Original research

Intraperitoneal administration of ascorbic acid attenuates hyperalgesia in a rat model of neuropathic pain

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Abstract: ***Background:*** Ascorbic acid is a well-known antioxidant but its antinociceptive effect on chronic pain is not known. The aim of this study is to evaluate the analgesic effect of intraperitoneal administration of different doses of ascorbic acid in a peripheral neuropathic pain model.
Methods: To investigate the efficacy of ascorbic acid on neuropathic pain, male rats were allocated to 5 acute administration and 2 chronic administration groups. Pain induced by chronic constriction injury of sciatic nerve (CCI). Different amount of ascorbic acid (1, 3, 5 and 10 mg/kg) and normal saline were injected in acute protocol (single injection two weeks after CCI). In addition, ascorbic acid was administrated with dose of 3 mg/kg (daily injection for three weeks; chronic administration). Hyperalgesia and allodynia were assessed.
Results: Chronic intraperitoneal injection of 3 mg/kg ascorbic acid for 3 weeks increase pain threshold from the second week after CCI. Acute administration of 1 mg/kg ascorbic acid did not produce any changes in pain threshold of neuropathic rats but acute injection of 5 and 10 mg/kg, significantly alleviate pain 30 minutes after injection in the second week following CCI. Similar result observed in chronic administration of ascorbic acid.
Conclusion: These data suggest that ascorbic acid produces analgesia in neuropathic rats.

Keyword: Ascorbic Acid; Hyperalgesia; Allodynia; Chronic Constriction Injury

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1. Introduction

Ascorbic acid (ascorbic acid) is an essential nutrient which accumulates in the blood supply and has high concentration in brain and adrenal glands (1, 2). It is transported into the brain and neurons via sodium-dependent vitamin c transporter 2 (SVCT2) which increases the concentration of ascorbic acid intracellulary to enhance its functions (3, 4). Dehydroascorbic acid (DHA) is transported into the brain via glucose transporters of the Glucose transporter family and is retained in brain in the form of ascorbic acid (5, 6). Among its antioxidant functions, ascorbic acid directly scavenges oxygen and nitrogen radical species generated during cellular metabolism, therefore in situations with high levels of oxidative stress it has an important neuroprotective role (7).

The non-oxidant functions of ascorbic acid in central nervous system focused on neurotransmitter release. It is proposed as a neuromodulator of glutamatergic, dopaminergic, cholinergic and GABAergic transmission and is responsible for their related behaviors (8, 9). Ascorbic acid is concentrated in glutaminergic synaptic vesicles of the glia and neural cells and is released spontaneously or in response to physical stimulation and or certain drugs as a part of glutamate re-uptake process (heteroexchange process) (10).

Glutamate and its N-methyl-D-aspartate receptor (NMDAR) are critically involved in nociceptive transmission, central sensitization and other mechanisms which could be the main cause of induction of neuropathic pain (11). Therefore, NMDAR antagonists and substances that can modulate glutamate release have

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been considered a target in the treatment of neuropathic pain.

Earlier studies have demonstrated that ascorbic acid has spare antioxidant effects and can modulate the redox state of NMDAR thus blocking NMDAR gated channels may prevent neural cells from glutamate-induced excitotoxicity, cell damage and death (12-14). Different assays identified that systemic, intraventricular or intraneostriatal administration of ascorbic acid, in a dose-dependent situation, may be neuroprotective in neurodegenerative disease such as Alzheimer (15, 16), Parkinson (17) and Huntington disease (18). It has been reported that, acute and chronic administration of ascorbic acid is capable of attenuating pain in shingles (19), complex pain syndrome (20) and postherpetic neuralgia (21). The aim of this study is to evaluate the analgesic effect of intraperitoneal administration of different doses of ascorbic acid in a peripheral neuropathic pain model.

2. Method

2.1. Animal

Male Wistar rats (250-300 g) were purchased from Pasteur institute and kept under a 12/12 light/dark cycle and room temperature of 23˚c with 50 to 60% humidity. The animals were free access to water and standard chow. All behavioral experiments took place during daytime between 9:00 am-3:00 pm. The animals belonging to the various treatment groups (n=8 in each group) were tested in randomized order. All animal experimental procedures were conducted in accordance with the guidelines of the International Association for the Study of Pain and were approved by the ethic Committee of the University for use of animal in experiments.

2.2. Induction of Chronic constriction injury model (CCI model)

Chronic constriction injury of left sciatic nerve was constructed according to the procedure explained by Bennett and Xie (22). Briefly, the rats were anaesthetized with pentobarbital (60mg/kg) intraperitoneally, the hair of the lower back and the right thigh was shaved. The skin of the mid-thigh surface and underneath muscles were incised to expose the common sciatic nerve. The sciatic nerve was loosely tied with four 4-0 chromic gut suture in almost 1 mm distance.

2.3. Treatment

Ascorbic acid was purchased as a powder from Sigma-Aldrich Company. Ascorbic acid was dissolved in normal saline immediately before use.

On the second week after CCI, different doses of ascorbic acid (1, 3, 5, and 10 mg/kg) or normal saline were injected intraperitoneally in a volume of 0.5 ml (acute administration). Pain behaviours were evaluated 30 minutes after injection.

In addition, injection of 3 mg/kg of ascorbic acid or saline started the day after induction of injury and continued every other day for 3 weeks (chronic administration). The animals were tested every week.

2.4. Behaviour evaluation

Prior to testing, all rats were handled and habituated to an open plexiglass chamber for 30 minutes before actual experimental sessions. Each test was conducted with regarding to its acceptable instruction.

Mechanical hyperalgesia, expressed as grams (gr), were measured using an Ugo Basile algesimeter (Ugo Basil Company, Italy). Pressure thresholds to trigger hind paw withdrawal or vocalization were determined. The test performed two times on injured hind paw with two minutes interval. The mean values was considered as a response to the mechanical pressure.

The thermal nociceptive threshold was detected using a Hargreaves apparatus (Plantar test, 7370, Ugo Basile, Comerio, Italy). Rats were placed in clear plastic cages on an elevated glass plate and allowed to acclimate to their surroundings for 30 min before testing. After acclimation, a radiant heat source of continuous intensity was located under the glass and aimed at the mid part of hind paw. A digital timer automatically read the duration between the start of stimuli and paw withdrawal. A cutoff time of 25 s of radiation was used to prevent any tissue injury. The experiment was repeated three times with at least three minutes was allowed to elapse between stimulations.

For the evaluation of mechanical allodynia, Von Frey filaments (4.56, 4.74, 4.93, 5.07, and 5.18) were used. Each filament was pressed until it bend and fixed in its bending position for 3 seconds. The test was repeated 5 times for each filament with an interval of 20 seconds on left hind paw. Licking or lifting the paw was considered as positive and the data was reported as percentage of the paw withdrawal.

2.5. Statistically analysis

All values are expressed as mean ± standard error. One way ANOVA and two-way repeated masseurs ANOVA followed by Bonferroni post hoc test analysis were used for determination of the significant differences between groups. Differences were considered significant at a P-value of less than 0.05.

3. Result

3.1. Acute administration

The effects of intraperitoneal administration of different doses of ascorbic acid (1, 3, 5, and 10 mg/kg) and

normal saline, 30 minutes after injection on mechanical allodynia, thermal and mechanical hyperalgesia in rats with CCI has been displayed in figure 1. Administration of ascorbic acid was ineffective on thresholds of mechanical allodynia (p>0.99), thermal (p>0.99), and mechanical hyperalgesia (p>0.99).

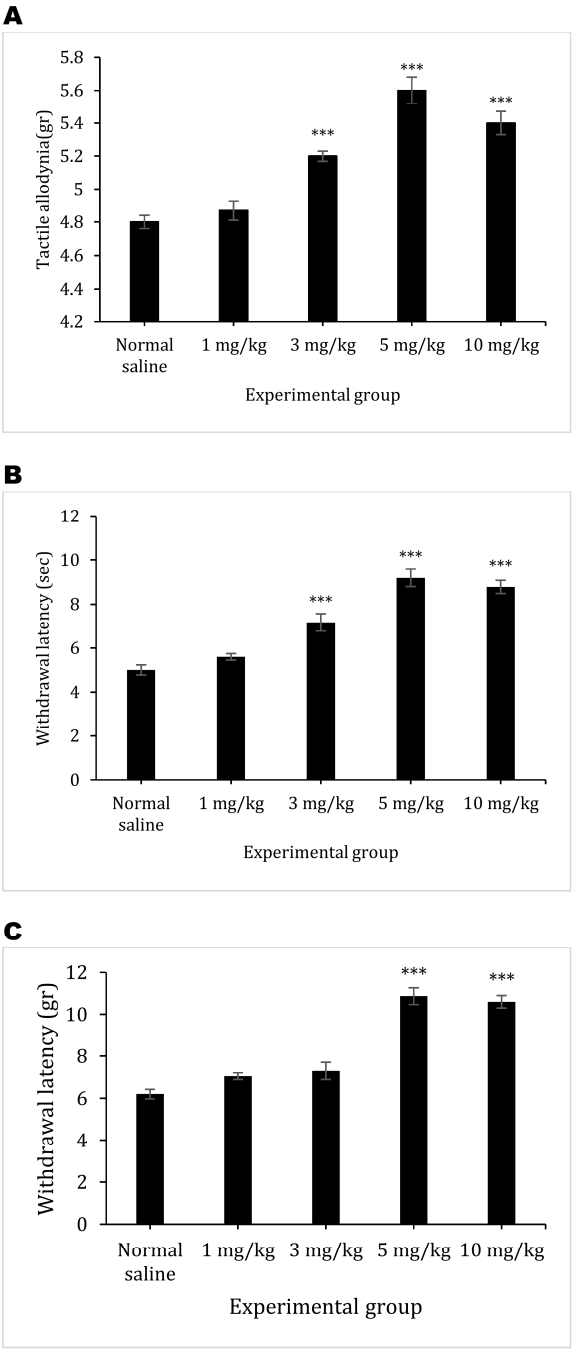

Figure 1: Effect of acute administration of different doses of ascorbic acid, (1, 3, 5 and 10 mg/kg) on tactile allodynia (A), thermal hyperalgesia (B) and mechanical hyperalgesia (C) in chronic constriction injury rats. Ascorbic acid was administered 30 min before the tests. Values are expressed as mean + Standard error (n=8). ***, P<0.001 compared with the saline-treated control animals.

However, the withdrawal thresholds of injured hind paw of animals injected with 3, 5 and 10 mg/kg ascorbic acid ,evaluated with Von Frey filaments, were significantly more than animals injected with normal saline (df: 4, 35, F= 33.4; p<0.001). The mean withdrawal thresholds were 5.2±0.3 gr, 5.6±0.09 gr, and 5.4±0.06 gr in animals received 3 mg/kg (p=0.0003), 5 mg/kg (p<0.0001) and 10 (p<0.0001) mg/kg of ascorbic acid, respectively. The withdrawal threshold of animals injected with normal saline was 4.8±0.04 gr (Figure 1A).

The same results were taken from radiant heat test. Intraperitoneal injection of 3 (p=0.001), 5 (p<0.001) and 10 (p<0.001) mg/kg of ascorbic acid increased the withdrawal threshold in radiant heat test which evaluated the thermal hyperalgesia (df: 4, 35, F= 29.2; p < 0.001). The withdrawal latency was 7.17± 0.47, 9.2±0.39 and 8.7±0.31, respectively which was significantly higher than animals injected with normal saline (5.0±0.23) (Figure 1B).

The results of Randall sellitto test showed no significant difference between ascorbic acid with a dose of 3 mg/kg comparing to normal saline (p>0.99). Withdrawal mechanical thresholds were 10.87±0.39 gr and 10.6±0.41 gr in animals injected with 5 mg/kg and 10 mg/kg, respectively. The withdrawal threshold of animals injected with normal saline was 6.2±0.71 gr which were significantly lower than animals injected with 5 and 10 mg/kg of ascorbic acid (df: 4, 35, F= 29.2; p < 0.001) (Figure 1C).

3.2. Chronic administration

Adminestration of ascorbic acid with dose of 3 mg/kg have increased paw withdraval thereshold to Von Fray test (df: 2, 42; F=14.97; P<0.0001) in the seconds and third weeks after CCI (p<0.0001). However, paw withdrawal threshold on the first week after injury had no significant differences (P=0.18) between animals injected with 3 mg/kg of ascorbic acid and animals injected with normal saline (Figure 2 A).

In thermal hyperalgesia, the withdrawal latency in animals injected with ascorbic acid significantly increased compared to animals injected with normal saline (P=0.02 on the first week and P=0.009 on the second and third week). The withdrawal latencies in animals injected with ascorbic acid were 7.9±0.63, 7.17±0.47, and 7.37±0.48 sec on the first, second and third weeks after injury respectively (df: 2, 42; F=13.07; P<0.0001) (Figure 2B).

In mechanical hyperalgesia evaluated with randall sellitto test, on the first week after injury no significant differences (P=0.18) observed in withdrawal threshold of injured hindpaw of animals injected with 3 mg/kg of ascorbic acid compared to animals injected with normal

saline. However, the difference in the withdrawal threshold was significant on the second (P=0.007) and third (P=0.03) weeks after injury (df: 2, 42; F=10.44; P<0.0001). The withdrawal thresholds were 7.05±0.7 gr and 8.2±0.3 gr on the second and thirds weeks after injury respectively (Figure 2C).

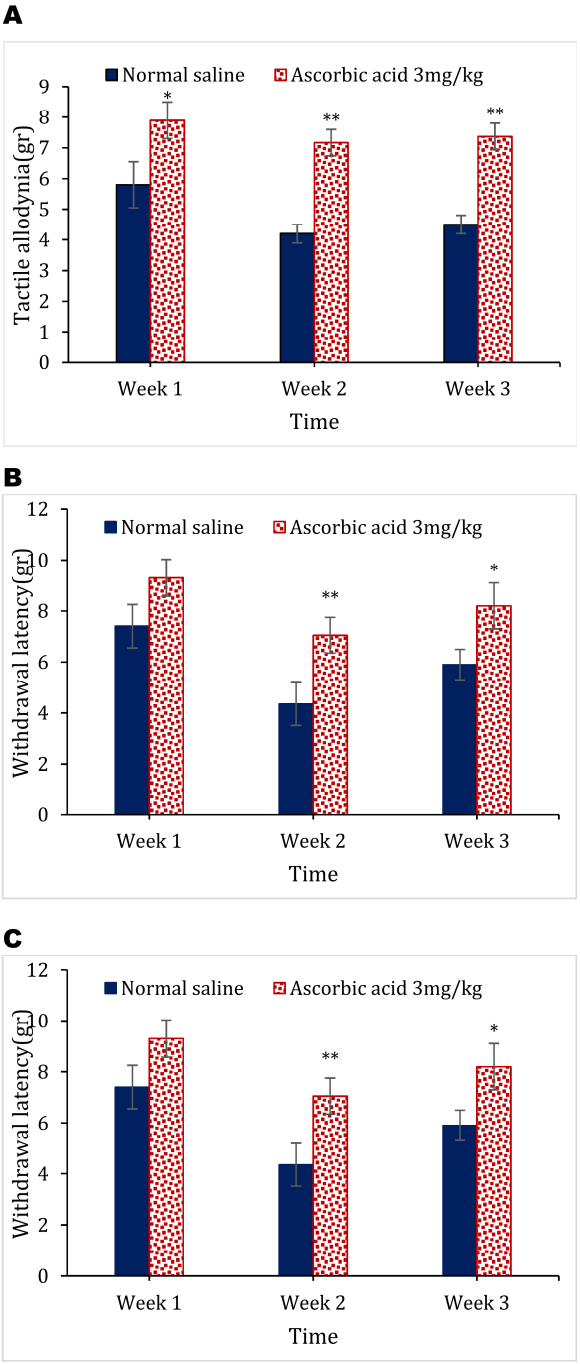

Figure 1: Effect of chronic administration of ascorbic acid, (3mg/kg) on tactile allodynia (A), thermal hyperalgesia (B), and mechanical hyperalgesia (C) in chronic constriction injured rats. Ascorbic acid administrated every other day for 3 weeks. Values are expressed as mean and standard error (n=8). *P<0.05, ** P<0.01 and ***P<0.001 compared with the saline-treated animals.

4. Discussion

In the present study we have demonstrated that intraperitoneal injection of ascorbic acid, reduces hyperalgesia and allodynia in the CCI model. The results showed that the acute administration of 3 mg/kg, 5 mg/kg, and 10 mg/kg but not 1 mg/kg of ascorbic acid, 30 minutes after injection attenuate hyperalgesia and allodynia.

Likewise, intraperitoneal injection of ascorbic acid 3 mg/kg every other day for 3 weeks, starting the day after injury, reduced withdrawal latency until the third week after injury. It is necessary to take into consideration that the analgesic effect of ascorbic acid on mechanical hyperalgesia and tactile allodynia is delayed. The pain alleviation effect of ascorbic acid on mechanical hyperalgesia and allodynia appeared on the second week after injury. While the analgesic effect on thermal hyperalgesia appeared on the first week after injury. This may be because of the delay in the appearance of mechanical pain compare to thermal pain. The reason that we did not observe the difference on withdrawal threshold on mechanical pain between animals injected with ascorbic acid and normal saline may be because mechanical threshold was not decreased on the first week. This results is similar to the results reported by Bennet and Xia (22). They reported a delay on appearance of mechanical pain compared to thermal pain in animals with chronic constriction injury of sciatic nerve.

It has also been reported that, mechanical and thermal hypersensitivity responded differently to pharmacological agents (23) which indicated that different mechanisms are responsible for thermal and mechanical hypersensitivity.

Consumption of ascorbic acid increases its plasma concentration. Glucose transporters family are responsible for transporting ascorbic acid into the cells. This increase in intracellular concentration of ascorbic acid results in increasing its performance as an enzyme cofactor, antioxidant and its other biological effects (3, 6). It has been reported that pharmacological concentrations of ascorbic acid is beneficial for the detoxification of reactive oxygen species, improving immune responses, neuroprotection and pain relief (24-27).

We have demonstrated that long term intraperitoneal injection of an effective dose of ascorbic acid (3mg/kg) could attenuate hyperalgesia and allodynia established following injury to the sciatic nerve. In Chronic constriction model symptoms of neuropathic pain gradually increased. It has been shown that two weeks after surgery, maximum pain sensation occurred. In this time a progressive wallerian degeneration was occurred and

almost 99% of fibers were degenerated. The fibers begin to regenerate from four weeks after injury and this is the time that pain gradually decreased in this peripheral model of neuropathic pain (28, 29). In the chronic study we started ascorbic acid injection once a day after CCI and observed the analgesic effects of 3mg/kg of vitamin C from the end of the first week until the third week, therefore it could be possible that, in the present of high concentration of ascorbic acid regeneration of the fibers was accelerated and this may relief the pain in chronic administration of ascorbic acid.

In this regard it is reported that the plasma concentration of ascorbic acid is related to pain sensation and administration of high dose of ascorbic acid for several days, could improve neuropathic pain condition such as post-herpetic neuralgia, chronic pancreatitis, shingles and complex regional pain syndrome (19-22, 30).

Ascorbic acid also appeared to modulate glutamatergic transmission and alter redox state of NMDARs thus block NMDA-gated channels (12, 13, 31). So it is possible that ascorbic acid acts as a NMDAR antagonists and in doses of 3, 5 and 10 mg/kg revealed antinociceptive effect. The role of NMDA receptors in central sensitization and pain sensation has well been proven. In our previous study we showed that by antagonizing the NMDA receptors, ascorbic acid inhibit the nociceptive effect of glutamate in neuropathic pain condition (32).

Recently, the activation of calcium channels (cav3.2) in both the peripheral and central ending of the primary afferent neurons leading to somatic and visceral hyperalgesia has been proven (33, 34). Calcium channels are involved in regulating the release of excitatory neurotransmitters such as substance P and glutamate (35). Ascorbic acid suppresses cav3.2 channels. Topical application of sodium isostraryl 2-o-L ascorbyl, an amphiphilic ascorbic acid derivative reduced hyperalgesia in rats. It is probable that analgesic effect of ascorbic acid were related to ascorbic acid-cav3.2 channels inhibition (25, 36, 37).

On the other hand, oxidative stress is a major contributing factor in the pathophysiology of neuropathic pain. Ascorbic acid has a strong oxidative capacity and is able to decrease NO synthase, scavenge and detoxify reactive oxygen species. So by attenuating oxidative damage it can improve neuropathic pain symptoms (36). The results of the research in our lab confirmed that ascorbic acid could decrease the allodynic and hyperalgesic effects of L-arginine (500 mg/kg) in rats with chronic constriction injury (38). While ischemia increased tissue release of reactive oxygen species, increasing in brain ascorbic acid concentration could be beneficial in ischemia or ischemia-reperfusion models of stroke (39-41).

In this study, ascorbic acid 1mg/kg couldn't attenuate CCI neuropathic symptoms, similarly Rosa (31) reported that 1mg/kg of ascorbic acid was ineffective in alleviating pain but 3 mg/kg and 5 mg/kg reduced the score of primary and secondary phase of formalin test. This results is similar to the resulted reported in this paper. While in the present study, no differences have been shown between analgesic effects of 5mg/kg and 10mg/kg of ascorbic acid, Rosa reported no analgesic effects for ascorbic acid in doses of 10mg/kg in formalin test (31). Of course formalin test is not able to generate neuropathic pain, it causes acute and chronic pain so this paradox may be partly related to utilizing different pain model.

5. Conclusion:

In summary, we hereby have identified that acute and long term administration of ascorbic acid dose-dependently can attenuate hyperalgesia and allodynia in neuropathic pain induced by chronic constriction injury of sciatic nerve in rats, so it is considered that the supplementation or treatment with vitamin C might be safe and effective in alleviating chronic pain.

6. Acknowledgment

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7. Conflict of interest

No conflict of interest was declared.

8. Funding source

None.

9. Author contribution

Conception and design of the work: FN; data gathering: SS; data analysis: FN; drafting the work: SS; critically revised the manuscript: FN. FN and SS approved final version of the paper to be published and agreed to be accountable for all aspects of the work.

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