

The effect of duration of compression on lipid peroxidation after experimental spinal cord injury

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Abstract

The present study was performed to evaluate the effect of duration of acute spinal cord compression on tissue lipid peroxidation in rats. A clip compression method (1) was used to produce acute spinal cord injury. Rats were divided into 3 groups, each consisting of 10. At 1 hour after trauma all rats were sacrificed, and MDA content of the injured spinal cord segment was measured. The tissue MDA contents were 3.922 $\mu\text{molMDA/gww}$ in group 1 (control), 10.192 $\mu\text{mol MDA/gww}$ in group 2 (30 seconds compression), and 12.147 $\mu\text{molMDA/gww}$ in group 3 (60 seconds compression). These results demonstrate that the length of duration of compression significantly enhances lipid peroxidation. Our study supported the view that persisting compression may cause progression of secondary mechanisms which may irreversibly eliminate any potential for recovery.

Keywords: Experimental spinal cord injury, lipid peroxidation.

1 Introduction

Blunt trauma to the spinal cord causes both immediate physical injury directly to nerve cells and blood vessels which is largely irreversible and the secondary injury which is a cascade of pathochemical events that is initiated by the primary injury. The secondary injury process involves excessive release of glutamate and aspartate [22], intracellular calcium overload [6, 7, 23, 28], activation of the arachidonic acid cascade [8, 24, 32], and the induction of free radical-induced lipid peroxidation [15]. Oxygen radical mediated lipid peroxidation has often been suggested to be an important factor in post-traumatic neuronal degeneration [19, 21]. Many researchers have shown that

agents inhibiting lipid peroxidation enhance functional recovery in experimental CNS injuries [20, 21, 25]. This concept was supported clinically by NASCIS II [10]. Available evidence suggests that evaluation of lipid peroxidation in injured spinal cord tissue indicates the severity of the trauma. Previous studies [16, 27, 29, 30] have shown the importance of the duration of spinal cord compression upon subsequent functional recovery. Increasing the duration of compression resulted in worsening of the functional recovery. The present study examines the relationship between the duration of compression and the lipid peroxidation levels in the injured spinal cord tissue.

2 Materials and methods

30 female Sprague-Dawley rats with weights ranging from 230–310 g were used. The rats were divided in three groups, each consisting of 10. The rats were anesthetized with an intraperitoneal injection of thiopentone sodium BP (pentothal sodium Abbott) 30 mg/kg and laminectomy was performed at C₇–T₁ using an operating microscope. The clip (Yaşargil aneurysm clip, Aesculap FE 752, force of closure 192 g (162–198 g), curved arms) was applied extradural to the spinal cord and remained compressing the cord for 30 seconds in group 2 and for 60 seconds in group 3. In group 1, laminectomy was performed, but the clip was not applied. One hour after the clip application, rats were sacrificed with large doses of pentothal sodium. The spinal cord was excised under the microscope, and dura, leptomeninges, and blood vessels were separated from the spinal cord tissue. The 1.5 cm long spinal cord segments within the trauma-

tized part in the middle were stored at -70°C . They were homogenized. In this study lipid peroxidation was assessed by measuring the tissue content of malonic dialdehyde (MDA), one of the end products of lipid peroxidation [12]. Tissue samples were homogenized in ice-cold trichloroacetic acid (TCA) (11 g tissue plus 1 ml 10%, wt/vol, TCA plus 8 ml 5%, wt/vol, TCA, or equivalent amounts) in an Ultra Turrax tissue homogenizer. After centrifugation, a volume of the supernatant was added to an equal volume of 0.67% (wt/vol) thiobarbituric acid, and the mixture was heated at 100°C for 10 minutes. The absorption spectrum was then recorded over 480–600 nm. The spectrum was quite similar to that obtained with an MDA standard produced by the acid hydrolysis of 1,1,3,3-tetraethoxypropane and run under the same conditions. The MDA concentration was calculated from the absorption at 532 nm (absorption maximum) of the difference spectrum with the use of a molar extinction coefficient of 1.56×10^5 , as reported by others and also recalculated from our standards. The results were analyzed by using an SPSS PC + statistical solving pocket. The student-t-test was used. The data shown are mean \pm standard deviations. A p value of less than 0.05 was considered statistically significant.

3 Results

The effect of duration of compression was evaluated by measuring lipid peroxidation levels of the injured spinal cord segments. Tissue lipid peroxidation was assessed by measuring the tissue MDA content. In a previous study [9], it was shown that lipid peroxidation levels reach their maximums at 1 hour after the compression and then decrease. We determined the tissue MDA content at 1 hour after the injury in all groups. The MDA content was 3.922 ± 0.933 $\mu\text{molMDA/gww}$ (gram wet weight) in group 1 (sham-operated rats), 10.192 ± 1.634 $\mu\text{molMDA/gww}$ in group 2 (30 seconds compression), and 12.147 ± 0.539 $\mu\text{molMDA/gww}$ in group 3 (60 seconds compression) (Figure 1). When the results were compared with each other, it was seen that the difference between group 1 and group 2 was statistically highly significant ($p < 0.0001$), and that MDA content in group 3 was significantly higher than in group 2 ($p = 0.001$).

4 Discussion

Spinal cord trauma can cause direct damage to nerve cells and axons and/or damage by a cascade

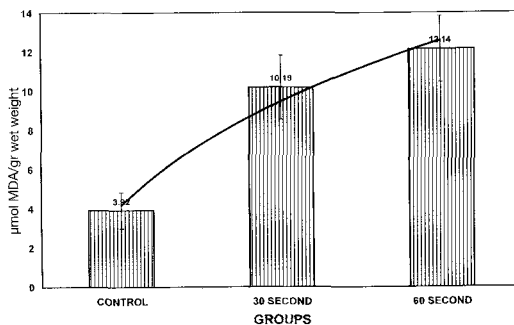


Figure 1. The effect of duration of compression on tissue lipid peroxidation. Each group consists of ten rats. Values above the bars are means, and vertical lines indicate standard deviations. Long duration of compression resulted in high MDA content of the injured segment. The trend line also shows that MDA content is related to duration of compression.

of pathochemical events. Lipid peroxidation, [3, 4, 5, 13, 14, 15, 18, 22, 26] phospholipid hydrolysis with production of eicosanoids [3, 5, 14, 22, 24], and depletion of energy stores with increased lactic acid formation [1, 2, 11] are the earliest biochemical events detected in injured spinal cord tissue. Lipid peroxidation is believed to be catalyzed by free radicals generated as the result of ischemic hypoxia and hemorrhage [15, 18]. The peroxidation of spinal cord lipids can lead to loss of certain enzyme activities that are critical for the maintenance of neuronal excitability and ultimately to tissue dissolution [18, 31].

The extent of lipid peroxidation is thus a useful parameter for evaluating the cellular disturbance caused by spinal cord injury.

The present study was performed to investigate the effect of duration of acute spinal cord compression on tissue lipid peroxidation, which is believed to be the most important determinant of secondary damage.

Tissue lipid peroxidation was evaluated by measurement of MDA content. It was shown that lipid peroxidation levels reach their maximums at 1 hour after injury under experimental conditions [9]. One hour after injury, we sacrificed all rats and determined tissue MDA content of the injured spinal cord segment. The difference between lipid peroxidation levels in group 1 and in group 2 was statistically highly significant ($p < 0.0001$) and between group 2 and group 3, statistically significant ($p = 0.01$). The

result of this study demonstrate that long duration of compression significantly enhances lipid peroxidation.

It is virtually impossible to perform surgical decompression within the time frame of our study. The value of decompression after spinal cord injury is still controversial. The extent to which the neurological deficit is due to primary injury and/or persistence of compression or whether persistence has any additive effect on the neurological deficit is not known. Tarlov [30] concluded that persisting compression adversely affected neurological function, and this led him to advocate immediate decompression as a treatment. Dolan et al [16] found that early relief of persisting compression produced improved neurological recovery for all the compression forces stu-

died. Guha et al [17] concluded that ultimate neurological deficit after experimental spinal cord trauma is determined by the severity of the initial impact and by the duration of persisting compression, with the magnitude of the initial impact force being the dominant factor. They also concluded that decompression of the spinal cord after duration of persisting compression of up to 4 hours may enhance neurological recovery. Although clip strength is the main determinant of neurological recovery, duration of compression is also an important factor for lighter compression forces.

The present study supported the view that persisting compression may cause progression of secondary mechanisms which may irreversibly eliminate any potential for recovery.

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