Effect of methylprednisolone, tirilazad mesylate and vitamin E on lipid peroxidation after experimental spinal cord injury

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Effect of methylprednisolone (MP), tirilazad mesylate (TM) and vitamin E on lipid peroxidation (LP) was evaluated in an experimental model of spinal cord compression injury in anesthetized rats. Forty rats, divided randomly into four groups, were injured by compressing on the spinal cord at Th 3 for 1 min. Bolus injections of saline solution, MP (30 mg/kg bolus and 5.4 mg/kg/h), TM (10 mg/kg four times per day), or vitamin E (30 mg/kg four times per day) were begun 1 h after the spinal cord injury (SCI). Twenty-four hours after treatment, the rats were killed, and malondialdehyde (MDA), a LP product, was measured in the spinal cord tissues. Rats treated with MP, TM and vitamin E had significantly decreased MDA levels ($P < 0.01$) than rats in the control group. The lowest MDA levels were found in the TM group. These results suggest that MP, TM and vitamin E may have a protective effect against SCI in rats by its antioxidant effect.

Keywords: lipid peroxidation; methylprednisolone; spinal cord injury; tirilazad mesylate; vitamin E

Introduction

Despite intensive preventive efforts, spinal cord trauma continues to be a significant public health problem with unacceptably high morbidity and mortality rates.¹ Secondary injury to the spinal cord after blunt trauma involves multiple biochemical and physiological mechanisms. These include influx of calcium ions, tissue lactic acidosis, free-radical generation and tissue peroxidation, production of prostaglandins and leukotrienes, and membrane depolarization by release of excitatory amino acid neurotransmitters.^{2–6} Lipid peroxidation is believed to be a major cause of posttraumatic cell damage and death. In patients with head and spinal injuries, this process is believed to contribute significantly to the development of permanent neurological dysfunction.⁷ LP can be inhibited by pharmacological agents^{7–11} such as the antioxidants methylprednisolone,^{12–18} tirilazad mesylate^{19–23} and vitamin E.^{24–26} MP is a synthetic glucocorticoid drug that has long been used by clinicians to treat brain oedema and spinal cord injury. The efficacy of an antioxidant dosing regimen of MP in acute SCI is consistent with the hypothesis that oxygen radical-induced LP is an important mediator of secondary spinal cord damage. The primary mechanism of the neuroprotective action of MP is likely to be the inhibition of posttraumatic LP^{13,16} and to protect the structural and functional integrity of biological membranes.^{9,27} Recently, a new class of compounds has been developed, the nonglucocorticoid 21-aminos-

teroid, TM (U74006F), which has been shown to be effective in reducing neurologic injury in animal models with head and spinal cord trauma.^{23,28,29} TM is a free radical scavenger, antioxidant, and LP inhibitor, and had a lack of side effects in volunteers.³⁰ Vitamin E (alpha-tocopherol) also known as an antioxidant has protective effects on central nervous system (CNS) trauma.^{24,25}

Based upon those results, the purpose of the present study was to compare the antioxidant neuroprotective effects of MP, TM and vitamin E after compression injury of the spinal cord in rats.

Materials and methods

The experimental procedure was approved by the Animal Experimentation Committee of Erciyes University at Kayseri, Turkey. Forty adult male, Swiss Albino rats, weighing 200–250 g were used in this experiment.

The animals were anesthetized with a mixture of ketamine (60 mg/kg) and diazepam (6 mg/kg) injected IP. Femoral arterial and venous lines were placed to record the mean arterial blood pressure (MABP) and arterial blood gas change, and to administer fluids and drugs IV. MABP was measured with a pressure transducer (TP-611 Nihon Kohden) before, during and 1, 2, 3 h after the injury. Arterial blood gas pressures were obtained before, during and 1 h after the injury. Body temperature was also monitored via a rectal probe and maintained between 36.5 and 37°C by an abdominal heating pad.

A laminectomy was performed at the Th 3 level and the spinal cord was exposed microsurgically, using the prominent spinous process of Th 2 as a surgical guide. A 52 g compression injury was produced for a 1 min period at Th 3 as described by Rivlin and Tator.³¹ This clip produces a severe SCI: all animals are completely paraplegic immediately after the injury. After the injury the wound was quickly closed. Bladders were manually expressed three times per day.

The rats were divided randomly and blindly to one of four groups (ten animals in each): (1) the control group rats received iv equal volumes of saline solution; (2) the MP group rats received an IV bolus injection of 30 mg/kg MP (Mustafa Nevzat Pharmaceutical Co.) 1 h after the injury, followed by infusion of 5.4 mg/kg/h for 23 h; (3) the TM group rats received IV bolus injections of 10 mg/kg TM (Upjohn Co.) 1, 7, 13, 19 h after the injury; (4) the vitamin E group rats received IV bolus injections of 30 mg/kg vitamin E (Eisai Pharmaceutical Co.) 1, 7, 13, 19 h after the injury. Each drug was administered through an infusion pump.

After 24 h of the treatment, each rat was killed with an intracardiac KCl injection. Then immediate removal of approximately 1 cm of the spinal cord in the Th 3 region was performed atraumatically. Spinal tissue specimens were weighed and immediately preserved in liquid nitrogen to stop enzymatic reactions and kept frozen until lipid peroxide determination, made within 1 week. Tissue malondialdehyde levels were directly determined by the ion-paired reversed phase HPLC method with UV detection as previously described by Esterbauer and Cheeseman.³² Following the removal of proteins from tissue homogenates by the acetonitrile treatment and centrifugation, clear supernatant is injected into the HPLC system. Malondialdehydbis-(dimethylacetal) (Merck) was used as external standards and concentrations were expressed as nmol MDA/g wet weight of the tissues.

All results were presented as mean $\pm$ SD. Statistical analysis of the results was evaluated using analysis of Variance (ANOVA), followed by post ANOVA (Fisher) test to compare changes among individual groups. A *P* value less than 0.05 was considered statistically significant.

Results

Preinjury MABP significantly increased (from an initial value of 120.2 ± 9.1 mmHg to 162.7 ± 11.2 mmHg) immediately after the compression injury and then fell below the baseline (89.7 ± 5.2 mmHg) and remained stable for the duration of the experiments (97.2 ± 11.2 mmHg). There was no significant difference between all the groups. Arterial blood gas did not show significant differences between all the groups. In all animals with SCI, paraplegia was observed at the time of euthanasia.

In all treatment groups with SCI, malondialdehyde levels were a significantly lower than in the control

Table 1 Malondialdehyde levels of traumatic spinal cord

Groups	nmol MDA/g wet wt
Control	121.2 ± 27.1
Methylprednisolone (MP)	$72.3 \pm 28.0^*$
Tirilazad mesylate (TM)	$54.6 \pm 22.5^*$
Vitamin E	$84.9 \pm 44.8^*$

Values are means $\pm$ SD. **P* < 0.01 (*F* = 7.78) compared with control

group (*P* < 0.01) (Table 1). In the TM group, though this level was lower than MP and vitamin E group, it was not statistically significant (*P* > 0.05). The lowest MDA levels were measured in the TM group.

Discussion

The results of this investigation show that the MDA levels fell to a significant degree following MP, TM and vitamin E treatment for a compression injury of the rat spinal cord.

Direct determination of the amount of free oxygen radicals is known to be related to LP. It can be measured indirectly by various methods which indicate LP. MDA is a main breakdown product of LP in the CNS.³³ Some investigators have indicated that much of the ischaemic and reperfusion injury to the CNS is secondary to the production of free radicals that alter lipid membranes, inducing LP with further destruction of cells and ultimately cell death.^{2,34,35} There is extensive experimental support for oxygen free radical formation occurring early.^{2,3} The free radical reactions and LP of neural, glial, vascular cell membranes and myelin are catalyzed by free iron released from hemoglobin, transferrin and ferritin by tissue hemorrhage, lowered tissue pH or oxygen radicals.^{3,4} LP is a geometrically progressing process that will spread over the surface of the cell membrane causing impairment to phospholipid-dependent enzymes, disruption of ionic gradients, and membrane lysis.³

The glucocorticoid steroids have been extensively employed in the clinical treatment of SCI since the 1960s. MP improves motor function in 14 muscle segments of spinal cord injured patients when administered in a 24 h intensive high dose regimen beginning within 8 h after SCI.¹³ Some investigators^{14,36,37} support the notion that lipid peroxide inhibition is the major mechanism for the beneficial effects of MP seen in the second National Acute Spinal Cord Injury Study (NASCIS). A 30 mg/kg iv dose of MP has been shown to attenuate posttraumatic LP.^{34,38} High dose MP can also exert a number of other actions on the injured spinal cord that may contribute to an attenuation of post-traumatic neuronal degeneration. Many of these are also due to the inhibition of peroxidation-related processes. The list includes the support of energy metabolism,³⁶ prevention of progressive posttraumatic ischaemia

development,³⁹ prevention of neurofilament degradation, and inhibition of membrane lipid hydrolysis and consequent vasoactive prostaglandin $F_{2\alpha}$ and thromboxan A_2 formation.³⁴ In the present study, MDA levels in the MP group were significantly lower than the control group ($P < 0.01$), and slightly lower than the vitamin E group. This finding supports the observation of other investigators that postinjury administration of high dose MP has consistently been shown to inhibit posttraumatic LP.

However, the more selective and effective antioxidant TM is introduced for a more clear-cut answer as to the therapeutic importance of inhibition of posttraumatic free radical reaction in the injured CNS. The 21-aminosteroid TM (U74006F), is a potent antioxidant and LP inhibitor, has been shown to be effective in animal models of SCI,^{19,20,23,40–43} and is currently being examined in the NASCIS III clinical trial.⁸ *In vitro* studies suggest that tirilazad exerts its antioxidant activity by multiple mechanisms including; increasing membrane stability, scavenging of lipid peroxyl radicals, reducing LP, induced arachidonic acid release, decreased formation or scavenging of hydroxyl radicals, and maintenance of the level of endogenous vitamin E.^{11,21,22} In contrast to MP, it has no steroidal side effects or peripheral vasodilator activity and is far more potent.^{3,29,30} These characteristics make TM an interesting compound for possible treatment of CNS injury. In this study, even short-term therapy with TM at a dose of 10 mg/kg significantly improved the LP after experimental SCI in the rats.

Vitamin E has been shown to have an inhibitory effect on ischaemic injury, possibility because of its lipid antioxidant action.^{44,45} Iwasa *et al*⁴⁶ reported that LP is increased in an injured spinal cord and that dietary vitamin E effectively protects the spinal cord from compression injury and subsequent LP in rats. Taoka *et al*⁴⁴ investigated the effect of vitamin E on experimental compression injury of the spinal cord in vitamin E-deficient rats. They showed that the amount of lipid peroxides increased with an increase in the compression weight and with decrease in the level of vitamin E intake. In our study, MDA levels in the vitamin E group significantly decreased compared to the control group, but it was not as effective as MP and TM.

This is the first study aiming to correlate the attenuation of LP by treatment with MP, TM and vitamin E on SCI. The doses of TM, MP and vitamin E were selected on the basis of previous studies.^{13,47,48} Our experimental study demonstrated that all three treatment regimens decreased the level of LP following spinal cord trauma in rats. Vitamin E was less effective than TM and MP. The beneficial effect of TM and MP in the treatment of traumatic SCI could be related to decreasing the peroxide lipid products produced as a result of the initial injury. However, further studies should be done in order to determine how long after the injury the antioxidant can be administered and still

produce beneficial effects, and further dose-response qualities of the drug are required.

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