

Effects of Ascorbic Acid and Alpha-Tocopherol on Superoxide Dismutase in Spinal Cord Injured Rats

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Abstract: ***Aim:** The purpose of this study was to determine the effects of ascorbic acid and alpha-tocopherol on superoxide dismutase (SOD) in spinal cord injured rats. **Methods:** A total of 90 male Sprague-Dawley rats (180–200 g) were used for this study. Sixty of them were subjected to spinal cord injury (SCI) and given different doses of ascorbic acid and alpha-tocopherol as treatment for 12 weeks (84 days). The remaining 30 rats were used as a control (without SCI, untreated), sham group (laminectomy only) and disease control (SCI, saline). The animals bodyweight (daily), and SOD were examined at the end of the study. **Results:** Evidently, at the end of the study, the level of SOD decline in rats after the injury. However, compared to the disease control, the treated rats (Groups 4, 5, 6, 7, 8 and 9) described progressive enhancements in the SOD by the end of the study. **Conclusion:** The findings of the study demonstrated that the administration of alpha-tocopherol and ascorbic acid increased the SOD level after SCI.*

Keywords: Superoxide Dismutase, spinal cord injury, ascorbic acid, alpha-tocopherol

1. Introduction

Spinal cord injury (SCI) is a distressing neurological injury, causing in stable disability producing terrific anguish to the patients and their families [1, 2]. The resultant neurological dysfunction and paralysis are related to the severity of the trauma itself and is a major problem in medicine, as it bases a high degree of mortality, severe disability, involving far-reaching rehabilitation and high expenses [3, 4]. In the meantime, the management of SCI remains to stance a challenge and no confident treatment has been acknowledged to solve it [5]. However, many studies comprising experimental modeling are being piloted to support a enhanced understanding of the biological and anatomical concerns of injury and repair, comprising examine the efficiency and the risk-to-benefit ratio of the proposed therapy to help resolve this important problem [3, 4, 6].

In injured spinal cords, potentially toxic elements are activated and released, comprising free radicals, phospholipases, lipid peroxidases and inflammatory cytokines, which lead to oxidative stress destruction, thereby causing in neuronal necrosis or apoptosis and progressive secondary nerve tissue damage. Among the principal mechanisms connected with post SCI cell death, excitotoxicity, oxidative stress, inflammatory response and apoptosis are documented as the possible targets to inhibit tissue destruction [7]. The vital role played by the

spinal cord in a broad range of physiological functions is obviously proven by the deficits detected post SCI and by the medical conditions that arise during the critical and chronic phases of post injury [8-10]. It is well agreed that spinal cord typically contains lipids and is certainly damaged by free radicals and the lipid peroxidation they induce. Hence, the secondary destruction of injured spinal cords. Free radical-induced lipid peroxidation is thought to be imperative. Antioxidants have been assessed as neuroprotective agents in stroke, as there is literature supporting the incidence of oxidative stress in the ischemic brain [11]. Pharmacological researches in animals indicated that antioxidant molecules are capable to cross the blood–brain barrier, such as polyethylene glycol-conjugated superoxide dismutase (SOD) and catalase, to reduce ischemic cerebral destruction [2, 11].

One of the body's key internal anti-oxidant defenses, SOD plays a vital part in diminishing the oxidative stress involved in SCI and other life-threatening health disorders. Earlier studies reported that SOD has been found to diminish internal inflammation and decrease the pain related to SCI and associated conditions [2, 11]. In the current study, the hypothesis that was tested the ascorbic acid and alpha-tocopherol as free radical scavengers, to explore their effectiveness in SOD changes of rats which had endured incomplete SCI, on a longstanding basis.

2. Methods

Animals

A total of 90 adult male Sprague–Dawley rats, 180-200 g in body weight were used for this present study. All the rats were retained housed in sawdust-lined polycarbonate cage with a stainless steel cover and kept in an environmentally controlled room with constant temperature ($23 \pm 20^{\circ}\text{C}$) and 12-hour light/dark cycles. The animals had free access to standard laboratory food and tap water.

Drugs

Ascorbic acid and alpha-tocopherol were the antioxidants used, (Aldrich chemical company, Germany) whereas chloral hydrate was the anesthetic used on the rats (Merck chemical company, Germany).

Spinal cord injury

A total of 70 animals (except group 1 and 2; weighing 180 - 200g) were anesthetized with chloral hydrate (450 mg kg^{-1} body weight) by intraperitoneal injection (IP). Laminectomy was done at the T 7-8 level without damaging the dura. To cause the incomplete SCI, a compression plate (2.2 x 5.0 mm) with a 35 g weight load was placed on the open spinal cord for 5 minutes. All the surgical instruments were sterilized prior to surgery. Throughout the surgery, the animals were located on a heating pad and the rectal temperature was maintained at 37°C to 38°C . With the support of a rectal probe inserted into the rectum, the body temperature was monitored and sustained between 37°C and 38°C using a thermal pad and a heating lamp [12].

Post-operative care and observations

Post-surgery, the rats were caged separately, on thick soft bedding. Heating pads were used during the first three post-surgical days. By manual compression the bladders were emptied twice a day. All the rats were maintained under cautious observation to prevent infection at the surgical site [13].

Groups

With similar functional behavior, animals were subjected into nine groups comprising ten rats each. The Group 1 rats were used as the control (no surgery, no treatment); Group 2 used as the sham-operated control group subjected to laminectomy with no spinal cord injury; Group 3 used as the disease control (SCI + Saline); Groups 4-9 rats were subjected to spinal cord transaction, and given different doses of alpha tocopherol and ascorbic acid. The Group 4 rats were given ascorbic acid intraperitoneal every day at a dose of 500 mg/kg body weight. Group 5 rats also given ascorbic acid daily, at a dose of 1000 mg/kg body weight. Group 6 rats were orally administered alpha tocopherol daily, at a dose of 500 mg/kg body. Group 7 animals were given the alpha tocopherol at a dose of 1000 mg/kg body weight. Group 8

rats were given the combination of ascorbic acid (intraperitoneal) and alpha tocopherol (orally) at 250 mg/kg each and finally Group 9 given the combination of ascorbic acid (intraperitoneal) and alpha tocopherol (orally) dose at 500 mg/kg each for the whole 84-day study period (12 weeks).

Body Weight and SOD measurement

During the study, the rat body weights (in grams) were recorded at baseline until day 84 [14].

Using the method employed by Sun et al., the SOD activity was identified [15].

Statistical Analysis

Data analysis was carried out using Microsoft Excel 2010 (Microsoft Corporation, Seattle, WA, USA) and the Statistical Package for Social Sciences version 22 (SPSS Inc., Chicago, IL, USA). The recovery scores of body weight were analyzed by analysis of variance with repeated measurements over time. The SOD differences were analyzed by univariate analysis of variance followed by Tukey post hoc test. P-values <0.05 were taken as statistically significant.

3. Results

Figure 1 showed the effects of ascorbic acid and alpha-tocopherol on the body weight of the control rats and those with SCI. Compared to the baseline value, group 3 (disease control group, SCI + Saline) a gradual decline was noted in the rat body weight from day 2 to day 7. However, the body weight gradually improved from day 8 (second week onwards) which was sustained until end of the study. All the groups examined (i.e. 4, 5, and 7) found similar effects, and the improvement for groups 7 and 9 began on day 6 onwards. Compared with the rats on low ascorbic acid (group 4) and alpha-tocopherol (group 6), positive body weight upsurge in rats was reported with high ascorbic acid (group 5) and alpha-tocopherol (group 7) doses. Also, while comparing the ascorbic acid treatment notable body weight improvement in the rats were documented in the alpha-tocopherol groups, mainly in rats which were given the high doses of alpha-tocopherol.

Superoxide dismutase (U/mg of protein)

Figure 2 displays the effects of the different doses of ascorbic acid and alpha-tocopherol on the SOD of the study groups. Compared with the sham (Group 2) and control groups (Group 1, normal rats), the SOD were found to be very low in the spinal cord tissue samples of the rats in the disease control (Group 3). However, when compared to the disease control, the treated rats (Groups 4, 5, 6, 7, 8 and 9) reported progressive improvements in the SOD by the end of study. Compared with the low-dose ascorbic acid and the alpha-tocopherol treated rat groups (Groups 4 and 6), strong differences in the SOD were identified in the rats given the high-dose ascorbic acid (Group 5) and alpha-tocopherol (Group 7). However,

when they were compared with the rats on ascorbic acid important added progress were seen in the alpha-tocopherol groups, Group 7, in particular (i.e. alpha-tocopherol 1000 mg/kg body weight).

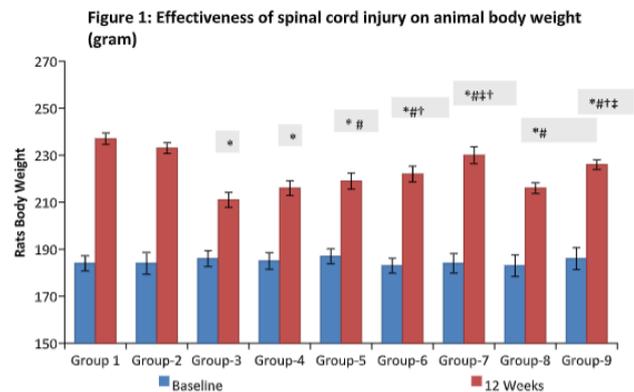
4. Discussion

Studies stated that the spinal cord experiences a series of pathological variations after a traumatic injury that causes the appearance of edema, hemorrhage, neuronal necrosis, axonal necrosis, demyelination, cyst formation, and also cavitation [16].

From this study it was obvious that post SCI the body weight of the rats declined during the first week (first 7 days) after which a slow retrieval was detected. This findings coincides with previous studies reported that SCI causes a decline in animal body weight. This weight loss could somewhat result from the massive stress put on the body at the time of the primary injury, which generates the body to respond with a higher metabolic rate speedily transporting energy and nutrients to assist in the healing process and inhibit infections. This is conceivably one of the causes for the decrease in the weight of the experimental animals [17-19]. Post injury weight loss was described in a recent research's to be a general result of any type of surgery, including SCI. In the initial phases of SCI the following symptoms are observed: an immediate appreciable drop in the energy expenditure, raised rate of catabolism, and huge nitrogen losses which may extend from many weeks to even months [19-21]. However, the present study revealed a steady recovery over a 12-week period post SCI when the baseline values were compared with the disease control group. These findings also concur with those reported earlier [22, 23].

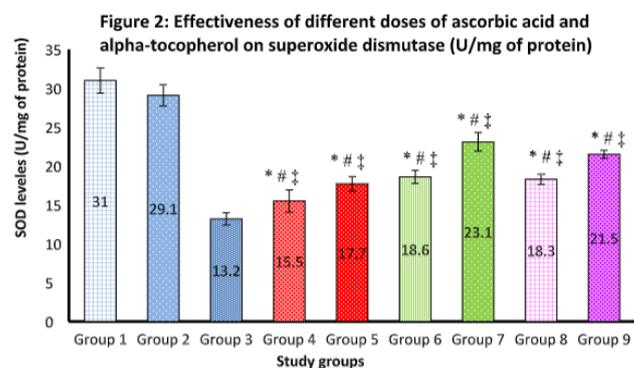
Among the leading internal anti-oxidant defenses in the body, SOD has a central part to play in decreasing the oxidative stress that is intrinsic to life-threatening illnesses and SCI [24]. It is obvious that SOD critically declines the internal inflammation and drops the intensity of pain associated to SCI and other associated conditions. [24] The SOD, which assists in tissue protection is an enzyme which catalyses the disproportionation process of the superoxide. Therefore, in the case of any damage to a tissue, the SOD is used up resulting in reduced activity [25-27]. In SCI rats the SOD concentration is seen to sharply increase in the spinal cord homogenate. A number of studies reported similar findings in neurological damages resulting from oxidative stress and the suppression of the antioxidant defense potential [28]. In the current study, SOD was found to be low in disease control rats at the end of study; however, when ascorbic acid and alpha-tocopherol were given, the SOD levels in the SCI rats were greater when compared with those of the untreated SCI rats (disease control). Further, when compared with the rat groups that were given low-dose ascorbic acid and alpha-tocopherol, positive differences in the SOD levels were noted in the rats on the high-dose ascorbic acid and alpha-tocopherol. However, when compared with those treated with the ascorbic acid considerable added increases were described in the alpha-tocopherol groups, mainly in the group with alpha-

tocopherol 1000 mg/kg body weight. From the research the severe drop in the antioxidant concentrations in the nerve, spinal cord and dorsal root ganglion due to neurological traumas in rat reveals that these tissues are greatly vulnerable to oxidative stress [28]. In a recent study the combined treatment of ascorbic acid and alpha-tocopherol was found to strongly nullify the effects of spinal cord contusion under oxidative stress [11]. This was specified the by the action of SOD, a fact confirmed by this study. In conclusion, the results of the present study showed that the administration of alpha-tocopherol and ascorbic acid increase the SOD level.



Groups Compared: * I Vs 4,5,6,7,8,9. # 2 Vs 4,5,6,7,8,9. ‡ 3 Vs 4,5,6,7,8,9,

One-way analysis of variance (ANOVA). Tukey–Kramer multiple comparisons test



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