THE EFFECTS OF DEFEROXAMINE ON LIPID PEROXIDATION IN AN EXPERIMENTAL SPINAL CORD INJURY

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ABSTRACT:

Some patients with an acute spinal cord injury (SCI) can immediately become paraplegic following the trauma. On the other hand, some patients with an incomplete trauma can be deteriorated by time as a result of the worsening pathology on the spinal cord. Consequently, great amount of sensorimotor functions are lost because of this delayed secondary (auto-destructive) response since many etiological factors are responsible for auto-destructive progression. A significant number of treatments are developed in the literature.

In this study, the effects of Deferoxamine (DFO) which inhibits iron dependent lipid peroxidation were investigated on 64 rats, equally divided into 2 subgroups, Control (C) and Treatment (T). Extradural clip compression at the thoracic 7 level was selected as a trauma model. 15 minutes before the trauma, 100 mg/kg DFO and same dosage of serum physiologic was injected intravenously to T and C subgroups respectively. Following the trauma, at the first, 30th, 60th and 120th minutes biologic activity was stopped with liquid nitrogen. Lipid peroxidation and the effects of the treatment were determined biochemically by the assessment of malonic dialdehid through the tiobarbituric acid test.

DFO reduced lipid peroxidations at the 60th and 120th minutes. This reduction was particularly more definite at the first 60 minutes. This study concludes that DFO can be effective on the secondary auto-destructive processes after an experimental SCI.

Key words: Deferoxamine, experimental spinal cord injury, iron chelates, lipid peroxidation, rat

INTRODUCTION

In acute trauma, primary damage to neuronal tissue occurs immediately following initial insult by flexion, deflexion, distraction, compression, rotation and combined forces. A large portion of neurons die and physical damage cannot be reversed at the lesion site. Human SCI has multiple morphologies: contusions, lacerations, hemorrhages, macerations, extensive cone-shaped necroses and cysts. The lack of expected improvement in trauma cases may be due to secondary or auto-destructive or continuing damage. These secondary mechanisms are

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vascular and biochemical changes, inflammatory reactions, edema, lipid peroxidation of the neuronal membrane, free oxygen radicals and loss of energy metabolism (15,19). The real auto-destructive mechanisms are not well understood. But, secondary injury is the cumulative effect of the events and occurs with numerous biochemical processes leading to further microvascular and neuronal degeneration. This cascade of events may be prolonged for as long as 24 hours. All these factors seem to have some effect in deterioration of the injured spinal cord. Clinicopathological results of the trauma are complete, incomplete and discomplete SCI. The term of "discomplete" is electrophysiological transmission of signals across the lesion in patients with clinically complete loss of all sensory-motor function below the level of the lesion.

Experimental studies demonstrated that some functional recovery occurs with correction of biomechanical malalignment and removal of the spinal cord compression. Despite the advances in therapeutical models, the practical approach to the SCI is limited to decompression, stabilization and rehabilitation (28). Some clinical benefit may be obtained by physical, electronic, neurophysiological (epidural stimulation, functional electrical stimulation, computer modelling), pharmacological or biological methods (21).

Clinical and experimental trauma may have different results. Generally, the trauma to the spinal cord is uniform, repeatable, occurs in sterilized condition and anesthetized animals without apparent bleeding whilst controlling the vital functions in experimental studies. But in the clinical setting, there may be multiorgan trauma with bleeding, infection, foreign body and other stressful factors. So, it is very difficult to have an animal model similar to human being. The study of auto-destructive mechanisms in experimental animals may have beneficial results for patients with acute SCI to prevent further damage.

We used DFO before SCI and discussed its properties as an agent expected to inhibit the auto-destructive mechanisms by suppression of iron dependent lipid peroxidation.

MATERIAL and METHODS

In this study, 3-4 months old, 296±24 g, sexually mature, healthy, 64 Spraque-Dawley female rats were used. Rats were equally divided into two main groups; Control (C) and Treatment (T). Each main group was divided into four subgroups consisting of 8 rats in each in terms of time of cessation of the biological activities with liquid nitrogen after the trauma (C1-C4 and T1-T4). Rats were anesthetized intraperitoneally in supine position with 40 mg/kg Thiopenthane Sodium BP (Pentotal sodium-Abbott). 100 mg/kg DFO (Desferal 500 Ciba-Geigy, deferoxamin methansulfonic 500 mg) and same dosage of serum physiologic were injected intravenously via left vena jugularis interna 15 minutes before the trauma to T and C groups respectively. Rats were turned to prone position and paravertebral muscles were microsurgically dissected free. Total laminectomy and bilateral facetectomy were performed from Thoracic (Th) 4 to Th 11.

The whole dural and neural anatomic entirety of the spinal cord and the roots were preserved during these procedure and traumatized animals were excluded from study. Horizontal extradural compression was applied at Th 7 level for 30 seconds with an aneurysm clip (Aesculap FE 716, closing pressure 130 ± 10 g) after laminectomy within 15 minutes. Circular subpial hemorrhage was observed in the traumatized cord after removal of the clip. The spinal cord between Th 5 -Th 10 was frozen to stop biological activities with

liquid nitrogen at the 1st, 30th, 60th and 120th minutes, for C1-T1, C2-T2, C3-T3 and C4-T4 subgroups respectively. Frozen vertebral column was removed and preserved in liquid nitrogen tank until the assessment of malonic dialdehid (MDA) through the tiobarbituric acid (TBA) test.

TBA test was used for the assessment of MDA for biochemical determination of the lipid peroxidation and the effects of the treatment (8). The injured part of spinal cord was taken from the liquid nitrogen tank and treated with trichlor acetic acid (TCA) in a form of mixture. Ultra-Turrax tissue homogenizator was used to obtain 10% homogenate of mixture within 5 minutes. The homogenate was centrifuged at 3000 rpm at +4°C for 15 minutes. 0.5 ml supernatant at the top of the tube was taken and mixed with equal amount of 0.67% of TBA. This mixture was kept in boiling water for 15 minutes and then left to cool to normal room temperature. It was examined by a spectrophotometer at the 532 nm to find out the absorbtion values. All absorbtion values are converted to values in nmol MDA/g tissue by using the specific coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$.

Nonparametric Kruskal-Wallis and Mann-Whitney U statistical tests were used for variance analysis and all parameters with mean values, and standard deviations were evaluated. The results were accepted as statistically significant at the P values less than 0.05.

RESULTS

The MDA values of the C and T groups are shown in the Table 1.

Table 1. MDA values of control and treatment group (nmol MDA/g tissue)

C Group	1. min.	30.min	60.min	120.min.
MEAN	15.737	12.788	18.974	18.509
STD	4.164	3.551	4.892	6.000
T Group				
MEAN	15.881	15.897	13.942	15.817
STD	6.102	3.672	6.012	5.636

In 4 control subgroups, there were statistically significant changes in terms of time ($p<0.05$) which revealed the existence of sufficient trauma to the spinal cord that caused lipid peroxidation. Multiple cross-match tests were used in the C group for determination of subgroup(s) that made differences. Constant number of 10.64 was found after estimation for significance of 0.05. In this way, subgroups were seen in C2-C3 and C2- C4 that made differences. There were no statistically significant changes ($p=0.52$) with Kruskal-Wallis analysis in the all T subgroups in terms of time (Table 2).

Table 2. Kruskal-Wallis variance analysis of control and treatment group

Subgroups	n	Mean rank (C)	n	Mean rank (T)
1	8	16.062	8	17.875
2	8	9.125	8	18.937
3	8	21.187	8	12.437
4	8	19.625	8	16.750
K-W test statistic = 7.850 p=0.04			K-W test statistic = 2.218 p=0.52	

Mann-Whitney U test was used to compare MDA values obtained at 1st, 30th, 60th and 120th minutes in the subgroups of C and T groups respectively (Table 3).

Table 3. Comprasion of subgroups according to MDA values

Subgroups	t values	df	Significance (p)
$C_1 - T_1$	0.577	14	0.56
$C_2 - T_2$	1.786	14	0.07
$C_3 - T_3$	-1.942	14	0.05*
$C_4 - T_4$	-0.840	14	0.40

d.f. Degree of freedom
* Statistically significant

The graphic obtained from the MDA values showed the decrease in treatment group (T) at 60th minute which was statistically significant ($p<0.05$) and continued to be lower than control group (C) till 120th minute. This difference at 120th minute was not statistically significant ($p>0.05$) (Figure 1).

Table 4. Kruskal-Wallis variance analysis of treatment group

Subgroups	n	Mean rank
1	8	17.875
2	8	18.937
3	8	12.437
4	8	16.750
K-W test statistic = 2.218	p=0.52	

Table 5. Comparison of subsgroups according to MDA values

Subgroups	t values	df	Significance (p)
C_1 - T_1	0.577	14	0.56
C_1 - T_1	1.786	14	0.07
C_1 - T_1	-1.942	14	0.05*
C_1 - T_1	-0.840	14	0.40

d.f. Degree of freedom * Statistically significant

(vasospasm, disturbance of the microcirculation etc.), ion disturbances, neurotransmitters, the metabolites of arachidonic acid (prostoglandins, leucotrienes, tromboxanes, etc.) and edema are some of the etiological factors of pathogenesis of auto-destruction (7,16,22,29,30). One of the important contributor to this secondary damage is oxygen radical-induced lipid peroxidation (12). Lipid peroxidation reactions cause disruption of cholesterol, proteins and polyunsaturated fatty acids within neuronal, myelin and microvasculature membranes decreased blood flow resulting in secondary hypoxic damage, inflammation, cell death and permanent neurological dysfunction. Hemorrhage and

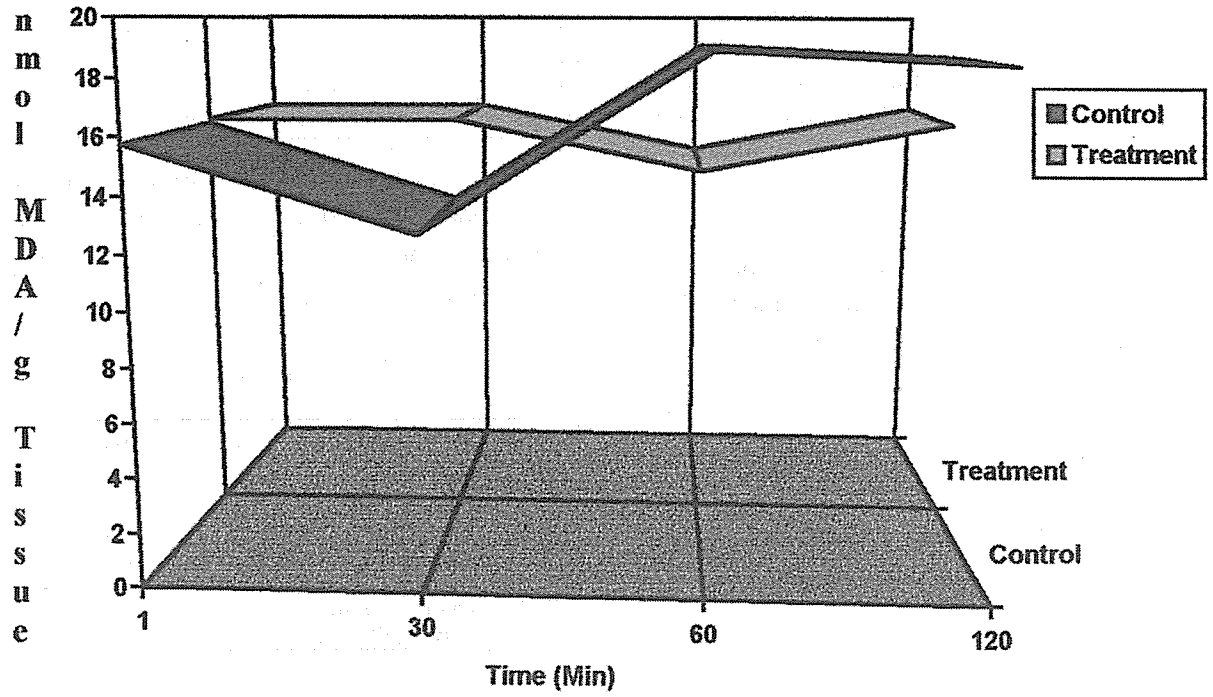

Figure 1. Lipid peroxidation curve of control and treatment groups according to time

DISCUSSION

Some experimental models have been developed to investigate the mechanisms of secondary injury (2,31). Vascular changes due to hypotension occur respectively after the disturbance of auto-regulation (16,25,31). Thus; p02 decreases, ATP production diminishes, lactic acidemia and acidosis occur. Anaerobic metabolism takes place instead of aerobic metabolism, the function of the enzymes of the

membrane are disturbed, permeability of the membrane changes and the dysequilibrium of the ions occurs. Arachidonic acid metabolism is followed by lipid peroxidation together with the increase of the calcium ions that cause damage. Irreversible changes occur by the direct effect of trauma, ischemia, hemorrhage and the free radicals secondary to leukocytes that come to place of event. All these dramatically changes may cause to a clinical progressive neurological deficit.

Many treatment modalities for clinical improvement of neurological functions in the treatment of acute SCI have been studied in clinical and experimental settings. Pharmacological agents used in secondary damages of SCI are glucocorticoids, phospholipases, inhibitors of cyclooxigenase and lipooxigenase, antioxidants, opioid antagonists, iron binding agents, radical scavengers, Ca channel blockers, prostacyclins and serotonin antagonists, hypothermia, direct-current field, epsilon amino caproic acid, heparin, hyperbaric 0_2, hypertonic solutions and phenitoin (3,9,10,14,15,16,18,20,25,33). Methylprednisolone (MP) is one of the most popular agents. MP has been widely used especially in all three National Acute Spinal Cord Injury Study (NASCIS) randomized controlled trials and results of treatment have been compared with naloxane and tirilazad mesylate (3,4,5,6). The removal of the injured part of the spinal cord and the transplantation of cultured Schwann cells, peripheral nerve grafts and lastly human fetal spinal cord tissue have been also recommended as surgical method (17,26,27,32).

The importance of iron in lipid peroxidation and free radicals reactions have been stressed in several studies (1,11). Iron may react directly with free radicals and/or catalyze these reactions and/or enter in the structure of the enzymes such as superoxide dismutase (SOD) (19). Ferric iron (Fe^{+++}) reacts with superoxide radicals (O_2^-) in Fenton reaction or catalyses Haber-Weiss reaction with iron salts (34). All these iron dependent reactions produce organic and inorganic radicals that cause demyelination and necrosis in the spinal cord.

DFO has a high affinity to ferric iron ($Kd\text{-}10^{31}$) but not to ferrous iron (13,24). DFO mobilizes iron from ferritin, hemosiderin, lactoferrin and transferrin, but can not mobilize it from Hgb (23). With the condensation of Fe^{+++}-DFO complex in the medium, ferric reduction decreases and ferrous reduction increases. DFO has anti-peroxidative effects with inhibition of the production of free radicals by binding of Fe^{+++} and also with scavenger effect of superoxide radical.

In this study, we aimed to find out the effects of DFO in lipid peroxidation as an iron binding and radical scavenger agent. DFO has not been used in SCI before the trauma in the literature. We preferred to use DFO intravenously before SCI to prevent the secondary damage. We used indirect method by assessing of the amount of MDA which is one of the end products of lipid peroxidation to reveal the changes of SCI.

All the differences in subgroups of the control groups (C1-C4) were found statistically significant. This result revealed that the trauma to the spinal cord was sufficient and caused lipid peroxidation 30 minutes after the injury ($p<0.05$). Similar trend were unlikely not observed in treatment group (T1 -T4) and MDA values were still in lower level than control at 120^{th} minutes of trauma. MDA differences in treatment subgroups were not statistically significant ($p>0.05$). So, prevention of lipid peroxidation by DFO may be said especially at 60^{th} and 120^{th} minutes although the effect was less at 120^{th} minutes. The decreased value of MDA at 60^{th} minute (C3-T3) was statistically

significant ($p<0.05$). The difference between C4 and T4 was not statistically meaningful although the lower value of T group. Presumably, insufficient blood supply due to the disturbance of auto-regulation may be responsible to inadequate penetration of DFO to injured area at the early stages of the injury (1st and 30th minute). Other possible factors might be due to the dosage of DFO and timing of apply.

We can conclude that sufficient trauma to the spinal cord that caused lipid peroxidation was obtained with this experiment. MDA values were reached the highest level at 60th minute and tend to decrease at 120th minutes. MDA was decreased by DFO especially at 60th and 120th minutes. DFO may be effective in inhibiting the lipid peroxidation especially at 60th minute and may have preventive effect in SCI. Different dosage, different applying time and long time usage are necessary to understand the exact effect of DFO.

This study has been performed in Microneurosurgery Laboratory of Istanbul University, School of Medicine, Department of Neurosurgery, Istanbul, Turkey.

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