EFFECT OF DEFEROXAMINE ON BLOOD-BRAIN BARRIER DISTURBANCES FOLLOWING SUBARACHNOID HAEMORRAGE IN CATS

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Turkish Neurosurgery 2 : 60 - 63, 1991

SUMMARY :

Disruption of the blood brain barrier plays a significant role in the pathophysiology of subarachnoid haemorrhage. Experimental studies concerning subarachnoid haemorrhage suggested the primary role of free radicals and subsequently lipid peroxidation in the pathogenesis of vasospasm. In this experimental study the role of deferoxamine on blood brain barrier disturbance in vasospasm was evaluated. Deferoxamine was used to inhibit the free radical reactions and lipid peroxidation in cats with subarachnoid haemorrhage. Despite the absence of morphological findings of vasospasm in cats with subarachnoid haemorrhage treated with deferoxamine, horseradish peroxidase particles were observed in the cytoplasm of vessels which indicates disturbance of blood-brain barrier. This result suggested that deferoxamine has no primary role in blood - brain barrier disturbance.

KEY WORDS :

Subarachnoid haemorrhage, vasospasm, horseradish peroxidase, blood-brain barrier, cat.

INTRODUCTION

Disruption of the blood brain barrier (BBB) may play a significant role in the pathophysiology of subarachnoid haemorrhage (SAH) (4,15,25,26). Permeability changes were seen on computerized tomography (CT) scans in SAH. These changes induced vasospasm in the intracranial arterial system (11). Increased free radical reactions and subsequent high lipid peroxidation level in SAH and experimental studies higly suggested the primary role of free radical products in the development of vasospasm (2,5).

Deferoxamine is powerful iron chelator which inhibits free radical reactions and subsequently lipid peroxidation. Deferoxamine has a remarkably high affinity for ferric iron and a low molecular weight and passes freely through the BBB and the cell membrane (13,14). Studies on BBB disturbance after SAH are few and so far there has been no experimental study to our knowledge to evaluate the role of deferoxamine on the BBB disturbance after SAH. We examined changes in the permeability of intraparenchymal vessels at 6 and 48 hours after SAH and deferoxamine treated SAH in cats, using horseradish peroxidase (HRP) as a tracer.

MATERIAL AND METHODS

Cats of either sex ranging in weight from 2-2,5 kg were anesthetized with 35mg/kg sodium pentobarbital administered intraperitoneally. Total 15 cats were divided 3 proups. Group 1 (6 cats) and group 2 underwent to operation for subarachnoid haemorage and group 1 treated with deferoxamine but Group 2 received only saline solution. Control group (3 cats) underwent to sham operation. The head of the cats in group 1 and group 2 were acutely extended and the cisterna magna was aseptically punctured by a 16 gauge butterfly needle. 1ml/kg autologous blood obtained from the femoral artery was injected to the cisterna magna via butterfly needle. The cats were head down position for 20 minutes to maintain distribution of the blood within the basal cisterns and around the basilar artery. Group 1 received 1.02 mg deferoxamine in 0.9% saline solution intratecally via cisterna magna, two hours and 36 hours later blood injection. Group 2 (6 cats) received only 1ml/kg saline solution instead of deferoxamine. Control group underwent to same operation but received only saline solution instead of blood. Six and 48 hours later cats in all groups received a 2% solution of horseradish peroxidase (200 mg/kg body wt, type II, Sigma Chemical Co.,) in saline injected intravenously 20 minutes before perfusion fixation. Thereafter the cats were deeply anesthetized the infusion. Craniectomy was performed immediately after infusion and the brain was quickly removed and immersed in a solution containin 2% paraformaldehyde in 0.1 M sodium cacodylate (pH 7.4) for 5 hours at 4°C. Samples for electron microscopy were postfixed in 1%

osmium tetroxide and embedded in Epon 812. Ultrathin section were examined unstained in a JEOL 100CX electron microscope.

RESULTS

In group 2, electron microscope observations showed degenerative changes in the endothelium of the brain stem arterioles and capillaries and revealed corrugation of the internal elastic lamina of the basilar arteries. Horse radish peroxidase extravasation was most noticeable in specimens taken from cats sacrified 2 days after SAH. Endothelial cell damage was not observed in the brain stem, but the endothelial cells were separated. Electron microscopy showed that the change in the permeability of the hemispheres was similar to that of the brain stem. In addition, electron microscopy revealed plasmolemmal vesicles labelled with tracer in the endothelium of the arterioles (Fig. 1).

There was no corrugation of the internal elastic membrane in all cats in the group 1 treated with deferoxamine. Despite this finding HRP particles were observed in the cytoplasm of the endothelium and subendothelial spaceses 6 and 48 hours post SAH (Fig. 2). The control group showed normal electron microscopy findings of vessels without HRP particles in cytoplasm.

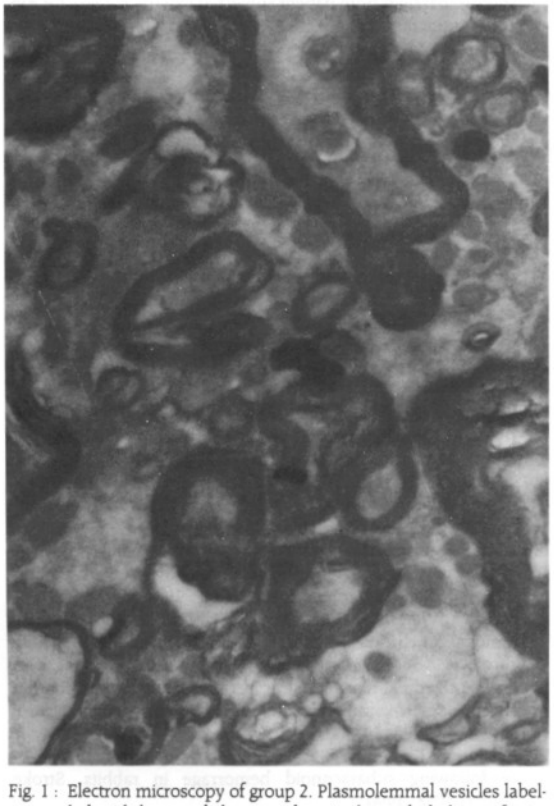

Fig. 1 : Electron microscopy of group 2. Plasmolemmal vesicles labelled with horseradish peroxidase in the endothelium of arterioles were seen.

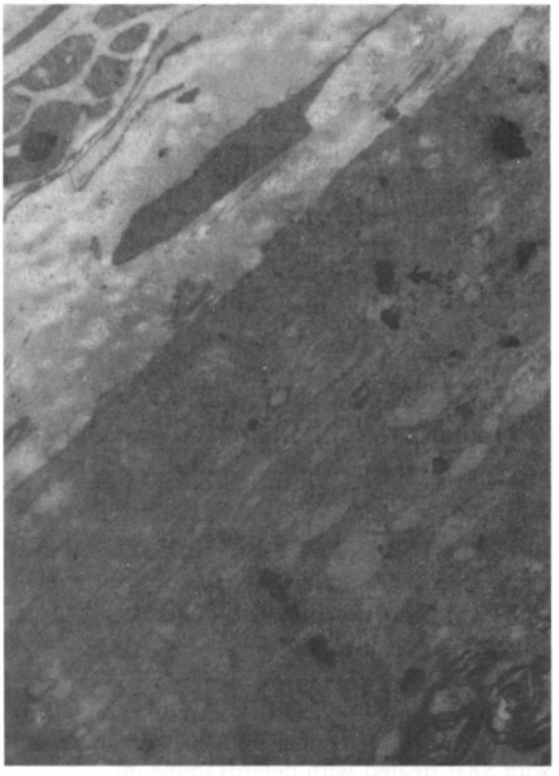

Fig. 2 : Electron microscopy of group 1. There was no corrugation of the internal elastic membrane in the first group of cats which indicate the absence of vasospasm. Despite this finding HRP particles were observed in the cytoplasm of the endothelium and supendothelial spaces.

DISCUSSION

Many studies of the pathophysiology of vasospasm have already demonstrated that many different factors may contribute to this pathology (3,9, 10,17-19,22-24) One of the attractive hypotheses is activation of the lipoxygenase pathway influenced by free radicals. It is demonstrated that free radical reactions, especially lipid peroxidation, are initiated by clot lysis in the CSF. Each constituent of free radical reactions (such as lipid hydroperoxides) has a vasocontractile capacity (2). Furthermore they have toxic effects on the endothelium and media of the artery, and increase the permeability of the endothelium which causes penetration of plasma constituents into the arterial wall (11,12,21) As a result, the state of chronic vasospasm with histological changes in observed in the vasospastic artery. Iron (Fe^{++}), degradation product of Hb, plays an important role in the initiation and propagation of free radical reactions, in which the hydroxyl radical, the most effective agent of lipid peroxidation, is produced (7,27). Therefore, most polyunsaturated fatty acids

in the CSF undergo peroxidation. It was demonstrated the efficiency of deferoxamine in prevention of ferric ion from undergoing the cyclic reduction and oxidation in vivo, which result in prevention of vasospasm (6). Deferoxamine was chosen in this study because it chelates iron with extremely high affinity ($K_d=10^3$), strong chelation would prevent ferric iron from undergoing the cyclic reduction and oxidation required to catalyze hydroxyl radical formation (1,8,14).

The deferoxamine was given 1.02 mg since 2-2.5 cc blood was given into the cisterna magna 2 cc blood has approximately 1 mg Fe^{+++} and 1 mg deferoxamine chelates 0.085 mg Fe^{+++}. Deferoxamine was given 2 hours and 36 hours after SAH since oxyhemoglobin is found within 2 hours of the ictus (owing the lysis of red cells), and peaks at about 36 hours (29)

Histological results are demonstrated significant narrowing of the diameter of spastic basilar arteries with folding and corrugation of lamina elastica in cats after SAH was mentioned with light microscopy previously (20,28). But, it was shown that there was no significant folding and corrugation of lamina elastica and no narrowing of the diameter in the cats with SAH treated with deferoxamine (6).

Therefore deferoxamine, an iron chelator, represented a very promising therapeutic tool in vasospasm after SAH if given intratecally before free radical and lipid peroxidation reactions take place in subarachnoid space.

In this study, subarachnoid clot produces constriction of the major cerebral arteries by corrugation of the elastic lamina in group 2. But there is no histopatological reveleance of vasospasm in grup 1. However marked disturbance of arterial permeability was observed in group 1 and group 2 cats. The corrugated lamina may squeeze the endothelial cells and inhibit their metabolism or vasoconstrictors released into the cerebrospinal fluid following SAH may also affect the permeability of the major cerebral arteries (16,30). Three different routes of HRP leakage into the vessel wall can be postulated: 1-opening of tight junctions, 2-endothelial cell pinocytosis and 3-trans endothelial channels (25,26). It was previously demonstrated that the HRP-reactive products, in the sub-endothelial space were generally localized in the area beneath the labeled interendothelial space. It may be that HRP, once having leaked into the labeled interendothelial space. It may be that HRP, once having leaked into the subendothelial space passes through the opened inter-endothelial junctions (26,30). But in the present study the HRP labelled plasmalemmal vesicles are also seen in the cytoplasm. This result suggests that SAH may activate endothelial cell pinocytosis in the wall barrier of the cerebral arteries.

However, both intracellular and subendothelial HRP granules did not change significantly in group 1 and group 2. Therefore it was suggested that, although deferoxamine can prevent vasospasm, it has no effect in preventing in BBB distruption seen after experimental SAH.

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