Ameliorating effects of dexpanthenol in cerebral ischaemia reperfusion induced injury in rat brain
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Abstract

Objective: To study the attenuating effect of Dexpanthenol (Dxp) provitamin B5 on neuronal damage after cerebral ischaemia/reperfusion.

Methods: This was a randomized, controlled experimental study conducted at the Islamic Azad University, Tabriz, Iran, from April to September 2008. Male wistar rats were divided into 4 groups randomly (n=13): 1- sham group, Group 2 :two hours occlusion of middle cerebral artery (MCA) and 24 hours reperfusion. Group 3: two hours occlusion of MCA and 24 hours reperfusion + Dxp (250mg/kg) since 3 days before ischaemia. Group 4: two hours occlusion of MCA and 24 hours reperfusion which had received Dxp (500mg/kg) since 3 days before ischaemia. Glutathione (GSH) and malondialdehyde (MDA) levels were studied in brain tissue and numbers of cornu ammonis (CA1 and CA3) pyramidal neurons were studied with light microscopy.

Results: The GSH levels were significantly higher in groups 3 and 4 as compared with group 2. In group 3 and group 4 animals, the MDA levels were significantly lower than in group 2 (P < 0.05). Numbers of CA1 and CA3 neurons were completely normal in appearance in the group 1. The surviving neurons in the CA1 and CA3 subfield were markedly decreased in number, in group 2 (P < 0.05).

Conclusion: Our pathologic and biochemical study has proven positive effect of Dxp on protection of cerebral tissue after I/R. The present findings correlate with previous studies on the protective effects of Dxp against cell and tissue injury by I/R.

Keywords: Dexpanthenol, Cerebral ischaemia, Reperfusion, Injury, Rat brain (JPMA 61:889; 2011).

Introduction

Cerebral ischaemia/reperfusion (I/R) is caused by a deficiency in blood supply to a part of the brain and often occurs during operative procedures such as carotid endarterectomy, extracorporeal circulation, temporary clipping of cerebral arteries, or deliberate hypotension and can cause permanent neurological damage, an area of infarcted tissue, severe functional impairments and death if not managed quickly. Therapeutic strategies to limit infarct size and improve functional outcome after acute stroke are aimed at rescuing this potential reversible ischaemic region.

Tissue hypoxia plays a critical role in the primary and secondary events leading to cell death after cerebral ischaemia. Oxidative damage due to overproduction of reactive free radicals plays a key role in the pathogenesis of ischaemic brain damage, and free radicals are generated during both ischaemia and reperfusion. Reactive oxygen species (ROS) are implicated in secondary brain damage as they damage proteins, DNA, and membrane lipids. The major sources of ROS are leakage from malfunctioning mitochondria, arachidonic acid metabolism and activated neutrophils. In addition, during ischaemia, NOS is up-regulated and the NO produced reacts with ROS to produce radicals, deleterious for neuronal survival. ROS are scavenged by superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase. Another small molecule antioxidant, glutathione (GSH) is involved in the detoxification of free radicals. Indeed, the overproduction of ROS and the consequent oxidative stress has a critical role in the development of brain injury.

Focal cerebral ischaemia models in rats have gained increasing acceptance in recent years for their relevance to human beings. Currently, treatment options for stroke are limited, and many promising drugs have failed in human clinical trials due to intolerable side effects or therapeutic limitations. Pharmacological modification of oxidative damage may protect against ischaemia. Cerebral I/R may lead to irreversible defects which cause death or plesia, so finding effective agents which could attenuate cerebral I/R induced injury is very important as previously some antioxidant drugs have been used for protection of cerebral /I/R injury.

Dexpanthenol (Dxp) provitamin B5, is the biologically active alcohol of pantothenic acid (PA) and
Experimental procedures:
Fifty three male wistar rats weighting 200-230 g were used. Animals were kept in the experiential animals’ laboratory of veterinary school of Islamic Azad University, Tabriz, Iran. The randomized controlled experimental study was performed during April - September 2008. Experiments were performed in conformity with the university research council guidelines for conducting animal studies. The animals were randomly placed in 4 groups (n=13): 1- sham group: operation without occlusion of MCA, group 2: 2 hours occlusion of MCA and 24 hours reperfusion, group 3: 2 hours occlusion of MCA and 24 hours reperfusion which received Dxp (250mg/kg) for 3 days, group 4: 2 hours occlusion of MCA and 24 hours reperfusion which received Dxp (500mg/kg) for 3 days. All the drugs were administered by intraperitoneal injection.

Focal cerebral ischaemia:
Rats were anaesthetized with ketamin (50 mg/kg) and xylazine (10 mg/kg) intraperitoneally. Rats were placed in supine position on a heated pad, with body temperature maintained at 37 ± 0.5°C using rectal thermometer. Right common carotid artery (CCA) and external carotid artery (ECA) were exposed. The internal carotid artery (ICA) was also dissected to the level of petrygopalatine artery. Thereafter, a silk thread was placed loosely around the ECA, and a nylon thread (3-0) was inserted through. While holding the thread around ECA tightly to prevent bleeding, the microvascular clip on ICA was removed, and the nylon thread was carefully and slowly pushed forward through ICA until a light resistance was felt. Such resistance indicated that the tip of nylon thread was wedged at the beginning of anterior cerebral artery (20-22 mm from CCA bifurcation), resulting in occlusion of the MCA. At 2 hours of induction of ischaemia, the filament was slowly removed. Animals were then recovered from anaesthesia, and kept in single cages for 24 hours, after which brains were removed for determination of injury.

Measurement of GSH Levels in Cerebral Tissue:
GSH levels were determined by a modification of the procedure described by Moron et al. Briefly, after homogenization of the tissue samples in 150 mm KCl, 0.5 ml of the resulting homogenate was mixed with 3 ml deproteinization solution (NaCl, metaphosphoric acid, EDTA in distilled water) and 1.5 ml 150 mm KCl solution. Each sample was centrifuged at 1,000 × g for 5 min, and 0.5 ml of the supernatant was added to 2 ml Na2HP04 and 0.5 ml Ellman solution (DTNB; dithiodinitrosobenzic acid, sodium citrate, distilled water). The absorbance of these supernatants were recorded at 412 nm and converted through those obtained from the GSH standards.

Light Microscopy Analyses:
After decapitation, the left hemispheres of the brains were stored in the 10% formaldehyde overnight at 4°C. The samples were then fixed in a 10% buffered formalin solution for 7 days. The left hippocampal regions were obtained from coronal sections of the frontal planes. Formaline-fixed, paraffin-embedded sections (4-μm thickness) were stained with haematoxylin eosin and cresyl violet. Intact hippocampal CA1 and CA3 pyramidal neurons were semiquantitatively counted in three consecutive 789 μm² areas outlined with a counting Gundersen’s frame under 40× magnification, and in three consecutive hippocampus
sections. A shrunken eosinophil cytoplasm and picnotic nucleus were accepted as criteria for definition of ischaemic neuronal damage. The histologist was blinded to the animal groups, and the procedure was conducted in a blinded fashion.\textsuperscript{18}

**Statistical Analysis:**

Data are presented as mean ± SD. Statistical comparisons were made by ANOVA, with SPSS software, version 11.5. A value of P<0.05 was considered statistically significant.

**Results**

**MDA Levels:**

As shown in the table, in group 3 and group 4 animals, the MDA levels were significantly lower than in group 2 (P < 0.05). In group 1, the MDA levels were lower than all other groups. MDA levels were significantly lower in group 4 compared to group 3 (p < 0.05) (Table).

<table>
<thead>
<tr>
<th></th>
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<th>CA3</th>
<th>MDA</th>
<th>GSH</th>
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<tbody>
<tr>
<td>Sham</td>
<td>208.46±5</td>
<td>134.30±6.2</td>
<td>107.53±5.1</td>
<td>185.07±3.9</td>
</tr>
<tr>
<td>I/R</td>
<td>121.69±5.4</td>
<td>89.23±6.5</td>
<td>218.69±6.3</td>
<td>78.00±4.3</td>
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<tr>
<td>I/R+250mg PA</td>
<td>132.30±5.4</td>
<td>97±7.9</td>
<td>181.15±11.3</td>
<td>107.84±6.4</td>
</tr>
<tr>
<td>I/R+500mg PA</td>
<td>155.46±5.9</td>
<td>117.38±3.1</td>
<td>150.30±6.7</td>
<td>129.61±5.9</td>
</tr>
</tbody>
</table>

The values are shown as a mean ± SD for rats in each group and difference of (P<0.05) considered significant. Tukey post hoc test was used for comparing the differences between groups. I/R (Ischaemia/reperfusion), PA (Pantothenic acid).

**GSH Levels:**

In group 2 the GSH levels were significantly lower when compared with other groups. The GSH levels were significantly higher in groups 3 and 4, as compared with group 2. GSH levels were significantly higher in group 4 as compared with group 3 (p < 0.05) (Table).

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**Histopathology:**

Under light microscopy, the pyramidal neurons in the CA1 and CA3 subfields of the hippocampus were completely normal in appearance in group 1. The surviving neurons in the CA1 and CA3 subfield were markedly decreased in number in group 2 compared to all other groups (P < 0.05), Table. Number of surviving CA1 and CA3 neurons were significantly higher in group 4 compared to group3 (P < 0.05).

**Discussion**

Brain ischaemia causes neuronal death in CA1 and CA3 region of hippocampus.\textsuperscript{19} The hippocampus is an essential part of the limbic system, and its function is associated with higher cognitive function. It is associated with cognitive mental processes such as learning, thinking, reasoning and remembering. The hippocampus has a highly selective vulnerability to hypoxaemia and ischaemia.\textsuperscript{20} I/R insult induces changes in the signal transduction pathways which lead to the degeneration of vulnerable cells.\textsuperscript{21}

ROS is one of the most important factors that induces neuron death in I/R insult. The generation of ROS and the free radical-mediates the changes of cellular signaling pathways and gene regulation and the oxidative damage of protein, lipid and DNA in the processes of cerebral ischaemia have been widely studied.\textsuperscript{22} ROS can be scavenged by endogenous antioxidants including SOD, GSH and CAT.

GSH is involved in the ultimate removal of detoxified oxidation products from the cell. Besides working as a scavenger of ROS, GSH is involved in a variety of metabolic functions such as DNA repair, activation of transcription factors, cell cycle regulation, modulation of calcium homeostasis and regulation of enzyme activities.

This study showed that the activities of GSH were decreased markedly after cerebral I/R. The results were completely consistent with a previous study,\textsuperscript{23} suggesting that a disturbance in endogenous antioxidant balance occurred in I/R. Dxp seems to balance the endogenous antioxidant by improving the activity of GSH. The protective effect of PA and its derivatives against cell damage produced by ROS are well documented.\textsuperscript{24} The cytoprotective effect of pantothenic acid is likely to be due to increased CoA and GSH levels.\textsuperscript{8} Pantothenic supplementation also increases hepatic GSH and liver resistance to irradiation, suggesting that GSH peroxidase and phospholipid-hydroperoxide GSH peroxidase may have detoxified any hydroperoxides formed. Pantothenic acid also prevents the collapse of mitochondrial membrane potential and restored ATP synthesis levels as well as the activity of antioxidant enzymes, such as catalase, GSH peroxidase, GSH reductase and the NADPH forming malic enzyme in vitro and in vivo.\textsuperscript{25,26} MDA is released during brain ischaemia followed by reperfusion, therefore the increase of post-ischaemic brain MDA levels is considered as an index of lipid peroxidation caused by free radical release.\textsuperscript{23} Previous studies show that pantethoine administration to rats prevented lipid peroxidation.\textsuperscript{8}

An obvious enhancement of the level of MDA was shown in the ischaemic group in this study, when treated with Dxp in the doses of 250mg/kg and 500 mg/kg, the level of MDA was significantly reduced, so it was valid to draw the conclusion that Dxp scavenged ROS mainly via increasing the activity of GSH-PX and, consequently, decreased lipid peroxidative damage and attenuated cell injury. Dxp significantly attenuated brain lipid peroxidation after
ischaemia/reperfusion. This alleviating effect was present in the dose of 250mg/kg and 500 mg/kg of Dxp.

Conclusions

In conclusion, to the best of our knowledge, the alleviating effect of Dxp on damage after cerebral ischaemia reperfusion has been shown for the first time. We understand that it depends on its antioxidant effect by increasing the reduced glutathione in cells. Our pathologic and biochemical study has proven positive effect of Dxp on protection of cerebral tissue after ischaemia reperfusion. This may be via positive effect of Dxp on cell CoA content.

The present findings correlate with previous studies on the protective effects of pantothenic acid against cell and tissue injury by Ischaemia reperfusion. Thus, pantothenic acid and some of its derivatives may be a valuable tool in treatment of ischaemia reperfusion induced injury related oxidative stress.

Acknowledgments

This research was supported by a grant from Islamic Azad University of Tabriz branch.

References