

CHANGES IN THE ACTIVITY OF ANTIOXIDANT ENZYMES (SOD, GPX, CAT) AFTER EXPERIMENTAL SPINAL CORD INJURY

MEHMET YASAR KAYNAR*, MURAT HANCI*, CENGİZ KUDAY*,
AHMET BELCE**, KORAY GUMUSTAS** AND EMINE KOKOĞLU**

**İstanbul University Cerrahpaşa Medical Faculty Department of Neurosurgery, Aksaray İstanbul Turkey.*

***İstanbul University Cerrahpaşa Medical Faculty Department of Biochemistry, Aksaray İstanbul Turkey.*

(Accepted: October 17, 1994)

We determined time dependent changes in the levels of the antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) at 1, 4 and 24 hours after standardized reversible spinal cord injury in rats. In each segment (rostral, lesion, caudal) enzyme activities at 1, 4 and 24 hours were not significantly different. Without time limitation we have found that SOD and GPx activities were not significantly different ($p>0.05$), but CAT activity was significantly high ($p=0.008$) in the lesion segment than the rostral and caudal segment. According to our results we suggest that one of the main reason for tissue damage during such a spinal cord trauma model may be neither H_2O_2 nor H_2O_2 derived radicals.

Key words: Experimental spinal cord injury — Superoxide dismutase — Glutathione peroxidase — Catalase

Trauma to the spinal cord produces problems which are difficult or impossible to solve for the patient and the physician. Primary effects of injury 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.

Recently, some studies put forward the importance of free radical production and lipid peroxidation secondary to spinal cord injury^{2,5,7,15}. In this study, we investigated the changes in the activity of antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT)) by time and among segments of injury.

MATERIALS AND METHODS

Twelve female Sprague-Dawley rats with weights ranging from 230 to 310 g were used. 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 (Yaşargil aneurysm clip force, of closure 192 g (162–198 g), curved arms) was applied extradurally to the spinal cord, and remained compressing the cord for 30 seconds. We have observed generalized tonic spasm during clip application for 2 seconds and then flaccid paraplegia. Rats were divided into 3 groups, each consisting of four. Rats were sacrificed with large doses of pentothal sodium at 1 hour after injury in group 1, at 4 hours in group 2 and at 24 hours in group 3.

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 the microscope. These spinal cord segments were stored at -20°C until homogenization procedure. In each segment superoxide dismutase, catalase and glutathione peroxidase 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 4°C . 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) method¹⁸⁾ and catalase activity was measured by using Beers and Sizer's method¹⁾ and Ransel RS 504 (Randox) was used by the method of Plagia and Valentin¹²⁾ 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 time-dependent enzyme activities were determined in each segment. The results were analyzed 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. Time-dependent changes of enzyme activity in each segment was not statistically significant. ANOVA was used to evaluate enzyme activity differences between the segments. The values were not limited with time and each segment was compared with

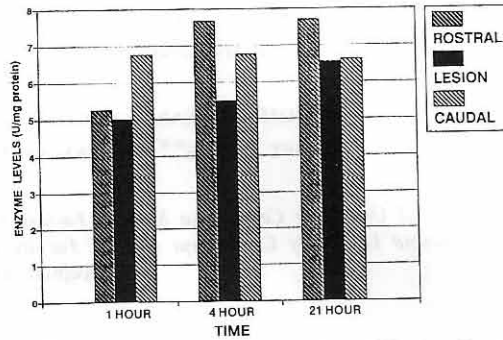


Fig. 1. SOD enzyme activity changes during first 24 hours.

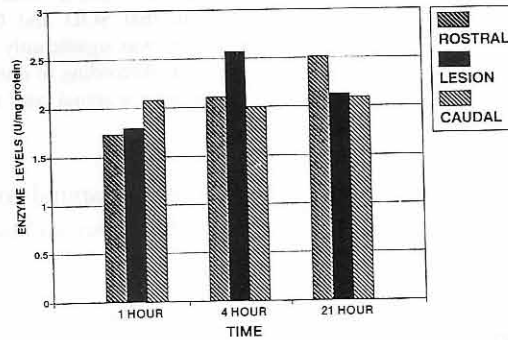


Fig. 2. GPx enzyme activity changes during first 24 hours.

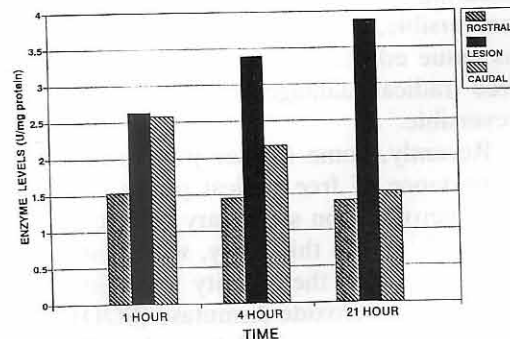


Fig. 3. CAT enzyme activity changes during first 24 hours.

another one as rostral/lesion, lesion/caudal, rostral/caudal. SOD (Fig. 1) and GPx (Fig. 2) activity differences between the segments of lesion and caudal and rostral were not statistically significant. CAT (Fig. 3) activity

was significantly high in the lesion segment.

DISCUSSION

In our study, we used a rat model spinal cord injury by the clip compression¹³. Studies were performed to determine the effects of trauma on enzymes (SOD, GPx, CAT) that catalyze various detoxication reactions. Traumatic neural injuries of both brain and spinal cord cause tissue damage through primary and secondary mechanisms. The injury sets in motion a series of molecular events that produce vascular and neuronal degeneration, thus destroying the anatomical substrate (e.g. long tracts) necessary for neurological recovery. Secondary injury is caused by the activation of endogenous substances such as monoamine, oxygen free radicals, neuropeptides, arachidonic acid metabolites and changes of extracellular calcium^{4,7}. Recent reports have suggested evidence for tissue damage induced by oxygen free radicals and lipid peroxidation in the primary and secondary process of acute traumatic injury of brain and spinal cord^{2-5,7,15}. Free radicals are molecules with a 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^{5,7,8,16,17}. Superoxide dismutase catalyzes the dismutation of superoxide anion radical. Cells are capable of increasing synthesis of superoxide dismutase in response to hyperoxidant stress¹⁶. Two enzyme systems catalyze breakdown of hydrogen peroxide^{7,16}. At low concentrations, most hydrogen peroxide is removed by reaction of glutathione peroxidase with reduced glutathione to form oxidized glutathione and water. At high concentrations of hydrogen peroxide, the enzyme catalase plays an important role. Tissues also have a variety of nonenzymatic antioxidants for

preventing their damage by free radicals^{5,7}. The brain and spinal cord may be prone to oxidative stress for several reasons^{5-7,9,10,14,17}. The membrane lipids are rich in cholesterol and polyunsaturated fatty acid which can be attacked by oxygen free radicals. Cerebrospinal fluid contains very little transferrin and therefore cannot easily bind released iron ions. The nervous system is rich in epinephrine and dopamine, all of which react with oxygen to form oxygen free radical and iron ions accelerate these oxidations. After application of an aneurysm clip for 30 seconds and performing a standardized reversible spinal cord injury, tissue SOD, CAT and GPx activities were determined at 1, 4 and 24 hours after injury. In each segment enzyme activities at 1, 4 and 24 hours were not significantly different. When enzyme activity differences between the segments were analyzed without time limitation, we have found that SOD and GPx activities were not significantly different but CAT activity was significantly high in the lesion segment compared with rostral and caudal segments. In such a spinal cord trauma model we found that the activities of primary antioxidant enzymes except SOD in lesion segment tend to be generally increased. The diminished activity of SOD causes to decline dismutation rate of superoxide radicals. H_2O_2 can be effectively removed by both GPx and especially CAT. According to our results we suggest that one of the main reason for tissue damage during such a spinal cord trauma model may be neither H_2O_2 nor H_2O_2 derived radicals.

ACKNOWLEDGMENT

The assistance of Emre Denizci Eng., in the preparation of this paper is gratefully acknowledged.

REFERENCES

- 1) Beers, R.F., & Sizer, I.W.: *J. Biol. Chem.* **195**: 133 (1952)
- 2) Demopoulos, H.B., & Flamm, E.S.: *Can. J.*

- Physiol. Pharmacol. **60**: 1415 (1982)
- 3) Hall, E.D., & Brauhler, J.M.: Res. Publ. Assoc. Res. Nerv. Ment. Dis. **71**: 81 (1993)
 - 4) Hall, E.D., & Braughler, J.M.: Surg. Neurol. **18**: 320 (1982)
 - 5) Halliwell, B.: Current concepts, pp. 4-27, Upjohn Company, Kalamazoo, Michigan, (1991)
 - 6) Halliwell, B., & Gutteridge, J.M.C.: pp. 135-153, Clarendon Press, Oxford, (1984)
 - 7) Ikeda, Y., & Long, D.M.: Neurosurgery **27**: 1(1990)
 - 8) Kavas, G.: Türkiye Klinikleri **1**: 1 (1989)
 - 9) Kontos, H.A., & Wei, E.P.: J. Neurosurg. **64**: 803 (1986)
 - 10) Levasseur, J.E., Patterson, J.L., Ghutak, N.R., & Kontos, H.A.: J. Neurosurg. **71**: 573 (1989)
 - 11) Lowry, O.H., Rosenbrough, N.J., Farr, A.L., & Randall, R.J.: J. Biol. Chem. **193**: 265 (1951)
 - 12) Plagia, D.E., & Valentine, W.N.: J. Lab. Clin. Med. **70**: 158 (1967)
 - 13) Rivlin, A.S., & Tator, C.H.: Surg. Neurol. **10**: 39 (1978)
 - 14) Schettini, A., Lippman, H.R., & Walsh, E.K.: J. Neurosurg. **71**: 578 (1989)
 - 15) Sonntag, V.K.H., & Douglas, R.A.: Neurosurgery Clinics of North America **1**: 729 (1990)
 - 16) Southorn, P.A.: Mayo Clin. Proc. **63**: 381 (1988)
 - 17) Southorn P.A.: Mayo Clin. Proc. **63**: 390 (1988)
 - 18) Sun, Y., Peterson, T.E., & Mc Cormick, M.L.: Clin. Chem. **35**: 1265 (1989)