Local Cerebral Blood Flow and Glucose Metabolism in Hydrostatic Brain Edema

Hitoshi UMEZAWA, Katsuji SHIMA, Hiroo CHIGASAKI and Shozo ISHII*

Department of Neurosurgery, National Defense Medical College, Tokorozawa, Saitama; *Department of Neurosurgery, Juntendo University School of Medicine, Tokyo

Abstract

This study investigated the effect of hydrostatic pressure gradient on the cerebrovascular dynamics and metabolism during the development of brain edema. Hydrostatic brain edema was induced by bolus injection of autologous blood through the common carotid artery in Sprague-Dawley rats. Rats were divided into two groups, with craniectomy (Cr+ group) and without (Cr− group). Animals were sacrificed 0, 24, and 48 hours after hypertensive insult. Brain water content was determined by the gravimetric method. Regional cerebral blood flow and local cerebral glucose utilization were measured by the quantitative autoradiographic method using [14C]iodoantipyrine and [14C]deoxyglucose, respectively. Hypertensive insult produced multifocal lesions stained by Evans blue. Brains from the Cr− group showed a transient increase in water content and no significant change in cerebrovascular dynamics and metabolism. Brains from the Cr+ group showed a pronounced increase in water content which persisted 48 hours later. Misery perfusion was also observed 24 hours after the insult and the cerebrovascular dynamics and metabolism were significantly decreased after 48 hours. These results indicate that an increased hydrostatic pressure gradient enhances tissue damage and causes reopening of the blood-brain barrier.

Key words: brain edema, hydrostatic pressure gradient, cerebral glucose utilization, cerebral blood flow

Introduction

Brain edema can be generally classified as cytotoxic or vasogenic according to whether functional changes occur in the plasma membrane or in the permeability of the cerebral vasculature. These two factors are closely related to the formation and development of brain edema. Clinically, they may both be present in granulocytic edema.¹ The biomechanics of formation and spread of edema are closely related to changes in the hydrostatic pressure gradient caused by altered intracranial pressure (ICP) and intracranial arterial pressure.⁹

The present study investigated the effect of the hydrostatic factors, hypertensive insult and decompressive craniectomy, on the occurrence and development of brain edema. The hydrostatic pressure gradient caused by hypertension and a combination of hypertension and decompressive craniectomy were evaluated, as well as the effect on net movement of edema resulting in changes in cerebrovascular dynamics and metabolism.

Materials and Methods

Ninety-six adult Sprague-Dawley rats weighing 300-400 gm were initially anesthetized with halothane (0.5-2%), intubated, and artificially ventilated. The left common carotid and external carotid arteries, and femoral artery and vein were cannulated using an aseptic technique (Fig. 1). The animals were divided into two groups, with craniectomy (Cr+ group) and without (Cr− group). The area of craniectomy was between the coronal suture anteriorly, and the rhomboid suture posteriorly. The medial and lateral borders were the sagittal suture and the superior temporal line, respectively (Fig. 2).

A 20-gauge needle was inserted transdurally into the cisterna magna of 10 animals (5 animals from each of the Cr+ and Cr− groups). Systemic blood pressure, ICP (cisterna magna pressure), and infu-
Pressure were recorded with pressure transducers (P10EZ; Nihon Koden, Tokyo) throughout the experiments. The blood gas parameters such as pH, PaO2, and PaCO2 were controlled within the normal range. Hypertensive insult was caused by bolus injection of 2 ml of fresh autologous heparinized blood over 5-8 seconds via the left common carotid artery.

The Cr+ and Cr- groups were divided into six subgroups according to differences in bolus carotid injection and intravenous Evans blue injection. Subgroups 1 (n = 20) and 2 (n = 14) from the Cr+ group both received a single bolus carotid injection. Evans blue injection was given prior to the hypertensive insult in Subgroup 1 and 24 hours later in Subgroup 2. Subgroups 3-6 came from the Cr- group. Bolus carotid injection and Evans blue injection in Subgroups 3 (n = 20) and 4 (n = 12) were performed as for Subgroups 1 and 2, respectively. Subgroups 5 (n = 16) and 6 (n = 14) received dual bolus injection separated by an interval of 30 minutes. Evans blue injection was given prior to the bolus carotid injection in Subgroup 5 and 24 hours later in Subgroup 6 (Table 1). Rats in Subgroups 1, 3, and 5 were killed at 0, 24, and 48 hours after the insult and those in Subgroups 2, 4, and 6 were killed 48 hours later under deep anesthesia with 5% halothane.

The rats were deeply anesthetized and perfused transcardially with 10% formalin. The brains were removed and cut in 1 mm thick coronal sections. The extent of Evans blue extravasation was examined macroscopically, sections were then stained with HE or cresyl violet and observed under the light microscope. The brain water content was assessed by the gravimetric method using a bromobenzene-kerosene linear density column (r > 0.997) calibrated by a standard solution of Na2SO4. Fifty-two rats (Subgroup 1, n = 15; Subgroup 3, n = 15; Subgroup 5, n = 16; sham control, n = 6) were decapitated under deep anesthesia with pentobarbital, and the brains were rapidly removed and cut coronally in about 1 mm sections. Samples from the gray and white matter, approximately 1 mm in diameter, were used for measurement.

The regional cerebral blood flow (rCBF) and local cerebral glucose utilization (1CGU) were also determined. Thirty-two rats (Subgroup 1, n = 16; Subgroup 3, n = 13; sham control, n = 3) were provided with food and water ad libitum until the experiments. After cannulation, the rats were mounted on a lead brick and kept at least 2 hours to avoid the effect of anesthesia. The blood gas, blood pressure, and hematocrit were measured, and the body temper-

Table 1 Subdivision of the rats in this experiment

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Evans blue injection</th>
<th>Bolus carotid injection</th>
<th>No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr+ group</td>
<td>1 P</td>
<td>S</td>
<td>20</td>
</tr>
<tr>
<td>2 A</td>
<td>S</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>3 P</td>
<td>S</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4 A</td>
<td>S</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>5 P</td>
<td>D</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>6 A</td>
<td>D</td>
<td>14</td>
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</table>

P: prior to bolus carotid injection, A: 24 hours after bolus carotid injection, S: single injection, D: dual injection with an interval of 30 minutes.
ature was maintained at 37°C with a heating lamp. Any animals with physiological parameters clearly beyond the normal range were excluded. Blood sampling and infusion of iodo-[\textsuperscript{14}C]-antipyrine (100 μCi/kg) (American Radiolabeled Chemicals Inc., St. Louis, Mo., U.S.A.) at a constant rate began simultaneously with a duration of 30 seconds. After six blood samples were taken, the animals were decapitated, and the brains rapidly removed and frozen in 2-methylbutane, cooled to −50°C with dry ice, then coated with chilled embedding medium. The brains were cut into 20 μm sections in a cryostat at −22°C. The sections were mounted on glass coverslips, dried, and placed in an x-ray cassette with calibrated [\textsuperscript{14}C]methyl-methacrylate standards (49-966 nCi/gm) on x-ray film (SB-5; Kodak, Rochester, N.Y., U.S.A.) for at least 7 days. The optical densities of 56 individual cerebral structures were determined using a microdensitometer (PDS-15; Sakura, Tokyo). Plasma \textsuperscript{14}C radioactivity was measured immediately by a liquid scintillation spectrometer (TRI-CARB; Packard, Meriden, Conn.; U.S.A.). The rCBF was calculated using Kety’s equation\textsuperscript{7} modified by Sakurada et al.\textsuperscript{22}

Thirty-two rats (Subgroup 1, n = 14; Subgroup 3, n = 14; sham control, n = 4) were fasted for 12 hours, cannulated, and mounted as described above. 100 μCi/kg of 2-deoxy-D-[\textsuperscript{14}C]glucose (American Radiolabeled Chemicals Inc.) was infused for 30 seconds at a constant rate through a venous catheter. Blood samples were obtained regularly through an arterial catheter over 45 minutes. The plasma concentrations of [\textsuperscript{14}C]deoxyglucose and glucose were measured by a liquid scintillation spectrometer and a glucose analyzer (Beckman Corp., Fullerton, Cal., U.S.A.), respectively. After 45 minutes, the animals were decapitated, the brains were rapidly removed, and the procedures described above carried out. The ICGU was calculated according to Sokoloff et al.\textsuperscript{23} using the plasma concentration of [\textsuperscript{14}C]deoxyglucose, glucose, and [\textsuperscript{14}C] density of individual cerebral structures. The lumped constant was 0.483 and the rate constants K\textsubscript{r*}, K\textsubscript{2*}, and K\textsubscript{3*} were 0.189, 0.245, and 0.052 in gray matter, and 0.079, 0.133, and 0.020 in white matter, respectively. The value of the lumped constant is presumably close to normal when the rCBF values are above the ischemic threshold and the delivery of glucose is maintained.\textsuperscript{16}

In all experiments, rats with left common carotid arteries ligated were used as the sham control.

All variables in these experiments are presented as the mean ± SEM. Significance was tested using Student’s t-test.

**Results**

The bolus infusion pressure in the Cr\textsuperscript{−} and Cr\textsuperscript{+} groups was 395.4 ± 12.30 and 408.2 ± 10.5 mmHg, respectively, with no statistically significant difference. Extravasation of Evans blue was recognized in nearly all animals in the Subgroups 1 and 3. Opacification appeared as patchy spots over the frontoparietal cortex, predominantly in the watershed areas of the anterior and middle cerebral arteries and the thalamus, the caudate putamen complex, and the hippocampus. Twenty-four and 48 hours after the insult, the extravasation of Evans blue was more diffuse and the opacification was uniform, becoming more pronounced with time. The extravasated Evans blue spots were denser and more widely distributed in the Subgroup 1 than Subgroup 3 (Fig. 3). The Subgroup 4 showed no Evans blue extravasation. The Subgroup 2, however, demonstrated Evans blue spots in the parietal cortex but confined to the area posterior to the craniectomy portion (Fig. 4). The Subgroup 5 showed an intensity of Evans blue extravasation nearly the same as that in the Subgroup 1. In some cases, the watershed areas of the contralateral hemisphere were also intensely stained with Evans blue. Evans blue spots were recognized at the parietal cortex in the Subgroup 6 in the same manner as that of the Subgroup 2.

![Fig. 3 Macroscopic photographs, showing the extravasation of Evans blue 0 (upper), 24 (middle), and 48 hours (lower) after the single hypertensive insult. left: Subgroup 3, right: Subgroup 1.](image-url)
The ICP in the resting state of the Cr+ group was significantly lower than that of the Cr− group (p < 0.005). During the hypertensive insult, the ICP value of the Cr+ group was 9.0 ± 1.65 mmHg, while that of the Cr− group reached 22.1 ± 5.38 mmHg (p < 0.025). After the hypertensive insult, the same trend was observed (p < 0.005) (Table 2).

Figure 5 shows the change in brain water content. The specific gravity of brains in the Subgroup 3 transiently decreased immediately after the hypertensive insult in the frontal cortex, the caudate nucleus, and thalamus (p < 0.01 vs. sham control). After 24 and 48 hours, the values were not significantly different from that of the sham control. In contrast, in the brains from the Subgroup 1, the value of these portions decreased with time. At 48 hours after the insult, the increase in water content was greatest (p < 0.01 vs. sham control). In the Subgroup 5, the water content had peaked immediately after the insult in the frontal cortex and caudate nucleus (p < 0.01 vs. sham control and Subgroups 1 and 3) and this trend continued for up to 24 hours, then slightly decreased 48 hours later to become similar to those in the Subgroup 1 (p < 0.01 vs. sham control and Subgroup 3). The water content of the pons and the medulla oblongata were not significantly different from those of the sham control for up to 48 hours after the insult, in Subgroups 1, 3, and 5.

The rCBF of the Subgroup 3 did not significantly differ from that of the sham control in all portions examined. In contrast, the Subgroup 1 showed significantly decreased values immediately after the insult, to 45% of the sham control in the frontal cortex (p < 0.01 vs. sham control, p < 0.01 vs. Subgroup 3). The value then increased 24 hours later, to 71% of the sham control (p < 0.05 vs. sham control, p < 0.01 vs. Subgroup 3). This trend continued 48 hours later (p < 0.01 vs. sham control, p < 0.05 vs. Subgroup 3) (Fig. 6 left). In other portions of the cerebral cortex and caudate nucleus, the change in rCBF was similar to that in the front-

**Table 2** Systemic blood pressure (SBP) and ICP before, during, and after the hypertensive insult

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>SBP (mmHg)</th>
<th>ICP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td>Cr− group</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>127.4 ± 2.98</td>
<td>145.8 ± 6.24</td>
</tr>
<tr>
<td>Cr+ group</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>126.8 ± 6.03</td>
<td>130.4 ± 6.37</td>
</tr>
</tbody>
</table>

*p < 0.025 and **p < 0.005 vs. Cr− group.
tal cortex. The rCBF in other basal ganglia such as putamen, globus pallidus, or brainstem showed no significant difference.

There was a transient decrease in ICGU immediately after the insult in the cortex, a part of the diencephalon, and the basal ganglia in the Subgroup 3. After 24 and 48 hours, no significant change was found. In contrast, the Subgroup 1 showed a marked decrease in ICGU immediately after the insult in these portions, to 38% of the sham control value in the frontal cortex (p < 0.01 vs. sham control and Subgroup 3). After 24 hours, the values returned to the control level and no significant difference was found as compared with the Subgroup 3. Forty-eight hours later, a second decrease in ICGU occurred, to 68% of the control value (p < 0.05 vs. sham control) (Fig. 6 right). This trend was recognized in other cortical portions, the lenticular nucleus, and part of the diencephalon.

Discussion

Arterial hypertension is commonly used as a method for the reversible opening of the blood-brain barrier (BBB). Transient opening of the BBB to proteins occurs with a 60 mmHg or more increase in blood pressure. The mechanism of parenchymal extravasation of Evans blue albumin involves the opening of a tight junction by the forced dilatation of the vessels and/or increased pinocytic vesicles. The patchy appearance of the extravasated Evans blue presumably results from an uneven distribution of medial muscle fibers. After extravasation, these patchy spots become distributed widely and uniformly with time, possibly with bulk flow. The extravasated plasma component is probably hydrophilic or degrades into smaller sub-units which may contribute to the formation of brain edema.

The hydrostatic pressure gradient seems to be the major factor in determining both the degree of the initial insult and subsequent deterioration, especially during and after hypertensive insult. In a previous report from our laboratory, hydrostatic brain edema is initiated by the hydrostatic pressure gradient either between blood vessels and tissue or between areas with different tissue pressures. In this study, the greater hydrostatic pressure gradient introduced by dual hypertensive insult or a combination of craniectomy and hypertension caused a denser and widely distributed Evans blue extravasation than caused by hypertension alone. Furthermore, a significant increase in water content occurred immediately after the insult and persisted for up to 48 hours in the former, while there was only a transient increase in water content in the latter. The development of brain edema was clearly different in the craniectomy followed by hypertensive insult or dual hypertensive insult group. Edema tended to

Fig. 6  rCBF (left) and ICGU (right) of the frontal cortex in the Subgroups 3 (○) and 1 (●) after hypertension. Numerals in parentheses indicate no. of animals. *p < 0.05 and **p < 0.01 vs. sham-operated group, †p < 0.05 and ‡p < 0.01 vs. Subgroup 3.
resolve as compared with the previous state in the dual hypertensive group.

In contrast, the craniectomy and hypertensive insult group showed the most pronounced increase in water content at 48 hours post-insult. Kogure et al. reported that protein leakage is accompanied by an equal bidirectional water movement, and that the extravasation of macromolecules per se does not cause brain edema while cerebral metabolism remains normal. Metabolic impairment may be related to the edematous process and tissue pressure changes derived from the hydrostatic factors.

After craniectomy, the pressure of the cranietomized portion is nearly equal to atmospheric pressure, so the tissue pressure gradient, once developed, is amplified and lasts longer. Kontos et al. noted that acute severe hypertension induced by angiotensin or norepinephrine caused a vasodilatation lasting at least 4 hours, and morphological and metabolic damage of the arteriolar endothelium. The prolonged tissue pressure gradient, either between cranietomized and non-cranietomized hemispheres, or between well-perfused and watershed areas, may be the major driving force (bulk flow) causing the extravasation of plasma-like fluid. This accounts for the observation of Evans blue extravasation at watershed areas of the anterior and middle cerebral arteries which became more diffusely and uniformly opacified with time.

Previous studies have shown that decompressive craniectomy causes a decrease in cortical tissue pressure and extravasation of plasma-like edema fluid from the microvasculature in the boundary zone of the decompressive area. Kontos et al. noted that acute severe hypertension induced by angiotensin or norepinephrine caused a vasodilatation lasting at least 4 hours, and morphological and metabolic damage of the arteriolar endothelium. In our model, with an amplified hydrostatic pressure gradient, the damaged vascular bed becomes leaky, resulting in profound cerebral edema. The microvasculature may be secondarily compromised with accumulated edema fluid and microcirculation impaired due to the direct compression of the vasculature, reducing cerebral metabolism in the acute phase. Misery perfusion occurred in the craniectomy and hypertensive insult group 24 hours after the insult. The mechanism remains obscure, but an inadequate circulation due to the edema and adrenergic reaction presumably enhances the anaerobic cerebral metabolism and possibly lactic acidosis.

Kuroiwa et al. reported that a unilateral hypertensive insult with a bolus carotid injection of autologous blood in rabbits induced a pure form of vasogenic edema, with no evident tissue damage. However, no information was obtained about chronological changes in the cerebral structures. Sokrab et al. caused a transient hypertensive insult by clamping the upper abdominal aorta in rats, resulting in permanent tissue damage 7 days from the onset, characterized by disintegration of neurons and proliferation of astrocytes. The pathological mechanism of the irreversible nerve cell injury was related to extravasation of plasma constituents. Extravasation of macromolecules in an induced hypertension model resulted in the main structural changes in cerebral tissue being increased permeability in both arterioles and capillaries, a moderate increase in pinocytic vesicles, dilatation of the arteriolar perivascular space with filling of amorphous exudate, and swelling of astrocyte and capillary endothelium. Presumably, these structural changes alter the microscopic environment of the essentially normal brain and lead to a relatively ischemic state and resultant metabolic damage.

In our study, both rCBF and ICGU were reduced in the craniectomy and hypertensive insult group and the spongy state of cerebral structures was apparent, mainly in that group and the dual infusion group. Under these conditions, re-opening of the BBB was recognized at the occipital part of the arterial boundary zone, and was confined to the area behind the craniectomized portion. Kuroiwa et al. noted biphasic opening of the BBB in cat brain where the rCBF fell below 15 ml/100 gm/min during transient middle cerebral artery occlusion. A marked reactive hyperemia after recirculation was also evident. They postulated that the initial BBB opening was induced by hydrodynamic force, due to reactive hyperemia, and the second BBB opening was induced by factors derived from the severely damaged tissue. In our experiments, the initial BBB opening was caused by the hydrostatic pressure alone and the induced edema was hydrostatic in nature.

This hydrostatic edema resulted in derangement of cerebral microcirculation and successive metabolic damage, presumably anaerobic glycolysis. The edema was prolonged or aggravated and accompanied by cytotoxic factors. Thus, a cycle of developing edema may occur in brain tissue when both cerebrovascular dynamics and metabolism are impaired. Hatashita et al. reported that tissue pressure was lowest at the occipital part of the arterial boundary zone after craniectomy, indicating that the hydrostatic pressure gradient is highest in this region. With the morphological and metabolic damage of the vascular wall, the second BBB opening was elicited by the amplified hydrostatic pressure gradient, demonstrated by patchy Evans blue spots in the parieto-occipital cortex, similar to those seen.
in vasogenic edema.

References

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Address reprint requests to: H. Umezawa, M.D., Department of Neurosurgery, National Defense Medical College, 3–2 Namiki, Tokorozawa, Saitama 359, Japan.