Effect of Hypertension on Blood-Brain Barrier Change after Restoration of Blood Flow in Post-Ischemic Gerbil Brains

An Electronmicroscopic Study

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SUMMARY The effect of induced hypertension on the blood-brain barrier (BBB) change in Mongolian gerbils exposed to various periods of ischemia was studied. Evans blue dye was used to determine the BBB change in animals subjected to different levels of hypertension after 3 h ischemia. Horseradish peroxidase (HRP) was used in electronmicroscopic studies of animals subjected to 30 min, 1, 3 or 6 h ischemia and subsequently exposed for 30 min to varying periods and sequences of normo- and hypertension. Furthermore, HRP-labeled vesicle counts were performed in animals from the 30-min ischemia group.

Our findings revealed that hypertension, after blood flow restoration following ischemia, induces and/or accelerates BBB damage by enhancing endothelial vesicular and/or tubulo-channel transport.

WHETHER AN INCREASE of blood pressure has a beneficial effect on the ischemic brain is still a matter of debate in clinical studies.1,2 The ischemic brain has been reported to be highly resistant to blood-brain barrier (BBB) change which induces vasogenic edema.3 However, it has been reported that a BBB change can occur after restoration of the blood flow to the ischemic brain.4-10 The present study was undertaken to determine how a slight change in blood pressure after blood flow restoration might affect the BBB after ischemia, and to elucidate the ultrastructural mechanisms of this BBB change.

The results of this line of inquiry may be of clinical importance in managing patients after the surgical restoration of blood flow to ischemic brain, and in the treatment of cerebral apoplexy without angiographic evidence of vascular obstruction11,12 and/or with evidence of rich collateral circulation.

Materials and Methods

Ischemia was produced in adult Mongolian gerbils of either sex under light ether anesthesia by clipping the left carotid artery with Scovill's aneurysmal clip. Polyethylene tubing (PE 10, Clay-Adams, USA) was inserted through the femoral artery to the abdominal aorta of all ischemia-sensitive animals, selected according to previously described criteria.13-15 Systemic arterial blood pressure was monitored continuously on a Statham transducer. Under pentobarbital anesthesia (1 mg/100 g body weight, i.p.), 0.1-0.5 mg/kg body weight metaraminol bitartrate was repeatedly injected i.v. to maintain the mean arterial blood pressure (MABP) at different levels of elevation. In each animal, MABP was measured at 5 min intervals after blood flow restoration.

BBB Change after 3 h Ischemia Demonstrated by Evans Blue Permeability

All animals received an i.v. injection of 0.1 ml/100 g body weight of 2% Evans blue dye immediately before clip release. They were sacrificed 5 min to 5 h thereafter by 10% buffered paraformaldehyde perfusion. Abnormal permeability of the BBB to the dye was assessed by visual inspection of coronal blocks of the perfused brains.

The animals were divided into 4 groups according to the different levels of MABP (table). Animals were considered to be positive for BBB change when a blue spot greater than 2 mm in diameter was noted in any of the coronal brain sections.

Electronmicroscopic (EM) Study of BBB Change as Traced by Horseradish Peroxidase (HRP)

Animals were subjected to 30 min, 1, 3 or 6 h of cerebral ischemia, the clips were released and the animals killed 30 min thereafter by fixative perfusion. Three animals of each of the 4 groups with ischemia were maintained under normotension between the time of clip release and sacrifice. In 3 other animals from each group, normotension was maintained for the first 10 min and hypertension (130-150 mm Hg) was induced for the subsequent 20 min after clip release. In addition, in 3 gerbils from the group with 30 min of ischemia, hypertension (130-150 mm Hg) was induced for the first 20 min after clip release and these animals were returned to normotension for the 10 min preceding sacrifice. Three normal gerbils without cerebral ischemia were used as the control.

One ml of saline with 25 mg HRP (Sigma Type II or IV) was injected i.v. into each animal a few seconds before clip release; 30 min later, the animals were perfused first for 5 min with diluted fixative (1% paraformaldehyde, 1.25% glutaraldehyde in 0.1 M cacodylate buffer) and then for 20 min with concentrated fixative (4% paraformaldehyde, 5% glutaraldehyde). Fixed...
TABLE Effective Induced Hypertension After Blood Flow Restoration on the Incidence of BBB Change Following 3 h Ischemia

<table>
<thead>
<tr>
<th>Time after blood flow restoration</th>
<th>Normotensive arterial blood pressure* (mm Hg) and ss</th>
<th>Induced hypertension</th>
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<tbody>
<tr>
<td></td>
<td>Group 1: 92.7 ± 1.8</td>
<td>Group 2: 110.4 ± 1.2</td>
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<tr>
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<td>Group 3: 132.8 ± 1.5</td>
<td>Group 4: 157.0 ± 2.0</td>
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*Mean arterial blood pressure was determined at 5 min intervals after blood flow restoration.

Results

BBB Change after 3 h Ischemia Demonstrated by Evans Blue Permeability

In 2 of 7 normotensive animals, the first change in BBB occurred 1 h after blood flow restoration. Thereafter, the incidence of BBB change increased. The table shows that the greater the degree of induced hypertension, the earlier the appearance of BBB change and the higher the incidence among the animals examined.

EM Study of BBB Change Traced by HRP

In normotensive animals, HRP extravasation was noted in all animals with ischemia of 6 h duration, in each of the 3 and 1 hr of ischemia animals and in none of those with 30 min of ischemia. On the other hand, all the hypertensive animals from all 4 groups showed marked HRP extravasation.

Macroscopically, HRP extravasation was visualized as an accumulation of spots (fig. 1). In the spots were small vessels, the walls of which showed segmental HRP extravasation (fig. 2). EM revealed HRP in the basement membrane of arterioles, venules and especially capillaries. Furthermore, a deeper HRP spread into the brain tissue through the intercellular spaces between neuropils, glial cells and neurons was seen (figs. 3, 4). In the endothelial cells there was a marked increase in the number of HRP-labeled vesicles. These were occasionally arranged in rows in the endothelial cytoplasm connecting the luminal and contraluminal sides (fig. 5a,b) and some showed tubulo-channel like structures, especially in obliquely sectioned cylinders (fig. 6). In the examined specimens, neither HRP extravasation via the intercellular spaces of adjacent endothelial cells (fig. 5c), nor membrane damage of endothelial cells was seen. Ultrastructurally, the endothelial cells were well preserved, even in areas where, after 6 h ischemia, severe cellular damage had been induced in the brain parenchyma around the vessels (fig. 3a,b).

In the 30 min ischemia group, induced hypertension after blood flow restoration resulted in drastic extravasation of HRP, associated with an increase in the number of HRP-labeled vesicles in the endothelial cells (fig. 4). However, no extravasation was observed in normotensive animals, although HRP-containing vesicles were seen in the endothelial cytoplasm. A vesicle count of the capillary endothelium revealed that there were 1.08 ± 0.23 HRP-labeled vesicles per μm² in normotensive animals. This number was 10.27 ± 1.43/μm² when hypertension was induced prior to

FIGURE 1. HRP extravasation on coronal brain slices from the left cerebral hemisphere of an animal subjected to a) 6 hr temporary ischemia and subsequent 30 min normotension. b) 30 min temporary ischemia and subsequent 10 min normotension and 20 min hypertension prior to sacrifice.
FIGURE 2. Light microscopy of an extravasation focus in Fig. 1-b. Segmental extravasation of the vascular wall is noted (arrows). X 200

sacrifice; the number decreased to 3.75 ± 0.65/μm², and HRP extravasation was conspicuous (fig. 6) when the blood pressure was normalized prior to sacrifice in hypertensive animals (fig. 7).

Discussion

Our previous study 7 revealed that change in BBB did not occur during cerebral ischemia, but that it was a relatively late event after the restoration of blood flow. There was a direct relation between the duration of the ischemic occlusion and the time and incidence of BBB change. The present observations indicate that a slight increase in systemic arterial blood pressure after blood flow restoration increases the incidence of BBB change via a marked enhancement of vascular endothelial vesicular transport.

Experimentally, an acute increase in systemic arterial blood pressure by more than 90 mm Hg has been reported to bring about BBB damage.14-16 Chronic hypertension in SHR17 and experimental renal hypertension in rats18 also produced BBB damage. In those studies, the BBB change may have been brought about by the increased vascular perfusion pressure which was due to hypertension beyond the cerebrovascular autoregulation limit.19-22

In regional cerebral blood flow (rCBF) studies,23-25 BBB change after restoration of blood flow to the ischemic brain of gerbils was concurrent with the appearance of luxury perfusion on 14C-antipyrine autoradiograms, suggesting that BBB change is associated with local cerebrovascular autoregulation disturbances.

Our previous autoradiographic assessment with 14C-antipyrine26 revealed a marked rCBF increase in the ischemic hemisphere of gerbils that had been exposed to 1 h ischemia when the systemic blood pressure was elevated to 130-150 mm Hg after blood flow restoration. In the same study, diffuse oligemia was noted in
normotensive animals. These findings suggest that a failure of the cerebrovascular autoregulatory system may have allowed the increase in rCBF in the post-ischemic brain, even with induction of slight systemic hypertension.

This led to the consideration that a slight increase in systemic arterial blood pressure may effect an increase in the perfusion pressure in the post-ischemic brain, because cerebrovascular autoregulation had been disturbed by the ischemic insult, and that this increased perfusion pressure may result in BBB change. Postischemic hypertension intensified brain edema and contributed to enhanced ischemic tissue injury.

In agreement with previous results, our EM study of the BBB change in ischemic brains revealed that marked HRP extravasation was not due to opening of endothelial tight junctions, but rather to enhanced vesicular transport which has been suggested as the underlying mechanism of BBB change.
induction by acute hypertension. According to the endothelial vesicle counts in animals with 30 min ischemia, vesicular transport is highly susceptible to a change in perfusion pressure (fig. 8).

We encountered many HRP-containing vesicles which had tubulo-channel-like structures arranged in a row-like pattern, suggestive of the existence of an intra-endothelial tubulo-vesicular network through which serum protein may extravasate passively without consuming energy. The susceptibility of HRP extravasation to a change in vascular perfusion pressure may be explained by these structures.

We suggest that a brief post-ischemic hypertensive flush is effective in preventing the transient no-reflow phenomenon following restoration of blood flow, but prolonged hypertension, even a slight increase in blood pressure, after surgical or spontaneous blood flow restoration to the post-ischemic brain may be potentially harmful by enhancing edema.

Acknowledgment

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References


![Figure 7. Electronmicrogram of an animal subjected to 30 min ischemia followed by 20 min hypertension and 10 min normotension. While HRP extravasation is conspicuous, the number of HRP-labeled vesicles is smaller than that seen in Fig. 4. X 7,500. en = capillary endothelium, bm = basement membrane, n = endothelial nucleus, as = astrocyte.](image)

![Figure 8. HRP-labeled vesicle counts in the endothelium of animals subjected to 30 min temporary ischemia followed by 30 min normotension, 10 min normotension, then 20 min hypertension. 20 min hypertension, then 10 min normotension.](image)


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