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Effect of 20% Mannitol-Saline Perfusion on Ruthenium Red Permeability through Endothelial Cell Junctions of Brain Capillaries in Rats

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Introduction

The blood-brain barrier (BBB) is known as a device which severely restricts cerebrovascular permeability to many chemical substances, above all, to water-soluble drugs. This restricted permeability is thought as one of major problems by which the efficacies of chemotherapy were limited in brain diseases, especially malignant brain tumors. Osmotic opening of the BBB induced by hypertonic solution infusion into the internal carotid artery may be expected as a method of increasing the cerebrovascular permeability and the efficacies in the chemotherapy of malignant brain tumors[1,2].

It was reported that infusion of hypertonic solution exceeding a certain osmotic pressure (threshold level) was necessary to increase cerebrovascular permeability[3]. Our previous studies on the osmotic pressure effects of some solutions such as mannitol, xylitol and urea also revealed that each solution was needed almost the same osmotic pressure as 20% mannitol-saline to prove the BBB opening[4,5]. The osmotic BBB opening is thought to be a result from disconnection of tight junctions between the endothelial cells[1,3]. Therefore, this experiment was carried out to examine by macroscopy and electron microscopy whether the osmotic opening of the tight junctions is proved by using ruthenium red as a tracer which enables to show visual evidence[6].

Materials and methods

Male Wistar-Kyoto strain rats (12 weeks old) were maintained on a normal chow and water ad libitum for more than 10 days before use. Under anesthesia of pentobarbital sodium (30 mg/kg, i.p.), the trachea was intubated, and finally a polyethylene catheter filled with heparinized saline (100 U/ml) was retrogradely inserted into the right external carotid artery just below the bifurcation of common carotid artery and utilized for infusion of test solutions into the internal carotid artery. The animals were allowed to rest for 30 min before experiment. Afterwards test solution (37°C) was infused into the right internal carotid artery with an infusion pump at a rate of 5 ml/min for 30 sec.

Test solutions used in this experiment was 20% mannitol-saline and the vehicle (saline).

On 5 min later, prefixation was carried out by perfusion from the left ventricle as following procedure. A catheter was inserted into the ascending aorta through the left

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ventricle. After the right atrium was cut to allow blood and fluid to be drained, Hanks' balanced salts solution (pH 7.4, 37°C) was infused at a pressure of 120 cmH_2O through the catheter until the fluid flowed out from the right atrium became clear. Then the infusing solution was switched to a mixed solution of 2.5% glutaraldehyde and 0.05% ruthenium red (Wako) in 0.1 M cacodylate buffer (pH 7.4). This solution was infused at 37°C for initial 5 min and under cooling with ice water for following 55 min.

After prefixation, the brain was removed and its macroscopic photograph was taken, and then the specimens for transmission electron microscopy were isolated from the area of right parietal lobe.

Ruthenium red dissolved in the prefixing solution served as a tracer not only for macroscopy but also for transmission electron microscopy, because it made the BBB opening visible by brain staining and it was observed as a high electron density area after postfixation in osmium tetroxide by transmission electron microscopy, which was utilized to see the localization of ruthenium red in the brain capillary walls.

The specimens were processed as follows ; sliced at 0.2 mm in thickness, rinced in 0.1 M cacodylate buffer (pH 7.4), postfixed for 2 hr in 1% osmium tetroxide in the same buffer at ice cold, dehydrated in a graded ethanol series, embedded in Epon 812, and cut with an ultramicrotome. Ultra-thin sections were not stained with uranyl acetate and lead citrate in this experiment to emphasize the contrast induced by ruthenium red.

Results

In saline infusion group, the brain was not stained with ruthenium red under macroscopy (Fig. 1, upper left). And the luminal surface of the endothelial cell membrane was stained with ruthenium red but the extravasation of ruthenium red beyond the monolayer of endothelial cells was not found under transmission electron microscopy (Fig. 1, lower left). It was shown that the extravasation of ruthenium red was restricted by the lining of endothelial cells.

In 20% mannitol-saline infusion group, on the other hand, the right hemisphere, which was directly perfused with the test solution, was stained with ruthenium red under macroscopy (Fig. 1, upper right). And ruthenium red was observed in a part of junctional area and basement membrane of cerebral capillaries under transmission electron microscopy (Fig. 1, lower right). Observing this area under a higher magnification, ruthenium red did not transfer into the endothelial cells. Therefore, it was suspected to penetrate tight junctions and to diffuse along the basement membrane (Fig. 2).

Discussion

Ruthenium red used in this experiment is useful as a tracer for macroscopic and electron microscopic observation[4]. Ruthenium red shows no transfer into the normal brain tissue, therefore the area stained with ruthenium red indicates opening of the BBB. Moreover, ruthenium red transferred into the brain tissue forms a high electron density area after postfixation in osmium tetroxide. Consequently, it makes possible to observe its localization by transmission electron microscopy. Therefore, we can trace the rout of extravasation of ruthenium red.

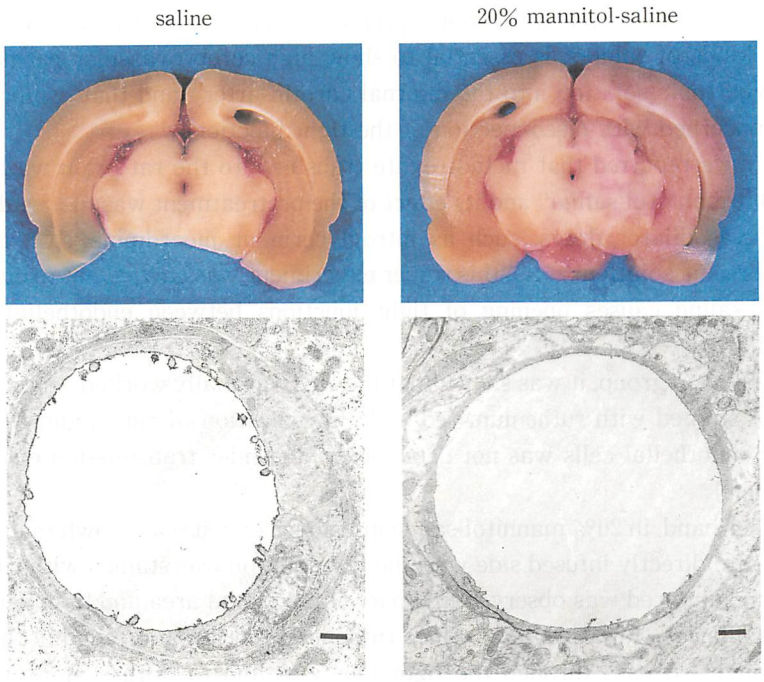

Fig. 1 Effect of 20% mannitol-saline infusion (5 ml/min, 30 sec) into the internal carotid artery on the blood-brain barrier in rats.
Macroscopic photographs of coronary sectioned brains just after perfusion fixation with a mixed solution of 2.5% glutaraldehyde and 0.05% ruthenium red and their capillary images obtained by transmission electron microscopy are shown. Bar=0.5 μm.

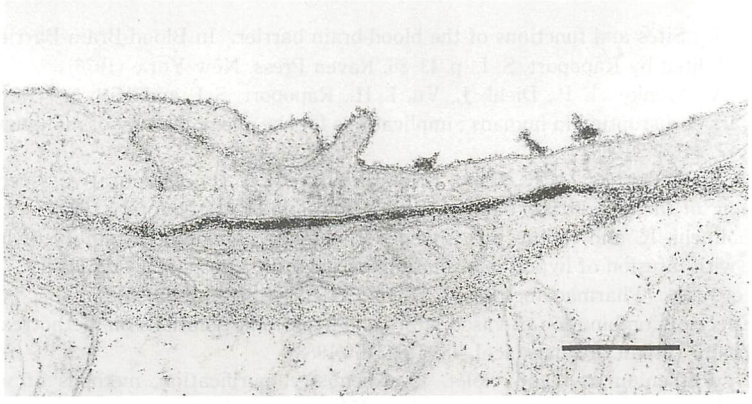

Fig. 2 Higher magnification image of the capillary shown on the lower right in Fig. 1. Bar=0.5 μm.

It has been assumed that an osmotic pressure exceeding a certain level (threshold), regardless of kinds of solutes, is essential to show high cerebrovascular permeability after infusion of hypertonic solution into the internal carotid artery and that osmotic opening of the BBB is concerned with disconnection of the tight junctions[1,3].

Previously we reported that methotrexate transfer into the rat brain was increased by infusion of 20% mannitol-saline[4] and its effect of the pretreatment was markedly augmented by adding of vasodilator drugs, such as nitroglycerin or nicardipine HCl, to the supra-threshold hypertonic solution[5]. So, this tracer experiment was carried out to clarify whether 20% mannitol-saline causes opening of tight junctions between endothelial cells in the cerebral capillaries.

In saline infusion group, it was shown that the BBB normally worked. Because the brain tissue was not stained with ruthenium red and extravasation of ruthenium red beyond the monolayer of endothelial cells was not detected either under transmission electron microscopy (Fig. 1, left).

On the other hand, in 20% mannitol-saline infusion group, it was shown that the BBB was opened. Because directly infused side with the test solution was stained with ruthenium red. Moreover, ruthenium red was observed in a part of junctional area and basement membrane of cerebral capillaries (Fig. 1, right). Since ruthenium red was not found in the endothelial cells, it is thought to pass through the tight junctions and to diffuse along the basement membrane (Fig. 2).

From these observation, it was considered that the increased transfer into the rat brain tissue by pretreatment with infusion of 20% mannitol-saline, a hypertonic solution, was resulted from opening of tight junctions between endothelial cells of brain capillaries. The present study suggests that the osmotic opening of BBB enables to increase the permeability to various kinds of drugs.

References

1) Rapoport, S. I. : Sites and functions of the blood-brain barrier. In Blood-Brain Barrier in Physiology and Medicine, Edited by Rapoport, S. I., p. 43-86, Raven Press, New York (1976)

2) Neuwelt, E. A., Frenkel, E. P., Diehl, J., Vu, L. H., Rapoport, S. I. and Hill, S. : Reversible osmotic blood-brain barrier disruption in humans ; implications for the chemotherapy of malignant brain tumors. Neurosurgery **7**, 44-52 (1980)

3) Rapoport, S. I., Fredericks, W. R., Ohno, K. and Pettigrew, K. D. : Quantitative aspects of reversible osmotic opening of the blood-brain barrier. Am. J. Physiol. **238**, R421-R431 (1980)

4) Abe, H., Inokuchi, K. and Fujita, K. : Changes in cerebrovascular permeability of methotrexate by pretreatment with infusion of hypertonic solutions through the internal carotid artery. (1) Experiments in Wistar-Kyoto rats. Pharmaçometrics **34**, 175-179 (1987) (Abs. in English)

5) Abe, H. : Effect of combination of vasodilators and hypertonic solutions on methotrexate distribution into the rat brain. Japan. J. Pharmacol. **48**, 373-376 (1988)

6) Luft, J. H. : Ruthenium red and violet. 1. Chemistry, purification, methods of use for electron microscopy and mechanism of action. Anat. Rec. **171**, 347-368 (1971)