Hemodilution and Hypertension Effects on Cerebral Hemorrhage in Cerebral Ischemia in Rats

Daniel J. Cole, MD, John C. Drummond, MD, Thomas S. Ruta, MD, and Norman H. Peckham, MD

We determined the effect of hemodilution and hypertension on cerebral hemorrhage and brain injury in 32 rats subjected to 180 minutes of middle cerebral artery occlusion and 120 minutes of reperfusion. We divided the rats into four groups. In the control group (n=8) neither hematocrit nor blood pressure was manipulated during occlusion, in the hemodilution group (n=8) 5% albumin was administered to maintain a hematocrit of 30% during occlusion, in the hypertension group (n=8) mean arterial blood pressure was increased to 30 mm Hg above baseline during occlusion with phenylephrine, and in the hemodilution/hypertension group (n=8) albumin and phenylephrine were employed simultaneously during occlusion. We assessed the amount of cerebral hemorrhage (as concentration of extravasated hemoglobin) spectrophotometrically and the extent of ischemic injury (as percentage of the hemisphere with deficient staining) histochemically using 2,3,5-triphenyltetrazolium chloride. Mean±SD hemoglobin concentration in the hemisphere ipsilateral to the occlusion in the hemodilution/hypertension group (71±14 μg/g brain tissue) was significantly (p<0.05) greater than that in the hemodilution and hypertension groups (25±5 and 29±7 μg/g, respectively), hemoglobin concentrations in these two groups were in turn significantly (p<0.05) greater than that in the control group (2±3 μg/g). Mean±SD percentage of the ipsilateral hemisphere with deficient staining was significantly (p<0.05) less in the hypertension and hemodilution/hypertension groups (8±3% and 11±6%, respectively) than in the control and hemodilution groups (26±8% and 26±7%, respectively). Our results are consistent with the hypothesis that, although hypertension decreases ischemic brain injury, it increases the risk of intraparenchymal hemorrhage in the injured area. (Stroke 1990;21:1333-1339)

Recent evaluations of the ability of hemodilution and hypertension to ameliorate focal cerebral ischemia have produced inconsistent results.1-6 A plausible explanation for this discrepancy is that, despite improving cerebral blood flow (CBF), these therapies act to worsen outcome via secondary injury processes such as cerebral hemorrhage. Following an ischemic event, the hemorrhagic process depends on sudden exposure of the vasculature to the full impact of blood pressure during reperfusion.7-11 However, there is evidence that hemorrhage can also be caused by hypertensive episodes during ischemia, which increase perfusion to and intraluminal hydrostatic pressure within the ischemic vasculature.12-14 Accordingly, we were concerned that physiologic therapies that increase perfusion to an ischemic area might also increase the risk of cerebral hemorrhage. Our study was designed to evaluate the effect of hemodilution and hypertension on cerebral hemorrhage following temporary middle cerebral artery occlusion (MCAo) in rats.

Materials and Methods

Following approval by the Institutional Animal Research Committee, 37 male spontaneously hypertensive rats weighing 350-400 g were anesthetized with 1.44% end-tidal isoflurane, orotracheally intubated, and mechanically ventilated. The femoral vessels were cannulated, and a small subtemporal craniectomy was made. Arterial blood (<100 μl) was sampled at 30-minute intervals and analyzed for pH,
Paco2 and PaO2 using an IL-1306 pH blood gas analyzer (Instrumentation Laboratory, Lexington, Mass.). Serum glucose concentration and hematocrit were analyzed using a YSI Model 23-A glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio) and an IEC MB centrifuge microhematocrit (DAMON/IEC Division, Needham Heights, Mass.). Mean arterial blood pressure (MABP) was recorded at 15-minute intervals on a Gould full-scale transducer and TA 2000 recorder (Gould Inc., Cerritos, Calif.). Rectal temperature was servo-controlled at 37°C with a heating blanket. The left middle cerebral artery was occluded in two locations with 10-0 monofilament nylon suture for 180 minutes. The first location was just proximal to the lenticulostriate arterial branch and the second was distal to the intersection of the middle cerebral artery and the inferior cerebral vein.

For spectrophotometric and histochemical analysis, each of 32 rats was randomly assigned to one of four groups. In the control group (n=8) neither blood pressure nor hematocrit was manipulated during MCAo. In the hypervolemic hemodilution (H-H) group (n=8) 5% human albumin (Travenol Laboratories, Glendale, Calif.) was administered to maintain a hematocrit of 29-32% during MCAo. In the hypertension (HTN) group (n=8), MABP was increased to 30 mm Hg above baseline with a phenylephrine infusion during MCAo. In the hypervolemic hemodilution/hypertension (H-H/HTN) group (n=8), hemodilution was achieved as in the H-H group and MABP was increased as in the HTN group by the simultaneous administration of albumin and phenylephrine during MCAo. Hemodilution and/or hypertension were maintained only during the 180 minutes of MCAo, after which 120 minutes of reperfusion was allowed.

After 120 minutes of reperfusion the rats were decapitated, and the brains were analyzed for ischemic injury and cerebral hemorrhage. In brief, the vascular space was cleared by aortic perfusion at 150 mm Hg with 100 ml of 0.9% NaCl at 37°C before decapitation. The brains were removed from the skulls and coronally sectioned 4.0, 6.0, and 8.0 mm posterior to the frontal pole (Figure 1).

In pilot studies, we determined that cerebral hemorrhage occurred predominantly in the segment 4.0-6.0 mm from the frontal pole. This segment was divided sagittally, weighed, and immersed for 72 hours in 2 ml Darkebin’s reagent (Sigma Chemical Co., St. Louis, Mo.) per 100 mg brain tissue to extract hemoglobin. Optical density of the diluted solution was measured using a Hitachi 100-80 computerized spectrophotometer (San Jose, Calif.) set at 540 nm and compared with a linear regression curve determined from Darkebin’s reagent/hemoglobin standards to calculate the concentration of hemoglobin as micrograms per gram of brain tissue in that hemisphere. Cerebral hemorrhage was also appraised by image analysis, which entailed photographing (Kodak Ektachrome, tungsten 160 ASA; Rochester, N.Y.) the anterior and posterior surfaces of the 4.0-6.0 mm coronal segment before hemoglobin extraction. The photographs were analyzed using a Drexel/DUMAS image processing system to determine the area in which hemoglobin was extravasated as a percentage of the total area in that hemisphere.

The extent of ischemic injury was assessed histochemically using 2,3,5-triphenyltetrazolium chloride (TTC). The brain segments 0-4.0 and 6.0-8.0 mm from the frontal pole were immersed in 2% TTC at 37°C for 30 minutes. The posterior surface of the 0-4.0 mm brain segment and the anterior and posterior surfaces of the 6.0-8.0 mm brain segment were photographed with Kodak Ektachrome, tungsten 160 ASA color slide film (Rochester, N.Y.). The photographs were analyzed using a Drexel/DUMAS image processing system to determine the area with deficient TTC staining as a percentage of the total area in that hemisphere. All image analysis was performed by an independent observer who was blinded to the study.

For histologic analysis, the remaining five rats were prepared as those in the H-H/HTN group. After 180 minutes of MCAo and 120 minutes of reperfusion, the rats were perfused with 10% buffered formalin and the brains prepared for hematoxylin and eosin staining and examined for qualitative indicators of ischemic injury.

We compared physiologic variables, concentration of hemoglobin extravasated, area in which hemoglobin was extravasated, and extent of ischemic injury among the control, H-H, HTN, and H-H/HTN groups using analysis of variance and, as appropriate, t tests with Bonferroni’s correction. A probability
Table 1. Physiologic Variables of Rats Subjected to Temporary Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=8)</th>
<th>H-H (n=8)</th>
<th>HTN (n=8)</th>
<th>H-H/HTN (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (units)</td>
<td>7.41±0.03</td>
<td>7.42±0.02</td>
<td>7.42±0.03</td>
<td>7.43±0.03</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>149±11</td>
<td>144±14</td>
<td>142±25</td>
<td>147±18</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>40.2±1.8</td>
<td>39.0±1.3</td>
<td>39.2±1.5</td>
<td>38.9±2.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47±3</td>
<td>31±1</td>
<td>47±2</td>
<td>31±1*</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>130±3</td>
<td>129±2</td>
<td>159±2†</td>
<td>160±2†</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>128±33</td>
<td>119±21</td>
<td>131±26</td>
<td>113±25</td>
</tr>
<tr>
<td>Reperfusion (120 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH (units)</td>
<td>7.40±0.03</td>
<td>7.42±0.02</td>
<td>7.41±0.02</td>
<td>7.42±0.03</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>151±15</td>
<td>145±26</td>
<td>147±28</td>
<td>163±30</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>39.0±1.5</td>
<td>38.0±1.3</td>
<td>38.5±1.5</td>
<td>37.8±1.6</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>46±3</td>
<td>33±3</td>
<td>44±4</td>
<td>33±3*</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>127±5</td>
<td>129±9</td>
<td>125±6</td>
<td>126±5</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>130±18</td>
<td>124±13</td>
<td>122±23</td>
<td>111±33</td>
</tr>
</tbody>
</table>

H-H, hypervolemic hemodilution; HTN, hypertension; H-H/HTN, simultaneous hemodilution and hypertension; MABP, mean arterial blood pressure. Values are mean±SD. *p<0.05 different from control and HTN groups by analysis of variance. †p<0.05 different from control and H-H groups by analysis of variance.

value of <0.05 was considered to indicate a significant difference. Results are reported as mean±SD.

Results

Except for expected differences in MABP and hematocrit (Table 1), there were no significant differences among groups for the physiologic variables overall or at any time. The mean±SD phenylephrine doses required in the HTN and H-H/HTN groups were 14±5 and 13±4 µg/kg/min, respectively.

In the hemisphere ipsilateral to the MCAo, hemoglobin concentration in the H-H/HTN group was significantly greater than those in the H-H and HTN groups, which in turn were significantly greater than that in the control group (Table 2). In general, the area in which hemoglobin was extravasated (Figure 2) in the H-H/HTN group was significantly greater than those in the H-H and HTN groups, which in turn were greater than that in the control group (Table 3). There was no area in which hemoglobin was extravasated in the hemisphere contralateral to the MCAo (data not shown).

The extent of ischemic injury was significantly less in the HTN and H-H/HTN groups than in the control and H-H groups (Table 4, Figure 2). The histologic findings show the early stages of infarction and intraparenchymal hemorrhage (Figure 3). In the hemisphere ipsilateral to the MCAo, the neuropil was edematous and vacuolated. Most neurons were surrounded by a clear halo. In some areas the capillaries were congested with blood cells, whereas in most areas the capillaries were empty. Some capillaries were surrounded by extravasated blood cells forming small ball hemorrhages. Most neurons displayed condensation and shrinkage of the perikaryon. The nuclei were pyknotic, and the nucleoli were not visible. The glia were distorted and vacuolated. The hemorrhages were focal and localized in infarcted regions.

Discussion

Our results are consistent with the hypothesis that, although hemodilution/hypertensive therapy augments CBF and decreases brain injury, it may have

Table 2. Hemoglobin Concentration in Brain Segment 4.0–6.0 mm Posterior to Frontal Pole in Rats Subjected to Temporary Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Control (n=8)</th>
<th>H-H (n=8)</th>
<th>HTN (n=8)</th>
<th>H-H/HTN (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral</td>
<td>3.0±4.8</td>
<td>6.2±5.0</td>
<td>6.8±4.4</td>
<td>6.6±6.8</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>2.0±2.6</td>
<td>24.6±4.9*</td>
<td>28.5±7.2*</td>
<td>70.7±14.1†</td>
</tr>
</tbody>
</table>

H-H, hypervolemic hemodilution; HTN, hypertension; H-H/HTN, simultaneous hemodilution and hypertension. Values are mean±SD µg/g brain tissue. *p<0.05 different from control group by analysis of variance. †p<0.05 different from control, H-H, and HTN groups by analysis of variance.
detrimental effects on cerebral hemorrhage. Methodologic concerns that should be addressed include the potential for trapped blood in the vasculature following saline perfusion, resulting in overestimation of intraparenchymal hemorrhage. Microscopic analysis revealed that <5% of the cerebral blood was of capillary rather than intraparenchymal origin. This should not have accounted for the extensive differences among groups. A second concern is the use of TTC as a histochemical marker of ischemic injury. Because TTC defines injury in terms of mitochondrial oxidative enzyme function, there are inherent limitations to the interpretation of TTC data. In certain settings the failure of neurons to stain with TTC is reversible. Accordingly, our TTC results should be viewed as indicating ischemic injury, not certain infarction. A final concern is temperature. We measured rectal temperature and kept the craniectomy site as clean as possible. In pilot studies with this model, brain temperature was closely correlated with rectal temperature (differed by <0.3° C).

We did not anticipate that a treatment (hypertension) that reduces ischemic injury when applied while the blood–brain barrier is intact would aggravate blood–brain barrier leakage to hemoglobin during a subsequent period of normotensive reperfusion. We consider the following theories to be plausible explanations for the discrepancy between the reduction in the extent of ischemic injury and the increase in the amount of cerebral hemorrhage achieved by hypertension. One hypothesis is that any reduction in the amount of cerebral hemorrhage due to a decrease in the area of blood–brain barrier injury was minor compared with the increase in the amount of cerebral hemorrhage consequent to an increase in hydrostatic pressure within the injured area. This theory is supported by data demonstrating that hypertension can disrupt the blood–brain barrier via increased intraluminal hydrostatic pressure. This mechanism might explain the peculiar finding that hemodilution failed to decrease the extent of ischemic injury but increased the amount of cerebral hemorrhage. The only explanation we can construct is speculative and contends that in this model of cerebral ischemia,
hemodilution increased perfusion to the ischemic area enough to increase the intraluminal hydrostatic pressure and worsen hemorrhage but not enough to increase CBF above the injury threshold.

A second hypothesis related to the first is that phenylephrine increases intraluminal hydrostatic pressure in both the arterial and venular beds. The following exaggerated example reveals such an event. All other factors being equal, if a 30 mm Hg increase in perfusion pressure increases arterial and venular intraluminal pressure by 30 mm Hg, then no net increase in CBF nor decrease in brain injury should occur. However, transfer of hemoglobin across the blood-brain barrier could increase secondary to the increase in intraluminal pressure. There is evidence of the cerebral arterial/venule vasoconstrictive and pressure-enhancing properties of phenylephrine, although the relative contribution of phenylephrine to each vascular bed is not known. Accordingly, this hypothesis requires further study.

Finally, phenylephrine may directly affect vascular permeability. Raichle et al demonstrated that intraventricular administration of the α-adrenergic blocking agent phentolamine decreased cerebral vascular permeability and concluded that one mechanism governing cerebral vascular permeability is a central noradrenergic system. The degree to which this permeability was operative in our study is not known. The extent of cerebral hemorrhage may become of greater clinical importance with the evolution of this property was operative in our study is not known.

In summary, we evaluated the effect of hemodilution/hypertensive therapy increases the risk of cerebral hemorrhage; our study does not prove that the negative effects of such therapy are greater than its positive effects.

Acknowledgments

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References

4. Cole DJ, Drummond JC, Shapiro HM, Hertzog RE, Brauer FS: The effect of hypervolemic-hemodilution with and without induced hypertension on cerebral blood flow following middle

TABLE 3. Area in Which Hemoglobin Was Extravasated in Coronal Sections 4.0 and 6.0 mm Posterior to Frontal Pole in Rats Subjected to Temporary Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Section</th>
<th>Control (n=8)</th>
<th>H-H (n=8)</th>
<th>HTN (n=8)</th>
<th>H-H/HTN (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.0 mm</td>
<td>2.8±1.2</td>
<td>3.8±1.6</td>
<td>3.2±1.9</td>
<td>6.2±2.2*</td>
</tr>
<tr>
<td></td>
<td>6.0 mm</td>
<td>1.9±1.1</td>
<td>4.5±1.9†</td>
<td>4.8±1.9†</td>
<td>8.8±2.2*</td>
</tr>
</tbody>
</table>

H-H, hypervolemic hemodilution; HTN, hypertension; H-H/HTN, simultaneous hemodilution and hypertension. Values are mean±SD % of total area in the hemisphere.

*p<0.05 different from control, H-H, and HTN groups by analysis of variance.

†p<0.05 different from control group by analysis of variance.

TABLE 4. Extent of Ischemic Injury in Coronal Sections 4.0, 6.0, and 8.0 mm Posterior to Frontal Pole in Rats Subjected to Temporary Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Section</th>
<th>Control (n=8)</th>
<th>H-H (n=8)</th>
<th>HTN (n=8)</th>
<th>H-H/HTN (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.0 mm</td>
<td>35.3±9.0</td>
<td>35.5±9.2</td>
<td>25.1±7.6*</td>
<td>20.7±6.4*</td>
</tr>
<tr>
<td></td>
<td>6.0 mm</td>
<td>25.6±7.9</td>
<td>26.3±6.7</td>
<td>8.1±3.3*</td>
<td>11.3±5.8*</td>
</tr>
<tr>
<td></td>
<td>8.0 mm</td>
<td>25.4±5.2</td>
<td>22.8±5.3</td>
<td>4.7±2.9*</td>
<td>6.9±3.1*</td>
</tr>
</tbody>
</table>

H-H, hypervolemic hemodilution; HTN, hypertension; H-H/HTN, simultaneous hemodilution and hypertension. Values are mean±SD % of total area in the hemisphere.

*p<0.05 different from control and H-H groups by analysis of variance.


**KEY WORDS** cerebral hemorrhage • hemodilution • hypertension • rats
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