Methylprednisolone reduces axonal impairment in the experimental model of brain oedema

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Abstract

OBJECTIVES: Objectives: In our earlier paper we demonstrated that opening of the blood-brain barrier with an osmotic insult induces brain oedema which represents a factor triggering axonal impairment accompanied with myelin disintegration. The aim of the present study was to find whether methylprednisolone can reduce such axonal impairment in our model of brain oedema.
METHODS: Brain oedema was induced by osmotic blood-brain barrier opening with 20% mannitol applied selectively into the internal carotid. Axonal changes were recognized as signs of myelin disintegration (oedematous vesicles, varicosity, myelin fragmentation) at histological sections stained with Black Gold in hippocampal areas CA1 and CA3 and in the dentate gyrus at time intervals of 30 minutes (acute group) or one week (chronic group) after the blood-brain barrier opening. At the same time intervals methylprednisolone was applied in two different ways – into peritoneal cavity or into the right carotid artery.
RESULTS: Impairment of the axonal integrity (changes of the myelin sheet integrity) was identified in all areas studied in both experimental groups. Whereas in the control group axons were of the uniform diameter, in the experimental groups various forms of myelin disintegration were observed. Methylprednisolone reduced the degree of myelin disintegration in both time intervals with the highest effect in the acute group with the intracarotic administration.
CONCLUSION: Methylprednisolone can effectively reduce myelin changes accompanying brain oedema induced by blood-brain barrier opening with an osmotic insult.

Abbreviations:

CNS	- Central Nervous System
BBB	- Blood-brain Barrier
ACC	- Arteria Carotis Communis
ACI	- Arteria Carotis Interna
ACE	- Arteria Carotis Externa
DG	- Dentate Gyrus

INTRODUCTION

The search for neuroprotective agents has been in the focus of systematic attention of experimental projects and clinical trials for a long time (Bullock *et al.* 1999). To this day, methylprednisolone has been the only neuroprotective substance for the control of the natural course of pathophysiological phenomena after serious CNS injury. Its positive

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effect was demonstrated in a randomised double-blind study (Hall 1992). The primary neuroprotective effect of methylprednisolone (MP) is attributed to its antioxidative ability which protects membrane lipids from peroxidation and against all subsequent adverse effects like changes in membrane fluidity or changes in the activity of membrane proteins (ion channels, transporters, enzymes). As the reactive oxygen species affect also other cellular systems (mitochondria, intracellular enzymes and co-factors, systems of transcription and translation) and thus alter various parameters of cell activity or induce the cell death in apoptosis, antioxidative ability of methylprednisone could interfere with pathologic processes in nerve cells at different levels (Faden & Salzman 1992, Hall 1992, Hall 1993). This phenomenon is employed in the standard treatment for spinal cord injury (Bracken *et al.* 1997). No neuroprotective effects have been proved in brain injury, and corticotherapy is not recommended in such cases either (Marshall 2000, Czekajlo & Milbrandt 2005). Though many other actions of MP in the damaged or normal central nervous system tissue have been recognized (Park 1998): 1) protective effects on axonal integrity in the face of various degenerative influences by preservation of lysosomal integrity and membrane-bound enzymes, 2) enhanced flow and preservation of microcirculatory pathways, 3) decrease in thromboxane and prostaglandin production, 4) maintenance of capillary endothelial membranes, 5) decrease in the permeability of the blood-brain barrier, and 6) anti-inflammatory effects, corticotherapy is recommended only in selected and specific brain pathologies.

In the present paper, effects of methylprednisolone on the axonal impairment (myelin integrity) in the hippocampus of rats with brain oedema induced by intracarotid injection of 20% Manitol have been studied. We evaluated the mode of administration (intraperitoneal versus intracarotid) and effects of time interval after the MP administration (30 minutes or 1 week).

MATERIAL AND METHODS

For the experiment, we used adult animals of both genders from the Wistar strain of laboratory rats (weight 350–450 g), all treated in accordance with the current Guidelines for the treatment of laboratory animals (EU Guidelines 86/609/EEC).

Microsurgical exposure of the internal carotid (ACI)

Animals were put into the state of general anaesthesia using intraperitoneal application of thiopental in the dose of 4 mg/100 g and allowed to ventilate spontaneously throughout the procedure. Starting from a skin incision along the midline between the upper end of the sternum and the mandible, the whole common carotid artery (ACC, *arteria carotis communis*) was exposed with a standard microsurgical technique and, before its bifurcation, also the proximal portions of the internal carotid (ACI, *arteria carotis interna*) and external carotid (ACE, *arteria carotis externa*), which was ligated close beyond the bifurcation. An intraluminal catheter was introduced into the ACC trunk from the arteriotomy for selective application of mannitol. With the application over and the catheter removed, the ACC was ligated distal to and proximal to the arteriotomy. The operation concluded with a single-layer suture (Kozler 2002).

Osmotic opening of the BBB –induction of oedema

Mannitol 20% (200 g in 1 000 ml of water for injection, 1 098 mosmol/l) in a dose of 5 ml/kg was selectively applied in the ACI at a rate of 0.12 ml/sec (Saris *et al.* 1988, Rapoport 2000). After the surgical intervention, the animals were placed in boxes offering standard access to food and drink.

Methylprednisolone administration

To study the neuroprotective effect of methylprednisolone, two groups of experimental animals and one control group were formed. Experimental groups for studying the acute effect (A) and chronic effects (C) consisted of three subgroups with different modes of animal treatment, each employing five animals. In the group A the brain oedema was induced by mannitol (A1) which was immediately followed by intraperitoneal administration of MP in the dose of 100 mg/kg (A2) or after 10 minutes by the intracarotid administration of MP (100 mg/kg) (A3). Perfusion fixation followed in 30 minutes in all three groups. The same pattern was used also in the group C (C1-brain oedema induced by mannitol, C2 – immediately after the administration of mannitol MP was administered intraperitoneally, C3- MP was injected into the carotid artery10 minutes after the mannitol administration). The dose of MP was the same as in the A group. Animals of the C group were perfused seven days after the brain oedema induction and MP treatment.

Perfusion and fixation

Animals were sacrificed in a deep anaesthesia via standard transcardial perfusion with a 4% solution of paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 15 minutes. After removal from the skull, the brain was fixed in the same solution for 24 hours. Serial coronary vibratome sections (30-μm thick) from each brain were placed on gelatine-coated slides and dried.

Neurohistology

The sections were rehydrated and axonal changes detected with the Black Gold II method of staining (Histo-Chem Inc., Jefferson, AZ, USA.) (Schmued *et al.* 1999). The hippocampal formation was the main part of the brain under study because of its known high sensitivity to various pathogenic stimuli. Analysis was centred on the CA1 and CA3 areas of the hippocampus and on the dorsal blade of the dentate gyrus (DG).

The neurohistological picture of the structural integrity of the axons was assessed with the aid of the following grades of myelin degradation: 1 = no change, 2 = sporadic oedematous distensions, solitary axonal swelling, 3 = multiple vesicles, varicosities, oedematous axons and helical course of axons, 4 = myelin fragmentation.

Neurohistological examination was done in two experimental groups (each with three different experimental arrangements) and one control group (altogether 35 rats). For the statistical analysis data from 10 sections in each brain were used.

Statistical analysis

The results were statistically evaluated using the t test and one-way analysis of variance (ANOVA) followed by Dunnett post hoc analysis. The statistical software GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses.

RESULTS

It was shown in the previous paper, that mannitol induced brain oedema brings about signs of myelin degradation which evidently progress, as evident from the higher intensity of myelin changes in the longer time intervals from the oedema induction. In the previous paper the shortest interval was one hour and the longest one week (Kozler *et al.* 2010). In the present study we aimed at the acute development – the group perfused 30 minutes after the mannitol administration (A) and chronic development – perfusion one week after the mannitol administration (C).

Results revealed an evident neuroprotective effect of MP. In the acute group myelin degradation was less extensive after the MP administration either it was given into the carotid artery or to peritoneum. Differences between the group with oedema only and MP treated animals as well as between groups with different administration strategy were significant ($p<0.0001$) (Figure 1).

In the chronic group with the level of myelin degradation higher, the neuroprotective effect of MP was well expressed in both modes of administration ($p<0.0001$). However; no difference was seen between the groups with different administration strategy (Figures 2 and 3).

DISCUSSION

The neuroprotective effect of methylprednisolone in brain injury was demonstrated in some experiments (Ildan *et al.* 1995, Park 1998) but not in others (Shapira *et al.* 1992). MP-induced reduction of vasogenic oedema was documented by Lin *et al.* (Lin *et al.* 1994). The neuroprotective effect of MP was also proved in some experimental models of cerebral ischaemia (Slivka & Murphy 2001). Different researchers use different doses of MP (30–105 mg/kg); the same applies to the dosage (at least one and at most five doses) and to the length of time of MP application (a few minutes up to 12 hours). This points not only to the lack of uniformity in testing the MP neuroprotective action on the brain but also to the fact that its primary effect on the brain cell has yet to be clarified, and that, for the time being, we have to do with the definition of MP as a nerve cell stabiliser (Hall 1992). In the experiments so far, MP was applied i.v. or i.p. and once intraarterially by way of the caudal artery.

Methylprednisolone is a synthetic steroid with four times higher glucotropic and one fifth of mineralotropic action of cortisol (hydrocortisone). Being a

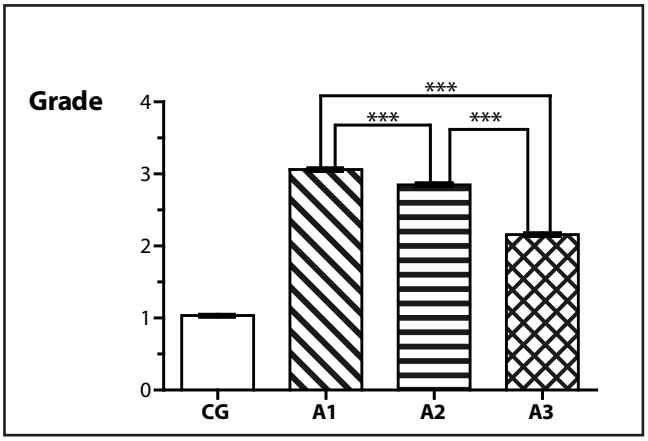

Fig. 1. Myelin changes in acute group (30 minutes after the mannitol administration).
CG control group; A1 animals with brain oedema; A2 animals with brain oedema treated with methylprednisolone intraperitoneally; A3 animals with brain oedema treated with injection of methylprednisolone into the internal carotid artery

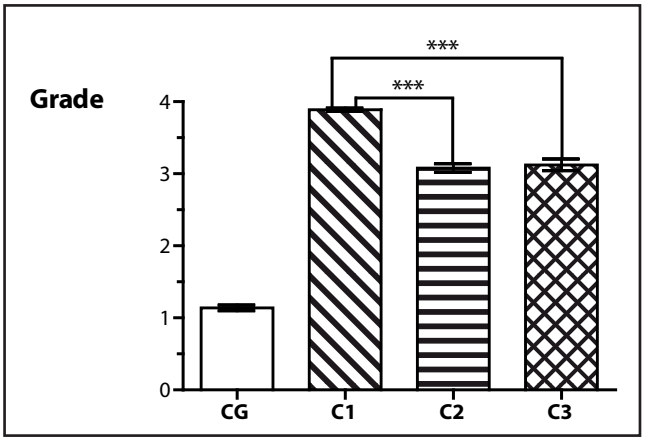

Fig. 2. Myelin changes in chronic group (one week after the mannitol administration).
CG control group; C1 animals with brain oedema; C2 animals with brain oedema treated with methylprednisolone intraperitoneally; C3 animals with brain oedema treated with injection of methylprednisolone into the internal carotid artery

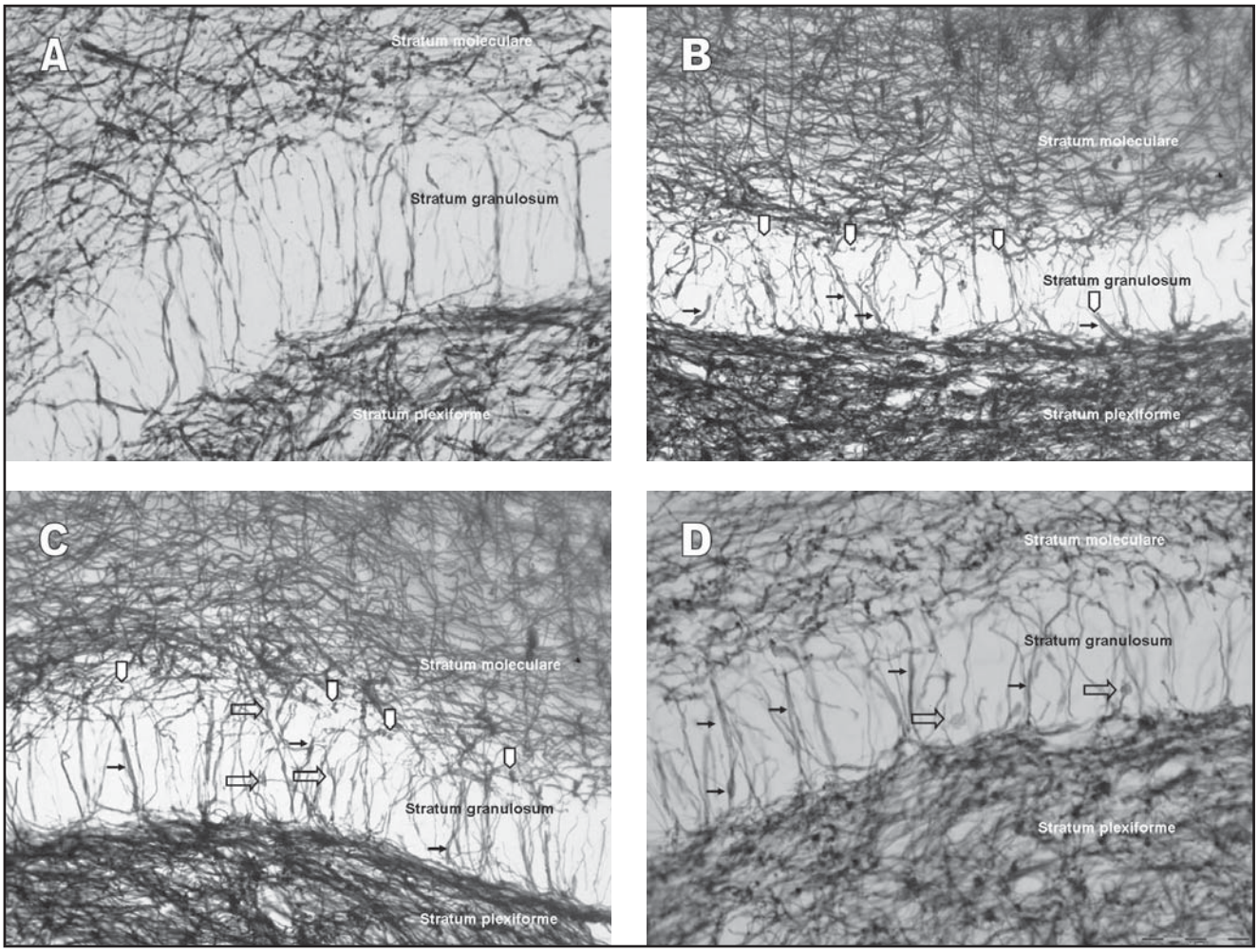

Fig. 3. Myelin changes in stratum granulosum and adjacent stratum moleculare and stratum plexiforme of gyrus dentatus of the acute group (30 minutes after the mannitol administration).
Empty arrow – oedematous vesicle; Full arrow – oedematous axon; Empty arrowhead – fragmented myelin; (original magnification 500 times, scale bar represents 50 μm)
a) Control animal – axons are of the uniform diameter without varicose distensions
b) animals with brain oedema
c) animals with brain oedema treated with methylprednisolone intraperitoneally
d) animals with brain oedema treated with injection of methylprednisolone into the internal carotid artery.

steroid, MP is of a lipophilic nature and is only little dissolved in water. To be distributed in the body fluids it has to be in the form of ester MPSS (methylprednisolone sodium succinate). MPSS is not stabile and due to activity of hepatic esterases MP is released and subsequently bound in 40 to 60% to plasma proteins. Contrary to the free liposoluble MP the high-molecular complexes cannot cross the blood-brain barrier. From the total MPSS administered intravenously or intraparietally only about one half do cross the blood brain barrier (Hall 1992). The studies referred above do not mention this important fact. For MP to act on the target structure – neuronal cell membrane – it would have to pass through the BBB regardless of the technique of application. Described dual effect of MP on the axonal deterioration induced by experimental brain oedema reflects pharmacokinetics of that substance. In the acute part of experiment, MPSS could cross the BBB during the first passage through the cerebral circulation. After 20 to 30 min the barrier becomes closed and therefore in the chronic group effective was only the MP released by hepatic esterases and results were similar in both modes of administration (i.p. and i.c.)

CONCLUSION

Results of our experiment revealed significant beneficial effect of methylprednisolone on the axonal and myelin deterioration induced by experimentally evoked brain oedema. Effect was demonstrated in axons of the hippocampal neurons both in the time interval of 30 minutes and 7 days after the oedema induction and MP

administration. MP had markedly higher effect on the acute oedema consequences if injected intracarotically (30 minutes after the oedema induction).

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