Brain Edema During Ischemia and After Restoration of Blood Flow

Measurement of Water, Sodium, Potassium Content and Plasma Protein Permeability

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SUMMARY The left cerebral hemisphere of Mongolian gerbils was used to elucidate the mechanisms of brain edema which develop during cerebral ischemia and after restoration of cerebral blood flow following temporary ischemia. Water content was measured by the tissue-drying method. Sodium and potassium ion concentration was measured by flame photometry. Passage of $^{125}$I-albumin (RISA) from blood to the cerebral parenchyma was measured on a gamma scintillation counter. Our findings indicate that pure cytotoxic edema develops during ischemia and during a short period after restoration of cerebral blood flow. Vasogenic edema, which is accelerated by the leakage of plasma constituents from blood due to blood-brain barrier damage, developed after restoration of the cerebral blood flow. After less than 1 hr of ischemia, restoration of the cerebral blood flow drastically reduced the degree of brain edema. However, restoration of the cerebral blood flow greatly worsened the brain edema following more than 3 hr of ischemia.

BRAIN EDEMA is one of the most important clinical complications in ischemic damage to the brain. Permanent ischemia rarely causes damage of the blood-brain barrier (BBB) in experimental animals.1, 2, 3 Our previous morphological study using Evans blue dye as a protein tracer confirmed that BBB damage in the ischemic brain of experimental animals occurred only after release of the carotid clip which restored cerebral blood flow and that during ischemia no BBB damage had occurred.4

In the present study we used Mongolian gerbils to 1) elucidate the mechanism(s) underlying brain edema which develops during ischemia and following the restoration of cerebral blood flow, 2) analyze the relationship between the degree of brain edema and the duration of temporary ischemia prior to cerebral blood flow restoration.

We found that pure cytotoxic edema developed during ischemia and for some time after the restoration of cerebral blood flow, while the onset of vasogenic edema occurred only after restoration of the cerebral blood flow. The degree of both cytotoxic and vasogenic edema depended on the duration of ischemic insult and ischemia of more than 3-hours duration caused more severe edema than did an insult of a shorter duration.

Materials and Methods

Five to six male adult non-fasting Mongolian gerbils weighing 60–80 g were used in each experiment in the present study.5 Only typically ischemia-sensitive animals were selected.6-7 In the permanent ischemia group, the left common carotid artery was exposed through a ventral midline incision in the neck under light ether anesthesia and the animals were sacrificed following occlusion with Scovill's clip for 30 min, 1, 3, 6, 9 and 20 hr. In the temporary ischemia group, restoration of the cerebral blood flow was effected by releasing the clip after 30 min, 1, 3, or 6 hr, and the animals were sacrificed 2, 3, 5, 8, 10, 20, 72 hr or one week after blood flow restoration. We avoided experiments exceeding 20 hr permanent ischemia and measurements more than 3 hr after clip release in the 3 and 6 hr temporary ischemia group because of an exceedingly high mortality rate. Severe rolling seizures during and/or after ischemia could be controlled by i.p. administration of pentobarbital (0.5 mg/100 g body weight). In control animals under the same anesthesia, the left common carotid artery was exposed but not occluded.

1. Measurement of Water, Sodium and Potassium Content

Brains were removed immediately after guillotine decapitation. The posterior two-thirds of the left cerebral hemispheres was separated from the brain, placed on a piece of aluminum foil and weighed. Approximately 30 sec were required for this procedure. Separate studies on control animals indicated that weight reduction due to desiccation during this 30 sec period, was less than 0.3% of the wet weight of the whole hemisphere. Therefore, artifactual weight loss during the procedure was considered to be negligible. The dry weight of the left hemisphere was established after complete dehydration at 90°C–100°C for 4 days and percentage water content was calculated as follows:

$$\text{water} (\%) = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

The dehydrated sections were homogenized with 10 ml of 0.75N nitric acid solution prepared with deionized water, the mixtures placed in thoroughly washed plastic tubes with tight plugs, and incubated at 4°C for 3 days. Sodium and potassium content of the
supernatant was measured by flame photometry. The value X mEq/l was converted into mEq/kg dry weight of the dehydrated sections according to the following formula:

$$X \times \frac{10}{1000} \times \frac{1}{\text{dry weight}} \times 1000$$

All labware used in the procedures was washed thoroughly with deionized water. 8-9

2. Passage of $^{131}$-Albumin from Blood into Normal and Ischemic Brains

The following animals were used in this experiment: 1) control animals, 2) animals from the temporary ischemia group with the left carotid artery occluded for 1 hr, and sacrificed 3, 5, 10, 20, 72 hr or 1 week after release of the clip, 3) animals from the temporary ischemia group sacrificed 3 hr after release of the clip following 6 hr occlusion, 4) animals from the permanent ischemia group following 9 hr occlusion. Carotid occlusion was released 15 min before sacrifice in all animals in order to avoid vascular inpatency during flushing of the cerebral vascular system.

RISA (8.7 $\mu$Ci/mg protein; protein 11.4 mg/ml; 2.12% free or loosely bound iodides, Dinabott, Tokyo) was passed through a Dowex 1-X8 (50-100 mesh) column charged with 1N HCl and buffered to pH 7.0 to remove free or loosely bound iodides. Each animal was injected intravenously with 10 $\mu$Ci of RISA in the left femoral vein 3 hr before termination of the experiment. Before sacrifice, 0.1 ml of venous blood was collected from the right femoral vein in a plastic tube with a tight plug. The vascular system of the animal was flushed with saline from the ascending aorta to the right atrium, at 130 cm H$_2$O pressure, until the outflow became clear and RISA $^{131}$I activity in the effluent clear saline was not above background activity. RISA $^{131}$I activity of the blood following an initial increase stabilized 1 hr post-injection (6 animals were examined).10 11

The posterior two-thirds of the left cerebral hemisphere was obtained as described above and placed in a plastic tube with a tight plug. The venous blood sample, as well as the hemisphere section, were placed into sealable plastic tubes and weighed. RISA $^{131}$I activity in each blood sample and section was counted for 10 min by the Aloka well-type gamma scintillation counter, and counts per gram for each specimen (wet weight) were calculated.

Results

1. Water, Sodium and Potassium Content

Following occlusion, water and sodium content (percentage wet weight) of the posterior two-thirds of the left ischemic hemisphere in the permanent ischemia group increased gradually until 20 hr, after a rapid initial increase during the first 9 hr. In contrast, while potassium content decreased as sodium content increased, the degree of change for potassium was less pronounced than the degree of change for sodium (fig. 1).

In the temporary ischemia group, restoration of blood flow following 30 min occlusion stopped the increase of water content for 8 hr post-restoration and, subsequently, water content decreased gradually until it reached the control value 3 days after release of the clip. Sodium content decreased during the first 6 hr post-restoration, increased slightly until 20 hr, and then decreased again until it reached the control value 3 days after release of the clip. Potassium content recovered instantly to control value after release of the clip (fig. 1).

In the 1 hr temporary ischemia group, following the restoration of blood flow, water content increased for 2 hr, subsequently decreased until 5 hr, gradually increased during the period of 8 hr to 3 days, and then decreased until 1 week after release of the clip. Sodium content decreased during the first 5 hr after which the sodium curve was similar to that for water content. However, water and sodium content never ex-
ceeded the permanent ischemia group values. Potassium content remained rather constant and recovered almost to the control value at 2 hr following restoration of the blood flow, decreased very slightly for 3 days and returned to the control values 1 week after release of the clip (fig. 2).

In the 3 and 6 hr temporary ischemia group, at 3 hr post-restoration of blood flow, water content increased markedly beyond the value obtained for the permanent ischemia group, indicating that restoration of the blood flow exacerbated the degree of edema. Sodium content also increased, but did not significantly exceed the value obtained for the permanent ischemia group. Potassium content showed a tendency to recover at 3 hr after release of the clip (fig. 3).

2. Passage of $^{131}$I-Albumin from Blood to Normal and Ischemic Brains

The left cerebral hemisphere sections were assayed for $^{131}$I-albumin and tissue-to-blood radioactivity ratios (counts/g) were calculated (ordinate on the left side of figs. 4 and 5). If one assumes specific albumin radioactivity of brain and blood to be equal, and if blood is assumed to contain 20 mg albumin per gram of blood, these ratios can be converted into milligrams of serum albumin per gram of tissue by multiplying the ratios by the blood albumin concentrations (20 mg/ml) (ordinate on the right of figs. 4 and 5).

In the 1 hr temporary ischemia group, RISA $^{131}$I activity of the ischemic brain section was assayed 3, 5, 10, 20 hr, 3 days and 1 week after release of the clip. Mean values and standard error of RISA passage into the control and ischemic left cerebral hemisphere are shown in fig. 4. A slight increase of RISA passage occurred at 3 and 5 hr, and a marked increase at 10 hr after release of the clip. Passage of RISA decreased for 3 days and returned close to the control value 1 week after release of the clip.

In the 6 hr temporary ischemia group, marked increase of RISA passage occurred at 3 hr after release of the clip. On the other hand, in the 9 hr permanent
ischemia group, only very slight RISA passage was observed (fig. 5).

Discussion

Brain edema, separated by Klatzo into the cytotoxic and vasogenic types, is defined as an abnormal water accumulation associated with increase in brain tissue volume. In cytotoxic edema, parenchymal structural elements are directly affected by noxious factors, resulting in intracellular swelling. In vasogenic edema, the increase in water content is accompanied by passage of serum protein from the blood into the brain. This type of edema is related to BBB damage which facilitates an escape of water and plasma constituents into the adjacent parenchyma.

We studied the mechanisms underlying brain edema during ischemia and after restoration of the blood flow and examined the relationship between the duration of ischemic insult and the severity of edema.

1. Reversibility of Edema with Restoration of Blood Flow

In the permanent ischemia group, brain edema became progressively worse with lengthening of the ischemic insult. In the temporary ischemia group, however, time is a critical factor in the recovery from brain edema after restoration of the blood flow. In the 3 and 6 hr temporary ischemia group, edema abruptly increased after restoration of the blood flow. However, in the 30 min and 1 hr ischemia groups, restoration of the blood flow brought about a drastic reduction in the rate of edema increase and the degree of edema never exceeded that of the permanent ischemia group. Normalization was noted at 72 hr in the 30 min and at 1 week in the 1 hr ischemia groups, after release of the clip (figs. 1 and 2).

2. Cytotoxic and Vasogenic Edema in the Ischemic Brain

There are several controversial experimental results concerning BBB change in ischemic brain edema. Among them, O'Brien stated that BBB change occurred during permanent ischemia, Hossmann, however, reported that BBB change did not occur in their ischemic model during and/or after 1 hr complete ischemia. The present findings, however, reconfirm the results of our previous morphological study. The permeation of Evans blue protein tracer revealed that no extravasation of Evans blue occurred in the cerebral hemisphere during ischemia and immediately after restoration of the blood flow, although marked diffuse edematous swelling was found in the ischemic hemisphere. Extravasation was noted at various times after restoration of the blood flow, depending on the duration of ischemia and appeared usually in confined areas of large severe tissue damage. These findings led us to conclude that cytotoxic edema occurs primarily in the ischemic brain and that vasogenic edema appears subsequent to the restoration of blood flow.
edema is mainly cytotoxic and that the ischemic derangement of cytomembrane function, especially of the glial cells, may interfere with the ion pump. Intracellular retention of sodium ion makes the glial cells particularly susceptible to taking up water.22-23

Following 30 min and 1 hr of ischemia, restoration of blood flow brought about a reduction in the rate of water increase and a reduction of sodium content up to 6 hr after release of the clip. Potassium content also recovered immediately after restoration of the blood flow (figs. 1 and 2). In the 1 hr ischemia group, water and sodium content increased again gradually from 8 to 72 hr after restoration of blood flow and returned to normal value at one week (fig. 2). The onset of vasogenic edema occurred at approximately 3 hr following blood flow restoration. Vasogenic edema gradually increased until 10 hr, the maximum was reached at 20 hr and then a decrease took place, until the control value was reached at one week. These results lead us to suggest that cytotoxic edema ceases due to the recovery of the sodium-potassium exchange pump mechanisms, and that this process is followed by a gradual increase in water content associated with vasogenic edema which subsequently subsides one week after release of the clip.24

On the other hand, following 3 and 6 hr ischemia, restoration of cerebral blood flow brought about an increase in water content during the 3 hours following release of the clip. In the 6 hr ischemia group, we observed a marked increase of RISA passage from blood into the posts ischemic brain parenchyma during the 3 hours following release of the clip (fig. 5). We estimated the amount of water which entered the brain associated with passage of albumin during this period and found that approximately 30% of the total water increase is due to vasogenic edema. The remaining 70% of water uptake may in part derive from cytotoxic edema. Therefore, it is possible that after an ischemic insult of 6 hr duration, cytomembrane function does not satisfactorily recover with the restoration of blood flow.

The control brains showed slight tissue uptake of RISA (fig. 5). This might be partially attributed to the slight transport of proteins across normal cerebral arterioles,26 and partly to an intravascular remnant of RISA even after saline perfusion.

3. Exchange of Na+, Water and K+

In cytotoxic edema during permanent ischemia, extracellular sodium enters the cell and potassium escapes into the extracellular compartment,22-23 resulting in low concentration of sodium and high concentration of potassium in the extracellular compartment. Sodium is known to permeate from blood to brain tissue at a rapid rate.26 Sodium ions are easily taken up from the blood into extracellular compartments of the brain due to the concentration difference, with the result that sodium concentration increased in the brain parenchyma.27 Cellular water increase, derived passively from the blood or via extracellular spaces, parallels sodium increase.27

Zimmerman and Hossmann,28 on the other hand, reported that there was no increase in water content but only intra- and extracellular water ion exchange in the complete ischemic model.

On the other hand, the uptake of $^{42}$K+ by the brain parenchyma from the blood is markedly slow.26 Therefore, extracellular potassium does not come from the blood, but drains instead into the CSF via the extracellular spaces.29-31 The total potassium content may decrease during ischemia, however, the change in potassium concentration is usually smaller than that of sodium.

In our ischemic model, following 30 min and 1 hr of ischemia, immediate reduction of sodium concentration occurred after restoration of the blood flow, accompanied by slight reduction of the water content. With the reactivation of the sodium-potassium exchange pump, retained intracellular sodium ions are moved into extracellular compartments and then drain into the blood. Intracellular water is moved...
passively into the blood via the extracellular space. As extracellular potassium ions are taken up intracellularly and drainage of potassium into the CSF ceases, the total potassium content in the ischemic brain recovers to the normal level.

However, sodium and water content increase again from 8 to 72 hr after release of the clip, and we suggest that this phenomenon is associated with vasogenic edema.

We found a rapid recovery of potassium content after restoration of the blood flow, following 30 min and 1 hr of ischemia (figs. 1, 2). Even in the 3 and 6 hr ischemia groups, potassium content showed a tendency to recover (fig. 3) after restoration of the blood flow, suggesting that the recovery of potassium content, particularly in the extracellular space, may be a valuable indicator for the prognosis of cerebral cell membrane recovery. Brain edema is considered to be one of the most important causes of mortality in ischemic brain disease.

In the present oligemic ischemia model, pure cytotoxic edema develops during ischemia and during a short period after restoration of cerebral blood flow. Vasogenic edema develops after restoration of the cerebral blood flow. After less than 1 hr of ischemia, restoration of the cerebral blood flow drastically reduces the degree of brain edema. However, restoration of the cerebral blood flow greatly worsens the brain edema following more than 3 hr of ischemia.

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