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Gallic acid prevents memory deficits and oxidative stress induced by intracerebroventricular injection of streptozotocin in rats

Mohammad Taghi Mansouri [a,*], Bahareh Naghizadeh [b], Behnam Ghorbanzadeh [c], Yaghoub Farbood [d], Alireza Sarkaki [d], Kowsar Bavarsad [d]

[a] Department of Pharmacology, School of Medicine, Physiology and Atherosclerosis Research Centers, Ahvaz Jundishapur Univ. of Med. Sciences (AJUMS), Ahvaz, Iran
[b] Department of Pharmacology, School of Medicine, Physiology and Pain Research Center, Ahvaz Jundishapur Univ. of Med. Sciences (AJUMS), Ahvaz, Iran
[c] Department of Pharmacology and Toxicology, School of pharmacy, Ahvaz Jundishapur Univ. of Med. Sciences (AJUMS), Ahvaz, Iran
[d] Department of Physiology, School of Medicine, Physiology Research Center, Ahvaz Jundishapur Univ. of Med. Sciences (AJUMS), Ahvaz, Iran

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ABSTRACT

In the present study, we evaluated the effects of gallic acid (GA; 30 mg/kg, orally, once daily for 26 days starting from day 5 prior to streptozotocin injection) on cognitive impairment and cerebral oxidative stress induced by intracerebroventricular-streptozotocin (ICV-STZ; bilaterally, two doses of 3 mg/kg) injection as an animal model of sporadic Alzheimer's type (SDAT) in rats. The results showed that ICV-STZ-injection reduced the passive avoidance and spatial memory performance associated with decreased non-enzymatic [total thiol concentration, −58.5%, −50.7%] and enzymatic [superoxide dismutase (SOD, −30.2%, −32.9%), catalase (CAT, −43.5%, −50.7%), glutathione peroxidase (GPx, −57.1%, −61.7%)] activities and increased the level of thiobarbituric acid reactive species (TBARS, +103.5%, +82.5%) in the hippocampus and cerebral cortex, respectively. In contrast, chronic administration of GA significantly prevented cognitive deficits and biochemical alterations in the ICV-STZ rats. These findings highlight the beneficial role of GA in the ICV-STZ rats *via* enhancement of cerebral antioxidant defense system. Thus, it may have a therapeutic value for the treatment of SDAT.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the aged brain and has become a major medical and social trouble for industrialized and developing countries. It is the most important cause of senile dementia of Alzheimer's type (SDAT) and is characterized by memory and cognitive impairment, the formation of beta-amyloid plaques, neurofibrillary tangles and degeneration of the cholinergic neurons. None of the several hypotheses proposed to explain AD etiology has been confirmed, but oxidative stress is often cited as an important factor (Weinstock and Shoham, 2004). Oxidative stress damages the polyunsaturated fatty acids leading to the disruption of cell membrane and its integrity, inactivation of antioxidant enzymes, and finally neuronal dysfunction and death (Javed et al., 2012).

Intracerebroventricular (ICV) injection of streptozotocin (STZ), in a sub diabetogenic dose in rat has been likened to sporadic dementia of Alzheimer's disease. It is characterized by cognitive impairment, impaired glucose metabolism oxidative stress (Sharma and Gupta, 2001) and a decrease in cholinergic markers in the brain (Lannert et al., 1998).

There has been much effort to develop beneficial agents from medicinal plants to achieve neuroprotection. Recent studies have shown that supplementation with some phenolic compounds such as crocin (Naghizadeh et al., 2013), rutin (Javed et al., 2012), co-enzyme Q10 (Ishrat et al., 2006), alpha lipoic acid, melatonine, resveratrol (Sharma et al., 2005), and epigallocatechin-3-gallate (Baluchnejadmojarad and Roghani, 2011) can prevent or treat the STZ-induced cerebral damage, and the ability of phenolic compounds might be related to their antioxidant properties.

Gallic acid (GA, 3,4,5-trihydroxybenzoic acid), is one of the most important polyphenolic compound in plants and is considered a putative active compound in tannin, namely gallotannin. GA is a polyphenolic substance present in grapes, different berries, mango, areca nut, walnut, green tea and other fruits as well as in wine. This compound possesses antioxidant and free radical scavenger, anti-cancer and anti-inflammatory properties (Isuzugawa et al., 2001; Kroes et al., 1992). Due to the antioxidant effects, GA-containing plant extracts have showed the antidiabetic, antiangiogenic and antimelanogenic effects and reduced heart infarction incidence and oxidative liver and kidney damage (Constat, 1997; Kim, 2007; Jadon et al., 2007). It has been reported that GA is involved in the protection of the neural cells against *in vitro* β-amyloid peptide (Aβ)-induced death (Bastianetto et al., 2006). GA also has a protective effect in the case of cerebral oxidative stress induced by diabetes induced by streptozotocin in rats through the modulation of antioxidant enzyme-dependent signaling systems

* Corresponding author at: School of Medicine, Atherosclerosis Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Tel.: +98 611 3330074.
E-mail addresses: mansouri_smt@yahoo.com, mansouri-m@ajums.ac.ir (M.T. Mansouri).

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(Kade and Rocha, 2013). Recently, our research indicated the neuroprotective effect of GA against oxidative stress induced by 6-hydroxydopamine (6-OHDA) in rat brain (Mansouri et al., 2013). In addition, Ferruzzi et al. (2009) demonstrated that repeated treatment of mice with grape seed extract significantly increased the bioavailability and brain deposition of GA which has previously been found to attenuate cognitive deterioration in a mouse model of Alzheimer's disease. Thus, GA may be a potential neuroprotective agent.

Considering all of these points, this study was intended to examine the efficacy of chronic GA administration on alleviation of learning and memory deficits in ICV-STZ rats using passive avoidance, and Morris water maze (MWM) tests and its effect on some markers of oxidative stress in the rat brain.

2. Materials and methods

2.1. Chemicals

TBA (2-thiobarbituric acid), n-butanol, tris base, Na_2EDTA, sodium acetate, glacial acetic acid, phosphoric acid, potassium chloride and 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).

2.2. Animals

Adult male Wistar rats weighing 250–300 g were used throughout the study. All of them were kept in the same room under a constant temperature (22 ± 2 °C), humidity (55–60%) and illuminated from 7:00 a.m. to 7:00 p.m., with food pellets and water available *ad libitum*. The rats were acclimatized to the laboratory conditions five 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 pharmacologic protocols.

2.3. 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 the brain (Paxinos and Watson, 2006). STZ (3 mg/kg) was injected ICV bilaterally on day 1 and 3 of the experiment (Sharma and Gupta, 2001). In the sham group, artificial CSF (147 mM NaCl, 2.9 mM KCl, 1.6 mM $MgCl_2$, 1.7 mM $CaCl_2$ and 2.2 mM dextrose) was injected (20 μl on each site) on the same days as STZ group. STZ was dissolved in the 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.

2.4. Experimental design

Animals were randomly divided into four groups (8 each) and were individually put in the cages. The treatment schedule and the intervals for estimation of various parameters have been presented in Fig. 1. 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-infused lesioned (L); group 4: GA-treated (30 mg/kg) and ICV-STZ-infused (GA + L). In the S and GA + S groups, the rats were injected ICV the same volume of artificial CSF. Groups 2 and 4 were administered with GA by gavage at a dose of 30 mg/kg (once/day) 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. During the behavioral test, GA was administered 60 min before the water maze training. The dose of GA used in this study has been obtained from previous experiments (Punithavathi et al., 2011; Dhingra et al., 2012) and the pilot study in our laboratory.

2.5. Learning and memory assessment

2.5.1. Passive avoidance test

On days 14 and 15 after the first dose of ICV-STZ infusion, the rats were tested for memory retention deficit using a passive avoidance task. The apparatus (Neuroscience, Inc., VA, USA) consisted of an illuminated compartment (20 × 10 × 2 cm) with a 6-W tungsten lamp and a dark compartment (30 × 30 × 30 cm) with a grid floor (15 parallel steel rods), separated by a guillotine door (8 × 8 cm). Electroshocks (0.2 mA, 75 V, 50 Hz) were delivered for 3 s through the grid floor in the dark compartment by a shock scrambler (Neuroscience, Inc., VA, USA). On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. During the acquisition trial, each rat was placed in the illuminated chamber. After initial habituation period of 60 s, the guillotine door was opened and the time taken by the rat to enter the dark chamber was noted. The latency to step into the dark compartment was recorded as initial trial or pre-shock latency (ITL). As soon as the rat entered the dark chamber, it was given a mild foot shock of 0.5 mA for 2 s through the grid floor. The rat was allowed to remain in the dark compartment for 5 s and then was taken out. After 24 h interval, retention trial was performed and retention trial or postshock latency (RTL) to step into the dark compartment was noted. The cut-off time was 600 s (Ishrat et al., 2006). Short latencies indicated poorer retention.

2.5.2. Morris water maze test

The water maze used was a black circular tank (136 cm in diameter and 60 cm in height) that was filled with water (20 ± 1 °C) to a depth

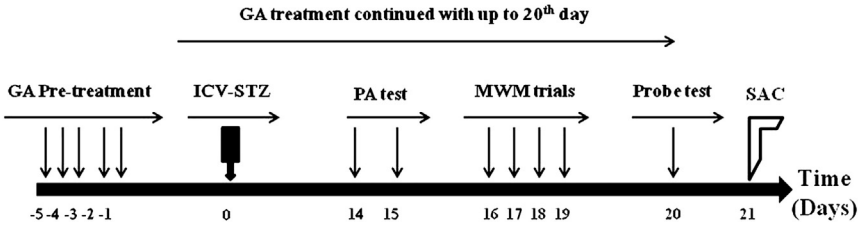

Fig. 1. The design of the treatment schedule and intervals for estimation of various parameters. GA: gallic acid; ICV-STZ: intracerebroventricular-streptozotocin; PA: passive avoidance; MWM: Morris water maze; SAC: sacrificed for biochemical parameters. Day 0 refers to the day of surgery (ICV-STZ infusion).

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