1). However, the hemoglobin group had higher hemoglobin concentration and CaO₂ than albumin-exchanged animals. After the exchange transfusion with the hemoglobin solution, plasma hemoglobin concentration remained constant at 2.2–2.4 g/dl and represented 25–30% of the total blood hemoglobin. Oxygen affinity remained unchanged after transfusion (P50 = 30 ± 2 mmHg). Mean values of PacO₂ were in the range of 140–160 mmHg. Arterial glucose concentration was in the range of 5–10 mmol/l. No differences were observed from baseline values or among the three groups throughout the protocol for PacO₂ and glucose concentration or for arterial pH, PaCO₂, or plasma osmolarity (Table 1). Colloid osmotic pressure increased by ~2 mmHg after albumin or hemoglobin transfusion.

Sustained increases of MABP occurred after transfusion of the hemoglobin solution (Fig. 1). With the use of the aortic balloon, increases in MABP in the control and albumin groups were matched to that of the hemoglobin group. Balloon inflation decreased renal blood flow by 37% in the control group but not in the albumin group. Differences in the response of renal blood flow may be related to the 25% decrease in blood viscosity previously observed after albumin exchange transfusion (45). Blood flow to the small intestine was not significantly altered in any group, thereby indicating that profound splanchnic ischemia did not occur with aortic balloon inflation.

Somatosensory-evoked potentials. Baseline amplitudes (control, 45 ± 19 μV; albumin, 35 ± 13 μV; hemoglobin, 37 ± 15 μV) of the primary cortical SEP were not different among groups. During left MCAO, ipsilateral SEP amplitude was suppressed to the same extent in all groups (Table 2). Transfusion did not increase SEP amplitude, consistent with a study in cats using saline or dextran hemodilution (17). In the contralateral somatosensory cortex, there was no change in SEP amplitude in any group. All cats had normal latency of the wave measured over the second cervical vertebra throughout the protocol, indicating intact peripheral nerve transmission.

Cerebral blood flow. During 6 h of MCAO, blood flow to the ipsilateral caudate nucleus was reduced to similar levels in all groups (Fig. 2). Blood flow in this end-artery region at 120–360 min of MCAO was not substantially greater than flow at 20 min of MCAO (before inflation of the aortic balloon or exchange transfusion) except for small significant increases in the control and albumin groups occurring at 120 min of MCAO. In the contralateral caudate nucleus, blood flow rose after albumin transfusion, whereas no flow change was observed after hemoglobin transfusion (Fig. 2). The lack of an increase in contralateral blood flow after hemoglobin transfusion, despite similar de-
There were no differences among groups.

In cortical gray matter, ipsilateral blood flow was equivalent among groups at 20 min of MCAO (Fig. 3). After induction of hypertension, blood flow increased slightly in the control group but remained below baseline values. Transfusion with the albumin solution combined with moderate hypertension increased ipsilateral cortical blood flow by 82% and restored flow to baseline levels in the contralateral cortex, flow increased by 110%. Transfusion with the hemoglobin solution also restored ipsilateral cortical blood flow and produced a greater blood flow compared with the control group, although the effect was not significant until 240 min of MCAO. In the contralateral cortex, blood flow was increased by 110%. Transfusion with the hemoglobin solution also restored ipsilateral cortical blood flow and produced a greater blood flow compared with the control group, although the effect was not significant until 240 min of MCAO.

### Table 1. Arterial blood analysis during 360 min of MCAO

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>20 min</th>
<th>120 min</th>
<th>240 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematocrit, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>29.9±2.0</td>
<td>29.4±1.6</td>
<td>29.3±2.0</td>
<td>30.3±2.0</td>
<td>29.8±2.3</td>
</tr>
<tr>
<td>Alb</td>
<td>30.1±2.1</td>
<td>28.0±3.4</td>
<td>17.8±0.5†</td>
<td>17.8±0.5†</td>
<td>17.5±0.7†</td>
</tr>
<tr>
<td>Hb</td>
<td>29.9±2.3</td>
<td>28.7±2.7</td>
<td>17.7±0.7†</td>
<td>17.7±0.7†</td>
<td>17.8±0.6†</td>
</tr>
<tr>
<td><strong>Hemoglobin, g/dl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>10.1±0.8</td>
<td>10.0±0.6</td>
<td>10.0±0.8</td>
<td>10.5±1.0</td>
<td>10.3±1.0</td>
</tr>
<tr>
<td>Alb</td>
<td>10.4±0.9</td>
<td>9.7±1.2</td>
<td>6.2±0.4†</td>
<td>6.1±0.4†</td>
<td>5.9±0.4†</td>
</tr>
<tr>
<td>Hb</td>
<td>10.3±0.9</td>
<td>9.9±1.0</td>
<td>8.4±0.4††</td>
<td>8.5±1.0††</td>
<td>8.6±0.5††</td>
</tr>
<tr>
<td><strong>O₂ content, ml O₂/dl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>13.7±1.1</td>
<td>13.6±0.9</td>
<td>10.7±2.2</td>
<td>14.2±1.4</td>
<td>14.0±1.5</td>
</tr>
<tr>
<td>Alb</td>
<td>14.0±1.1</td>
<td>13.1±1.6</td>
<td>8.3±0.5†</td>
<td>8.2±0.6†</td>
<td>7.9±0.6†</td>
</tr>
<tr>
<td>Hb</td>
<td>13.9±1.3</td>
<td>13.4±1.4</td>
<td>11.0±0.6††</td>
<td>10.9±1.3††</td>
<td>11.1±0.6††</td>
</tr>
<tr>
<td><strong>Arterial pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>7.42±0.03</td>
<td>7.43±0.03</td>
<td>7.44±0.03</td>
<td>7.43±0.03</td>
<td>7.44±0.02</td>
</tr>
<tr>
<td>Alb</td>
<td>7.42±0.03</td>
<td>7.42±0.03</td>
<td>7.44±0.03</td>
<td>7.44±0.02</td>
<td>7.43±0.03</td>
</tr>
<tr>
<td>Hb</td>
<td>7.42±0.03</td>
<td>7.42±0.04</td>
<td>7.44±0.03</td>
<td>7.43±0.02</td>
<td>7.43±0.02</td>
</tr>
<tr>
<td><strong>Paco₂, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>35.5±2.2</td>
<td>35.7±2.3</td>
<td>34.6±2.2</td>
<td>35.6±2.6</td>
<td>35.5±1.9</td>
</tr>
<tr>
<td>Alb</td>
<td>35.1±1.7</td>
<td>35.4±2.1</td>
<td>35.9±2.6</td>
<td>35.6±2.7</td>
<td>36.3±1.9</td>
</tr>
<tr>
<td>Hb</td>
<td>36.0±1.9</td>
<td>34.2±2.5</td>
<td>35.1±2.1</td>
<td>36.2±1.8</td>
<td>36.2±2.5</td>
</tr>
<tr>
<td><strong>Plasma osmolarity, mosmol/l</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>321±10</td>
<td>328±19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alb</td>
<td>328±8</td>
<td>322±6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>320±13</td>
<td>323±13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Colloid osmotic pressure, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>17.6±1.5</td>
<td>17.1±2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alb</td>
<td>17.8±1.2</td>
<td>19.2±1.2†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>16.9±2.3</td>
<td>19.5±1.8†</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of cats. Con, control group (n = 16); Alb, exchange transfusion with albumin solution at 30 min of middle cerebral artery occlusion (MCAO) (n = 16); Hb, exchange transfusion with hemoglobin solution at 30 min of MCAO (n = 15). P<0.05 from the baseline value; †P<0.05 from the control group; ‡P<0.05 from the albumin group.

In cortical gray matter, ipsilateral blood flow was equivalent among groups at 20 min of MCAO (Fig. 3). After induction of hypertension, blood flow increased slightly in the control group but remained below baseline values. Transfusion with the albumin solution combined with moderate hypertension increased ipsilateral cortical blood flow by 82% and restored flow to baseline levels in the contralateral cortex, flow increased by 110%. Transfusion with the hemoglobin solution also restored ipsilateral cortical blood flow and produced a greater blood flow compared with the control group, although the effect was not significant until 240 min of MCAO. In the contralateral cortex, blood flow was increased by 110%. Transfusion with the hemoglobin solution also restored ipsilateral cortical blood flow and produced a greater blood flow compared with the control group, although the effect was not significant until 240 min of MCAO.

### Table 2. Somatosensory-evoked potential amplitude during 360 min of MCAO

<table>
<thead>
<tr>
<th>Group</th>
<th>20 min</th>
<th>120 min</th>
<th>240 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ipsilateral Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1±1</td>
<td>1±1</td>
<td>1±1</td>
<td>1±1</td>
</tr>
<tr>
<td>Alb</td>
<td>1±2</td>
<td>2±2</td>
<td>2±5</td>
<td>1±2</td>
</tr>
<tr>
<td>Hb</td>
<td>3±7</td>
<td>1±2</td>
<td>1±3</td>
<td>1±1</td>
</tr>
<tr>
<td><strong>Contralateral Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>96±21</td>
<td>100±19</td>
<td>100±42</td>
<td>106±38</td>
</tr>
<tr>
<td>Alb</td>
<td>100±17</td>
<td>87±28</td>
<td>83±28</td>
<td>90±31</td>
</tr>
<tr>
<td>Hb</td>
<td>96±20</td>
<td>97±29</td>
<td>86±30</td>
<td>91±29</td>
</tr>
</tbody>
</table>

Values are means ± SD. Somatosensory-evoked potential amplitudes are shown as a percentage of baseline.

Fig. 1. Time course of mean arterial blood pressure before and during 360 min of middle cerebral artery (MCA) occlusion (MCAO) in the control group and in groups undergoing albumin or hemoglobin exchange transfusion between 30 and 45 min of MCAO. At 40 min of MCAO, an aortic balloon was inflated in the descending aorta to increase arterial pressure for the remainder of the experiment. There were no differences among groups.
flow in the hemoglobin group was similar to that in the control group and less than that in the albumin group at 120 and 240 min of MCAO.

For the 12 coronal sections, cortical blood flow was analyzed separately for the four anterior, middle, and posterior coronal sections. The middle four sections were subdivided further into the superior parietal, lateral temporal-parietal, and inferior temporal cortex. In the superior parietal cortex, the blood flow changes were similar to those seen in the total ipsilateral cortex (Fig. 4). In both the lateral temporal-parietal cortex and inferior temporal cortex, albumin transfusion restored blood flow to preischemic levels, although the values were variable and not significantly different from those in the control group during MCAO (Fig. 4).

With hemoglobin transfusion, blood flow in both these regions increased above preischemic baseline by 240 min of MCAO and the values were greater than those in the control group. Unexpectedly, blood flow was not significantly reduced at 20 min of MCAO before albumin or hemoglobin transfusion in the lateral temporal-parietal cortex or before hemoglobin transfusion in the inferior temporal cortex.

In anterior and posterior gray matter, the blood flow pattern was similar to that of total ipsilateral gray matter; albumin transfusion increased blood flow above that of the control group, and hemoglobin transfusion produced a delayed increase in flow (Fig. 5). In the ipsilateral hippocampus, blood flow was not severely reduced by MCAO and flow was augmented by albumin transfusion (Fig. 5).

Oxygen transport, calculated as $CaO_2 \div CBF$, was not different between the control and albumin groups in the ipsilateral MCA distribution cortex (Fig. 6) despite the greater CBF in the albumin group. Oxygen transport was not significantly decreased in the hemoglobin group at 20 min of MCAO. However, oxygen transport increased above those in the control and albumin groups at 240 min of MCAO. In the contralateral MCA distribution cortex, oxygen transport was similar among groups (Fig. 6).

**Brain injury.** The total volume of injury in the ipsilateral hemisphere (gray plus white matter) measured by TTC staining at 6 h of permanent MCAO was not different among groups (Fig. 7). Much of the injury was present in the caudate nucleus and lateral and inferior cerebral cortex. However, there was no difference in the volume of injury among groups in either the superior parietal cortex, lateral temporal-parietal cortex, inferior temporal cortex, or caudate nucleus, whether expressed in units of absolute volume or as a percentage of the volume of each region. No cat in either group demonstrated injury in the right caudate nucleus or right hemisphere.

**DISCUSSION**

The present study describes the time course of changes in regional CBF in the cat during 6 h of

![Fig. 2. Blood flow (means ± SD) to the caudate nucleus ipsilateral (A) and contralateral (B) to MCAO in the control group and in groups transfused with albumin or hemoglobin solutions at 30 min of MCAO. *P < 0.05 from the within-group preischemic baseline; †P < 0.05 from the control group; ‡P < 0.05 from the albumin group; §P < 0.05 from the within-group value at 20 min of MCAO (before transfusion or aortic balloon inflation).](image)

![Fig. 3. Blood flow (means ± SD) to cortical gray matter in the whole hemisphere ipsilateral (A) and contralateral (B) to MCAO in the control group and in groups transfused with albumin or hemoglobin solutions at 30 min of MCAO. *P < 0.05 from the within-group preischemic baseline; †P < 0.05 from the control group; ‡P < 0.05 from the albumin group; §P < 0.05 from the within-group value at 20 min of MCAO (before transfusion or aortic balloon inflation).](image)
permanent MCAO when an isovolumetric exchange transfusion with a solution of albumin or tetrameric cross-linked hemoglobin is performed 30 min after MCAO. Moderate hypertension (\(\text{MAP} = 110 \pm 20 \text{ mmHg}\)) was induced in the control and albumin groups, and the time course of changes in MAP was well-matched to that produced after hemoglobin transfusion. The major findings are 1) albumin and hemoglobin transfusion failed to substantially increase CBF in the caudate nucleus, an end-artery region in the ischemic core; 2) albumin transfusion increased CBF in the ischemic cortex, but the increase was insufficient to increase bulk \(\text{O}_2\) transport or reduce the volume of acute ischemic injury; and 3) hemoglobin transfusion increased both CBF and \(\text{O}_2\) transport in ischemic cortex, but the increase was delayed to 240 min of MCAO and failed to significantly reduce injury volume.

In nonischemic regions, CBF increased after albumin transfusion, such that bulk \(\text{O}_2\) transport remained constant. These results agree with other studies in nonischemic rats and cats in which hemotocrit was reduced but decreases in the \(\text{O}_2\)-carrying capacity were attenuated by plasma-based hemoglobin. For example, the percent increase in CBF was found to be approximately equal to the percent decrease in \(\text{CaO}_2\) after exchange transfusion with either albumin or hemoglobin transfusion \((10, 45)\) even when hypoxic hypoxia was produced after transfusion \((46)\) or when plasma viscosity was increased at a hematocrit below 3% \((50)\). Consequently, cerebral \(\text{O}_2\) transport appears to be reg-
ulated effectively by adjustments in vascular hindrance when changes in viscosity are imposed. Constriction of pial arterioles after transfusion of a solution of the same cross-linked hemoglobin used in the present study supports the concept of an active regulation of cerebral O2 transport (2). This arteriolar constriction is probably not due to scavenging of nitric oxide because nitric oxide-dependent dilation of pial arterioles to acetylcholine and ADP was unaltered after hemoglobin transfusion (2).

In regions with severe ischemia, vasodilation occurs at the onset of ischemia and is expected to limit further dilation when hematocrit and CaO2 are reduced. Thus blood flow in ischemic regions is thought to become passively dependent on viscosity and perfusion pressure (10). In the ischemic caudate nucleus, however, CBF was not significantly augmented after hemoglobin transfusion and only a slight increase occurred after albumin transfusion or with hypertension in the control group. The lack of a large increase in perfusion is presumably related to the caudate nucleus being an end-artery region with few collateral vessels. A portion of the caudate is perfused via the anterior cerebral artery, accounting for a small portion of viable tissue in TTC-stained sections. For the microsphere measurements of caudate nucleus blood flow, the viable and nonviable portions were pooled because of the small tissue volume. Thus the slight increase in caudate blood flow seen in the control and albumin groups may have been localized in the nonischemic portion of the caudate nucleus.

In contrast to the caudate nucleus, CBF in ischemic cortical gray matter was restored to preischemic baseline values after albumin transfusion. The decrease in viscosity and moderate hypertension are presumed to have caused a passive increase in collateral blood flow. However, the increased CBF was inadequate for augmenting cerebral O2 transport above that of the control group. The lack of improved O2 transport after albumin transfusion may account for the lack of reduced injury volume. Hypervolemic hemodilution with albumin may be required to augment O2 transport sufficient for ameliorating injury.

After the hemoglobin solution was transfused, CBF in ischemic gray matter was increased to values above preischemic baseline and cerebral O2 transport was restored. Thus an amelioration of acute injury volume was anticipated. However, the improvement in CBF occurred between 120 and 240 min of MCAO. This improvement may have occurred too late to reduce the acute injury volume. The reason why CBF did not increase more at 120 min is unclear. Transfusion of DCLHb increases the plasma concentration of endothelin-1 in rats (21, 22) and in acute stroke patients (36). Endothelin antagonists can increase intracerebral CBF and reduce infarct volume in some models of
MCAO without hemoglobin transfusion (32, 33, 42). Thus it is possible that endothelin initially limits increased CBF after hemoglobin transfusion in ischemic regions. Because clinical trials of DCLHb in acute stroke (35) and traumatic hemorrhagic shock (38) were halted because of safety concerns, understanding the potential role of endothelin in the vascular response to ischemia may be important.

The DCLHb used in clinical trials and the sebacoyl cross-linked hemoglobin used in the present study are similar in that they are both tetramers stabilized by covalent intramolecular cross-links to prevent dimerization but differ in their sites of cross-linking. The DCLHb is cross-linked between the two 99-lysines on the α-subunits by a four-carbon chain fumaryl cross-linker (39). The sebacoyl cross-linked hemoglobin is cross-linked between the two 82-lysines in the β-subunits by the 10-carbon chain sebacoyl cross-linker, confirmed by both peptide mapping and crystallographic analysis (7). In addition, about one-half of the α99-lysines on the two α-subunits are cross-linked (8). Molecular modeling of the β82-lysine cross-linking site indicated that the 10-carbon cross-linker fits well with some degree of freedom between the β82-lysines in the deoxygenated state but becomes sterically restricted in the oxygenated state when the β82-lysines move closer together (7). A negative cooperativity between the first and second step of oxygenation results in a much lower O2 affinity (P50 = 34 mmHg) than native hemoglobin without 2,3-diphosphoglycerate. The P50 is slightly greater than the 29- to 30-mmHg values reported for DCLHb and overall cooperativity is similar (16, 39, 48).

The sebacoyl cross-linked hemoglobin is highly purified by the use of affinity chromatography, has a low rate of autoxidation, and has a normal Bohr factor (8).

The effect of DCLHb transfusion on CBF and injury volume after MCAO has been investigated in the spontaneously hypertensive rat by Cole et al., who observed early increases in CBF (10, 16) and decreased injury volume both acutely (14, 15) and at 72 h (11, 12). Our observations in the cat with sebacoyl cross-linked hemoglobin differ from these results in the rat with fumaryl cross-linked hemoglobin in that the increase in CBF in the cat was delayed and no decrease in injury volume was detected. Several differences in experimental design may contribute to differences in the results and could prove instructive for the design and interpretation of clinical studies. First, the transfusion in rats was performed before MCAO in the acute survival studies or at the onset of MCAO in the chronic survival studies, whereas the transfusion commenced at 30 min of MCAO in our study. Increasing CBF with hemoglobin transfusion at 30 min of MCAO may be less effective if cells are already energy depleted and swollen and if the endothelin concentration is already increased (3, 32).

Second, hypervolemic transfusion was usually used in the studies on rats, whereas an isovolumetric exchange transfusion was used in the present study. Although normovolemic hemoglobin transfusion can decrease injury volume in the spontaneously hypertensive rat, the decrease was less than that obtained with hypervolemic transfusion and the associated hypertension (14). In rats without spontaneous hypertension, hypervolemic DCLHb transfusion at 15 min of MCAO enhances tolerance to more prolonged transient ischemia (1). However, the effect of DCLHb-induced hypertension was not controlled in this study. In the cat, the benefit of normovolemic hemoglobin transfusion may have been small and masked by the greater variability in infarct volume in the cat compared with the ischemia models in the rat. In addition, we elected to increase MAP in the control and albumin groups to match that occurring after hemoglobin transfusion to determine if a benefit of hemoglobin transfusion on O2 transport was independent of hypertension. This moderate hypertension tended to increase regional CBF in the control group. Likewise, in the albumin group, ipsilateral and contralateral cortical blood flow increased 82–110% with moderate hypertension compared with intraischemic blood flow before transfusion. This percent increase is greater than the −50% increase in CBF previously reported in the cat without induced hypertension (25, 45, 46). Thus the moderate hypertension used in the present study appears to have augmented blood flow and may have reduced any potential differences in injury volume in the control and albumin groups from that in the hemoglobin group.

Another consideration in the use of induced hypertension in the control and albumin groups is that the segmental arteriolar responses may be different from those with hypertension associated with hemoglobin transfusion. Induced hypertension in the cat produces selective constriction of large pial arteries (30), whereas exchange transfusion with sebacoyl cross-linked hemoglobin produces constriction of both small and large pial arterioles (2). Constriction of small arterioles in the presence of decreased blood viscosity could conceivably result in a greater maldistribution of red blood cells among capillaries and a decrease in functional capillary density (27, 43). If plasma flow as well as red blood cell flux were reduced in a subset of individual capillaries, the benefit of a plasma-based O2 carrier could be mitigated. It is also possible that the use of aortic balloon inflation in control and albumin-transfused cats would produce different neurohumoral effects on the cerebral vasculature than hypervolemia. However, the degree of aortic balloon inflation used in the present study did not produce profound renal or splanchnic ischemia.

Third, hematocrit was decreased from 30% to 18% in the cat. This 40% decrease in hematocrit produces about a 25% decrease in blood viscosity (45). Cole et al. (14–16) found greater effects on viscosity, CBF, and injury volume in the rat when hematocrit was reduced from 44% to 16% or 9% with hemoglobin transfusion than when reduced to 30%. Thus larger decreases in hematocrit may be required in the cat to produce increases in CBF sufficient for reducing infarct volume. In addition, the rheological effects of reducing hematocrit on the microcirculation may be different in the rat.
with a baseline hematocrit of 44% than in the cat with a baseline hematocrit of 30%.

Fourth, the concentration of hemoglobin in the infusate was 6 g/dl in the cat and 10 g/dl in the rat. Increasing the hemoglobin concentration from 10 g/dl (43 mmHg oncotic pressure) to 20 g/dl (129 mmHg oncotic pressure) produced a greater reduction in infarct volume in the rat (11). A 20 g/dl solution of albumin provides protection when administered at reperfusion (5). In our experiments, colloid osmotic pressure increased by only 2 mmHg after albumin and hemoglobin transfusion. Perhaps a hyperoncotic solution would have been more beneficial in the cat.

Fifth, studies showing reduced injury in the rat with DCLHb used transient focal ischemia (12, 14, 15), whereas we used permanent focal ischemia. Transfusion of DCLHb was ineffective in a model of permanent multifocal ischemia in the rabbit (6) or when the duration of transient focal ischemia was increased beyond 3 h (1). Thus permanent focal ischemia may be too severe for hemoglobin transfusion to be beneficial. Surprisingly, isovolemic hemodilution with dextran at 3 h of focal ischemia in the dog provided protection (51). However, cortical CBF is reduced by ~35% in this model (44), which is considerably less than the reduction seen in the present study. Less severe focal cerebral ischemia presumably acts to enhance the therapeutic window for hemodilution.

Another limitation of the present study is that injury volume was assessed at 6 h of MCAO, which is before the infarction process has fully matured. However, the delay in maturation of the TTC-determined injury volume is most profound when ischemic duration is <90 min (19). Prolonged recovery was not studied because mortality from brain swelling can be high with permanent MCAO in the cat. It is possible that enhanced O2 supply to the penumbra by hemoglobin would limit the progression of the infarction process beyond 6 h and that long-term injury would be reduced. Alternatively, hemoglobin transfusion may be beneficial only with less severe ischemia. We chose to study permanent MCAO so that the effects of transfusion on regional CBF could be studied over a prolonged period of stable ischemia rather than designing a study with less severe, brief ischemia in which long-term histopathology and behavior could be evaluated. It is possible that other short-term outcome measures, such as local tissue Po2 and ATP, would show more specific improvements in O2 delivery on a paired basis than TTC-determined injury volume, which exhibits considerable interanimal variability.

In summary, replacing red blood cell-based hemoglobin with plasma-based hemoglobin after the onset of MCAO in the cat promoted blood flow selectively in the ischemic cortex with little change in the caudate nucleus or in contralateral regions. However, augmentation of O2 transport was delayed unexpectedly until 240 min of MCAO and a decrease in acute injury volume could not be demonstrated. Perhaps newer generations of modified hemoglobin solutions employing higher O2-carrying capacity polymers or liposomal encapsulation, which produce less peripheral vasconstriction (34), will be more effective than tetrameric cross-linked hemoglobin.

The authors thank Y. Wu, K. Blizard, and J. Klaus for technical assistance.

This investigation was supported by National Institutes of Health Grants NS-38684 (to R. C. Koehler) and HL-48517 (to E. Bucci), by the Curt-Engelhorn-Stipendium, Ruprecht-Karls Universität, Heidelberg, Germany (to A. Rebel), and by the Deutsche Forschungsgemeinschaft (to A. Rebel).

REFERENCES


