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Original Article

Effects of Ascorbic Acid on Streptozotocin-induced Oxidative Stress and Memory Impairment in Rats

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ABSTRACT *Introduction.* **Streptozotocin (STZ) is a glutamine-nitrosourea compound that shows selective cytotoxicity to pancreatic β-cells. Though the mechanisms of the induction of diabetes are not clear, it is proposed that STZ is related to the generation of free radicals which results in DNA fragmentation. If injected intracerebroventricularly (i.c.v.), STZ can produce oxidative stress in the brain and cognitive impairment that probably cause sporadic Alzheimer's disease. Since the ascorbic acid (AA) has been shown with high antioxidant probabilities in many studies. The present study was undertaken to assess the potential of AA as memory-enhancer via the mechanisms of reduction of free radical and increase in reduced glutathione (GSH) as the free radical scavenger.**

Method. **The experiment was performed in male Wistar rats weighing 250 g. The animals were divided into 2 groups: the first group as the vehicle-treated group and the second group as the STZ-treated group. The STZ was stereotaxically injected into the lateral ventricle of the brain, 3 mg/kg BW, according to the method of Sharma and Gupta, 2001. The animals in each treatment group were sub-divided into 4 subgroups in accordance with the dose of AA treated (0, 50, 100, 200 mg/kg BW). AA was injected intraperitoneally daily for 21 days. On the 19th and 20th day of AA treatment, the animals were tested for memory retention using an elevated plus-maze. After completing the experiments, the brains of all animals were collected and homogenized for further biochemical determination of malondialdehyde (MDA) and GSH.**

Results. **It was observed that all grouped of vehicle-treated with STZ were not different in memory retention and biochemical aspects. However, STZ-treated animals showed decreased memory retention accompanying with oxidative stress, indicating by, the increased MDA activity and decreased GSH activity. The beneficial effects of AA treatment were shown in STZ-treated animals that the significant recovery cognitive function was observed in accompanying with reduced oxidative stress. The effects are likely in dose-dependent manner.**

Conclusion. **This study indicates that AA is effective in decreasing oxidative stress and help recovery of damages in cognitive function caused by i.c.v. STZ injection. Supported by Faculty of Medicine Research Fund, Chiang Mai University, Chiang Mai (2006).**

Key words: **Ascorbic Acid, Vitamin C, Oxidative Stress, Streptozotocin, Memory Impairment**

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by progressive loss of memory and personality changes in the population over the age of 65 years. The pathology of AD is characterized by the secretion of β-amyloid (Aβ) that condenses to form amyloid plaques and the formation of neurofibrillary tangles consisting mainly of tau protein[1]. Although it is not known whether oxidative stress is primary in the disease process or a result of the disease, it does appear that it is a part of the pathophysiologic process[2]. During the progression of AD, lipid

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peroxidation, protein oxidation, and DNA oxidation have been reported[3]. There is increasing evidence that the very earliest neuronal and pathological characteristic of AD are evidences of oxidative damage indicating that oxidative stress represents a very early contributor to the disease. Indeed, this early role is borne out by clinical management of oxidative stress which appears to reduce the incidence and severity of AD[4].

Streptozotocin (STZ) is a functional nitrosourea derivative. STZ has broad spectrum antibiotic activity and is oftenly used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β cells. Besides its antibiotic and diabetogenic properties, STZ is genotoxic in a variety of assays, including microbial mutagenesis, unscheduled DNA synthesis, micronucleus, chromosomal abberations and sister chromatid exchanges. If injected intracerebroventricularly (i.c.v.) with subdiabetogenic dose, STZ can produce oxidative stress in the brain and cognitive impairment that probably cause sporadic Alzheimer's disease.

Ascorbic acid (AA) or vitamin C is the most abundant water-soluble vitamin found in many plants and fruits and is also an essential nutrient for humans and a few other animals[5]. AA readily undergoes reversible oxidation and reduction and plays an important role as a redox agent in biological systems. AA is an electron donor and therefore a reducing agent. All known physiological and biochemical actions of AA are due to its action as an electron donor. AA donates two electrons from a double bond between the second and third carbons of the 6-carbon molecule. AA is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized. However, by the very nature of this reaction, AA itself is oxidized in the process[6]. AA is an effective antioxidant because it can donate two electrons from one AA molecule to reduce free radicals and it has low reduction potentials and can react with most other biologically relevant radicals and oxidants. Therefore, AA may be useful for application on the treatment of AD and other neurodegenerative disorders caused by oxidative stress[7].

In this study, we hypothesized that AA can prevent oxidative damage and cognitive impairment by its antioxidant properties in i.c.v. STZ-induced oxidative stress animals which referred to the model of AD.

METHODS

Animals

The experiments were performed in male Wistar rats, weighing between 230-250 g. The animals were obtained from the National Laboratory Animal Center, Salaya Campus, Mahidol University. All animals were housed in rodent cages in the animal room where the temperature was maintained approximately at 24-25°C with 12 hours dark-light cycle (lights on 06.00 - 18.00 h). Laboratory food and water were given ad libitum. After adapting to the new environment for at least 7 days, the animals were randomly divided into eight groups.

CA0: ACSF + vehicle
CA50: ACSF + AA 50 mg/kg
CA100: ACSF + AA 100 mg/kg
CA200: ACSF + AA 200 mg/kg
SA0: STZ + vehicle
SA50: STZ + AA 50 mg/kg
SA100: STZ + AA 100 mg/kg
SA200: STZ + AA 200 mg/kg

Each group of animals consisted of 8 rats. All of them were injected intracerebroventricularly (i.c.v.) by stereotaxic method using the method of Sharma and Gupta[8]. The rats were anaesthetized with pentobarbital sodium, 50 mg/kg body weight, by intraperitoneal injection and positioned in stereotaxic apparatus. A sagittal incision was made in the scalp and two holes were drilled through the skull for injection with microsyringe into the lateral cerebral ventricles. Animals received either i.c.v. artificial cerebrospinal fluid (ACSF; 20 μl/site) or STZ (3 mg/kg body weight) on days 1 and 3 of the experiment. The composition of the ACSF was (in mmol/Litre): NaCl 147; KCl 2.9; $MgCl_2$ 1.6; $CaCl_2$ 1.7; dextrose 2.2. To ensure diffusion of the administered drug, the microsyringe was place for 2 min. following i.c.v. injection. The stereotaxic coordinates for i.c.v. injection were 0.8 mm posterior to bregma, 1.5 mm lateral to the sagittal suture and 3.6 mm beneath the cortical surface. AA was dissolved in saline at concentrations of 50, 100 and 200 mg/kg respectively and administration by intraperitoneal injection once daily for 21 days.

Cognitive Function Test

An elevated plus-maze was used to test the cognitive function of animals. Elevated plus-maze consists of

two opposite open arms (50 X 10 cm), crossed with two closed arms of the same dimensions with 40 cm high walls. The arm are connected by a central square (10 X 10) and all of these are elevated 70 cm from the floor. On day 19 after i.c.v. injection, rats were place individually at one end of the open arm, facing away from the central square. The time taken for the rat to move from the open arm and enter into one of the closed arms was recorded as "initial transfer latency" (ITL). The animal was allowed to explore the maze for 30 s after recording ITL and returns to its home cage. Then, 24 h after ITL, the rat was placed again on the open arm and the retention latency was noted as "retention transfer latency" (RTL). The rat that spent on the open arm more than 5 min or fell out of the maze was excluded from the experiment.

Biochemical Tests

At day 21, animals were decapitated under pentobarbital sodium anaesthesia and their brains quickly removed, weighed, cleaned with ice-cold saline and stored at -80 °C.

Tissue preparation

Brain tissue samples were thawed and homogenized with 10 times (w/v) ice-cold 0.1 mol/l phosphate buffer (pH 7.4). The homogenates were centrifuged at 15,000 g for 60 min and the supernatant was then used to determine lipid peroxidation and glutathione.

Measurement of Lipid Peroxidation

Malondialdehyde, a measure of lipid peroxidation, was determined as described by Jainkang et al.[9]. Trichlorocetic acid (2 ml; 10%), 1.5 ml thiobarbituric acid (0.8%) and 0.1 ml butylated hydroxytoluene (0.2%) were added to 1 ml processed tissue samples, then heated at 100 °C for 60 min. The mixture was cooled with tap water. After centrifugation at 3000 g for 10 min, the organic layer was separated and absorbance was measured at 532 nm using a spectrophotometer. The concentration of MDA is expressed as μM/g tissue.

Measurement of Reduced Glutathione

Glutathione was measured according to the method of Ellman[10]. An equal quantity of homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.01 ml of this supernatant, 2 ml phosphate buffer (pH 7.4), 0.5 ml 5û5-dithiobis (2-nitrobenzoic acid) and 0.4 ml distilled water were added. The mixture was vortexed and the absorbance read at 412 nm within 5 min. The concentration of reduced glutathione was expressed as μg/g tissue.

Statistical Analysis

All data were presented as means ± S.E.M. Comparison of data within the same group of animals were analyzed by Wilcoxon Signed Rank test, while those data between different groups were analyzed by Mann-Whitney U test. A level of $p < 0.05$ was considered as statistical significance.

RESULTS

Effects of AA on Cognitive Function in i.c.v. STZ-Treated Rats

It was observed that the animals in all i.c.v. ACSF and i.c.v. STZ groups had no significant difference in transfer latency on first trial or ITL. As shown in figure 1, ITL of all groups were 47.59 ± 6.89, 48.19 ± 7.32, 37.06 ± 5.35, 35.78 ± 4.60, 41.07 ± 6.23, 40.79 ± 4.93, 38.65 ± 5.95 and 36.57 ± 3.94 second in CA0, CA50, CA100, CA200, SA0, SA50, SA100 and SA200 groups, respectively. The transfer latencies on second trial or RTL of i.c.v. ACSF groups were 23.19 ± 4.72, 22.57 ± 5.29, 16.50 ± 4.03 and 14.84 ± 4.96 second in CA0, CA50, CA100 and CA200 groups, respectively. RTL of all i.c.v. ACSF groups were significantly lower than ITL of the same group ($P<0.05$). RTL of i.c.v. STZ groups were 32.25 ± 5.93, 20.12 ± 5.77, 13.69 ± 3.44 and 12.68 ± 2.41 second in SA0, SA50, SA100 and SA200, respectively. The RTL of SA50, SA100 and SA200 groups were significantly lower than ITL of the same group ($P<0.05$) but SA0 group was not different between RTL and ITL.

Effects of AA on MDA Level in i.c.v. STZ-Treated Rats

To confirm that the antioxidant effect of AA can decrease oxidative stress, the oxidative stress markers were investigated by using spectrophotometry colorimetric method. MDA is one of the product of lipid peroxidation which has high level when oxidative stress occurs. As

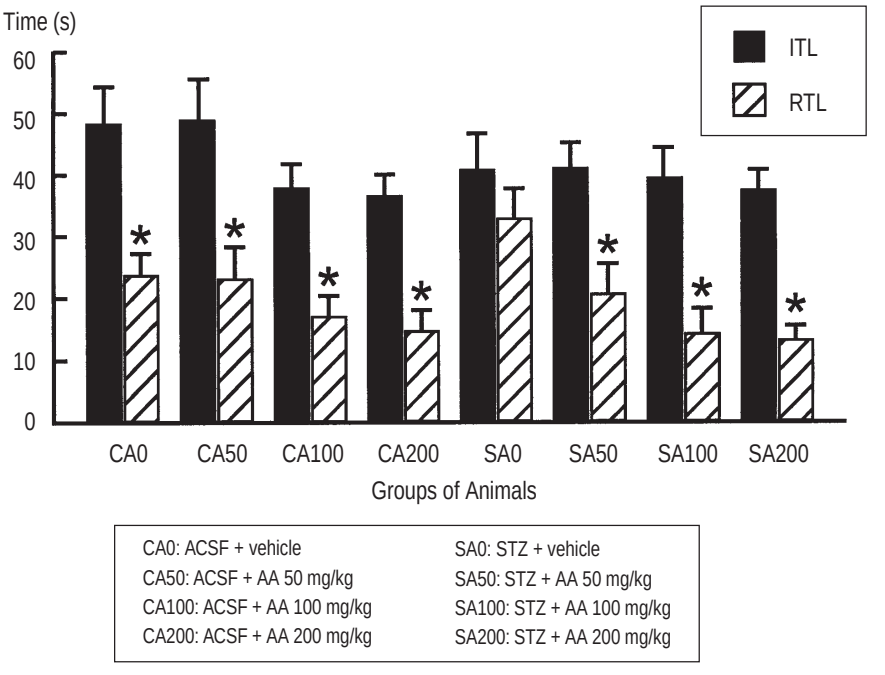

Fig. 1 Effect of intracerebroventricular STZ injection on transfer latency in elevated plus-maze paradigm. The columns show mean values of transfer latency in seconds and bars represent S.E.M. On the abscissa are the groups of animals. *P<0.05 significant decrease in the retention transfer latency (RTL) as compared to initial transfer latency (ITL) of the same groups of animals.

shown in figure 2, the MDA levels of i.c.v. ACSF animals were 405.31 ± 16.71, 382.58 ± 11.76, 388.75 ± 18.52 and 391.25 ± 24.78 μM/g tissue in CA0, CA50, CA100 and CA200 group, respectively. There were no significantly different in MDA levels among groups. The MDA levels of i.c.v. STZ animals were 1,505.63 ± 29.04, 537.31 ± 13.58, 419.77 ± 14.16 and 410.91 ± 8.04 μM/g tissue in SA0, SA50, SA100 and SA200 group, respectively. The MDA concentration in brain appeared to be higher in the SA0 group than other i.c.v. STZ groups and CA0 group (P<0.05). This indicates that AA can significantly reduce the oxidative damage caused by i.c.v. STZ. However, the SA200 group also had the MDA level lower than SA50 group (P<0.05), indicates that the effects of AA were seemingly in dose-dependent manner.

Effects of AA on GSH Level in i.c.v. STZ-Treated Rats

GSH is the antioxidant in the body and usually be the biomarker for measuring the severity of oxidative stress. If the GSH markedly decrease as compared to the normal physiological level, the oxidative stress will be indicated. As shown in figure 3, the GSH levels in the brain of i.c.v. ACSF-injected animals were 434.65 ± 15.08, 445.93 ± 12.36, 448.40 ± 21.27 and 449.11 ± 18.44 μg/g tissue in CA0, CA50, CA100 and CA200 groups, respectively. There were no significant difference in GSH levels among groups. The GSH levels of i.c.v. STZ animals were 256.68 ± 26.81, 416.33 ± 14.92, 451.82 ± 29.43 and 452.45 ± 19.74 μg/g tissue in SA0, SA50, SA100 and SA200 groups, respectively. The GSH level of SA0 group was significantly lower than other i.c.v. STZ-injected groups and CA0 group (P<0.05). This indicates that AA at all dose can rise the GSH level up the the physiological range compared to normal animals.

DISCUSSION

The process of aerobic respiration of the cells can cause the releasing of reactive oxygen species (ROS) such as O_2^-, H_2O_2 or OH^-. ROS are involved in normal biochemical processes, including the control of cell proliferation and cell signaling, but they can also be detrimen-

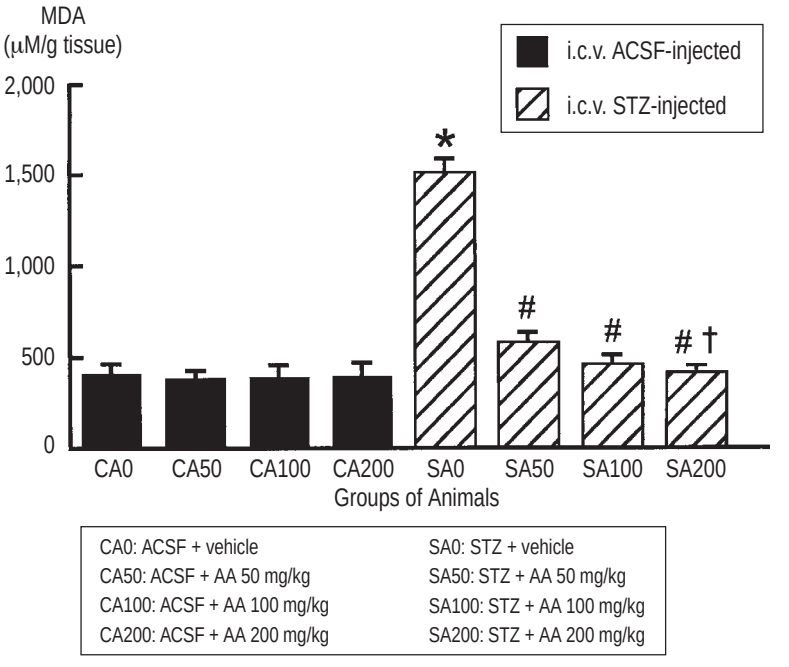

Fig. 2 Effect of intracerebtroventricular STZ injection on MDA level in the brain. The columns show mean values of MDA in μM/g tissue and bars represent S.E.M. On the abscissa are groups of animals. **P*<0.05 significant decrease in MDA of SA0 group as compared to CA0 group. #*P*<0.05 significant decrease in MDA of SA50, SA100 and SA200 groups as compared to SA0 group. #*P*<0.05 significant decrease in MDA of SA200 group as compared to SA50 group.

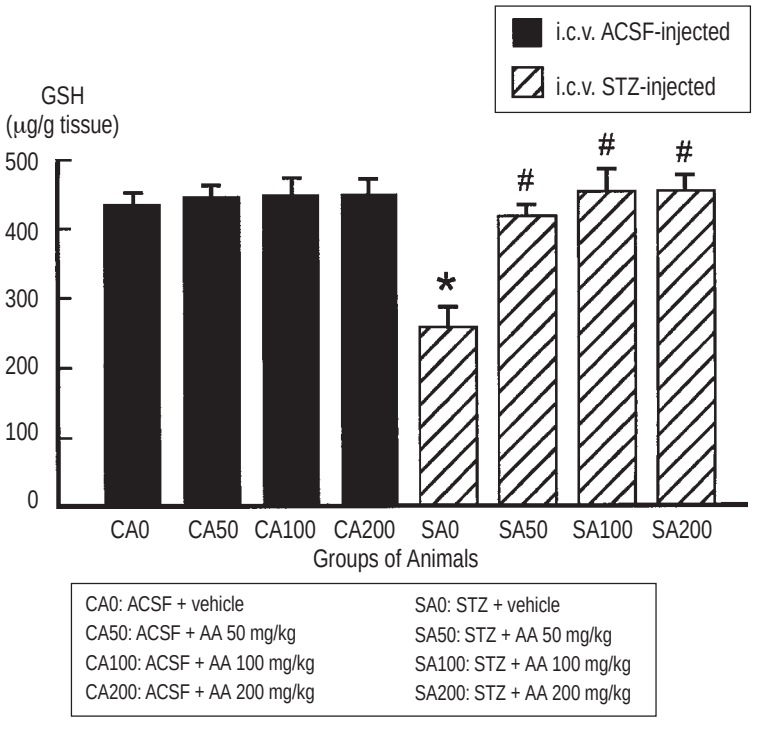

Fig. 3 Effect of intracerebroventricular STZ injection on GSH level in the brain. The columns show mean values of GSH in μg/g tissue and bars represent S.E.M. On the abscissa are groups of animals. **P*<0.05 significant decrease in GSH of SA0 group as compared to CA0 group. #*P*<0.05 significant decrease in GSH of SA50, SA100 and SA200 groups as compared to SA0 group.

tal to cells by damaging cellular biomolecules, including DNA, proteins and lipids[11]. ROS also activate enzymes that cause any symptoms or diseases. The production of ROS can, however, be balanced by the existence of cellular antioxidant defences, including enzymes that remove ROS (superoxide dismutase, catalase, peroxidase, etc), proteins that sequester transition metal ions (ferritin, transferrin), low molecular weight peptides and cofactors (glutathione, NADPH, thioredoxin, etc.) and lipid and water-soluble low molecular weight dietary agents that scavenge reactive oxygen and nitrogen species (e.g. vitamin E, vitamin C and β-carotene)[12].

Oxidative stress may initiate various cascades leading to neurodegenerative disorders such as Alzheimer's Disease (AD), Parkinson's Disease (PD) and amyotrophic lateral sclerosis. This study showed that i.c.v. STZ injection in rats can cause the progressive deterioration of both cognitive function and oxidative stress in the brain. So, it has been postulated that it may provide a relevant model of sporadic AD. However, the oxidative stress caused by i.c.v. STZ injection only increase in MDA level and decrease in GSH level, but do not affect the enzymes that remove ROS such as superoxide dismutase and catalase[13]. The mechanisms by which STZ induces oxidative stress in the brain are not clear understood, it may be possible that free radicals generated by STZ might be involved in the toxic action of STZ with the energy impairment caused by the disturbance of glucose metabolism. When administered parenterally, STZ induces diabetes by destroying pancreatic β cells. One potential mechanism may be inhibition by STZ of β cell O-GlcNAcase[14]. Another potential mechanism is the generation of hydrogen peroxide thereby preventing the release of insulin[15]. ROS can be produced by microglia, which are activated by i.c.v. STZ injection[16].

The oxidative stress caused by i.c.v. STZ injection can be prevented or reduced by the administration of antioxidants such as melatonin[17], trans resveratrol[18], alpha lipoic acid[19] or herbal plant such as *Centella asiatica*[13]. The present study showed the effects of AA, a water-soluble vitamin which has been found in many plants and fruits such as pepper and guava. AA also has an antioxidant effect in the body which efficiently scavenges toxic free radicals and other ROS formed in cell metabolism. It was found in the present study that AA decreased the level of MDA in i.c.v. STZ-treated animals and they were seemingly in dose-dependent manner because the dose of AA at 200 mg/kg BW can decrease MDA level more than dose of AA at 50 mg/kg BW. However, the effect of AA on GSH level does not show the dose-dependent response. There were no significantly difference of GSH level in all of AA-treated i.c.v. STZ group and i.c.v. ACSF only group. This result may be that the AA has reached the maximal antioxidant effect and changed oxidative stress to the normal physiological condition. Although AA 50 mg/kg BW may not be enough to eradicate the exceeding free radicals and led to the production of MDA by lipid peroxidation, it can raise GSH to the normal level but AA 100 mg/kg BW is just enough to treat oxidative stress caused by i.c.v. STZ injection. AA, when injected intraperitoneally (i.p.) or by oral administration, it is possible that the dose we had given to the animals could not transport all of AA directly to the brain. This based on pharmacokinetic dose-response curve for plasma AA level. In human, the limitation of concentration of AA in plasma are about 80 μmol/l. though the dietary intake of AA are over than this[6, 20]. The other limitation of AA transport to the brain is that AA could not diffuse across the blood-brain barrier (BBB). The transportation of AA across BBB into the brain is mediated by glucose transporter GLUT1 in the form of DHA. DHA is expected to compete partially for endogenous glucose uptake across BBB. Both DHA and glucose are substrates of the glucose transporters under physiologic conditions. When DHA are carried across GLUT1 into endothelial cells, it may be converted to AA or not. DHA may first be reduced to AA inside the cell, and then be oxidized for export through GLUT1, although there are little data concerning this step. The DHA in the brain is then reduced to AA, which is trapped in the brain because AA cannot be transported through GLUT1 again. The mechanism of DHA reduction is not known, but it is believed to be related to glutathione and/or glutathione-dependent reductases. DHA reduction requires electron donation, and the mechanism of donor regeneration is not known. It is possible that a large amount of DHA in the brain may overwhelm the brain's reductive capacity, but there is no evidence to this point[21].

The antioxidant properties of AA to deplete the oxidative stress are being that AA can detoxification of ROS by non-enzymatic reaction. AA itself can donate

electrons directly to the free radicals such as O_2- or H_2O_2 and become semidehydroascorbate or DHA[22]. The regulation of endogenous AA and GSH via the GSH-AA interrelationship. The liver, being the main source of synthesis and export of both antioxidants, must accomodate to the increased demand. The falling AA level alone could favour AA synthesis decreasing its feedback inhibition. However, the tight connection between GSH and AA suggests that these two compounds can mutually regulate the synthesis of each other. In accordance with this assumption, GSH deficiency produced by the *in vivo* administration of buthionine sulfoximine increases AA synthesis in mice[23]. In isolated murine hepatocytes similar effect could be brought about by the addition of other GSH depleting agents (menadione, diamide, acetaminophen). Several previous observations have suggested that GSH depletion regulates AA synthesis through the alteration of glycogen metabolism. First, the dependence of AA synthesis on glycogenolysis has been shown[24]. Second, glycogenolysis is also induced by various agents known to provoke GSH deficiency. Third, gulonolactone oxidase, another possible target of this regulation was not influenced by GSH level or by different GSH/ oxidized glutathione (GSSG) ratios[25]. According to this hypothesis, all the glutathione depleting agents investigated (buthionine sulfoximine, menadione, diamide, acetaminophen) increased glycogen breakdown and AA production in isolated murine hepatocytes[26].

In this study, the reduction of MDA level in AA-treated i.c.v. STZ animals was the antioxidant effects of both AA and GSH. In i.c.v. STZ group had shown the high level of MDA and low level of GSH, these were the results of oxidative stress. When oxidative stress occcured in the brain of i.c.v. STZ animals, MDA, the product of lipid peroxidation rose up and we can detect by TBARS method. Thus, GSH, the endogenous antioxidant scavenge the free radicals and this led to the falling level of GSH in the brain. The administration of AA in i.c.v. STZ animals were reduced the level of MDA by eradication of free radicals by AA and decrease the reduction of GSH, this process brought the rising level of GSH in AA-treated i.c.v. STZ animals.

The present study, we used elevated plus-maze to test cognitive function of the animals. The elevated plus-maze was first used to test anxiety level and then this equipment was modified to use for testing cognitive function[8]. The rationale of using elevated plus-maze for cognitive function test relies on the movement of animal to avoid danger or harm into the closed place. In the first trial, the animal spends some times on open arm of elevated plus-maze and it then moves to safe place (the closed arm of elevated plus-maze) by trial and error condition. At the second trial, the animal knows where the safe place is, so it moves to the closed arm faster than the first trial. Since it is realized that the test results depends on movement, Automex-II was used to test the locomotor activity of the animals. No effects of AA or STZ on the locomotion in any group of animals were observed.

In this study used STZ to impair the cognitive function. Although the mechanism of STZ on cognitive impairment is still unclear, the effects of i.c.v. STZ injection on cognitive function has been reported in many studies. Thirteen days after a single i.c.v. injection of STZ in rats retention of passive avoidance behaviour was decreased[27]. Bilateral injection of i.c.v. STZ on days 1, 3 and 20 induced a significant impairment in spatial memory 20 days later in older rats[28]. The i.c.v. STZ injection on days 1 and 3 induced cognitive impairment in elevated plus-maze test in rats and this can be improved by administration of antioxidants such as melatonin[17], trans resveratrol[18], alpha lipoic acid[19] or herbal plant *Centella asiatica*[13]. The results of this study showed that all i.c.v. ACSF-injected group had good cognitive function. However, i.c.v. STZ only-injected group had the same RTL as ITL, while other i.c.v. STZ-injected animals chronically treated with AA at 50, 100 and 200 mg/kg BW doses showed significantly shorter RTL time. The results indicated that i.c.v. STZ only-injected animals had cognitive impairment which can be improved by administration of AA.

CONCLUSION

The present study, AA was shown to reduce oxidative stress and improve the cognitive function in i.c.v. STZ-treated animals. The data also suggest a close-association between oxidative stress and cognitive function impairment induced by the i.c.v. injection of STZ. This study provides a support for the STZ injection model as a tool for inducing oxidative stress. AA may possibly be a useful target for development of AD treatment in the future.

REFERENCES

1. Sullivan GP, Brown MR. Mitochondrial aging and dysfunction in Alzheimer's disease. Prog. Neuro-Psychopharm. Biol. Psy. 2005; 29:407-10.
2. Tuppo EE and Forman LJ. Free radical oxidative damage and Alzheimer's disease. JAOA. 2001; 101:11-5.
3. Zhu X, Rhina AK, Lee H. Oxidative stress signalling in Alzheimer's disease. Brain Res. 2004; 1000:32-9.
4. Perry G, Cash AD, Smith MA. Alzheimer's disease and oxidative stress. J. Biomed. Biotech. 2002; 23:120-3.
5. Hancock RD, Viola R. Improving the nutritional value of crops through enhancement of L-ascorbic acid (vitamin C) content: rationale and biotechnological opportunities. J. Agrig. Food Chem. 2005; 53:5248-57.
6. Padayatty SJ, Katz A, Wang Y. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J. Am. Col. Nutr. 2003;
7. Bsoul SA, Terezhalmy GT. Vitamin C in health and disease. J. Contemp. Dent. Prac. 2004; 5:1-13.
8. Sharma M, Gupta YK. Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. Life Sci. 2001; 68:1021-9.
9. Jainkang L, Rei E, Hideaki K, et al. Antioxidant action of guilingi in the brain of rats with FeCl3 induced epilepsy. Free Radic. Biol. Med. 1990; 9:451-4.
10. Ellmann GL. Tissue sulfhydryl group. Arch. Biochem. Biophys. 1959; 82:70-7.
11. Duarte TL, Lunec J. When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. Free Radic. Res. 2005; 7:671-86.
12. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidant, oxidative damage and oxigen deprivation stress. Ann. Bot. 2003; 91:179-94.
13. Kumar MHV, Gupta YK. Effect of Centella asiatica on cognition and oxidative stress in an intracerebroventricular streptozotocin model of Alzheimer's disease. Clin. Exp. Pharm. Physiol. 2003; 30:336-42.
14. Konrad RJ, Mikolaenko I, Tolar JF, et al. The potential mechanism of the diabetogenic action of streptozotocin: inhibition of pancreatic beta-cell O-GlcNAc-selective N-acetyl-beta-D-glucosaminidase. Biochem. J. 2001; 356:31-41.
15. Takasu N, Komiya I, Asawa T, et al. Streptozotocin- and alloxaninduced H2O2 generation and DNA fragmentation in pancreatic islets. Diabetes 1991; 40:1141-5.
16. Colton CA, Gilbert DL. Microglia, an in vivo source of reactive oxygen species in the brain. Adv. Neurol. 1993; 59:321-6.
17. Sharma M, Gupta YK. Effect of chronic treatment of melatonin on learning, memory and oxidative deficiencies induced by intracerebroventricular streptozotocin in rats. Pharmacol. Biochem. Behav. 2001; 70:325-31.
18. Sharma M, Gupta YK. Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. Life Sci. 2002; 71:2489-98.
19. Sharma M, Briyal S, Gupta YK. Effect of alpha lipoic acid, melatonin and trans resveratrol on intracerebroventricular streptozo-tocin induced spatial memory deficit in rats. Indian J. Phys. Pharmacol. 2005; 49:395-402.
20. Frei B. To C or not to C, that is the question! J. Am. Col. Cardiol. 2003; 42:253-5.
21. Agus BD, Gambhir SS, Pardridge WM. Vitamin C crosses the blood-brain barrier in the oxidized form through the glucose transporters. J. Clin. Invest. 1997; 100:2842-8.
22. Arrigoni O, Tullio MC. Ascorbic acid: much more than just an antioxidant. Biochim. Biophys. Acta. 2002; 1569:1-9.
23. Martensson J, Meister A. Glutathione deficiency increases hepatic ascorbic acid synthesis in adult mice. Proc. Natl. Acad. Sci.USA. 1992; 89:11566-8.
24. Braun L, Garzo T, Mandl J, et al. Ascorbic acid synthesis is stimulated by enhanced glycogenolysis in murine liver. FEBS Lett. 1994; 352:4-6.
25. Banhegyi G, Csala M, Braun L, et al. Ascorbate synthesis dependent glutathione consumption in mouse liver. FEBS Lett. 1996; 381:39-41.
26. Braun L, Csala M, Poussu A, et al. Glutathione depletion induces glycogenolysis dependent ascorbate synthesis in isolated murine hepatocytes. FEBS Lett. 1996; 388:173-6.
27. Mayer G, Nitsch R, Hoyer S. Effects of changes in peripheral and cerebral glucose metabolism on locomotor activity, learning and memory in adult male rats. Brain Res. 1990; 532:95-100.
28. Lannert H, Hoyer S. Intracerebroventricular administration of streptozotocin causes longterm diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. Behav. Neurosci. 1998; 112:1199-208.