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Protective effects of gallic acid against chronic cerebral hypoperfusion-induced cognitive deficit and brain oxidative damage in rats

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ABSTRACT

Free radical-induced neural damage is implicated in cerebral hypoperfusion disorders and antioxidants have protective effects. In the present study, we examined the effect of gallic acid (GA; 100 mg/kg, p.o. for 10 days) on cognitive deficit and cerebral oxidative stress induced by permanent bilateral common carotid artery occlusion (2VO) as an animal model of vascular dementia (VD). The results showed that 2VO significantly reduced the spatial memory performance in Morris water maze as well as non-enzymatic (total thiol) and enzymatic [glutathione peroxidase (GPx)] antioxidant contents and increased the level of malondialdehyde (MDA) in the hippocampus and frontal cortex of vehicle-treated group as compared to sham-operated rats. Furthermore, chronic administration of GA significantly restored the spatial memory, total thiol and GPx contents and also decreased MDA levels in these tissues. GA alone did not show any change neither in the status of various antioxidants nor behavioral tests over sham values. The results demonstrate that GA has beneficial activity against 2VO-induced cognitive deficits *via* enhancement of cerebral antioxidant defense. Taken together, the present study suggested that GA might be useful in the treatment of VD.

1. Introduction

Ischemic brain injury is caused by the interruption of cerebral blood flow leading to excessive production of reactive oxygen species which is associated with behavioral dysfunction and also cognitive deficits (Li et al., 2011). The formation of reactive oxygen species represents the first key step to initiate tissue oxidative stress (Silva-Adaya et al., 2008). Oxidative stress is defined as an imbalance between cellular levels of reactive oxygen species (*e.g.*, superoxide and hydroxyl radicals) and cellular antioxidant defense. Reactive oxygen species are produced by a free radical chain reaction caused tissue injury by reacting with biomolecules such as lipids, proteins, and nucleic acids as well as by depleting enzymatic and/or non-enzymatic antioxidants in the brain. In order to scavenge reactive oxygen species, various defense systems such as glutathione, antioxidant enzymes [glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT)] exist in the brain (Akdag et al., 2010).

Gallic acid (GA, 3,4,5-trihydroxybenzoic acid), is one of the most important polyphenolic substances in plants which is present in grapes, different berries, mango, areca nut, walnut, green tea and other fruits as well as in wine. Also, it is considered as putative active compound in tannin, namely gallotannin (Singh et al., 2004). This compound possesses antioxidant, free radical scavenging, anti-cancer, anti-inflammatory (Bhouri et al., 2012), anti-fungal (Choi et al., 2010) and anti-human rhinovirus activities (Nguyen et al., 2013). Gallic acid has also been used in food, cosmetics, and in pharmaceuticals as an antioxidant (Bhouri et al., 2012). GA can be used to treat human albuminuria and diabetes (Hsieh et al., 2007). Moreover, it has been reported that GA produced anti-anxiety (Dhingra et al., 2012), anti-depressant (Chhillar and Dhingra, 2013) and anti-epileptic (Huang et al., 2012) effects in animal models.

Due to the free radical scavenging property, GA-containing plant extracts have showed anti-diabetic, anti-angiogenic and anti-melanogenic effects and reduced heart infarction incidence and the oxidative damage of liver and kidney tissues (Priscilla

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and Prince, 2009). It has been reported that GA is involved in the protection of neural cells against *in vitro* β-amyloid peptide (Aβ)-induced death (Ban et al., 2008). Recently, our research indicated the neuroprotective effect of GA against cerebral oxidative stress induced by 6-hydroxydopamine and or streptozotocin in rat brain (Mansouri et al., 2013a,b). In addition, Ferruzzi et al. (2009) demonstrated that repeated treatment of rodents 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. Thus, GA as a major constituent of grape seed extract may be a potential neuroprotective agent.

To the best of our knowledge, there is no published scientific report on the protective role of GA against cognitive deficits and cerebral oxidative stress induced by chronic cerebral hypoperfusion. Therefore, the present study intended to examine the effects of GA on learning and memory impairments induced by permanent cerebral artery occlusion (2VO) and determined whether this effect was modulated through antioxidant mechanisms in the brain.

2. Materials and methods

2.1. Chemicals

DTNB (2,2′-dinitro-5,5′-dithiodibenzoic acid), TBA (2-thiobarbituric acid), *n*-butanol, tris base, ethylendiamine tetraacetic acid disodium, sodium acetate, glacial acetic acid, phosphoric acid, potassium chloride, tetramethoxypropane were obtained from Merck Company (Darmstadt, Germany) and GA was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and prepared from Merck Company (Darmstadt, Germany).

2.2. Animals

Adult male Wistar rats weighing 200–250 g were used throughout the study. All animals were obtained from the Animal House of Shahrekord Medical School (Shahrekord, Iran). Animals were allowed free access to standard laboratory chow and water, *ad libitum*. A 12-h light/dark cycle at 22 ± 2 °C and 50% humidity conditions were maintained. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals adopted by National Institute of Health and the Federation of Iranian Societies for Experimental Biology.

2.3. Surgery

Permanent cerebral hypoperfusion (PCH), that mimics the vascular dementia model, was induced by occlusion of the bilateral common carotid arteries (two vessels occlusion; 2VO) (Xu et al., 2012). Briefly, a neck ventral midline incision was made and the common carotid arteries were then exposed and gently separated from the vague nerve. The bilateral common carotid arteries were tied with silk threads, whilst the rats were under an appropriate level of ketamine/xylazine (50/5 mg/kg) anesthesia (Roohbakhsh et al., 2007).

2.4. Experimental design

The animals were randomly divided into 4 groups (seven each). Group 1 was the sham-operated (sham) in which normal saline (2 ml/kg) was given by oral gavage. Group 2 was 2VO in which bilateral common carotid artery was ligated and received normal saline as the same of sham group. Group 3 was sham + GA which treated with GA at dose of 100 mg/kg (Hsu and Yen, 2007). Group 4 was 2VO + GA in which bilateral common carotid artery was ligated and received GA at dose of 100 mg/kg. GA administration was started 5 days before surgery and continued for 10 consecutive days.

2.5. Assessment of spatial memory via Morris water maze test

The spatial memory performance was evaluated using a Morris water maze (MWM). The water maze used was a black circular tank (136 × 60, diameter × height) which filled with water (20 ± 1 °C) to a depth of 25 cm. The maze was located in a room containing extra-maze cues (posters). The pool geographically divided into four quadrants [northeast (NE), northwest (NW), southeast (SE), southwest (SW)] and starting positions [north (N), south (S), east (E), west (W)] that were equally spaced around the perimeter of the pool. A hidden circular platform (diameter: 13 cm) was located 2 cm below the surface of the water on a fixed location in one of the four quadrants of the pool. A video Camera was mounted directly above the water maze to record the rats' swim paths. An automated tracking system (EthoVision®, Noldus, Wageningen, Netherlands) was used to measure the escape latency and percentage of the time in the target quadrant. 6 days after 2VO (Kim et al., 2006), rats were given four training trials each day for 4 consecutive days. For each training trial, the rats were placed in the water facing the pool wall at one of the four starting in a different order each day and allowed to swim until they reached the platform. The latency to reach the platform was recorded for up to 60 s. The rats remaining on the platform for 30 s were removed. One day after the last training, a probe trial was conducted by removing the platform. Rats were allowed to swim freely into the pool for 60 s. The time spent in the target quadrant, which had previously contained the hidden platform, was recorded. The time spent in the target quadrant indicated the degree of memory consolidation that has taken place after learning. After the trials, the rats were dried with a towel and placed in a holding cage under a heating lamp before returning to the home cage. A visible platform trial was performed with the platform placed on the side of the pool opposite its location during hidden platform training to check the vision of all rats (Itoh et al., 1999; Morris, 1984). In order to determine whether the group differences in escape latency and swimming distance were due to their differences in swimming ability, the swimming speed was also evaluated.

2.6. Brain sample collection and biochemical assays

At the end of behavioral experiments, the animals were decapitated and the hippocampus and frontal cortex 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 thiobarbituric acid reactive substances value (expressed as malondialdehyde (MDA) equivalents), total thiol contents and GSH-Px activity (Naghizadeh et al., 2008).

2.6.1. Thiobarbituric acid reactive species measurement

MDA levels, an index of lipid peroxidation (LPO), produced by free radicals were measured. 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 rpm for 20 min. The colored layer was transferred to a fresh tube and its absorbance was measured at 532 nm. MDA levels were