Protective Effects of Gallic Acid against Streptozotocin-induced Oxidative Damage in Rat Striatum

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Abstract

The present study aimed to investigate the protective effects of gallic acid (GA) against ICV STZ-induced oxidative damage in the rat striatum. Animals were randomly divided into 4 groups (8 each). Group 1 (Sham), were injected ICV on day 1 and 3 with artificial CSF and treated with normal saline (2 ml/kg, p.o.). Group 2 (sham + GA), were injected ICV on day 1 and 3 with artificial CSF and treated with GA (30 mg/kg, p.o.) for 26 days. Group 3 (lesion) were injected with ICV-STZ (3 mg/kg bilaterally, on day 1 and 3) and received normal saline (2 ml/kg, p.o.) as vehicle. Group 4 (lesion + GA), were injected with ICV-STZ (3 mg/kg bilaterally, on day 1 and 3) and treated with gallic acid (30 mg/kg, p.o.) once daily for 26 days starting 5 days before the first injection of ICV STZ. The homogenized striatum was used for measuring thiobarbituric acid reactive species (TBARS) and total thiol contents, glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) activities. The results showed that ICV STZ-injection increased the level of TBARS (+ 69.3 %) and decreased total thiol concentration (- 48.8 %), GPx (- 47.3 %), CAT (- 47.1 %) and SOD (- 30.7 %) activities. In contrast, chronic administration of GA significantly prevented the biochemical alterations in the ICV-STZ rats. These findings highlight the beneficial role of GA via enhancement of cerebral antioxidant defense system.

Introduction

Sporadic Alzheimer’s disease (SAD) has been known as a chronic debilitating neurodegenerative disorder characterized by progressive cognitive impairment, memory loss, and behavioral disturbances and is considered as the most common cause of dementia in elderly patients. Free radicals and oxidative stress have been implicated as the prime candidates mediating the behavioral impairments and memory deficits in such neurodegenerative disorders. Some of this damage may include lipid and protein peroxidation, increase in DNA oxidation products, and deficits in calcium regulatory mechanisms that may eventually lead to cell death. Since oxidative damage is implicated in the etiology of neurological complications, treatment with antioxidants has been used as a therapeutic approach in various types of neurodegenerative diseases [1]. Intracerebroventricular (ICV) injection of streptozotocin (STZ), in a sub-diabetogenic dose in rat, has been found to cause prolonged impairment of brain glucose and energy metabolism and presence of oxidative stress [2].

Gallic acid (GA, 3,4,5-trihydroxybenzoic acid), is one of the most important polyphenolic compounds in plants and is considered as a putative active compound in tannin, namely gallotannin. It is found in grapes, different berries, mango, areca nut, walnut, green tea and other fruits as well as wine. This compound possesses antioxidant and free radical scavenging, anti-cancer and anti-inflammatory properties [3]. Due to the antioxidant effects, GA-containing plant extracts have shown the antidiabetic, antiangiogenic and antimelanogenic effects and reduction in heart infarction incidence and oxidative liver and kidney damage [4, 5]. It has been reported that GA is involved in the protection of the neural cells against in vitro β-amyloid peptide (Aβ)-induced death [6]. GA also has protective effect in case of cerebral oxidative stress induced by diabetes in rats through the modulation of antioxidant enzyme-dependent signaling systems [7]. Recently, our research indicated the neuroprotective effect of GA against oxidative stress induced by 6-hydroxy dopamine (6-OHDA) and, also ICV STZ in rat brain [8, 9]. Also, our study has shown the anxiolytic effect of GA [10]. In addition, Ferruzi
et al. demonstrated that repeated treatment of mice with grape seed extract significantly increased the bioavailability and brain deposition of GA which previously found to attenuate cognitive deterioration in a mouse model of Alzheimer’s disease (AD) [11]. Thus, GA may be a potential neuroprotective agent.

It has been observed that the use of antioxidants as well as dietary improvements with regard to the consumption of fruits and vegetables, high in antioxidant activity and neuroprotective agents, may decrease the risk of memory deficits of AD [1]. Thus, the present study was designed to investigate the protective effects of gallic acid on biochemical markers of oxidative stress induced by ICV STZ administration in the rat striatum.

Materials and Methods

Chemicals

TBA (2-thiobarbituric acid), n-butanol, tris base, Na₂EDTA, sodium acetate, glacial acetic acid, phosphoric acid, potassium chloride, tetramethoxypropane were obtained from Merck Company (Darmstadt, Germany). Streptozotocin and gallic acid HCl were purchased from Sigma-Aldrich (St. Louis, MO, USA). SOD and GPx kits were purchased from Randox (Randox Labs, Crumlin, UK) and CAT kit from Oxis Research. All other chemicals were of analytical grade and prepared from Merck Company (Darmstadt, Germany).

Animals

Adult male Wistar Albino rats weighing 250–300 g were used throughout the study. All animals were obtained from the Animal House of Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran). Animals were put separately in the cages in an air-conditioned unit and allowed free access to standard laboratory chow and water, ad libitum. A 12-h light/dark cycle at 22 ± 2 °C and 50% humidity condition was maintained. The rats were acclimatized to the laboratory condition 5 days before the experimental session. All animal experiments were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals. The Institutional Animal Ethical Committee of Jundishapur University, formed under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Reg. No. PRC98) approved the pharmacological protocols.

Intracerebroventricular administration of streptozotocin

Rats were anesthetized with combination of ketamine/xylazine (60/6 mg/kg, i.p.). The head was positioned in a stereotactic frame (Narishige, Japan) and a midline sagittal incision was made in the scalp. Burr holes were drilled in the skull on both sides over the lateral ventricles using the following coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture, and 3.6 mm beneath the surface of brain. STZ (3 mg/kg) was injected ICV bilaterally on day 1 and 3 of the experiment [2]. In the sham group, artificial CSF: 147 mM NaCl, 2.9 mM KCl, 1.6 mM MgCl₂, 1.7 mM CaCl₂ and 2.2 mM dextrose was injected (20 μl on each site) on the same days as STZ group. STZ was dissolved in artificial CSF. All microinjections were performed by delivering drug or vehicle solution slowly over a 1-min period and the needle remained in position for a further 5 min to prevent reflux along the injection tract. The progress of the injection was continuously monitored by following the movement of an air bubble in the tubing.

Experimental design

Animals were randomly divided into 4 groups (8 each) and individually put in the cages. Group 1: vehicle-treated (normal saline, 2 ml/kg, p.o.) and sham-operated control (S); group 2: GA treated (30 mg/kg, p.o.) and sham-operated (GA+S); group 3: vehicle-treated and ICV-STZ-lesioned (lesion); group 4: GA-treated (30 mg/kg) and ICV-STZ-lesioned (lesion + GA). In the S and GA+S groups, the rats were injected ICV the same volume of artificial CSF. Groups 2 and 4 were administered GA by gavage at a dose of 30 mg/kg (once daily) for 26 days starting 5 days before the first injection of ICV-STZ. On the day of ICV injections (days 1 and 3), GA or normal saline was administered 1 h prior to ICV injection. Kade and Rocha have shown that oral gallic acid at dose 25 mg/kg modulates cerebral oxidative stress conditions and activities of enzyme-dependent signaling systems in STZ-treated rats [7]. Also, in our previous study orally administered gallic acid at dose 30 mg/kg indicated the neuroprotective effects against ICV infused STZ in the hippocampus and cerebral cortex in rats [9]. Hence, we have chosen the dose of 30 mg/kg for our study.

Brain sample collection and biochemical assays

At the end of the experiments, the animals were decapitated and the striatums were removed quickly, rinsed with saline, and then frozen in a freezer (~80°C) until used. The tissues were homogenized in cold KCl solution (1.5%) to give a 10% homogenate suspension used for measuring TBARS content and SOD, CAT, and GPx activities. Antioxidant enzyme activities were assayed using commercial kits (RANSOD assay kit for SOD activity, Randox Labs, Crumlin, UK; catalase assay kit for CAT activity, Oxis International, Oregon, USA; and glutathione peroxidase assay kit for GPx activity, Randox Labs, Crumlin, UK).

Thiobarbituric acid reactive species (TBARS) measurement

TBARS levels, an index of lipid peroxidation (LPO), produced by free radicals were measured. Meldonialdehyde (MDA) reacts with thiobarbituric acid to produce a red colored complex that has peak absorbance at 532 nm. Briefly, 3 ml phosphoric acid (1%) and 1 ml TBA (0.6%) were added to 0.5 ml of homogenate in a centrifuge tube and the mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml n-butanol was added to the mixture and vortex-mixed for 1 min followed by centrifugation at 2000 g for 20 min. The colored layer was transferred to a fresh tube and its absorbance was measured at 532 nm. TBARS levels were determined using 1,1,3,3-tetramethoxypropane as standard. The standard curve of MDA was constructed over the concentration range of 0–20 μM [13].

Total thiol (-SH) groups assay

Total SH groups were measured using DTNB (5,5′-dithiobis-2-nitrobenzoic acid) as the reagent according to the method previously described [15]. This reagent reacts with the SH groups to produce a yellow colored complex which has a peak absorbance at 412 nm. Briefly, 1 ml Tris-EDTA buffer (pH 8.6) was added to 50 μl brain homogenate in 2 ml cuvettes and absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then, 20 μl DTNB reagents (10 mM in methanol) was added to the mixture and after 15 min (stored in laboratory temperature), the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mM)
was calculated from the following equation: Total thiol concentration (mM) = \( (A_2 - A_1 - B) \times 1.07/0.05 \times 13.6 \).

**Glutathione Peroxidase (GPx) activity assay**

GPx catalyses the oxidation of glutathione (at a concentration of 5µmol) using cumene hydroperoxide. In the presence of glutathione reductase (at a concentration > 0.75. 10^{-3} U) and 0.35µmol of NADPH, the oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to NAPDH. The decrease in absorbance measured at 340 nm and 37°C. The assay was performed on a supernatant. The necessary enzyme activity to convert one µmol of NADPH to NAPDH in 1 min defined as the GPx unit and the results expressed as GPx U/g wet tissue [16].

**Catalase (CAT) activity assay**

The activity of CAT was measured using a Bioxytech Catalase-520 Assay Kit (Oxis Research, Portland, OR, USA), according to the manufacturer’s procedure. Summarily, aliquots of sample (30 µl) was added to phosphate buffer (20 mM, pH 7.4) containing 10 mM H2O2 as substrate and the change in absorbance was noted at 520 nm using spectrophotometer. Enzyme activities were determined from standard curve of purified catalase and one unit of catalase equals to the decomposition of 1 µmol of H2O2 per min at pH 7.0 at 25°C. The enzymatic activity was expressed as U/g wet tissue.

**Superoxide desmutase (SOD) activity assay**

Measurement of SOD activity was performed using Ransod reagents (Randox Lab, UK) according to the manufacturer’s procedure. This method is based on the formation of red formazan from the reaction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) and superoxide radical (produced in the incubation medium from xanthine oxidase reaction), which is assayed in a spectrophotometer at 505 nm. The inhibition of the produced chromogen is proportional to the activity of the SOD present in the sample. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of formazan dye formation by 50%. The enzymatic activity was expressed as U/g wet tissue.

**Statistical analysis**

The results are expressed as mean ± SEM. Data was analyzed by one-way ANOVA followed by Newman Keul’s test. Data analysis was performed using the Prism 5.0 (SanDiego, CA, USA) statistical package program. Significance was ascertained at \( P < 0.05 \).

**Results**

**Effect of GA on MDA levels in the striatum**

The degree of free radical damage following STZ injection was assessed using lipid peroxidation (LPO), which was measured as MDA levels. According to \( \circledast \) Fig. 1 there was an increase in MDA levels of STZ-lesioned group (\( P < 0.01 \)) as compared to sham-operated rats in the striatum. Oral administration of GA resulted in a significant reduction of MDA levels in the striatum of Lesion+GA animals as compared to STZ-lesioned group (\( P < 0.05 \)). GA per se did not influence the MDA levels.

**Fig. 1** Effect of gallic acid (GA, 30mg/kg/day, p.o., 26 days) on the malondialdehyde (MDA) levels in the striatum of ICV STZ-treated rats. Values are expressed as Mean ± SEM (\( n = 8 \)). ** *P<0.01, lesion vs sham group; #P<0.05, lesion+GA vs. lesion group (One-way ANOVA and Newman Keul’s test).

**Effect of GA on total thiol levels in the striatum**

The total thiol concentration (mM) was measured to evaluate the non-enzymatic defense potential of the cells against oxidative stress. According to \( \circledast \) Fig. 2 total thiol levels in STZ-lesioned animals were found to be significantly depleted as compared to sham group in the striatum (\( P < 0.05 \)). Chronic treatment with GA in lesion + GA group was able to raise total thiol levels significantly as compared to STZ-lesioned animals (\( P < 0.05 \)). GA per se did not influence the total thiol contents.

**Fig. 2** Effect of gallic acid (GA, 30mg/kg/day, p.o., 26 days) on the total thiol group (-SH) levels in the striatum of ICV STZ-treated rats. Values are expressed as Mean ± SEM (\( n = 8 \)). ** *P<0.05, lesion vs. sham group; #P<0.05, lesion+GA vs. lesion group (One-way ANOVA and Newman Keul’s test).

**Effect of GA on glutathione peroxidase (GPx) activity in the striatum**

GPx activity (u/L) was measured to evaluate the enzymatic defense potential of the cells against oxidative stress. According to \( \circledast \) Fig. 3a the GPx activity was significantly (\( P < 0.01 \)) decreased in STZ-lesioned group as compared to sham-operated animals in the striatum. However, the decrease of GPx activity was significantly restored by GA treatment in the striatum of lesion+GA group (\( P < 0.05 \)). GA per se did not influence the enzyme activity.
Effect of GA on catalase (CAT) activity in the striatum

CAT activity (u/g tissue) was measured to evaluate the enzymatic defense potential of the cells against oxidative stress. According to Fig. 3b the CAT activity was significantly (P<0.05) decreased in STZ-lesioned group as compared to sham-operated group in the striatum. However, the decrease of CAT activity was significantly restored by GA treatment in the striatum of lesion + GA group (P<0.05). GA per se did not influence the enzyme activity.

Effect of GA on superoxide dismutase (SOD) activity in the striatum

SOD activity (u/g tissue) was measured to evaluate the enzymatic defense potential of the cells against oxidative stress. According to Fig. 3c the SOD activity was significantly (P<0.05) decreased in STZ-lesioned group as compared to sham-operated group in the striatum. However, the decrease of SOD activity was significantly restored by GA treatment in the striatum of lesion + GA group (P<0.05). GA per se did not influence the enzyme activity.

Discussion

The present study was intended to evaluate the protective effect of GA, a natural polyphenolic compound, on the ICV-STZ-induced oxidative damage and biochemical alterations in rat striatum. ICV-STZ infusion markedly increased the oxidative stress in rat brain. However, chronic pre-treatment with GA significantly restored oxidative stress markers in ICV-STZ-treated rats. These results are in consistent with previous studies [7, 9, 17].

Model of memory deficit induced by ICV injection of STZ in rodents is well accepted because it mimics various pathological aspects of AD like progressive deterioration of memory, cerebral glucose and energy metabolism, oxidative stress and cholinergic dysfunction [2].

Free radical-induced oxidative stress has been expressed as an important factor mediating the behavioral impairments and memory deficits in age related neurodegenerative disorders such as AD. The brain processes large amounts of O2 in relatively small mass, and has a high content of substrates available for oxidation in conjunction with low antioxidant activities, making it extremely susceptible to oxidative damage. In addition, certain regions of central nervous system (CNS), such as the striatum, may be particularly sensitive to oxidative stress because of their low endogenous levels of vitamin E, an important biochemical antioxidant, relatively to other brain regions. Such a depressed defense system may be inadequate under normal circumstances. However, in pro-oxidative conditions, these low antioxidant defenses can predispose the brain to oxidative stress [18].

Oxidative stress is defined as a cytological consequence caused by a mismatch between the production of free radicals and the ability to scavenge them. Increase in free radical generation due to impaired endogenous antioxidant mechanism is an important factor that has been implicated in neuronal damage [19]. Free radicals and reactive oxygen species (ROS) are generated during oxidative metabolism and can inflict damage on all classes of cellular macromolecules, eventually leading to cell death. Therefore, antioxidant substances can play an important role in prevention and cure of neurodegenerative diseases. Antioxidants scavenge the free radicals directly or activate the protective mechanisms inside the cells that prevent the damage reaction on deoxyribonucleic acid, proteins and lipids. Almost all organisms have natural antioxidant defenses such as glutathione peroxidase, catalase and superoxide dismutase that prevent against oxidative stress. However, these systems are insufficient to prevent the damage entirely. The imbalance between antioxidants and free radicals in the body can cause degenerative and neurodegenerative diseases [18].
In this study, the results from the biochemical experiments showed a significant increase in MDA levels and a simultaneous decrease in total thiol contents and GPx, CAT and SOD activities in STZ-lesioned rats indicating neuronal damage caused by oxidative stress. While, chronic pre-treatment with GA restored the oxidative damage via reduction in MDA levels and increment in total thiol contents and GPx, CAT and SOD activities.

The increase in levels of MDA, a marker of lipid peroxidation (LPO), indicates increased free radical generation in STZ-lesioned rats. The significant decrease in MDA levels in the striatum of GA treated rats indicates attenuation of LPO. Similar results showing the reducing effect of GA on TBARS formation have been reported by other investigators [20].

There was a simultaneous significant decrease in total thiol contents and GPx activity in STZ-lesioned rats. Glutathione is an endogenous antioxidant largely present in its reduced form within cells which reacts with free radicals and prevents the generation of hydroxyl radicals. During this defence process, reduced glutathione is converted to the oxidized form with the help of the enzyme glutathione peroxidase [18]. However, chronic treatment with GA significantly restored total thiol contents and GPx activity.

A steady level of superoxide anion (O$_2^-$) and H$_2$O$_2$ is always present within neurons as a result of a normal metabolism. SOD and CAT are responsible for degradation of O$_2^-$ and H$_2$O$_2$, respectively [21]. There was a significant decrease in SOD and CAT activities in STZ-lesioned rats. GA pretreatment could increase the activity of these enzymes.

A growing body of research indicates that GA has the neuroprotective effect through some possible mechanisms. GA is a natural product of tannins hydrolysis found abundantly in grapes, tea, different berries, and other fruits as well as in wine [3]. GA received much attention because of its ability to scavenge reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, hydroxyl radicals and hypochlorous acid and also because of its antimutagenic and anticarcinogenic activities [4,20]. Neuroprotective effect of GA is further supported by its beneficial effects against β-amyloid, sodium fluoride and or lead nitrate induced neurotoxicity and brain oxidative damage [22,23]. Also GA has been reported to improve the cerebral antioxidant status of diabetic animals induced by STZ [7]. Lu et al. have shown that pre-treatment of SH-SYSY human cells with GA and its derivatives suppress 6-OHDA induced oxidative stress in vitro [24]. Also, Li et al. denoted that when GA was used to treat the 9-month-old male senescence accelerated mice not only reinstated the activities of CAT and GPx but also significantly reduced the amount of TBARS in the brain, liver and kidney [25]. Ferruzzi et al. demonstrated that repeated treatment of mice with grape seed extract significantly increased the bioavailability and brain deposition of GA which previously found to attenuate cognitive deterioration in a mouse model of AD [11]. Additionally, GA provided effective protection against kainic acid-stressed PC12 cells via reducing the Ca$^{2+}$ release, ROS, and LPO [26]. Moreover, we have recently shown the protective effect of GA against memory deficit and brain oxidative damage caused by 6-OHDA and also ICV STZ in rats [8,9].

All together, ameliorating effect of potent antioxidant chemicals like crocin, rutin, co-enzyme Q10, alpha lipoic acid, melatonin, resveratrol and epigallocatechin-3-gallate on ICV-STZ cognition deficits consolidate the hypothesis antioxidant action of GA on brain oxidative impairment [2,17]. So, a diet containing gallic acid may be effective to restore the neurological deficits in AD and other age-related neurodegenerative disorders where oxidative stress is involved.

Conclusions

The present findings provide evidence that GA has a possible neuroprotective effect against oxidative stress induced by STZ which is supported by inhibition of lipid peroxidation and upregulation of endogenous antioxidant defense system. These beneficial effects of GA might be attributed partly due to its antioxidant capacity. Nevertheless, further studies are required to establish the potential and mechanism of GA.

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Conflict of Interest

There is no conflict of interest.

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