THE EFFECTS OF SELENIUM AND ALPHA-TOCOPHEROL ON THE FREE RADICALS IN AN EXPERIMENTAL MODEL OF ACUTE SPINAL CORD INJURY

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SUMMARY :

Ischemia-induced lipid peroxidation is one of the most important factors producing tissue damage in spinal cord injury. In this study a standard spinal cord injury model in rats was used and the effects of chronic administration of Selenium (3 ppm/lt water orally) before and alpha-tocopherol management (100 mg/kg, intraperitoneally) just after the trauma were investigated. For this purpose animals were divided into three groups:

A (untreated controls), B (selenium-treated), C (alpha-tocopherol-treated). In group-A lipid peroxide levels increased significantly one hour after the trauma, and then decreased. In group-B, this increase was not seen ($P<0.05$). This study has demonstrated that chronic selenium administration is effective in preventing lipid peroxide formation in spinal cord injury. For grup-C further investigations have been planned.

KEY WORDS :

alpha-tocopherol, free radicals, posttraumatic ischemia, selenium, spinal cord injury.

INTRODUCTION

The treatment of spinal cord injury is still a problem for today's neurosurgeon. Recently there have been many experimental studies on the pathophysiology of acute spinal cord injury. The results of these studies, show that the injured spinal cord suffers not only from the direct mechanical effects of trauma on the neuronal and neurovascular components, but even more from the secondary effects of trauma on the vasculature and blood flow in the spinal cord (1,3,7,9,10,22). It might be possible to treat patients with spinal cord injury by preventing or reversing the undesirable effects of posttraumatic ischemia.

During the past decade, a considerable research effort has been focused on the role of free radicals and lipid peroxidation in the occurence of ischemic damage (2,14,16,26).

In this study, selenium and alpha-tocopherol (Vitamin E) known as free radical scavengers were used to investigate their therepeutic effects on spinal cord ischemia related to trauma.

MATERIALS AND METHODS

One hundred and twenty female Wistar rats weighing 180±20 gr were used. Three main groups each containing 40 rats were investigated; Group A (untreated controls), Group B (animals given 3 ppm selenium per liter of tap water, approximately 30 ml/24 hours, started 20 days before the trauma), and Group C (animals administered pure alpha-tocopherol emulsion 20 mg, intraperitoneally, just after the trauma). Each main group was also divided into five subgroups of eight animals (AO-4, BO-4, CO-4).

Surgical procedure

Rats were anesthetized with intraperitoneal administration of thiopental sodium (35 mg/kg), and immobilized in prone position. Following a T_2-T_{11} midline incision, the paravertebral muscles were dissected free, then spinous process and laminar arcs were removed between T_3 and T_9 under the surgical microscope and the spinal cord was exposed.

In the zero groups (AO, BO, CO), spinal cord trauma was not performed after laminectomy.

The spinal cord was compressed at the midthoracic level extradurally by a Yaşargil aneurysm clip (Aesculap FE 760, closing force:180±18 gr) for one-minute. By this procedure it was possible to standardize spinal cord trauma in all rats as described by Tator and Dolan (8). After the trauma the whole length of the thoracal vertebral column was cooled by applying liquid nitrogen and was removed at the first

minute in the first subgroups (A_1, B_1, C_1) at the fiftienth minute in the second subgroups (A_2 , B_2, C_2) one hour later in the third subgroups (A_3, B_3, C_3) and two hours later in the fourth subgroups (A_4, B_4, C_4). All these specimens were cooled and preserved in liquid nitrogen to stop enzymatic reactions until lipid peroxide determination.

Lipid peroxide determination

The degree of lipid peroxide formation was assayed by determining thiobarbituric acid reactive substance formation (5). Spinal cord specimens were taken out from the liquid nitrogen and transported in ice-cold receptacles so as not to break the cold-chain, and then homogenized with tricloracetic acid (10 ml % 10 TCA for 1 gr. neural tissue) using an Ultra Turrax tissue homogenizer. After 15 minutes centrifugation (4000 tr/min, at4°C) from each specimen a volume of the supernatant fluid was added to equal volume of 0.67 % thiobarbituric acid and the mixture was kept in a boiling water bath for 15 minutes. Samples were warmed to room temperature and absorbance values were measured at 532 nm spectrophotometrically. Tissue free radical leves were expressed in terms of malondialdehyde equivalents using an extinction coefficient of $1.56x10^5$ $M^{-1}cm^{-1}$.

Results were evaluated statistically by one-way analysis of variance and for the multiple range test (the Tukey method) was used. Statistical significance was taken at the 5 % level.

RESULTS

The lipid peroxide mean values ± standard deviation in nmol/gr tissue for all groups and subgroups are summarized in Table 1.

the alpha-tocopherol-treated group, the values of lipid peroxide formation were high from the beginning to the 120th minute. Although there was no significant value change among the subgroups, the values of this group were globally significant compared with groups A and B (Fig.1).

There was no statistically significant difference between the subgroups without trauma (AO, BO, CO) and those just after trauma (A_1, B_1, C_1).

Bu there was a statistical significant difference between the lipid peroxide formation values of the untreated control group and selenium-treated group at the 60th minute after trauma (A_3 and B_3 subgroups). This significance was also evident between the B_3 and C_3 alpha-tocopherol treated subgroups.

In the subgroups two-hours after trauma (A_4, B_4, C_4), statistical significancy was obtained in only between B_4 and C_4 (Fig.2).

DISCUSSION

Ischemia-induced pathological processes are most frequently responsible for the cause of death. Cerebral and spinal cord ischemia, aging, cardiovascular diseases and many other ischemic disorders make a major contibution to morbidity and mortality. Recent evidence suggests that toxic oxygen free radicals areproduced abundantly in ischemic tissues and they are thought to be important pathologic mediators in many clinical disorders (4,6,17,18,19,20,26). Since this theory was first put forward various free radical scavengers have been studied as possible protective agents in ischemia. And fortunately, many of them have proved to have protective effects on the ischemic tissue by radical scavenging action. For examp-

Table : 1 The malondialdehyde mean valves + standard deviation in nmol/gr tissue.

Groups	0	1	2	3	4	Total
A	53.0±10,4	33,5±20,4	31,3±12,5	64,8±31,4	33,9±19,1	43,0±23,2
B	43.5±15,4	44,5±33,3	26,8± 6,9	19,1±14,0	26,7± 9,3	32,7±20,1
C	58,3±19,1	58,6±20,5	52,7±25,6	56,9±19,3	50,4±230,0	55,2±20,9

In group A (untreated controls), the level of lipid peroxide formation was highest at the 60th minute (A_3) and this elevation was found statistically significant when compared with A_1, A_2 and A_4 subgroups. On the other hand in the selenium-treated group (group B) no elevation of lipid peroxide formation was seen at any time and there was no significance among the subgroups of this main group (BO-4). However in

le, Mannitol, Vitamin E, Glucocorticoid, Phenytoin (16,24,25,26), and Allopurinol (20,21) were found to be to be useful in cerebral ischemia.

However, free radical reactions are not the sole precipitating factors in the pathophysiology of ischemia. Energy failure, cellular acidosis and disruption of normal tissue ion homeostasis are all the known to result in tissue ischemia (16,19,20,26).

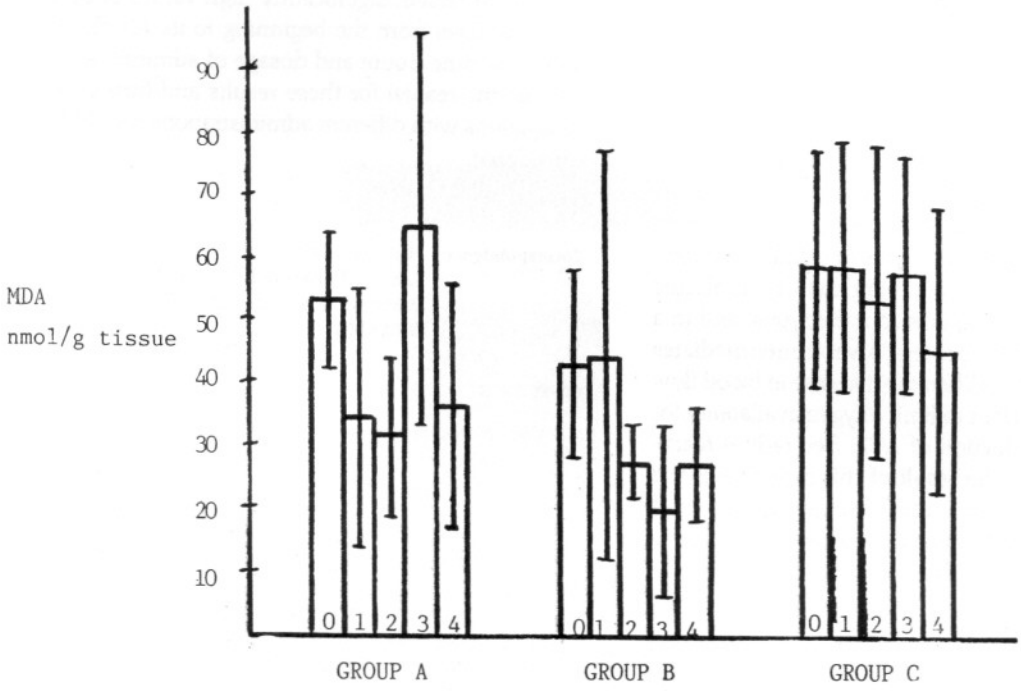

Fig.1 : The values of malondialdehyde formation for the groups.

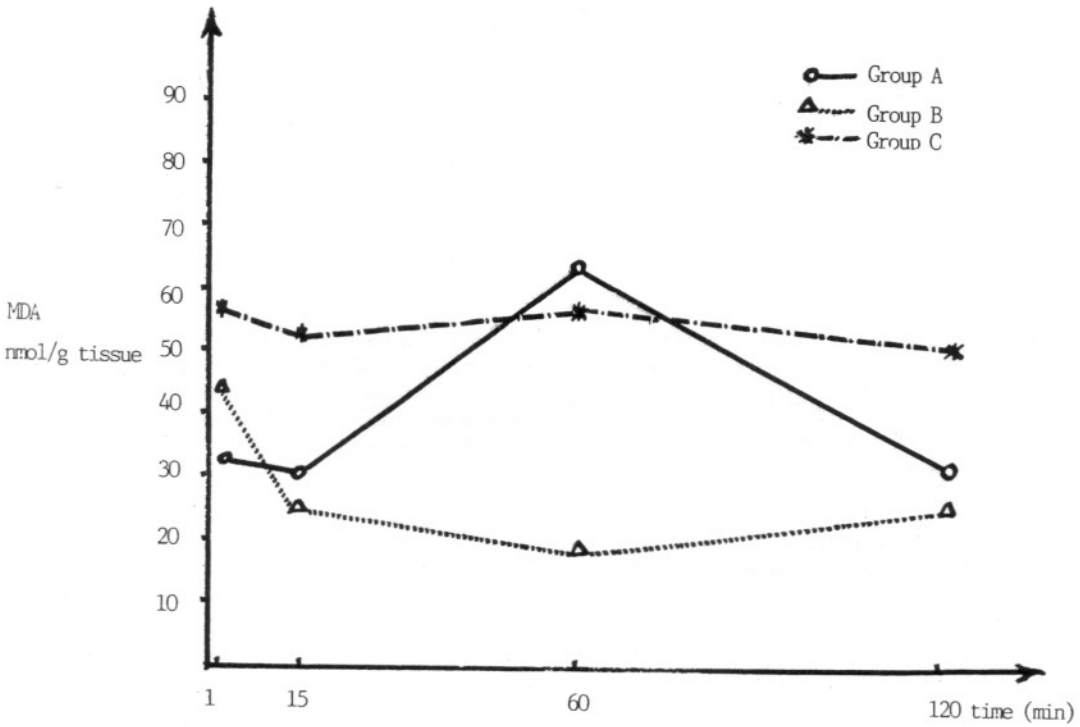

Fig.2 : The malondialdehyde formation trend in the groups related with time.

A free radical is any molecule that has an odd number of electrons, and can occur in both organic and inorganic molecules. Free radical reactions are critical for the normal course of a wide spectrum of biological processes. Free radicals which can be highly reactive and toxic are generated in vivo, but eliminated by specific enzymes to be well tolerated within living systems (11,13,15,17). Because of the ubiquity of molecular oxygen in aerobic organisms and its ability to accept electrons, oxygen-centered free radicals are often mediators of cellular free radical reaction (11). Complete reduction of a molecule of oxygen to water requires four electrons, and in a sequential univalent process several intermediates will be released (13). When the decrease in blood flow to a tissue is sufficient to limit oxygen availability for the requisite production of ATP, free radical reactions are initiated. The result of this sequence is the production of large amounts of superoxide (0_2^-) and secondarily derived cytotoxic radicals (for example hydroxyl radical OH^-) capable of causing massive tissue damage (13,17,20).

A few available "antioxidant" strategies can be mentioned here. First, some specific enzymes e.g.; superoxide dismutase for a superoxide radical, glutathione peroxidase for hydrogen peroxide) may be administered to remove free radicals, or some agents may be given to increase the activiy of the specific enzymes scavenging some free radicals. In the second strategy, free radical production is prevented or an agent may be used for scavenging (12,14,20).

The element selenium is located at the active site of the Glutathione peroxidase enzyme (GSH). This enzyme is found in both the cytosol and mitochondria of human cells and one of its function is to keep the thiol (-SH) groups of enzymes and proteins in the reduced state. GSH and related glutathione-requiring enzymes are also involved in the protection of cell membranes against lipid peroxidation. It removes H_2O_2 and thus prevents OH^- formation, alpha -tocopherol (Vitamin E) breaks radical chain reactions by trapping peroxy and other reactive radicals (14,23).

This study has confirmed that the lipid peroxide level after ischemia-induced spinal cord trauma reaches its highest value one hour after trauma and then decreases (3).

Chronic administration of Selenium as a free radical scavenger is efficient in preventing this increase of lipid peroxidation but selenium has some known toxic effects especially on the myocardium and liver in over-dosage (14). Although, there are many reports of the free radical scavenging effect of alpha -tocopherol, in this study, the rats given alpha -tocopherol just after trauma did not show a typical increase of lipid peroxide formation but showed instead unexplained, significantly high values of lipid peroxide level from the beginning to its 120 th minute. The time, route and dosage of administration may be the reason for these results and further investigations with different administrations should be considered.

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