EFFECT OF DIFFERENT SEVERITY OF EXPERIMENTAL SPINAL CORD INJURY ON ANTIOXIDANT ENZYME ACTIVITIES

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ABSTRACT:

Eight female Sprague-Dawley rats with weights ranging from 230 to 310 g were used to perform a rat model of spinal cord injury by the clip compression. After application of an aneurysm clip for 30 seconds in group 1 and 60 seconds in group 2, we determined superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities at 24. hours following trauma. Each segment was compared with the same segment of the other group (lesion with lesion, rostral with rostral and caudal with caudal). The enzymes' activity changes were not statistically significant. This lack of response of the enzymes to the increased severity of trauma may explain a part of the secondary damage due to oxygen free radicals in traumatic injury.

Key Words : *Experimental spinal cord injury, superoxide dismutase, glutathione peroxidase, catalase*

INTRODUCTION

Primary effects of injury on spinal cord such as axonal shearing and vascular disruption are largely irreversible, however secondary effects such as tissue edema, ischemia, ionic fluxes and free radical damage are preventable or reversible. Despite many researches about the mechanism of secondary tissue damage in spinal cord injury, these mechanisms still remain obscure. Demopoulos et al (1) has shown the importance of free radicals and lipid peroxidation in progressing of secondary injury. Free radicals are molecules with a single, unpaired electron in their outer orbit. These molecules are chemically very active, reacting with other molecules to form more free radicals. The most extensively studied free radical system is oxygen. Active oxygen free radical species include the superoxide anion, hydrogen peroxide and the hydroxyl radical. Aerobic organisms have evolved enzymes such as catalase, superoxide dismutase and peroxides, and substances such as vitamin E, betacarotene, cholesterol, ascorbic acid to scavenge the excess free radicals (2, 3, 4, 5, 6). When these systems are overwhelmed, secondary injury may occur.

Following our previous study (7) on changes in the activity of antioxidant enzymes by time and among segments after spinal cord injury in rats, we planned to study effects of injury severity on the activity of antioxidant enzymes (superoxide dismutase: SOD, glutathione peroxidase:GPx, catalase : CAT).

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MATERIALS AND METHODS

Eight female Sprague-Dawley rats with weights ranging from 230-310 g were used. Rats were divided in two groups, each consisting of four. Rats were anesthetized with intraperitoneal injection thiopentone sodium BP (pentothal sodium Abbot) 30 mg/kg, and laminectomy was performed at C7-T1 by using an operating microscope. The clip (Yaşargil enaurysm 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 1 and for 60 seconds in group 2. Rats were sacrified with large doses of pentothal sodium at 24. hours after injury. The spinal cord was excised under the microscope and then divided into rostral, lesion and caudal segments of 6-7 mm in length each. Dura, leptomeninges and blood vessels were separated from the spinal cord tissue under microscope. These spinal cord segments were stored at -20C until hoomgenization procedure. In each segment SOD, CAT and GPx activities were determined.

Tissue homogenization was performed with a tissue grinder fitted with a teflon pestle. Tissues were homogenized with 0.05 M phosphate buffer at pH 7.5 to give a 5% W/V homogenate. Homogenization procedures were performed at 4C. Homogenate was centrifuged at 10000 g for 15 minutes. Then the supernatant was removed and used for enzyme and protein assays. SOD activity was assayed by using nitroblue tetrazolium (NBT) method (8) and catalase activity was measured by using Beers and Sizer's method (9), and Ransel RS 504 (Randox) was used by the method of Plagia and Valentin (10) to measure glutathione

peroxidase activity. The protein content of supernatant was determined by the method of Lowry et al (11). The results were given as enzyme unit per mg protein.

RESULTS

The spinal cord was divided into rostral, lesion and caudal segments and enzyme activities were determined in each segment. The results were anlyzed by using SPSS PC+ statistical solving pocket. ANOVA test (analysis of variance) was used. A p value less than 0.05 is considered statistically significant. The results were shown in Fig. 1, 2 and 3. Each segment was compared with the same segment of the other group. For each segment, the activities of antioxidant enzymes has not significantly changed by the increasing severity of trauma.

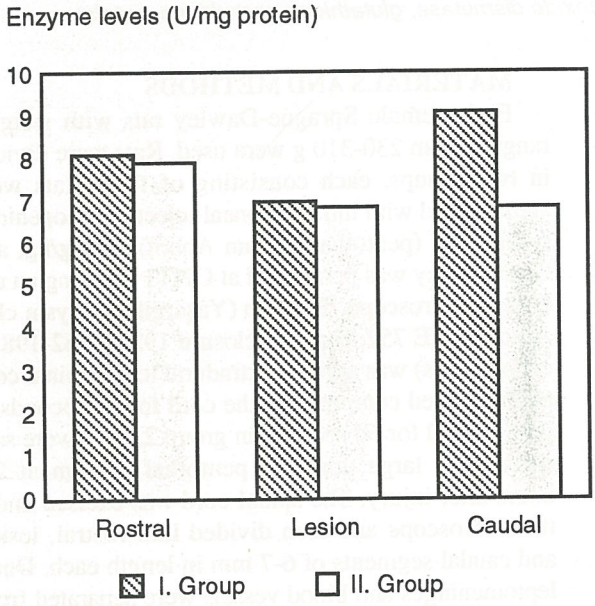

Fig. 1: Superoxide dismutase (SOD) activity in different segments of the two different injury severity groups at 24 hours after trauma.

DISCUSSION

It was the purpose of this experiment was to study the changes in the activities of antioxidant enzymes as a result of varying degrees of spinal cord trauma. We used a rat model of spinal cord injury by the clip compression (12). By application of an aneurysm clip for 30 seconds in group 1 and 60 seconds in group 2, we performed a standardized spinal cord injury. 24 hours after injury, tissue SOD, CAT and GPx activities were determined. Each segment was compared with the same segment of the other group.

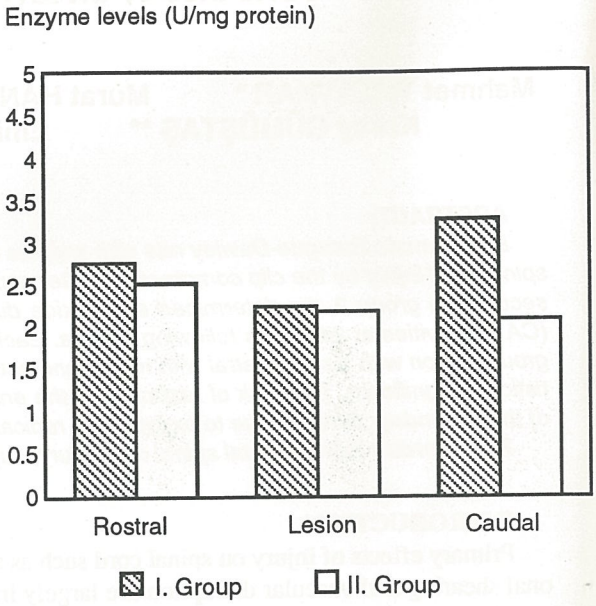

Fig. 2: Glutathione peroxidase (GPx) activity in different segments of the two different injury severity groups at 24 hours after trauma.

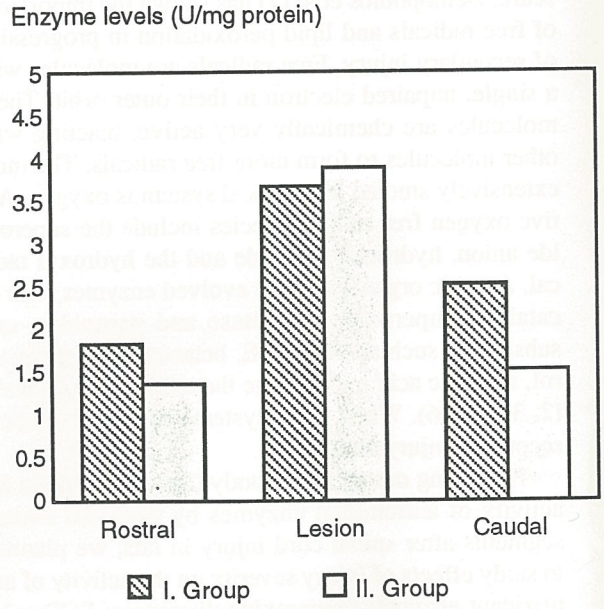

Fig. 3: Catalase (CAT) activity in different segments of the two different injury severity groups at 24 hours after trauma.

Traumatic neural injuries of both the brain and spinal cord cause tissue damage through primary and secondary mechanisms.

Substances such as free radicals, calcium and leukotrienes have been implicated as contributing to the development of secondary injury (3, 13). Kontos et al (14) reported that oxygen free radicals play a key role in the primary and secondary process of acute traumatic neural injuries. Demopoulos et al (1) and Ortega et al (15) emphasized that the brain and nervous system are especially prone to tissue damage induced by oxygen free radicals. Olesen (16) also reported that oxygen free radicals induce a decrease of the electrical resistance of pial venules in frogs, indicating an increase of ionic permeability that could be prevented by SOD and catalase. Recent data (17, 18, 19, 20, 21, 22) are consistent with the view that the mechanism of oxygen free radicals is a critical pathophysiological process in traumatic central nervous system injury.

Post-traumatic necrosis in spinal cord is a consequence of the initial physical insult and a series of melocular events that promote progressive damage. The secondary events are targets of therapeutic investigation to reduce the permanent damage.

The first therapeutic approach against harmful effects of oxygen free radicals may be to reduce the superoxide radicals by using SOD. Ando et al (23) have used an SOD derivative which was reversibly bound to albumin, circulated with a half life of 6 hours, and accumulated at the site of an injured tissue whose local pH was decreased. Intravenous administration of this SOD derivative markedly suppressed the increase in vascular permeability and the early development of brain edema.

The second therapeutic approach may be the inhibition of the iron catalyzed Haber-Weiss reaction by reducing the availability of iron ions by using iron chelating agents. The hydroxyl radical reacts with almost every type of molecule found in living cells: sugars, amino acids, phospholipid, DNA bases and organic acids (24). A major source of hydroxyl radical in biological systems is the reactive sequence of superoxide radical with hydrogen peroxide in the presence of the iron ion.

In our spinal cord trauma model, we have found that SOD, GPx and CAT activities have no any significant response to the increased severity of trauma. When each segment was compared with the same segment of the other group, the enzyme activity changes were not statistically significant. This lack of response of the enzymes to the increased severity of trauma may explain a part of the secondary damage due to oxygen free radicals in traumatic injury. If these protective enzymes cannot scavenge oxygen free radicals produced by trauma, then harmful effects of free radicals will contribute to the progression of the damage.

According to our results and previously reported data, we suggest that efforts in developing agents to increase oxygen free radical scavengers at the injury site will have a major role in preventing secondary damages following neurotrauma in the future.

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