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Original Article

Effectiveness of ascorbic acid and alpha-tocopherol in functional recovery of spinal cord injured rats: an experimental study

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ABSTRACT

The objective of this study was to examine the effects of the ascorbic acid and alpha-tocopherol on rats with incomplete spinal cord injury (SCI). A total of 80 male Sprague-Dawley rats (180–200 g) were used in this study. Of these 70 were subjected to SCI and received various doses of ascorbic acid and alpha-tocopherol treatment for 84 days (12 weeks), 10 rats were used as a control (without SCI, untreated). Spontaneous coordinate activity (vertical and horizontal movements) was used to assess functional recovery. Compared to baseline value, all of the test groups (i.e 3,4,5,6,7 and 8) showed a gradual improvements in vertical and horizontal movements at week 1, and such improvements were maintained at all-time points until the end of the trial period (week 12). In addition, when compared to disease control, positive improvement were observed in all test groups in vertical and horizontal movements throughout the trial period. Compared to low dose ascorbic acid and alpha-tocophoral groups, positive differences in vertical and horizontal activity were observed in high dose ascorbic acid and alpha-tocophoral groups. When compared with ascorbic acid treatment a notable added improvements were observed in alpha-tocopherol groups, especially in high dose alpha-tocopherol group. In conclusion, the administration of high dose alpha-tocopherol enhances the functional activity against SCI and it is more effective than ascorbic acid.

Key words: Antioxidants, Ascorbic acid, Alpha-tocopherol, Spinal cord injury, Functional recovery.

INTRODUCTION

Spinal Cord Injury (SCI) is a condition where the neural elements suffer acute trauma, causing in short-term or permanent sensory and motor complications [1, 2]. Motor and sensory deficits after SCI cause in functional reformation of the sensorimotor network [3]. The contribution of various mechanisms injuring spinal cord is known presently rather well, with isolation of two groups of causes that are primary SCI as a result of direct force acting on it during trauma and the secondary damage caused by vascular changes following trauma, free radicals' overproduction, increased inflammation, glial scarring and neuronal cell death [4-6]. Secondary injury after the primary impact comprises various pathophysiological and biochemical events. In addition, SCI results in loss of sensory and motor functions because injured axons do not regenerate and neurons that die are not replaced [5].

Studies have shown that functional recovery, however, differ greatly, depending upon size and location of injury, type and timing of intervention, and type of recovery and plasticity evaluated [7]. Spontaneous regeneration of damaged axons or plastic rearrangements of spared fiber systems after SCI in mammals are inadequate [8]. The most important reasons for this poor spontaneous repair capacity seem to be the inadequate growth response of neurons to injury, the growth-inhibitory components of the adult central nervous system tissue, and the formation of cysts and scar tissue at the injury site [8]. Attempts to overcome local barriers by grafting peripheral nerve bridges, Schwann cells or olfactory ensheathing cells have led to regenerative fiber growth and in some instances to behavioral recovery in animal models of SCI, although the mechanistic understanding of this recovery remains partial because of the complexity of these interventions [9-11]. Previous

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research shows that varying degrees of neurologic function spontaneously recovers in humans and animals during the days and months after SCI [12].

Over the last two decades, an understanding the underlying functional and structural biological repairs of the SCI mechanisms has strongly increased. However, compared with the other fields in medicine, the present level of treatment and care for SCI are quite insufficient [13]. Also, currently, treatment options are inadequate, but substantial advances have been made in understanding the pathophysiology of SCI. Also, pharmacological approaches focus on the control of secondary injury processes, primarily lipid peroxidation, and the salvage of as much white matter as possible [14-16]. Although, during the past decade, extensive research efforts have been focused on the role of free radicals in the occurrence of ischemic damage [14, 15]. In this present study, ascorbic acid and alpha-tocopherol known as free radical scavengers were used to investigate their effectiveness in functional recovery of incomplete spinal cord injured rats.

METHODS

Animals: Eighty adult male Sprague-Dawley (SD) rats weighing 180-200 g were used in the study. The rats were housed in polycarbonate cages with sawdust bedding. They were kept in a temperature controlled room (23 ± 1^{0C}) and maintained on 12-hours light/dark cycles, with free access to standard laboratory food and tap water.

Drugs: Ascorbic acid and alpha-tocopherol used as an antioxidants were purchased from Aldrich chemical company, Germany and chloral hydrate was purchased from Merck chemical company, Germany for anaesthetizing the rats.

Spinal cord injury: Out of 80, seventy SD rats were anaesthetized with chloral hydrate (450 mg kg^{-1} body weight) by intraperitoneal (IP) injection and laminectomy performed at T 7-8 level leaving the dura intact. A compression plate (2.2 x 5.0 mm) was loaded with a weight of 35 g placed on the exposed cord for 5 minutes in order to create the incomplete SCI.

Groups: Eighty SD rats were divided into eight groups of ten rats each with similar functional activity. The rats in group 1 served as control (without SCI, untreated), group 2 served as disease control (SCI + saline). Rats in group 3 received intraperitoneal administration of ascorbic acid daily with the dose of 500 mg/kg body weight and group 4 rats received ascorbic acid daily with the dose of 1000 mg/kg body weight. Group 5 rats received oral administration of alpha-tocopherol daily with the dose of 500 mg/kg body and group 6 rats received alpha tocopherol daily with the dose of 1000 mg/kg body weight. Group 7 rats received ascorbic acid and alpha-tocopherol 250 mg/kg each and group 8 received ascorbic acid and alpha-tocopherol 500 mg/kg each for 84 days (12 weeks).

Behavioral studies: Rats were carefully observed for any behavioral abnormality before the daily administration of drugs. The infrared beam-array activity cage-7431 (Ugo Basile Biological Research Apparatus, Comerio, Varese, Italy) was used to monitor the horizontal and vertical activity of the rats for 10 minutes each day. The activity cage basically relies on horizontal and vertical sensors. The movements of the animal inside the cage interrupt one infrared beam per second. The beam interruptions are counted and monitored by the electronic unit, which allow the examiners to assess and analyze the animal activity. The horizontal and vertical movement were collected daily for 84 days and every 7 days (week) mean are displayed in the results. The study was approved by an institutional ethics committee.

Statistical analysis: The activity scores were analyzed by one-way analysis of variance (ANOVA). Tukey–Kramer multiple comparisons test was used for comparing the activity score with test and control groups. *p*-values <0.05 were taken as statistically significant.

RESULTS

Body weight: Group 2 (disease control group, SCI+saline) showed a gradual decrease in rats body weight at day 2 until day 7 as compared with the baseline value. However, there was a gradual increase in the body weight from day 8 (second week onwards) and this improvement was maintained until the end of the study (Table 2). Similar results were observed in all test groups (i.e 3,4,5,7), the improvements were started on day 6 for group 6 and 8. Compared to low doses ascorbic acid (group 3) and alpha-tocophoral groups (group 5), positive improvements in rats body weight were observed in high doses of ascorbic acid (group 4) and alpha-tocophoral (group 6). Further, when compared with ascorbic acid treatment a notable improvements in rats body weight were observed in alpha-tocopherol groups, especially rats received high dose of alpha-tocopherol.

Vertical activity: Compared to baseline value, group 3,4,5,6,7 and 8 showed a gradual improvement in vertical movement at week 1, and such improvements were maintained at all-time

points until the end of the trial period (week 12). Compared to disease control positive improvements in vertical activity were observed in all test groups. Compared to low doses of ascorbic acid (group 3) and alpha-tocophoral groups (group 5), positive vertical activity improvements were observed in high doses of ascorbic acid (group 4) and alpha- tocophoral groups (group 6). In addition, when compared with ascorbic acid treatment, notable added improvements were observed in alpha-tocopherol groups mainly group 6 (i.e alpha tocopherol 1000 mg/kg body weight). Compared to initials weeks, the recovery rates of all test groups rats were less in last four weeks (i.e week 9-12).

Horizontal activity: Compared to baseline value, group 3,4,5,6,7 and 8 showed a gradual improvement in horizontal movement at week 1, and such improvements were maintained at all-time points until the end of the trial period (week 12). Compared to disease control positive improvements in horizontal activity were observed in all test groups. Compared to low doses of ascorbic acid and alpha- tocophoral groups (group 3, 5), positive differences in horizontal activity were observed in high doses of ascorbic acid (group 4) and alpha-tocophoral groups (group 6). However, when compared with ascorbic acid treatment notable added improvements were observed in alpha-tocopherol groups, specially group 6 (i.e alpha tocopherol 1000 mg/kg body weight). Compared to initials weeks, the recovery rates of all test groups rats were less in last four weeks (i.e week 9-12).

DISCUSSION

In the present study we investigated the effects of ascorbic acid and alpha-tocopherol on rats with incomplete spinal cord injury. The findings of the study showed that SCI reduced the rats body weight for the first week (first 7 days) followed by a slow recovery. This is in agreement with earlier studies stated that SCI decrease animal body weight. This decrease in weight may partly due to the great stress exerted on the body at the time of the initial trauma, so the body's metabolism works quicker to offer energy and nutrients to help in healing the body and fighting infections. As a result, it helps in decreasing the weight of the animals [17-19]. However, the results of this study showed that there was steady recovery over a period of 12 weeks after SCI compared with baseline readings and disease control group. These observations are in agreement with previous findings [20, 21].

Studies have reported that ascorbic acid and alpha-tocopherol are endogenous antioxidants that are thought to have protective effects by decreasing or inhibiting oxidative damage [22, 23]. The ascorbic acid is found in the cytosol and extracellular fluid and can directly interact with free radicals to inhibit oxidative damage [24, 25]. Alpha-tocopherol is prevents lipid peroxidation chain reactions in cellular membranes by interfering with the propagation of lipid radicals [24, 25]. In the present study, we found all test groups that received alpha-tocopherol, showed gradual improvements in both vertical and horizontal movements at week 1, and such improvements were maintained at all-time points until the end of the trial period (week 12) as compared with the baseline values. Similarly, when compared to disease control, positive improvements were observed in both vertical and horizontal movements of alpha-tocopherol groups especially rats received high dose of alpha-tocophoral. Previous studies were conducted to assess the effectiveness of alpha-tocopherol on compression injury in the spinal cord of rats described that the motor disturbance induced by SCI was greatly reduced by alpha-tocopherol supplementation [20, 21]. After injury, the spinal cord evoked potentials that revealed major recovery of both amplitude and latency in the alpha-tocopherol supplemented group than in the control group [20, 21]. Acute SCI produces tissue damage that continues to evolve days and weeks after the initial insult, with corresponding functional impairments. Reducing the extent of progressive tissue loss (neuroprotection) after SCI should result in better recovery [26]. It has been suggested that the generation of free radicals and subsequent lipid peroxidation has been proposed to contribute to postponed tissue destruction after traumatic SCI. Ascorbic acid and alpha-tocopherol are endogenous antioxidants; therefore decreases in the tissue levels of these compounds may therefore reflect ongoing oxidative reactions [27]. In the present study, the functional disorder induced by SCI was impressively decreased by alpha-tocopherol. This observation is in agreement with other studies, which have also reported that alpha-tocopherol may have reparative effects on SCI [20, 21].

Similar to alpha-tocopherol treatment, rats that received ascorbic acid also showed gradual improvements in both vertical and horizontal movements compared with the baseline value; these improvements were detected at week 1, and maintained at all-time points until the end of the trial period (week 12). In addition, when compared to disease control positive improvements in both vertical and horizontal movements were observed in all test groups that received ascorbic acid. Furthermore, notable additional improvement was

observed in the rats that received a high dose of ascorbic acid compared to those that received a low dose. A recent study also found that the administration of high-dose of ascorbic acid is effective for treating the SCI. Further they reported that motor disturbance induced by SCI was found to be greater in the ascorbic acid deficient rats [28]. In contrast to our finding, few studies also reported that the use of ascorbic acid and alpha- tocopherol did not improve the neurological performance in SCI rats. However, their histopathological examination showed that the inflammatory response was less intense following administration of the combination of ascorbic acid and alpha-tocopherol, despite the discrepancy regarding this issue in scientific literature [28-30].

Our data clearly demonstrate that the vertical and horizontal scores of SCI rats treated with either ascorbic acid or alpha-tocopherol had improvements in functional recovery of the SCI rats. However, less positive difference were observed in ascorbic acid treated animals compared to alpha-tocopherol treated animals. Earlier, studies reported that the antioxidative function of ascorbic acid and its effect on reducing ischemia after experimental compression injury were reported [27]. A pretreatment of spinal cord injured animals with a single dose of ascorbic acid has been reported to support spinal cord blood flow, although 'not as effectively as alpha- tocopherol treatment [28, 31]. It should be noted here that in our study we found better positive horizontal and vertical activity when treating with high doses of ascorbic acid or alpha-tocophoral than with lower doses of the same treatment. A recent study also reported that high-dose of ascorbic acid administration during the acute phase post SCI significantly decrease secondary injury induced tissue necrosis and enhanced functional recovery in rats [32].

In conclusion, the results of this study clearly indicate that both alpha-tocopherol and ascorbic acid increases the functional activity of SCI rats, which suggests both ascorbic acid, alpha-tocopherol have reparative effects for SCI because of its antioxidants effects. In addition, the study also concluded that administration alpha-tocopherol has more reparative effects than ascorbic acid.

Table 1: Group descriptions

Groups	Treatment
1	Control (without SCI, untreated)
2	Disease control (saline)
3	Ascorbic acid 500 mg/kg body weight
4	Ascorbic acid 1000 mg/kg body weight
5	Alpha-tocopherol 500 mg/kg body weight
6	Alpha-tocopherol 1000 mg/kg body weight
7	Ascorbic acid 250 mg/kg body weight+ Alpha- tocopherol 250 mg/kg body weight
8	Ascorbic acid 500 mg/kg body weight+ Alpha- tocopherol 500 mg/kg body weight

Table 2: Effectiveness of spinal cord injury on rats' body weight (gram)

	Group-1	Group-2	Group-3	Group-4	Group-5	Group-6	Group-7	Group-8
Baseline	184 ± 2.1	186 ± 2.1	185 ± 3.1	187 ± 3.4	183 ± 2.9	184 ± 3.51	183 ± 2.8	186 ± 4.20
Week-1	188 ± 3.20	173 ± 6.04*	174 ± 5.71*	176 ± 6.05*	175 ± 5.30*	174 ± 5.75*	174 ± 5.28*	175 ± 5.88*
Week-2	196 ±1.95	179 ± 4.27	181 ± 5.15	181 ± 6.75	182 ± 5.81	181 ± 6.21	181 ± 5.15	182 ± 5.81
Week-3	201 ± 1.34	188 ± 1.79	184 ± 1.23	185 ± 3.55	186 ± 6.04	190 ± 4.19	185 ± 3.40	188 ± 6.02
Week-4	207 ± 1.73	191 ± 0.89	189± 0.75	191± 1.90	192± 1.29	196 ± 1.34	189 ± 1.01	194± 2.76
Week-5	211 ± 1.57	193 ± 0.57	191 ± 0.81	194 ± 0.76	196 ± 1.11	199± 0.81*	191 ± 1.11	197 ± 1.25
Week-6	216 ± 1.29	194 ± 0.95	194± 1.81	196 ± 0.75	198 ± 1.11	204 ± 3.64#*	195 ± 0.57	201± 0.59*
Week-7	220 ± 1.57	197 ± 0.97	197 ± 0.75	200± 0.48	201± 0.53*	208± 0.53* #†	198± 0.78	204± 1.21*#
Week-8	224 ± 1.21	200 ± 1.11*	202 ± 1.06*	203 ± 0.78*	205 ± 0.81*	213± 0.488* #†	202 ± 0.53*	209 ± 2.30*#
Week-9	229 ± 1.79	203 ± 0.53*	205 ± 0.69*	207 ± 0.53*	208 ± 0.57 *	216 ± 0.69* #†‡	206 ± 1.13*	212 ± 0.75*#
Week-10	233 ± 0.89	206 ± 0.78*	209 ± 0.48*	210 ± 0.57*	213 ± 0.89 *#	220 ± 0.57 *#†	209 ± 2.16*	217 ± 0.81*#†
Week-11	235 ± 0.75	208 ± 1.60*	213 ± 0.48*	215 ± 0.53* #	217 ± 0.81*#	226 ± 0.81 *#†‡	214 ± 0.75*#	221 ± 1.21*#†
Week-12	237 ± 0.48	211 ± 0.97*	216 ± 0.53*	219 ± 0.48* #	222 ± 0.81*#†	230 ± 0.53*#‡†	216 ± 0.48*#	226 ± 2.26*#†‡

Values are presented as mean ± standard deviation.
Groups compared: * Compared to baseline, **#** 2 vs 3, 4, 5, 6, 7, 8.
† 3 vs 4, 5, 6, 7, 8. ‡ 4 vs 5, 6, 7, 8. ***f*** 5 vs 6, 7, 8. ¶ 6 vs 7, 8,
One-way analysis of variance (ANOVA). Tukey–Kramer multiple comparisons test.

Table 3: Effectiveness of different doses of ascorbic acid and alpha-tocopherol on vertical activity of spinal cord injured rats

	Group-1	Group-2	Group-3	Group-4	Group-5	Group-6	Group-7	Group-8
Baseline	1356 ± 23.4	178 ± 32.7	181 ±21.7	190 ±19.6	185 ± 23.8	186 ± 16.5	185 ± 24.5	186 ± 21.5
Week-1	1280 ±27.8	243 ± 34.8	248 ± 38.5	251 ± 63.9	241 ± 59.0	241 ± 56.1	241 ± 56.1	242 ± 59.0
Week-2	1159 ±19.4	472 ± 56.4*	487 ± 33.7*	502 ± 61.9*	502 ± 49.1*	534 ± 85.8*	482 ± 34.1*	519 ± 83.7*
Week-3	1154 ± 18.5	419 ± 28.2*	514 ± 22.2*	552 ± 12.2*	564 ± 18.1*	660 ± 45.5 *	524 ± 13.1*	625 ± 23.6 *
Week-4	1143 ± 11.6	495 ± 17.0*	536 ± 29.6*	592 ± 11.5*	601 ± 32.2 *	756 ± 18.7 *	564± 37.4	668 ± 19.5 *
Week-5	1120 ± 42.7	547 ± 20.3*	572 ± 56.0 *	612 ± 13.1 *	632 ± 12.1 *	776 ± 14.2 *	603 ± 22.5 *	683 ± 26.2 *
Week-6	1139 ± 15.89	594± 18.5*	602 ± 38.9 *	643 ± 18.1 *	682 ± 13.1*	786 ± 13.2*	632 ± 14.6*	746 ± 16.5*
Week-7	1177 ± 14.0	626 ± 32.6*	642 ± 17.9*	671 ± 13.4*	752 ± 40.5*	798 ± 15.3*	652 ± 14.2*	783 ± 19.2*
Week-8	1169 ± 21.1	646 ± 11.3*	674 ±16.2*	752 ± 21.4*	812± 22.9*	866 ± 26.4*#† *f*	694 ± 16.2*	854 ± 10.2*
Week-9	1161 ± 21.3	695 ± 19.2*	694 ± 11.4*	786 ± 13.6*	842 ± 31.2*	953 ± 24.5*#†‡ *f*	762 ± 15.7*¶	897 ± 28.4*#†
Week-10	1149 ± 21.3	703 ± 16.4*	712 ± 17.4*	816 ± 13.5*	886 ± 16.4*	1007±18.2*#†‡ *f*	782 ± 12.8*¶	912 ± 25.2*#†
Week-11	1167 ± 19.4	726 ± 16.9*	762 ± 21.4*	827 ± 17.4*	892 ± 18.4*#†	1012±16.2*#†‡ *f*	794±15.4*¶	932 ± 22.4*#†
Week-12	1150 ± 18.6	746 ±17.2*	768 ± 22.3*	834 ± 19.5*	894 ± 21.5*#†	1016±21.4*#†‡ *f*	798±17.4*¶	947 ± 21.6*#†

Values are presented as mean ± standard deviation.
Groups compared: * Compared to baseline, **#** 2 vs 3, 4, 5, 6, 7, 8.
† 3 vs 4, 5, 6, 7, 8. ‡ 4 vs 5, 6, 7, 8. ***f*** 5 vs 6, 7, 8. ¶ 6 vs 7, 8,
One-way analysis of variance (ANOVA). Tukey–Kramer multiple comparisons test.

Table 4: Effectiveness of different doses of ascorbic acid and alpha-tocopherol on horizontal activity of spinal cord injured rats

	Group-1	Group-2	Group-3	Group-4	Group-5	Group-6	Group-7	Group-8
Baseline	972 ± 9.3	186 ± 9.4	180 ± 13.5	181± 10.5	178 ±11.5	185 ± 9.2	190 ± 12.4	185 ± 12.4
Week-1	984 ± 21.4	230 ± 17.8	248 ± 21.1	248 ± 13.6	242 ± 16.3	242 ± 12.5	251 ± 12.6	243 ± 14.4
Week-2	991 ± 13.5	332 ± 21.1	482 ± 17.3	482 ± 24.1	402 ± 15.1	475 ± 21.2	468 ± 14.5	412 ± 15.3
Week-3	977 ± 18.9	378 ± 21.4	384 ± 15.8*	525 ± 21.4*	493 ± 18.5*	564 ± 17.5*	445 ± 17.4 *	532 ± 23.1*
Week-4	991 ± 18.9	413 ± 21.5*	462 ± 21.4*	556 ± 16.7*	567 ± 19.5*	633 ± 16.4*	469 ± 15.3*	598 ± 21.5*
Week-5	992 ± 17.5	453 ± 17.5*	512 ± 18.4*	577 ± 21.2*	602 ± 21.3*	711 ± 21.4*#†	533 ± 19.3*	632 ± 18.9*
Week-6	977 ± 21.5	479 ± 18.9*	547 ± 17.5*	638 ± 19.4*	657 ± 18.2*	804 ± 19.5*#†	603 ± 21.3*	702 ± 15.4#†*
Week-7	991 ± 23.1	501 ± 17.8*	586 ± 17.4*	712 ± 17.5*#†	726 ± 18.4*#†	845 ± 15.3*#†	654 ± 21.4*#	794 ± 19.4*†#
Week-8	991 ± 19.2	528 ± 17.4*	612 ± 14.2*	753 ± 17.4*#†	793 ± 16.5*#†	860 ± 18.4*#†	702 ± 18.4*#	814 ± 19.6*†#
Week-9	991 ± 18.4	553 ± 18.5*	654 ± 14.3*	772 ± 21.7*#†	826 ± 16.7*#†	864 ± 12.5*#†	735 ± 21.6*#	834 ± 17.4*†#
Week-10	991 ± 17.4	570 ± 19.2 *	712 ± 19.4*#	812 ± 19.5*#†	834 ± 18.9*#†	878 ± 16.5*#†	772 ± 18.4*#	849 ± 21.3*†#
Week-11	992 ± 18.4	586 ± 21.4*	752 ± 18.4*#	816 ± 18.5*#†	839 ± 14.8*#†	882 ± 15.7*#†	784 ± 18.5*#	865 ± 16.4*†#
Week-12	977 ± 18.5	611 ± 17.5*	772 ± 18.6*#	817 ± 21.4*#†	842 ± 17.4*#†	896 ± 17.2*#†	796 ± 19.4*#	879 ± 18.5*†#

Values are presented as mean ± standard deviation.
Groups compared: * Compared to baseline, **#** 2 vs 3, 4, 5, 6, 7, 8.
† 3 vs 4, 5, 6, 7, 8. ‡ 4 vs 5, 6, 7, 8. ***f*** 5 vs 6, 7, 8. ¶ 6 vs 7, 8,
One-way analysis of variance (ANOVA). Tukey–Kramer multiple comparisons test.

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