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Enhanced memory function in young rats following ascorbic acid administration

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Abstract: The phenomenon of aging is associated with different variations in physiological, behavioral and neurochemical processes. The aging brain goes through a considerable amount of alterations resulting in changes in neuronal structure and functioning that finally leads to cognitive impairment. Ascorbic acid is the major water-soluble antioxidant and acts as a first defence against free radicals in plasma and brain and thus prevents onset of aging. The aim of present study was to evaluate the antioxidant effects of ascorbic acid in growing rats. Twelve young (3-4 months) female rats were taken and randomly assigned into Control (saline 0.9% injected) and test groups (50 mg/kg ascorbic acid injected Intraperitoneally i.p.) for 4 weeks. The learning and memory of rats were assessed by passive avoidance (PA) test. Locomotor activity of rats was monitored by open field test and anxiety was monitored in light/dark transition test. Food intake and growth rate were also monitored daily for 4 weeks. Results of behavioral tests showed significant ($p<0.01$) increase in working memory of vitamin C treated rats as compared to control rats. In light-dark test treatment with ascorbic acid showed significant ($p<0.01$) increase in time spent in light-box and the administration of vitamin C did not produce a significant effect on the locomotor activity in test rats as compared with their control rats. Hence ascorbic acid has a significant positive influence on young rats and may prove to be a useful memory enhancing agent to treat neurodegenerative disorders seen in elderly individuals.

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INTRODUCTION

Aging is associated with alterations in brain size, vasculature and cognition, therefore, the brain size shrinks with increased age and changes are found from the level of molecules to morphology[1]. The free radical theory of aging suggested that aging occurs as a result of harmful effects of free radical which are produced during cellular metabolism[2]. Cells which utilize oxygen and consequently produce reactive oxygen species develop naturally complex antioxidant defence systems to neutralize reactive oxygen species and guard themselves against free radical damage. Thus, the enhanced oxidative stress in aging appears to be a consequence of the imbalance between free radical production and antioxidant defence with a higher production of free radicals[3].

Ascorbic acid is the most widely cited form of water-soluble antioxidant and prevents oxidative damage to cell membranes induced by aqueous radicals[4]. Several studies have verified an age-dependent decline in ascorbic acid levels in both plasma and most tissues[5]. Reports have illustrated that the devastating consequences of aging which includes delayed amnesia and impairment of learning and memory are the major cause of cognitive decline[6] and other deficits are reduced ability of learning, psychological changes[7], slow thinking and memory impairment. It is reported that treatment with ascorbic acid significantly improved cognitive function in aged rats[8] and it can enhance learning and memory processes[9]. In current years, the behaviorally advantageous effects of the antioxidant ascorbic acid suggesting that they may slow down age-related deterioration of the CNS and related cognitive declines particularly in the presence of neurodegenerative diseases[10]. Moreover, ascorbic acid being an antioxidant prevents oxidative stress and associated damage in a number of neurodegenerative diseases including Alzheimer's disease[11].

Not many studies have seen the effect of vitamin C on young rats. In the light of above discussion the present study was designed to investigate the effect of Vitamin C on different behaviors like memory, anxiety, and locomotion on young rats.

MATERIALS AND METHODS

Animals

Twelve young female Albino Wistar rats weighing 100-150gm purchased from the Aga Khan University and Hospital was used in the study. All animals were housed individually under a 12h light/12h dark cycle (lights on at 06:00h) and controlled room temperature (22 ± 2^{o}C) with free access to cubes of standard rodent diet for at least 5 days before experimentation. All experiments were carried out according to the guidelines approved by Local Animal Care Ethical Committee.

Experimental procedure

Animals were randomly divided into control and ascorbic acid treated groups. Each group composed of 6 rats. Control rats were injected saline (0.9%) while the rats in test group were injected a dose of Ascorbic Acid 50 mg/kg intraperitoneally, daily for 4 weeks. During the last week of experiment different behavioral tests were performed such as open field

activity, light dark transition test and the passive avoidance test. Food intake was monitored daily by giving rats weighed amount of food and weighing the remaining food in the hopper of the cages. Body weights of the rats were also monitored daily during the 4 weeks of the treatment. Growth rate was calculated in terms of percentage of initial body weight.

Behavioral analyses

Passive avoidance memory testing

Passive avoidance model consists of two Compartments as an illuminated 'safe' and a dark 'punishable' one. Both compartments were connected with a door that allow free crossing from one compartment to another. Both compartments had a grid floor. The diameter of rods was 5mm with 0.5cm distance between the rods. In the training session, rat was placed in an illuminated box. Once the rats entered into the dark compartment, rats received 1.5mA foot shock through the grid floor to its paws for 5 seconds. After receiving the foot shock, it instantaneously came back to illuminated safe compartment. During the test period (90 minutes later), rats were placed in the light compartment again for a maximum of 5 minutes. The step-through latency that indicates the time elapsed before the rat entered the dark compartment was recorded in the test session as described earlier[12].

Locomotor activity

The locomotor activity of both young and old treated rats were monitored in an open field apparatus which consisted of a square area measuring 76x76cm with walls 42cm high. The floor was divided by lines into 25 equal squares. To determine activity, a rat was placed in the central square of the open field and the number of square crossed with all four paws was scored for 5 minutes as described earlier[13]. Activities of both young and old group of treated rats were monitored in a balanced design to avoid order effect.

Light-dark model of anxiety

The light dark box consist of two compartment-box with one box brightly lighted (having transparent walls and the other dark (black) box, thus differing in their sensory properties. Both the compartments have lids which were kept closed during the conduction of experiment. Both the compartments are of equal sizes (26×26×26cm each) skinners boxes, with as access (12×12 cm) between the compartments. In the test session the animal was placed in the light compartment of the box and left to render freely between the two compartments of the apparatus for 5 minutes (cut off time).

During this time the number of crossings between the light and the dark compartment, along with the amount of time spent in each was monitored[14].

Statistical analyses

The behavioral data following the vitamin C administration were analyzed by Student's *t*-test. P values <0.05 were taken as significant.

RESULTS

Effects of ascorbic acid administration on food intake and % growth rate

Figures 1 and 2 show the weekly effect of vitamin C administration on food intake and % growth rate. Analysis by student's *t*-test revealed that Vitamin C administration produced no significant difference in food intake, and % growth rate in test group compared to control rats.

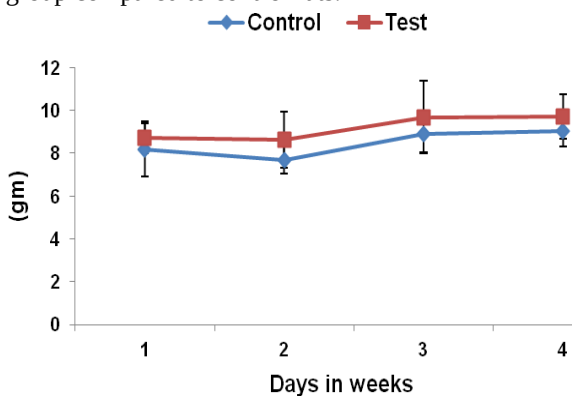

Figure 1: Effect of ascorbic acid (50mg/kg daily for 4 weeks) administration on weekly food intake of young rats. Values are means±SD (n=6). Data analyzed by student's *t*-test were non-significant.

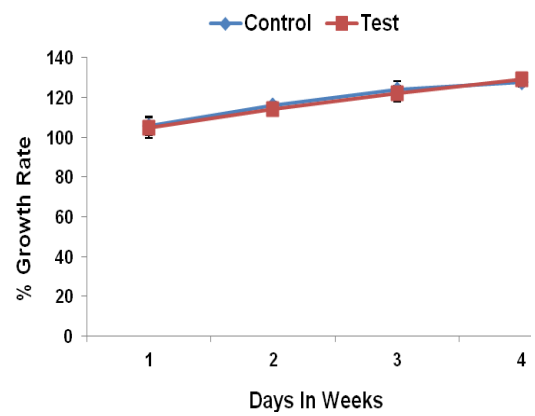

Figure 2: Effect of ascorbic acid (50mg/kg daily for 4 weeks) administration on % growth rate of young rats. Values are means±SD (n=6). Data analyzed by student's *t*-test were non-significant.

Effects of ascorbic acid administration on learning and memory

Figure 3 shows the effect of vitamin C administration on memory. Data analyzed by student's *t*-test showed that vitamin C treated rats exhibited enhanced memory which was evident by the increase time to enter the dark box.

Effects of ascorbic acid administration on anxiety

Figure 4 shows the effect of vitamin C administration on anxiety. Data analyzed by student's *t*-test revealed a significant ($p<0.01$) increase in time spent in light box by vitamin C treated rats.

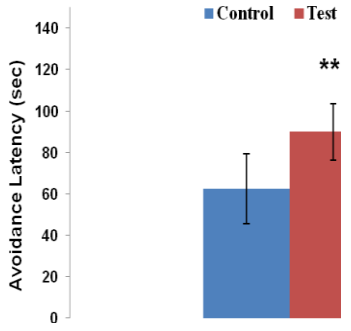

Figure 3: Effect of ascorbic acid (50mg/kg daily for 4 weeks) administration on working memory in PA task of young rats. Values are means±SD (n=6). Significant difference by student's t-test; *p<0.01 vs control rats.

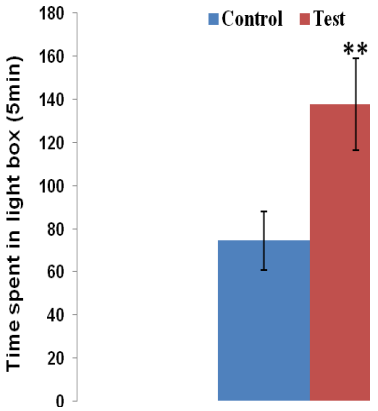

Figure 4: Effect of ascorbic acid (50mg/kg daily for 4 weeks) administration on anxiety in Light/Dark transition test of young rats. Values are means±SD (n=6). Significant difference by student's *t*-test; *p<0.01 vs control rats.

Effects of ascorbic acid administration on locomotor activity

Figure 5 shows the effect of vitamin C administration on locomotor activity in rats. Data analyzed by student's *t*-test revealed no significant difference in locomotor activity of vitamin C treated rats compared to control group.

DISCUSSION

There are few and conflicting studies regarding the usefulness of ascorbic acid in the treatment of dementia and cognitive dysfunction. Some reports indicated that ascorbic acid may protect against cognitive dysfunction[15,16] whereas other studies suggest that no correlation exists[17,18]. In the present study Ascorbic acid (Vitamin C, 50mg/kg) was administered to young rats (3-4 months) for 4 weeks to determine whether chronic antioxidant treatment had any effect on behavioral performances in young rats.

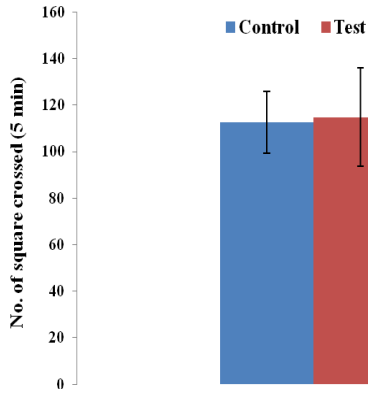

Figure 5: Effect of ascorbic acid (50mg/kg daily for 4 weeks) administration on open field activity of young rats. Values are means±SD (n=6). Data analyzed by student's *t*-test were non-significant.

In the present study, passive avoidance task was used to measure memory function of young rats. The result showed significant increase in working memory of vitamin C treated rats as compared to control rats. Previously it has been reported that ascorbic acid administered for a short time period improved acquisition and retention of passive avoidance task[8,19]. In most studies, researchers believed that ascorbic acid prevents memory deficits by its antioxidant effects[20,21]. Ascorbic acid is an antioxidant[22] which is highly concentrated in the central nervous system[23] and reverses the tissue dysfunction induced by any process which might promote oxidative stress[24]. Many studies have reported that the oxidative stress is more common to develop in aging individuals than in young people[25]. Moreover the young rats suggested a higher rate of oxidative metabolism and increased production of reactive oxygen metabolites[26]. Therefore the response of Vitamin C supplementation in test group was more enhanced compared to control rats. This probably may be the optimal antioxidant protection system in young group which is able to lower oxidative stress[25].

Thus the results of the present study which show an enhancement in memory may be due to the antioxidant action of ascorbic acid. Thus, ascorbic acid has facilitatory effects on passive avoidance learning and memory in healthy young rats.

On the other hand ascorbic acid has a modulatory action on brain neurotransmitters like cholinergic, serotonergic, and dopaminergic system[20,27]. These neurotransmitter systems are important in learning and memory processes[28]. It has been reported that local application of ascorbic acid enhanced the response of neurons to glutamate[22]. Glutamate is a neurotransmitter which has a critical role in learning and memory processing[29]. Therefore it is possible that ascorbic acid effects may be due to its neuromodulatory functions. Ascorbate is an important component of neuronal antioxidant network and an enzyme cofactor in the synthesis of catecholamines[30]. Ascorbate is required for the release of noradrenaline and acetylcholine from synaptic vesicles[31]. Even though no estimations of neurotransmitters were made in the present study, but based on the previous research may be suggested an involvement of these neurotransmitters in improved cognitive function in growing rats after vitamin C supplementation. So in addition to the many beneficial effects of ascorbic acid, it can be considered to be a memory enhancer in healthy subjects.

Present results showed that ascorbic acid treated rats spent more time in light box as compared to control rats. It has been reported that vitamin C reduced the time spent by mice in the dark versus aversive light compartment[32]. It was reported that 14 days of treating healthy young adults with vitamin C resulted in reduced anxiety in response to a psychologically stressful experience[33]. The antioxidant properties of ascorbic acid might also be responsible for anxiolytic effects because they have been shown to modulate immobilization-induced oxidative stress in the rats brain as indicated by increased activity of superoxide dismutase, glutathione-s-transferase and catalase and decreased lipid peroxidation[34]. It was also reported vitamin C and E normalized brain serotonin[27] and produce anxiolytic effect. So the involvement of any of these systems is possibly related to the effects of vitamin C on anxiety.

In the present study, open field test was performed to study the effect of vitamin C on exploratory and locomotor activity. The result showed that administration of vitamin C did not increase the locomotor activity in test rats as compared to control rats. The present results are in general in conformity with previous study which reported that long-term decreased vitamin C levels led to hyperactivity in transgenic mice[35]. It has been suggested that the age-related deficits in motor performance are due to alterations in the striatal[36] and cerebellum[37] dopamine system. Studies have been suggested that ascorbic acid can behave as a dopamine receptor antagonist[11] as the effects of vitamin C treatment were similar to those of haloperidol (a DA receptor antagonist) and the effects were more enhanced when both were combined[38]. The lack of any effect on motor activity in present study may be attributed to antagonistic effect of vitamin C on DA. With age, brain DA undergoes increasing oxidation[39], which in turn leads to the formation of free radicals[40]. Following these observation it can be suggested that vitamin C might be helpful in oxidative damage by neutralizing dopamine which produces free radicals. Vitamin C is an essential co-factor in the synthesis of norepinephrine from dopamine and vitamin C deficiency in guinea pigs has been shown to increase brain dopamine level in proportion to the change in brain ascorbate[41]. It was also reported that Vitamin C is released in the striatum with behavioral activation[42] and both locomotor activity and vitamin C release increase in response to dopamine agonists[43].

CONCLUSION

The present study investigated the effect of ascorbic acid on different behaviors of growing rats. From the present findings it may conclude that vitamin C supplementation is also beneficial in reducing anxiety and improving memory function in growing rats.

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