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Effect of nimodipine and N-acetylcysteine on lipid peroxidation after experimental spinal cord injury

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Abstract

The effectiveness of nimodipine and N-acetylcysteine in experimental spinal cord injury was evaluated by measuring tissue lipid peroxidation levels of the damaged spinal cords 1 hour after the injury. We used the clip compression method to produce acute spinal cord injury in 40 female Sprague-Dawley rats were used. The rats were divided into four groups of 10 each. Lipid peroxidation was assessed by measuring the tissue content of malonil dialdehyde (MDA). In group 3, nimodipine, and in group 4, N-acetylcysteine, was administered i.p. as a single dose immediately after the injury. The rats were sacrificed 1 hour after clip application. The tissue mean MDA content was 3,992 µmol MDA/gww in group 1 (sham operated), 10,192 µmol MDA/gww in group 2 (trauma), 10,449 µmol MDA/gww in group 3 (nimodipine treatment) and 9,009 µmol MDA/gww in group 4 (N-acetylcysteine treatment). These results demonstrated that a single dose of nimodipine and N-acetylcysteine had no effect on peroxidation of lipid membranes in the early period of experimental spinal cord injury.

Keywords: Lipid peroxidation, N-acetylcysteine, Nimodipine, Spinal cord injury.

1 Introduction

Excessive entry of Ca^{2+} into cells has been proposed as a major contributor to neuronal death in the injured spinal cord [2, 3, 23, 33]. As it was previously reported, there is a marked increase in the intracellular concentration of calcium in the spinal cord after spinal cord injury [23, 29, 33, 36]. The effect of influx of Ca^{2+} on neuronal death has not been definitely established. Membrane lipid peroxidation is potentially one of the principal side effects of excessive calcium entry into cells [25]. A second pathway to lipid peroxidation is through free radical production [11–13]. Inhibition of Ca^{2+} ionic influx has been a rational target for pharmacological intervention. Agents used in spinal cord injury models include nicardipine [7, 26], nimodipine [15–19], verapamil, diltiazem, and nifedipine [22]. Most of the studies showed no significant effects of these agents on spinal cord blood flow, electrophysiological changes, or histopathologic and behavioral outcomes.

The formation of free radicals after spinal cord injury has been a target of pharmacological control. Megadose steroids, vitamins C and E, selenium and high doses of opiate antagonists have been used to scavenge the free radicals, thereby preventing lipid peroxidation and membrane destabilization. Studies in animals have shown that N-acetylcysteine inhibits free radical formation [6, 30].

The purpose of this study was to investigate the effect of single-dose nimodipine and N-acetylcysteine on lipid peroxidation, which is a useful parameter for evaluating the cellular disturbance caused by the spinal cord injury.

2 Materials and methods

Forty female Sprague-Dawley rats with weights ranging from 230 to 310 g were used. Rats were divided into four groups of 10. Rats were anesthetized with intraperitoneal injection of thiopentone sodium BP (pentothal sodium Abbott) 30 mg/kg, and laminectomy was performed at C7-T1 by using an operating microscope. The clip (Yasargil aneurysm clip, Aescu-

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lap FE 752, force of closure 192 g [162–198 g]) was applied extradurally to the spinal cord and compressed the cord for 30 in group 2, group 3 and group 4. In group 1 (sham-operated group) laminectomy was performed, but the clip was not applied. In group 3, nimodipine was administered intraperitoneally as a single dose of 0.05 mg/kg immediately after spinal cord injury. In group 4, N-acetylcysteine was adminstered i.p. as a single dose of 163 mg/kg immediately after the injury. Results of group 1 and group 2 rats were used in another study (28) which was conducted simultaneously with this study, in order to avoid sacrificing more animals. Rats were sacrificed with large doses of pentothal sodium at 1 hour after the clip application. The 1.2 cm of spinal cord which contained the traumatized part in the middle was excised under the microscope and stored at m – 70°C until the homogenization procedure. Lipid peroxidation was assessed by measuring the tissue content of malonic dialdehyde (MDA) [9], one of the end products of lipid peroxidation. Tissue samples were homogenized in ice-cold trichloracetic acid (TCA) (1 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 give an exual volume of 0.67 % (wt/vol) thiobarbituric acid, and the mixture was heated to 100°C for 10 min. 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 × 100 000, as reported by others and also recalculated from our standards.

3 Results

The results were analyzed by using SPSS PC+ statistical solving pockage. Student's-**t** test was used. A p-value less than 0.05 was considered statistically significant. The effect of treatment was evaluated by assessing MDA formation in the spinal cord at 1 hour after injury. The MDA content is listed in Table I by group. The MDA content was higher in group 2 (without treatment) than in group 1 (sham-operated). The difference between group 2 and group 3 (nimodipine treatment) and group 4 (N-acetylcysteine treatment) was not statistically significant. A single dose of nimodipine and N-acetylcysteine had no effect on MDA formation in the spinal cord homogenates.

4 Discussion

There is a rapid change in the extracellular concentration of Ca^{2+} and K^+ ions in the injured spinal cord [36]. Excessive entry of ca^{2+} into cells is a major contributor to neuronal death [2,3,23,33]. The possible mechanisms of calcium toxicity are activation of intracellular proteases and lipases, free radical formation, depletion of energy, and impairment of metochondrial oxidative phosphorylation [10]. Membrane lipid peroxidation, with its deleterious offects on membrane integrity and on the production of arachidonic acid metabolites, is potentially one of the principal harmful side effects of excessive calcium entry into cells [25].

Table I. MDA levels (micromoles MDA per gram of wet weight)

Control groups		Treatment groups	
Sham-operated Group 1 $n = 10$	Trauma Group 2 $n = 10$	Nimodipine Group 3 $n = 10$	N-Acetylcysteine Group 4 $n = 10$
2.69	12.05	8.46	10.58
3.53	8.65	11.22	7.18
4.08	12.50	10.32	12.45
5.32	9.23	11.54	10.71
2.44	8.85	9.36	7.95
3.97	11.03	9.68	6.73
3.40	9.74	11.86	8.33
4.62	10.26	7.95	8.21
4.17	11.26	10.45	11.22
5.00	7.69	13.65	6.73
(mean) 3.9220	(mean) 10.1920	(mean) 10.4490	(mean) 9.0090
±0.933	±1.634	±1.700	±2.056

Lipid peroxidation occurs as a result of ischemia and reperfusion, which increases the permeability of cell membranes to calcium. Increased intracellular concentration of Ca^{2+} might either enhance lipid peoxidation reactions further or stimulate the degradation of phospholipid, which further disrupts membrane permeability [21].

Calcium antagonists have been shown to interfere with voltage-activated inward displacement of Ca^{2+} into neurons; hence they can potentially minimize calcium toxicity following injury to the central nervous system [14].

In our study, the ability of a single intraperitoneal dose (0,05 mg/kg) of nimodipine to attenuate lipid peroxidation at 1 hour after spinal cord injury in rats was examined. It was previously reported [5] that the lipid peroxidation level in the injured spinal cord tissue reaches its maximum at 1 hour after trauma. Therefore, we sacrifficed the rats 1 hour after trauma. Our results show that nimodipine has no effect on lipid peroxidation in experimental spinal cord injury. This is probably due to (1) inffectiveness of nimodipine to prevent Cay^{+2} entry into traumatized cells and/or (2) presence of other mechanisms causing lipid peroxidation and/or (3) dose and method of administration of nimodipine.

Previous reports have shown that nimodipine can effectively increase cerebral and spinal blood flow [16, 17, 24, 31]. GUHA et al. [17] found approximately 40 % increase in spinal cord blood flow following administration of nimodipine in a dose of 0.05 mg/kg.

Several studies in traumatic spinal cord injury models have shown that nimodipine has no beneficial effect. FORD and MALIN [15] found no benefit in terms of functional recovery from spinal cord injury. HAGHIGHI et al. [19] reported that nimodipine has no beneficial effect on axonal function as measured by the evoked cortical activities. HOLTZ et al [27] reported hat nimodipine was of no benefit either functionally or in terms of spinal cord blood flow.

A second pathway to lipid peroxidation is through free radical production secondary to injury. DEMOPOULOS et al. [11] reported that cell membrane damage in the central nervous system following cerebral ischemia and spinal cord injury may be induced by free radical reaction and lipid peroxidation.

Free radicals are molecules with single, unpaired electron in their outer electron orbit. These molecules are chemically very active, reacting with other molecules to form more free radicals. Endogenous enzyme systems and antioxidants control the production and effects of free radicals. When these systems are overwhelmed, secondary injury may occur.

ARUOMA et al. [1] have shown that N-acetylcysteine scavenges hydroxyl radicals and hypochlorous radicals. Studies in animals have shown that N-acetylcysteine inhibits free radical formation [6, 30].

Oxygen radical mediated lipid peroxidation has been suggested to be an important factor in post-traumatic neuronal degeneration [20]. Many studies have evaluated the neuroprotection efficacy of pharmacological agents with lipid antioxidant activity in models of spinal cord and brain injury. The results of NASCIS II [8] support the significance of lipid peroxidation as a post-traumatic degenerative mechanism. As a part of the present study, the efficacy of a single intraperitoneal dose (163 mg/kg) of N-acetylcysteine was investigated by measuring MDA content of the traumatized spinal cord 1 hour after the injury. Our results show that N-acetylcysteine has no effect on lipid peroxidation.

It has been shown that N-acetylcysteine inhibits lipid peroxidation caused by doxorubicin in vivo [32], whereas it enhances lipid peroxidation in the presence of iron in vitro [34, 35]. BARTH et al. [4] has shown that N-acetylcysteine slightly increases cyclosporin A-induced lipid peroxidation when iron is added to the medium of incubation.

In our opinion the probable causes of the inability of N-acetylcysteine to decrease lipid peroxidation are (1) the amount of free Fe^{3+} available to oxidize the sulfhydryl group of N-acetylcysteine and (2) the dose and method of administration were inadequate; and (3) there are many pathways to lipid peroxidation the prevention of the progression of only one pathway, may not be sufficient to decrease lipid peroxidation.

N-acetylcysteine may be effective if used with chelators to reduce the availability of iron in spinal cord injury. It may be more effective to use a combination of agents that affect the pathways of the secondary injury cascade.

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