Mechanisms of Edema Formation After Intracerebral Hemorrhage

Effects of Extravasated Red Blood Cells on Blood Flow and Blood-Brain Barrier Integrity

Guohua Xi, MD; Ya Hua, MD; R. Rick Bhasin, BS; Steven R. Ennis, PhD; Richard F. Keep, PhD; Julian T. Hoff, MD

Background and Purpose—Red blood cell (RBC) lysis contributes to brain edema formation after intracerebral hemorrhage (ICH), and RBC hemolysate (oxyhemoglobin) has been implicated to be a spasminogen in subarachnoid hemorrhage. Whether cerebral ischemia contributes to brain edema formation after ICH remains unclear, however. The aims of this study were to test whether extravasation of RBCs induces cerebral ischemia and/or blood-brain barrier disruption in a rat ICH model characterized by perihematomal brain edema.

Methods—In this study, 87 pentobarbital-anesthetized Sprague-Dawley rats were used. In each animal, saline, packed RBCs, or lysed RBCs were injected into the right caudate nucleus. Sham injections served as controls. Regional cerebral blood flow, brain water and ion contents, blood-brain barrier integrity, and plasma volume were measured.

Results—Intraparenchymal infusion of lysed RBCs caused severe brain edema by the first day but did not induce ischemic cerebral blood flows. In contrast, blood-brain barrier permeability increased during the first day after infusion of lysed RBCs (a 3-fold increase) and 3 days after infuison of packed RBCs (a 4-fold increase).

Conclusions—These results suggest that ischemia is not present at 24 or 72 hours after hematoma induction by injection of intact or lysed RBCs. RBC constituents that appear after delayed lysis, however, increase blood-brain barrier permeability, which contributes to edema formation. (Stroke. 2001;32:2932-2938.)

Key Words: cerebral hemorrhage ■ brain edema ■ cerebral blood flow ■ blood-brain barrier permeability

Brain edema formation after intracerebral hemorrhage (ICH) causes herniation-related death and severe neurological deficits. Although the mechanisms of edema formation after ICH are not fully determined, our previous study indicated that erythrocyte lysis and hemoglobin toxicity contribute to delayed brain edema. Thus, an intracerebral injection of packed red blood cells (RBCs) into the caudate nucleus in a rat causes brain edema formation 3 days later, when the cells lyse. If the RBCs are lysed before injection, brain edema forms within 24 hours. Hemolysate from erythrocyte lysis after subarachnoid hemorrhage contributes to cerebral vasospasm and ischemia. Whether hemolysate causes vasospasm and cerebral ischemia after ICH is unknown, however. Indeed, whether ischemia contributes to brain edema formation after ICH remains controversial. For example, we found that the anatomic site of an intracranial hematoma may determine whether or not cerebral blood flow (CBF) is affected. Hemoglobin and its degradation products (oxyhemoglobin, deoxyhemoglobin, or methemoglobin) can have adverse effects on the brain. Oxyhemoglobin is a spasminogen after subarachnoid hemorrhage. An intracortical injection of hemoglobin causes chronic focal spike activity and gliosis at the injection site. Hemoglobin activates lipid peroxidation and kills neurons in culture. In addition, oxyhemoglobin can induce apoptosis in cultured endothelial cells, and apoptosis has been found in ICH.

The aims of present study were to determine whether an intraparenchymal infusion of RBCs reduces perihematomal CBF and whether blood-brain barrier (BBB) disruption after the infusion contributes to brain edema formation.

Materials and Methods

Animal Preparation and Intracerebral Infusion

The protocols in this study were approved by the University of Michigan Committee on the Use and Care of Animals. Male Sprague-Dawley rats (n=87, Charles River Laboratories, Portage, Mich) weighing between 350 and 400 g were used in the experiments. Animals were anesthetized with pentobarbital (40 mg/kg IP). The right femoral artery was catheterized for blood pressure monitoring and blood sampling for ICH induction and blood gases. The rats were positioned in a stereotactic frame (Kopf Instruments), and a cranial burr hole (1.0 mm) was drilled in the skull (0.2 mm anterior, 5.5 mm ventral, 1.0 mm lateral to the bregma). Saline, packed RBCs,
or lysed RBCs were microinfused into the right caudate nucleus at a rate of 10 μL/min through a 26-gauge needle. Controls for needle insertion received no infusion. To prepare packed RBCs, blood (0.5 mL) was centrifuged at 14 000g for 2 minutes, and the plasma and buffy coat were discarded. The RBCs were then washed 3 times in saline to obtain packed RBCs (hematocrit = 86%). To prepare lysed RBCs, the packed cells were frozen in liquid nitrogen for 5 minutes and then allowed to thaw in containers immersed in water at room temperature.

**Experimental Groups**

This study was divided into 4 parts.

**Part 1.** Animals received an intracerebral infusion of either saline (20 μL, n=4), packed RBCs (50 μL, n=4), or lysed RBCs (20 μL, n=5). Brain water and sodium contents were measured 24 hours later.

**Part 2.** There were 3 sets of experiments in this part. In the first set, animals had either needle insertion only (sham, n=5) or infusion of lysed RBCs (20 μL, n=5). CBF was measured at 2 hours. In the second set, animals had either sham (n=5), infusion of packed RBCs (50 μL, n=5), or infusion of lysed RBCs (20 μL, n=6). CBF was measured 24 hours later. In the third set, animals received needle insertion (n=5) or infusion of packed RBCs (50 μL, n=5), with CBF measured at 72 hours.

**Part 3.** Animals received either needle insertion (n=10), packed RBCs (50 μL, n=11), or lysed RBCs (20 μL, n=5). BBB disruption was assessed by the permeability surface area product of [14C]aminoisobutyric acid (AIB). Measurements were made at 24 hours, except for 5 animals with packed RBCs and 5 animals with needle insertion only, which were measured at 72 hours.

**Part 4.** Two groups of rats had either an infusion of lysed RBCs (20 μL, n=5) or packed RBCs (50 μL, n=7). These were used to assess BBB integrity by the [3H]inulin method at 24 and 72 hours, respectively.

In our previous studies, we found that 50 μL of packed RBCs did not cause brain edema after 1 or 2 days but induced edema 3 days later. We used 20-μL injection volumes in this study rather than 50 μL of lysed RBCs because preliminary studies showed that 6 of 6 rats died within 24 hours after intracerebral infusion of 50 μL of lysed RBCs.

**Brain Water and Sodium Contents**

Rats were decapitated under deep pentobarbital anesthesia (60 mg/kg IP). The brains were immediately removed, and a 3-mm-thick coronal brain slice was cut (4 mm from the frontal pole). The slice was divided into 4 samples, ipsilateral and contralateral basal ganglia and ipsilateral and contralateral cortex. The cerebellum was also taken as control tissue distant from the site of injection. Tissue samples were weighed on an electronic analytical balance (model AE 100, Mettler Instrument Co) to the nearest 0.1 mg to obtain the wet weight (WW). The tissue was then dried at 100°C for >24 hours to determine the dry weight (DW). Tissue water contents (%) are calculated as [(WW–DW)/WW]×100. The dehydrated brain samples were digested in 1 mL of 1N nitric acid for 1 week. Sodium content was measured by flame photometry (Instrumental Laboratory, Inc.). Ion contents were expressed in microequivalents per gram of dehydrated brain tissue (μEq/g DW).

**CBF Measurement**

Rats were reanesthetized with pentobarbital, and CBF was determined. CBF was measured by the indicator fractionation technique with [14C]N-isopropyl-p-iodoamphetamine ([14C]IMP; American Radiolabeled Chemicals) as the blood flow marker. This method uses an intravenous bolus injection of the blood flow indicator and a continuous rate (0.25 mL/min) of blood withdrawal through a femoral artery catheter to obtain the integral of the arterial isotope concentration. The withdrawals were started 3 seconds before the intravenous injection of 3 μCi of [14C]IMP and 10 μCi of [3H]inulin, the latter used to measure cerebral plasma volume. Two minutes later, the animal was killed by decapitation, and blood withdrawal was stopped. The sample of withdrawn arterial blood was bleached with 30% H2O2 before addition of scintillation fluid and determination of radiochemical content with a Beckman 3801 liquid scintillation counter. The brain was sampled as described above for brain edema measurements and digested in methylbenzethonium hydroxide before counting.

Blood flow rates for the individual pieces of brain tissue were calculated with the following equation:

$$F_b/M_b = Q_s(T) \times F_s/Q_s(T) \times M_s$$

where $F_b$ is brain blood flow, $M_b$ is brain mass (g), $Q_s(T)$ is quantity of indicator in the tissue at time $T$, $F_s$ is rate of blood withdrawal from $t=0$ to $t=T$, and $Q_s(T)$ is quantity of indicator present in the withdrawal at time $T$. The CBF is expressed as mL · 100 g$^{-1}$ · min$^{-1}$.

**BBB Permeability**

BBB integrity was initially assessed with [3H]AIB. The permeability of the BBB to [3H]AIB was determined as described previously in our studies of cerebral ischemia and cerebral hemorrhage. [3H]AIB (25 μCi; American Radiolabeled Chemicals) was injected 10 minutes before the end of the experiment, and [3H]inulin (8 μCi; American Radiolabeled Chemicals) was given as a second injection 8 minutes later. Simultaneous with the [3H]AIB injection, a peristaltic pump was started, and blood was withdrawn from an arterial cannula. At the end of the experiment, a terminal plasma sample was obtained, and the rat was killed by decapitation. The entire contents of the arterial cannula were emptied, and an aliquot was pipetted for counting. Blood samples were digested in methylbenzethonium hydroxide, bleached with H2O2, and counted in an aqueous-based liquid scintillation cocktail. Brain was sampled according to the earlier description of water content measurement. Brain tissue was also digested in methylbenzethonium hydroxide and counted. The influx rate constant ($K_i$) for [3H]AIB was calculated from the following formula:

$$K_i = (C_{br} – (CPV \times C_i))/C_i \times dt$$

where $C_{br}$ is the counts per gram of brain, CPV is the plasma volume of the brain determined from the [3H]inulin space, $C_i$ is the terminal plasma concentration of [3H]AIB, and $\int C_{br} \cdot dt$ is the integral of the [3H]AIB plasma concentration ($C_{br}$) for the experiment. The latter was calculated from the radiosotope content of the continuously withdrawn arterial blood sample.

In the experiments with intracerebral infusion of lysed RBCs (24 hours) and packed RBCs (72 hours), it became evident that the degree of BBB disruption was such that there was some extravasation of [3H]inulin, our plasma volume marker, despite the short circulation time of 2 minutes. For these 2 groups, therefore, further experiments were undertaken to assess cerebral plasma volume more accurately and to assess the influx rate constant for inulin. These experiments followed the same format as those for [3H]AIB, except that [3H]inulin (50 μCi) was circulated for 10 minutes and [3H]inulin (10 μCi) was circulated for 20 seconds. Cerebral plasma volume was determined from the [3H]inulin space, and $K_i$ for [3H]inulin was determined from Equation 2.

**Statistical Analysis**

Data were analyzed by t test or ANOVA with a Scheffé’s multiple comparison test. Significance levels were measured at $p < 0.05$. Values were mean ± SD.

**Results**

For brain water content and BBB measurements, blood pH (7.48 ± 0.04), glucose (6.1 ± 1.0 mmol/L), hematocrit (40 ± 2%), $P_{O_2}$ (78 ± 5 mm Hg), $P_{CO_2}$ (42 ± 5 mm Hg), and blood pressure (112 ± 11 mm Hg) were in the normal range. During measurements of the CBF, there were no significant differences in the physiological parameters among any of the groups (Table 1).
Brain Water Content

Twenty-four hours after intracerebral infusion, brain water contents in the ipsilateral basal ganglia were 84.7 ± 0.2%, 78.7 ± 0.8%, and 79.1 ± 0.3% for the lysed RBCs, packed RBCs, and saline groups, respectively. The marked edema formation in the lysed RBC group (P < 0.01 versus other 2 groups) was associated with a marked (P < 0.01) increase in brain sodium content (sodium contents were 659 ± 102, 200 ± 14, and 229 ± 19 μEq/g DW for the lysed RBCs, packed RBCs, and saline groups, respectively). There were no differences in water or sodium contents in contralateral tissues or the cerebellum between the various groups of animals.

Cerebral Blood Flow

Although intracerebral infusion of lysed RBCs was associated with marked ipsilateral edema, it did not reduce CBF to levels expected to cause ischemic brain damage in the ipsilateral basal ganglia or cortex (Figure 1). Blood flows in the ipsilateral basal ganglia were 53 ± 7, 49 ± 3, and 37 ± 17 mL · 100 g⁻¹ · min⁻¹ in the sham, packed RBC, and lysed RBC groups, respectively, 24 hours after intracerebral infusion. CBF in the ipsilateral basal ganglia was decreased significantly (P < 0.05) at 2 hours after infusion of lysed RBCs, but this reduction was again modest (45 ± 7 versus 62 ± 12 mL · 100 g⁻¹ · min⁻¹ in shams). To test lysis of RBCs in vivo, packed RBCs were injected into the right basal ganglia, and CBF levels were measured 72 hours later. Packed RBCs failed to reduce CBF at 72 hours in either the ipsilateral basal ganglia (64 ± 24 versus 55 ± 10 mL · 100 g⁻¹ · min⁻¹ in sham control) or the ipsilateral cortex (72 ± 26 versus 54 ± 9 mL · 100 g⁻¹ · min⁻¹ in sham control) (Figure 2).

BBB Permeability

Initial experiments indicated that an infusion of lysed RBCs resulted in a marked increase at 24 hours (P < 0.05 for all) in the [³H]AIB influx rate constant to 7.93 ± 1.87 μL · g⁻¹ · min⁻¹ in the perihematomal basal ganglia compared with 2.63 ± 0.50 μL · g⁻¹ · min⁻¹ in sham-operated rats or 1.96 ± 0.31 μL · g⁻³ · min⁻¹ in the contralateral hemisphere (Figure 3). There was also an increase in the ipsilateral cortex (Figure 3). In contrast, an infusion of packed RBCs did not cause an increase in the [³H]AIB influx rate constant at 24 hours in the ipsilateral basal ganglia or cortex (Figure 3).

Our [³H]AIB experiments used a 2-minute [¹⁴C]inulin space to correct for cerebral plasma volume. Examination of

<table>
<thead>
<tr>
<th>Time and Group</th>
<th>MABP, mm Hg</th>
<th>pH</th>
<th>PO₂, mm Hg</th>
<th>Pco₂, mm Hg</th>
<th>Hematocrit, %</th>
<th>Glucose, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>111 ± 11</td>
<td>7.44 ± 0.04</td>
<td>81 ± 10</td>
<td>46 ± 5</td>
<td>45 ± 2</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>Lysed RBCs</td>
<td>113 ± 12</td>
<td>7.46 ± 0.02</td>
<td>81 ± 12</td>
<td>45 ± 3</td>
<td>44 ± 2</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>1 day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>102 ± 13</td>
<td>7.44 ± 0.06</td>
<td>77 ± 3</td>
<td>48 ± 4</td>
<td>40 ± 1</td>
<td>6.1 ± 1.0</td>
</tr>
<tr>
<td>Packed RBCs</td>
<td>111 ± 5</td>
<td>7.48 ± 0.03</td>
<td>77 ± 3</td>
<td>45 ± 2</td>
<td>40 ± 1</td>
<td>5.8 ± 0.7</td>
</tr>
<tr>
<td>Lysed RBCs</td>
<td>103 ± 15</td>
<td>7.49 ± 0.04</td>
<td>79 ± 7</td>
<td>47 ± 4</td>
<td>41 ± 2</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>3 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>106 ± 16</td>
<td>7.49 ± 0.05</td>
<td>78 ± 4</td>
<td>46 ± 6</td>
<td>41 ± 3</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td>Packed RBCs</td>
<td>100 ± 8</td>
<td>7.45 ± 0.05</td>
<td>81 ± 7</td>
<td>47 ± 6</td>
<td>40 ± 3</td>
<td>5.8 ± 0.9</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial blood pressure. Values are expressed as mean ± SD.
the [14C]inulin space, however, indicated that this was much greater in the ipsilateral basal ganglia of the animals infused with lysed RBCs than in the contralateral tissue or the other 2 groups (Table 2), suggesting that there was BBB disruption allowing significant [14C]inulin leakage into the brain during the 2-minute circulation time. Therefore, a second set of experiments on inulin permeability was performed to assess BBB with cerebral plasma volume determined by use of [3H]inulin circulation for 20 seconds and [14C]inulin circulation for 10 minutes. In animals infused with lysed RBCs, contralateral cerebral plasma volume was not significantly different from those experiments that had used a 2-minute circulation time. The apparent plasma volume in the ipsilateral basal ganglia (and cortex) was greatly reduced (P<0.01), however, and was no longer different from the contralateral hemisphere (Table 2). The influx rate constant for [14C]inulin in these lysed RBC experiments was 0.774±0.442 μL·g⁻¹·min⁻¹ in the ipsilateral basal ganglia, 11-fold greater than in contralateral basal ganglia (0.069±0.057 μL·g⁻¹·min⁻¹; Figure 4A) and 3-fold higher in the ipsilateral cortex than in the contralateral cortex.

As with the BBB disruption found with lysed RBCs at 24 hours, this increase in [3H]AIB permeability to the ipsilateral side, P<0.05) and cortex (3.95±0.34 μL·g⁻¹·min⁻¹ (compared with 1.95±0.31 μL·g⁻¹·min⁻¹ when corrected with data from a 2-minute inulin space), whereas that in the contralateral basal ganglia was 1.94±0.34 μL·g⁻¹·min⁻¹ when corrected with data from a 2-minute inulin space), a 4-fold increase.

In contrast to the 24-hour results, an intracerebral injection of packed RBCs produced an increase in the influx rate constant for [3H]AIB after 72 hours in the ipsilateral basal ganglia (5.07±3.12 versus 1.34±0.49 μL·g⁻¹·min⁻¹ in the contralateral side, P<0.05) and cortex (3.95±1.34 versus 2.09±0.90 μL·g⁻¹·min⁻¹ in the contralateral side, P<0.05). As with the BBB disruption found with lysed RBCs at 24 hours, this increase in [3H]AIB Kᵢ was associated with an increase in the 2-minute [14C]inulin space in the ipsilateral basal ganglia and cortex compared with contralateral tissues (Table 2). Again, therefore, experiments were performed in which cerebral plasma volume was assessed with [3H]inulin circulated for 20 seconds and [14C]inulin was circulated for 10 minutes to correct BBB inulin permeability. There were no differences in the 20-second [3H]inulin spaces between the ipsilateral and contralateral tissues in these experiments.

**TABLE 2. Plasma Volumes**

<table>
<thead>
<tr>
<th></th>
<th>Ipsilateral Basal Ganglia</th>
<th>Ipsilateral Cortex</th>
<th>Contralateral Basal Ganglia</th>
<th>Contralateral Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>7.3±1.8</td>
<td>7.4±2.0</td>
<td>9.1±1.9</td>
<td>8.9±2.4</td>
</tr>
<tr>
<td>Packed RBCs</td>
<td>7.4±1.0</td>
<td>5.9±0.5</td>
<td>8.0±0.3</td>
<td>7.1±1.0</td>
</tr>
<tr>
<td>Lysed RBCs</td>
<td>14.4±2.7*</td>
<td>5.8±1.0</td>
<td>12.4±3.2*</td>
<td>8.2±1.7</td>
</tr>
<tr>
<td>(6.1±0.8)</td>
<td>(6.4±0.7)</td>
<td>(7.4±0.3)</td>
<td>(7.7±0.9)</td>
<td></td>
</tr>
<tr>
<td>72 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>6.2±0.7</td>
<td>6.0±0.6</td>
<td>8.5±1.2</td>
<td>8.2±1.1</td>
</tr>
<tr>
<td>Packed RBCs</td>
<td>10.6±4.7†</td>
<td>6.0±0.7</td>
<td>9.1±1.4</td>
<td>7.4±1.1</td>
</tr>
<tr>
<td>(6.1±0.6)</td>
<td>(6.3±0.8)</td>
<td>(8.1±0.9)</td>
<td>(8.4±0.9)</td>
<td></td>
</tr>
</tbody>
</table>

Plasma volumes (in μL/g) were calculated using a 2-minute circulation time for inulin except for the measurements in parentheses for lysed and packed RBCs, which were calculated using a 20-second circulation time. Values are mean±SD, n=5 to 7.

*P<0.05 vs packed RBCs and Sham.
†P<0.05 vs sham.
(Table 2), but there was a marked increase in the influx rate constant for $[^{14}\text{C}]$inulin in the ipsilateral basal ganglia and cortex (Figure 4B).

**Discussion**

Our present studies indicate that although an intracerebral infusion of RBC hemolysate induces marked edema, CBF in the vicinity remains close to normal. Rather than ischemic damage, it appears that the edema formation is related to marked BBB disruption from the toxic effect of lysed RBCs. Such disruption occurred in response to artificially lysed RBCs or natural lysis after the infusion of packed RBCs.

Whether ischemia contributes to brain edema formation after ICH and if so how much remains controversial. Experiments have shown that CBF adjacent to a hematoma decreases. Perihematomal CBF falls below 25 mL $\cdot$ 100 g$^{-1} \cdot$ min$^{-1}$, but the reduction lasts <10 minutes and returns to baseline within 3 hours. Previous work in our laboratory also showed that a 50% reduction of CBF develops around the hematoma only during the first hour and returns to control levels within 4 hours. Generally speaking, the CBF threshold for ischemic injury is 15 to 20 mL $\cdot$ 100 g$^{-1} \cdot$ min$^{-1}$.

These results indicate that critical levels and durations of hypoperfusion do not occur after experimental ICH and that perihematomal brain edema is not associated with cerebral ischemia, at least with hematomas of the size used in this study. Recently, Wagner et al. measured ATP and phosphocreatine levels in the perihematomal edema zone at 1, 3, 5, and 8 hours after ICH. Although severe brain edema was present around the hematoma at all time points, ATP levels remained in the normal range and brain phosphocreatine contents increased with time, indicating that an energy deficit was not present around the hematoma. Interestingly, Qureshi et al. reported that an ischemic penumbra is not present around the clot in a canine ICH study.

In humans, Mayer et al. using single photon emission computed tomography (SPECT), investigated perihematomal CBF. An early hypoperfusion zone was found around the hematoma, with resolution by 48 hours. Because this low-CBF zone was correlated with a perihematomal edema rim, they suggested that reperfusion injury might contribute to edema formation. CBF reduction was also reported by Tanaka et al. In a positron emission tomography (PET) study, however, Diringer et al. could not find secondary ischemic brain injury after ICH in 12 patients. Using diffusion-weighted MRI and proton magnetic resonance spectroscopic imaging, Carhuapoma et al. reported that widespread ischemia was not found around the hematoma. Most recently, Zazulia et al. measured perihematomal CBF, cerebral metabolic rate of oxygen, and oxygen extraction fraction in 19 ICH patients with PET. They found that both cerebral metabolic rate of oxygen and CBF were reduced in the perihematomal zone, resulting in reduced oxygen extraction fraction. Thus, an apparent reduction in CBF may result from reduced metabolism around the clot.

Some of the differences in CBF data between patients and animal models may be due to the absolute volume of the hematoma. First, the average hematoma size in humans is $\approx 30$ to 40 mL, whereas in the animal models, the hemorrhage volume is proportionately smaller. For example, a 50-$\mu$L clot in a rat or a 2-mL clot in a piglet corresponds to a 30-mL clot in a human. Second, measurement of the hematoma volume from CT scans is complex. Reports show that the hematocrit in the clot may be as high as 90% because of clot retraction. Hence, the initial hemorrhage volume may be greater than the hematoma size shown by CT.

The anatomic localization of an intracranial hematoma is a major factor affecting CBF. Patel et al. injected equal volumes of blood into the subdural space or into the caudate nucleus of rats. They measured CBF by $[^{14}\text{C}]$iodoantipyrine autoradiography, infarct volume by TTC staining, and brain edema. Subdural hemorrhage induced significant reductions in CBF, but ICH alone induced a modest reduction in CBF around hematoma. Ischemic brain injury was observed in the cerebral cortex after subdural hemorrhage, but minimal perihematomal infarct was detected after ICH. Although ICH did not cause marked brain ischemia, it did induce significant brain edema.

Although it is generally accepted that reductions in CBF of $>\approx 70\%$ are necessary to induce ischemic brain damage, some recent evidence suggests that more modest reductions ($\approx 60\%$) may cause delayed neuronal death. This may raise the question of whether the modest reduction (30%) in CBF with lysed erythrocytes might cause some brain injury. It should be noted, however, that infusion of lysed erythrocytes causes very marked edema formation (85% water content in the ipsilateral basal ganglia) within 24 hours. This edema is similar to the 85% water content that occurs in the core of the infarct after 24 hours of middle cerebral artery occlusion, where CBF is reduced by $\approx 80\%$. Thus, because of timing and magnitude, it is very unlikely that such lysate-induced edema could result from the observed change in CBF. It should also be noted that the study by Zazulia et al. on human ICH patients suggests that modest declines in CBF may reflect a decline in metabolism around the clot.

Rather than ischemia, perihematomal edema development may involve damage to the vascular endothelium. Iron is a potent catalyst of lipid peroxidation, and the release of iron (a breakdown product of hemoglobin) after erythrocyte lysis may contribute to BBB dysfunction. It has also been shown that oxyhemoglobin can induce apoptosis in cultured endothelial cells, possibly through free radical damage to the endothelial vessel wall tissue. The temporal pattern of edema formation after infusion of lysed or packed erythrocytes is similar to the pattern of BBB disruption. In our study, intracerebral infusion of lysed RBCs caused marked BBB disruption demonstrable by both $[^{1}\text{H}]$AIB (a 3-fold increase compared with sham) and $[^{14}\text{C}]$inulin. Infusion of packed RBCs also caused a marked disruption, but this occurred 72 hours later and not after 24 hours, corresponding to the time when lysis occurs in vivo. The variation in the degree of disruption was greater in the packed RBC experiments than in the studies using lysed RBCs. This may reflect the fact that the onset of lysis and the rate of lysis in the packed RBC experiments may vary between animals. Yang et al. examined $[^{1}\text{H}]$AIB permeability in the same rat ICH model as used in this study. Their studies extended only to 2 days after ICH. They found
progressive disruption with an \( \sim 2.5 \)-fold increase in \([^{3}H]\)AIB 2 days after injection of autologous blood into the caudate nucleus. Their findings may reflect the onset of erythrocyte lysis. Indeed, RBCs contribute only to delayed edema formation after ICH (>24 hours).

A second mechanism that might contribute to edema development after erythrocyte lysis is direct damage to neurons and astrocytes involved in maintaining extracellular homeostasis. Intracortical infusion of lysed blood but not unlysed blood induced strong expression of heat shock protein 70, a neuronal injury marker, in both ipsilateral and contralateral neocortex and hippocampus.\(^{30}\) Exposure of cultured rat spinal cord cells to hemoglobin produces concentration-dependent cell toxicity, which can be measured by lactate dehydrogenase release.\(^{8}\) Hemoglobin may induce brain injury via its degradation products, because our previous studies indicate that such products play a major role in the formation of brain edema.\(^{31}\)

Thrombin has been implicated in brain injury and edema formation after ICH.\(^{32–34}\) Most attention has focused on thrombin formation during clotting. The fact that erythrocyte lysis causes marked BBB disruption, even to large molecules such as inulin, however, suggests that there may be a significant entry of prothrombin into the brain after erythrocyte lysis. If activated factor X is present, either because of significant entry of prothrombin into the brain after erythrocyte lysis or through expression of factor X by brain parenchymal cells,\(^{35}\) an influx of prothrombin will result in the generation of thrombin within the brain, which may also induce edema formation.

Intracerebral injection of thrombin also causes BBB disruption. Infusion of 5 U of thrombin, a dose that causes marked cerebral edema, however, causes only an \( \sim 50\% \) increase in BBB \([^{3}H]\)AIB permeability, compared with the 300\% increase caused by lysed RBCs.\(^{15}\) In a time-course study of BBB disruption after ICH, Yang et al\(^{44}\) found only a modest increase in BBB at 12 and 24 hours but a more marked increase at 2 days. Their data may reflect the difference in the capacity of thrombin and erythrocyte lysis to disrupt the BBB. The increase in BBB \([^{3}H]\)AIB permeability after intracerebral infusion of lysed RBCs is also greater than that which occurs in focal cerebral ischemia, in which increases of 70\% to 100\% occur 24 hours after middle cerebral artery occlusion in the rat.\(^{14,36}\) Similarly, we found that BBB inulin permeability is increased by a factor of 3 at 6 hours after middle cerebral artery occlusion, compared with an 11-fold increase induced by lysed RBCs reported in this study.\(^{37}\)

Because the adverse effect of an ICH seems to result from a "toxic" effect of blood components on the brain, the question arises as to whether clot removal is the best therapeutic strategy, because it would result in the removal of all the toxic components. Six randomized trials have been carried out, but clot evacuation by surgery remains controversial.\(^{38–43}\) Recently, Wagner et al\(^{44}\) infused tissue plasminogen activator (tPA) into hematomas in a pig model at 3 hours after induction and aspirated the liquefied clots 1 hour later. Clot removal after tPA treatment resulted in a 72\% reduction in hematoma volume compared with unevacuated controls. Clot removal also reduced brain edema volume and BBB disruption and improved cerebral tissue pressure. Thus, early and complete hematoma removal, which prevents the contribution of erythrocyte lysis to an already edematous brain, may be beneficial.

We conclude that a cascade of events triggered by erythrocyte lysis is critical for the delayed development of edema after ICH. Cerebral ischemia does not play a significant role in edema formation after ICH. Rather, edema formation results from a toxic effect of RBCs and/or hemoglobin breakdown products, with BBB disruption contributing to increased cerebral water content in the region of the hemorrhage. Erythrocyte-induced BBB disruption and edema formation after ICH are delayed and should therefore be susceptible to therapeutic modification. Such therapies should focus on preventing hemoglobin-induced toxicity rather than ischemia.

Acknowledgments

This study was supported by grants NS-17760 (Dr Hoff), NS-34709 (Dr Keep), and NS-39066 (Dr Xi) from the National Institutes of Health.

References

2. Ropper AH, King RB. Intracranial pressure monitoring in comatose patients with cerebral hemorrhage. \( \text{Arch Neurol} \), 1984;41:725–728.
14. Menzies SA, Betz AL, Hoff JT. Contributions of ions and albumin to the formation and resolution of ischemic brain edema. \( \text{J Neurosurg} \). 1993;78:257–266.
Guohua Xi, Ya Hua, R. Rick Bhasin, Steven R. Ennis, Richard F. Keep and Julian T. Hoff

Stroke. 2001;32:2932-2938
doi: 10.1161/hs1201.099820

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/32/12/2932

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/