EFFECTS OF LECITHINIZED SUPEROXIDE DISMUTASE AND METHYLPREDNISOLONE ON THE SPINAL CORD INJURY IN RATS

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Introduction
The secondary pathological changes following spinal cord injury (SCI) cause deterioration of its function. Inflammatory reactions occur, and oxidative stress is known to be important in this condition. Recent studies showed that in oxidative circumstances, redox-sensitive nuclear factor-kappa B (NF-κB) is activated and dysfunction of glucocorticoid-receptor (GR) occurs in various cells (1,2). Thus anti-oxidant treatment may down-regulate transcriptional factors through inactivation of NF-κB or reversal of GR dysfunction, which may enhance the effect of methylprednisolone (MP) on SCI. In this study, we used lecithinized superoxide dismutase (PC-SOD) to improve the oxidative condition, and examined the effect of a high dose of MP in combination with PC-SOD on the expressions of mRNAs of pro-inflammatory substances (IL-1β, ICAM-1 and i-NOS) and neurotrophin-3 (NT-3). We also examined the effects by studies on lipid peroxidation (LPO) and hindlimb motor function.

Methods
Young adult male specific-pathogen-free Wistar rats (7 - 8 weeks old) weighing 280-310 g were used. After laminectomy was performed at Th10, incomplete compression injury was induced by placing a weight of 30 g extradurally on the spinal cord at Th10 for 5 min. PC-SOD (40,000 units/kg), MP (30 mg/kg), or a combination of these two drugs in the same dosage was given intradurally on the spinal cord at Th10 for 5 min. PC-SOD (40,000 units/kg), MP (30 mg/kg) or saline only (2 ml/kg) was used for controls. The motor functions of 33 rats (6 rats in a control group, 8 in an MP-treated group, 10 in a PC-SOD-treated group and 9 in a PC-SOD+MP-treated group) subjected to compression injury were assessed in a blind manner using Multicenter Animal Spinal Cord Injury Study (MASCIS) open field scores. LPO in spinal cord tissues of rats were measured and expressed as TBA-reactive substances (TBARS). The level of TBARS was measured before and in each group 1 h after SCI. The expressions of mRNAs of IL-1β, ICAM-1, i-NOS, NT-3 and G3PDH were measured by RT-PCR, and expressed as IL-1β, ICAM-1, i-NOS, NT-3 / G3PDH ratios. In three rats in each group, IL-1β, ICAM-1 at 6 h, i-NOS at 24 h and NT-3 at 48 h to evaluate peak levels after SCI.

Results
Motor disturbances after SCI
MASCIS open field scores (Fig.1) in the three groups treated with PC-SOD, MP and PC-SOD+MP increased with time until 3 days after SCI, and were significantly higher than that of the control (p<0.05). Thereafter the score of PC-SOD group increased and was significantly higher than that of the control at all times after SCI (p<0.05). The score of the MP group decreased from day 3 to 5 and then recovered gradually, and was not significantly higher than that of the control after 4 days. The scores in all groups reached plateaus at about 18 days after SCI. The PC-SOD+MP group did not show any synergism and was similar tendency with the MP group.

Changes in TBARS levels in the spinal cord tissue
The control value of TBARS in the normal rats was 66.2 ± 12.8 nmol MDA/g tissue, and the value increased to 192.2 ± 16.8 in the control rats. Intravenous infusion of 30 min after SCI reduced increase in the TBARS value in the injured spinal cord to 143.2 ± 57.0 with MP, 142.9 ± 8.2 with PC-SOD and 83.2 ± 10.6 with PC-SOD+MP. Expressions of IL-1β, ICAM-1, i-NOS, NT-3 mRNAs after SCI
IL-1β, ICAM-1 and i-NOS mRNA expressions were reduced by all three treatment. The relative mRNA levels of IL-1β, ICAM-1 and i-NOS in treated groups to that in control group were 14.9 %, 67.1 % and 27.8 % in the MP-treated group, 61.3 %, 88.2 % and 40.9 % in the PC-SOD-treated group and 73.3 %, 48.2 % and 22.0 % in the PC-SOD+MP treated group (Fig.2). We investigated change with time in NT-3 mRNA expression, therefore the mRNA level increased from 6h, reached a maximum at 48h, and returned to the basal level 1 week after SCI. Figure 3 shows that MP treatment alone suppressed the expression of NT-3 mRNA slightly (-21.5 % at 6 h, - 33.9 % at 24 h, and -27.4 % at 48 h in the control group). The expression of NT-3 mRNA was enhanced by PC-SOD treatment at 6 h (+12.3 % in the control group) and remarkably at 24 h and 48 h (+55.9 % and +51.3 % in the control group, respectively) after SCI.

Discussion
Inhibition of lipid peroxidation and scavenging of oxygen radicals block post-traumatic pathophysiological changes, which lead promoting functional recovery and survival after SCI (3). In the injured spinal cord, the repair processes may be activated, and neurotrophic factors may be an important in the recovery of motor function. It was reported that spontaneous and orientated regenerative events could be induced after spinal cord compression injury. On the other hand, MP treatment is reported to attenuate the levels of IL-1β, BDNF and NT-3 mRNAs compared with those of an MP untreated group 6 h after SCI (4), suggesting that MP treatment may inhibit the spontaneous and orientated regenerative events after SCI. In this study, PC-SOD and MP were for functional recovery after SCI in correlation with suppression of the increase of pro-inflammatory genes and LPO following SCI. No synergistic effect of PC-SOD and MP was observed on functional recovery, although PC-SOD and MP had synergistic suppressive effects on pro-inflammatory genes. Besides these similar antioxidative effects of PC-SOD and MP, enhanced expression of NT-3 mRNA was observed on PC-SOD treatment but not on MP treatment. The present results should be considered in the planning of new therapeutic means to enhance neurotrophic function in addition to suppress the oxidative stress following SCI.

References

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Fig. 1

Fig. 2

Fig. 3

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