

# Neuroprotective Effect Of Ascorbic Acid In Experimental Blunt Sciatic Nerve Injury In Rats

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## Abstract

Ascorbic acid (AA) is a potent natural antioxidant. The aim of this study was to investigate the antioxidant effects of AA in experimental sciatic nerve (SN) injury in rats.

Forty rats were used for the study in which SN injury was produced using aneurysmal clips. The rats were randomly divided into five groups. In the control group nontraumatized SN samples obtained. In the trauma group injured SN was removed after trauma. The vehicle group received distilled water. The fourth group received 100 mg/kg AA and the fifth group received 500 mg AA. Lipid peroxidation was estimated by measuring malondialdehyde (MDA) content of the injured SN.

In the fourth and fifth groups, MDA was found to decrease significantly in comparison to vehicle and trauma groups. Injection of AA after trauma reduced MDA content of injured SN. Ascorbic acid has been shown to be effective in protecting the injured SN from secondary injury.

## INTRODUCTION

Peripheral nerve trauma, with an estimated incidence of, 1/1000 in population per year, remains a major cause of morbidity and social disruption.<sup>1</sup> Even with optimal surgical repair, cutaneous innervation will remain reduced, normal sensation is seldom reattained, and clinically the sensory outcome remains very poor.<sup>1,2</sup> Poor sensory function adversely impacts upon motor function, particularly fine manipulative movements, since adequate sensory feedback is a vital component of normal proprioception and therefore of motor control.<sup>3</sup> Peripheral nerve trauma therefore carries a high cost in healthcare, as consequences may lead to significant constraints in personal and professional life.

Secondary injury that occurs after a primary peripheral nerve injury further contributes to worsening of nerve function. The secondary injuries are the result of a number of autodestructive phenomena, the pathomechanisms of which in some cases have been partially elucidated. Neutrophilic infiltration increases significantly in the traumatized nerve. The activated neutrophils are shown to be implicated in the worsening of nerve injury. Oxygen radicals released from

neutrophils are toxic to the cell membrane component and free radical-induced lipid peroxidation is important in the autodestruction of the injured nerve.<sup>4,5</sup>

Increased production of free radicals and reactive oxygen species leading to oxidative stress appears to play an important role in the pathogenesis of traumatic peripheral nerve injury.<sup>3</sup> Lipid peroxidation may alter the fluidity and permeability of neuronal membranes and impair cellular functioning, or damage membrane bound receptors and enzymes.<sup>6</sup> In addition, tissue lactic acidosis can dramatically enhance reactive oxygen species formation and lipid peroxidation in neuronal tissue.<sup>7</sup> Because free radicals are short-lived and usually present at low concentrations, they are difficult to measure in biological samples.<sup>8</sup> However, there are indirect indexes that can be used to examine sequelae of free radicals production such as malondialdehyde (MDA) concentration measurement.

Ascorbic acid (vitamin C) appears to be particularly important in limiting oxidative lipid damage in biological systems. Numerous studies have demonstrated that under many different types of oxidizing conditions, Ascorbic acid

(AA) forms the first line of antioxidant defense and effectively protects the lipids in plasma and lipoproteins against detectable peroxidative damage.<sup>9</sup>

The objective of the current study was to ascertain the content of thiobarbituric acid reactive substances (TBARS) in the damaged sciatic nerve as an indicator of free radicals activity and lipid peroxidation and investigate the effects of AA on the prevention of experimental sciatic nerve injury.

### MATERIALS AND METHODS

#### ANIMALS AND SURGICAL TECHNIQUE

Forty male Wistar rats weighing 250 to 300 grams were used according to the Guide for Care and Use of Laboratory Animals (DHEW Publication No. 78-23, NIH revised 1978) and local guidelines for humane use of animals in research. The animals were housed three per cage and provided with free access to rat food and water. They were kept under constant laboratory conditions of 18 to 21°C room temperature, and illumination (12 hours each of light and darkness; darkness beginning at 7:00 PM). Under Ether (Merck Inc.) anesthesia, groups of young adult male rats underwent unilateral sciatic nerve exposure at the upper border of quadratus femoris. Acute constriction injury was initiated by clipping the right sciatic nerves with Yasargil standard temporary aneurysmal clip (41-5112.TI, Geister, Tuttlingen, Germany) for 5 minutes.

#### DRUG PREPARATION AND ANIMAL GROUPS:

The rats were randomly allocated into five groups: The first group was a control (sham-operated) group of eight rats in which only sciatic nerve exposure was performed and nontraumatized SN samples were obtained. The second group (trauma group) consisted of eight rats in which, following surgical and traumatic interventions, injured SN samples were removed 6 hours after trauma. The third group (vehicle group) received distilled water intraperitoneally as the AA vehicle after injury. In the fourth group rats received low dose AA (100mg/kg) intraperitoneally immediately after trauma and the fifth group received high dose AA (500mg/kg) intraperitoneally. Ascorbic acid (Merck Inc.) was dissolved in distilled water and administered via the intraperitoneal route. A 0.5 cm sample of the injured sciatic nerve was derived 6 hours after trauma.

#### SAMPLE PREPARATION AND DETERMINATION OF LIPID PEROXIDES

Lipid peroxidation in the sciatic nerve of the rat was measured as thiobarbituric acid-reactive material.<sup>10</sup> After

isolation of sciatic nerve, tissue homogenates (10% w/v) were prepared by homogenizing tissue in cold potassium phosphate buffer (PH=7.4), using a glass/glass homogenizer. In a test tube 0.5 ml of tissue homogenates were mixed with 3 ml of 1% orthophosphoric acid. After addition of 1 ml of 0.67% thiobarbituric acid, the mixture was heated in boiling water for 45 minutes. The color formed was extracted into 4 ml of n-butanol and the absorption was measured at 532 nm, using tetramethoxypropan as the standard. Malondialdehyde (MDA) as indicator of tissue lipid peroxide levels was calculated as nanomol per gram (nmol/gr) of wet tissue.<sup>11,12</sup>

#### METHOD OF STATISTICAL ANALYSIS

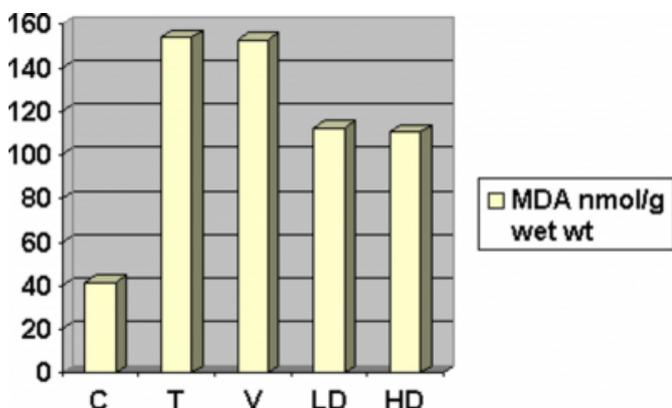
Levels of MDA in the trauma and vehicle groups were almost the same as shown in Fig-1. For this reason in statistical analysis these two groups were mixed and were compared with another three groups. The distribution of MDA was skewed, so logarithmic transformation of values was performed to make a good approximation to the normal distribution. Analysis of variance was used to estimate the differences and probability of the first type error. Measures of effects were relative, so relative values and percent changes in values were desired and reported. Since variance of MDA level in different groups was somehow different, P-values were calculated based on unequal variances assumption. In all data analysis, P-values of 0.05 or less were considered significant. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS version 11, SPSS, Inc., Chicago, IL).

#### RESULTS

The mean content of MDA in the control group was 41.725nmol/g (SD=6.3073 nmol/g). Trauma was found to produce a significant elevation in lipid peroxidation (153.85nmol/g tissue and SD=52.9830nmol/g). Intraperitoneal administration of AA (both low dose and high dose) produced a significant decrease in lipid peroxidation at 6 hours post-injury compared with trauma groups. In summary MDA contents differ significantly between injured and non-injured groups while MDA contents remain similar within both dosage AA groups. MDA contents were also nearly alike in injured groups with and without placebo injection (Fig. 1)

**Figure 1**

Figure 1: MDA content in sciatic nerve in different groups. C=control group, T=trauma group V=vehicle group, LD=low dose AA group, HD=high dose AA group.



Tab-1 shows effects of procedures and statistical significances. Differences between groups are highly significant (P=0.000). Trauma was found to produce a significant 3.6 times elevation in MDA content (P=0.000). Intraperitoneal administration of distilled water had no effect on MDA content. Early treatment with low dose AA (100 mg/kg) after trauma significantly (P=0.018) decreased the MDA content about 26 percent. High dose AA in the injured nerve also decreased the MDA content by about 28 percent (P=0.014). The two percent difference between efficacy of low dose and high dose of AA was not statistically significant (P= 0.88). Although both doses of AA were effective in lowering MDA contents, they failed to bring the MDA down to non-injury levels. The MDA contents in the non-injured and treated-injured differ significantly (2.6 times above normal and P=0.000).

**Figure 2**

Table 1: Differences between groups and effect of procedures on MDA contents of injured sciatic nerve. C=control group, V+T=vehicle and trauma groups, LD=low dose AA group, HD=high dose AA group.

Groups	Relative difference	P value *
C versus (V+T)	3.59	0.000
LD versus (V+T)	1.36	0.018
HD versus (V+T)	1.38	0.014
HD versus LD	0.98	0.88
LD & HD versus C	0.38	0.000

\* Unequal variances assumed.

**DISCUSSION**

Oxygen free radical-mediated lipid peroxidation has been increasingly suggested to be an important factor in posttraumatic neural tissue degeneration.<sup>13</sup> As is known, neural tissue does not contain highly active oxidative

defense mechanisms. Neurons, unlike many other cells, are generally considered to be incapable of mitosis, and thus, damage to these cells by any means, including that by free radicals, is especially devastating and may cause permanent lesions.<sup>14</sup> Free oxygen radicals not only damage phospholipids of the neural membranes, but it has been proposed that in situ myelin proteins in the membrane are highly susceptible to the attack of reactive oxygen species as well.<sup>15</sup> Thus, authors of numerous studies have evaluated the neuroprotective efficacy of pharmacological agents with lipid antioxidant activity in central and peripheral nervous system injury.<sup>4,11,12,13,16,17,18,19,20,21,22,23,24,25</sup> Some studies reveal the role of vitamin E as an antioxidant agent in peripheral nerve injury.<sup>24,26</sup> The role of antioxidant effect of AA has been studied in diabetic neuropathy in rats as well as in cold injury of peripheral nerves.<sup>17,21,23,25</sup>

To our knowledge this is the first study in which the antioxidant effect of AA is shown in an experimental blunt sciatic nerve injury. Intraperitoneal administration of AA at different doses was shown to significantly decrease MDA contents. Although high dose AA decreased lipid peroxidation more than the low dose the difference was not statistically significant. We chose the sciatic nerve clipping technique to induce acute blunt sciatic nerve injury because it allows us to perform a standard blunt trauma in each rat, and it also results in a lesion similar to those seen in patients with peripheral nerve injury. Factors like duration and severity of injury as well as dose selection can alter results. On the other hand uniform distribution of rats among different groups and random allocation provide some control of confounding factors. In our study MDA levels were measured at 6 hours post-injury. In similar studies investigating effects of antioxidant agents on nervous tissues after trauma, lipid peroxidation was measured at different times post-injury and different effects of a variety of antioxidant agents were also compared.<sup>17,24</sup> Our study was designed to simply evaluate the effect of an antioxidant agent on an experimental peripheral nerve injury. We believe that further studies are warranted and should investigate the effects of different agents, doses, and latency of administration in peripheral nerve injuries. Lipid peroxidation should also be measured at different times after trauma.

**CONCLUSION**

In summary, data obtained from the present study reveals that AA (Vitamin C) has a significant effect on lowering MDA levels after nerve trauma in rats and potentially could

have major therapeutic benefits. The present study reveals that AA provides protection of sciatic nerve against lipid peroxidation, occurring as a result of trauma, and there is no dose dependent level of protection. Evaluation of motor function and measuring nerve conduction velocity could be performed to confirm the therapeutic benefits of AA in future studies.

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