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Ellagic acid prevents cognitive and hippocampal long-term potentiation deficits and brain inflammation in rat with traumatic brain injury

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ABSTRACT

Aims: Traumatic brain injury (TBI) remains one of the main clinical problems globally and is a common cause of death among youth. Cognitive defects such as thinking, memory and behavior or mental health disorders are considered as the most frequent effects of severe and moderate TBI. It has been reported that ellagic acid (EA), a natural polyphenol, exhibits protective effects against oxidative damage. This study was performed to examine the EA preventive effects on cognitive impairments, long-term potentiation (LTP) deficits in hippocampus and brain inflammation induced by diffuse TBI in rat.
Main methods: Subchronic oral administration of 100 mg/kg EA, 7 consecutive days before induction of trauma (once daily) was used to elucidate the EA effects on passive avoidance memory and hippocampal LTP following TBI. To illustrate the possible mechanisms related to the preventive effects of EA on brain function following TBI, brain content of IL-1β, IL-6 and blood–brain barrier (BBB) permeability were determined.
Key findings: EA pretreatment significantly ($P < 0.001$) prevented TBI-induced memory and hippocampal LTP impairments in rat. Furthermore TBI induced elevation in brain content of IL-1β, IL-6 and BBB permeability were decreased significantly ($P < 0.001$) due to EA pre-treatment.
Significance: Our findings suggest that EA can prevent cognitive and LTP deficits and also prevent brain inflammation following TBI.

Introduction

Traumatic brain injury (TBI) remains one of the main clinical problems globally and is a common cause of death among youth [17,25]. In the USA, about 1.7 million people survive a TBI annually, among whom 275,000 are hospitalized [17]. TBI occurs in two stages: 1) primary injury, indicated by destruction of the brain tissue and blood vessels, which initiates complex physiological processes involving cellular and molecular events; 2) secondary injury, the processes that occurs hours to days after TBI which lead to further injury on neurons and axons [2,4]. Therapeutic policies have focused on preventing secondary injury [25]. Inflammation has a main role in secondary brain injury [50]. Previous studies have shown many examples of cytokine production after TBI [25].

Cognitive defects such as thinking, memory, and reasoning problems as well as behavior or mental health disorders are among the most frequent sequelae after severe and moderate TBI [3,24,72]. The hippocampal areas especially dentate gyrus (DG) neurons are vulnerable to TBI [27]. It has been shown that neuronal death in the DG following TBI leads to learning and memory impairments in adult rodents [26]. Long-term potentiation (LTP) in hippocampal synapses has been proposed as a model for the cellular changes that underlie learning and memory [64].

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Naturally occurring polyphenols are known as capable alternatives for use as pharmaceuticals ([38]). Previous studies suggested a relation between phenolic food intake and the protection against several diseases [41]. Polyphenols are plant metabolism products and they have antioxidant functions [32,43]. Ellagic acid (EA) (2,3,7,8-tetrahydroxybenzopyrano[5,4,3-cde]benzopyran-5-10-dione) is a polyphenol present in many plant species such as pomegranate plants, grapes, raspberries, blackberries, strawberries and walnuts [14,75]. Several line of studies have shown that EA has different pharmacological effects such as anti-bacterial, anti-inflammatory, immune regulatory and inhibition of tumorigenesis and also it is considered as a potent antioxidant [6,12,18].

The pretreatment approach has long been successfully employed for the neuroprotection against TBI [36,46,79,80]. This recognition has led us to choose the pretreatment strategy in this study. To the best of our knowledge, there is no published scientific report on the effects of EA on brain inflammation, learning and memory deficits induced by TBI. Therefore, the present study intended to examine the preventive effects of EA on avoidance memory and hippocampal LTP deficits induced by closed head injury and determined whether these neuroprotective effects were modulated through anti-inflammatory mechanisms in the brain.

Materials and methods

Animals and experimental groups

The Ahvaz Jundishapur University of Medical Sciences Institutional Animal Care and Use Committee approved all experiments, and the procedures followed the NIH Guide for the care and use of experimental animals [20]. Adult male Wistar rats (250 ± 20 g) purchased from Ahvaz Jundishapur University of Medical Sciences Animal House (Ahvaz, Iran) were housed in clear cages in temperature (22 ± 2 °C) and humidity (50%) controlled conditions and 12/12 h light/dark cycle. Animals had free access to food and water ad libitum and allowed to adapt to the laboratory conditions for at least 7 days before the study. The rats were randomly assigned to Control, Sham-injury, Veh + TBI and EA + TBI groups (n = 24 for each group). Both Sham-injury and Veh + TBI rats received EA vehicle (10% DMSO in normal saline in a total volume of 10 ml/kg, once daily) orally for 7 consecutive days before induction of trauma (Gavage needle about 11 cm long with a 15° curved blunt ended needle). Animals in the Sham-injury group underwent TBI procedures but were not exposed to TBI while the Veh + TBI animals were exposed. Control and EA + TBI rats orally received a dose of 100 mg/kg EA and 10% DMSO in normal saline as solvent (in a total volume of 10 ml/kg, once daily) for 7 consecutive days before induction of trauma using the Gavage needle (as described above). Animals in the EA + TBI group were exposed to trauma while the rats in the Control group did not undergo any procedure (naive rats). To habituate the animals to oral administration, all rats received normal saline (10 ml/kg, by gavage) daily for three days prior to the experiments. We used 8 rats in each experimental group to perform passive avoidance memory and electrophysiological tests, 8 rats for determination of blood–brain barrier permeability and 8 rats for determination of brain IL-1β and IL-6 content. Every possible effort was made to minimize animal suffering.

Chemicals

We purchased ellagic acid (purity ≥ 95%), Evans blue, dimethyl sulfoxide (DMSO) and Triton X-100 from Sigma-Aldrich Co. (St. Louis, MO, USA), protease inhibitor cocktail from Roche (Basel, Switzerland), T-PER™ Tissue Protein Extraction Reagent from Pierce Biotechnology Inc. (Rockford, IL, USA), and a Bio-Rad protein assay kit from Bio-Rad Laboratories (Hercules, CA, USA). Tris base, sodium phosphate, sodium chloride, potassium phosphate and potassium chloride were of analytical grade and obtained from Merck Co. (Darmstadt, Germany). EA was dissolved in 10% DMSO in normal saline. Drug was freshly prepared so that the necessary dose could be given in a total volume of 10 ml/kg orally route. EA dose and administration schedules were selected based on our pilot studies and previous reports [22,54,66].

Induction of brain trauma

Tracheal intubation was performed while the rats were under an appropriate level of ketamine/xylazine (50/5 mg/kg, IP) anesthesia before TBI [60]. Then animals in the Veh + TBI and EA + TBI groups were exposed to diffuse traumatic brain trauma using an instrument made in the Physiology Research Center of Ahvaz Jundishapur University of Medical Sciences with the Marmarou method [29,39]. As it is instructed in this method, a 200 g weight was dropped from a 2-m height through a free-falling tube onto the head of an anesthetized animal while a steel disk was attached to the animal's skull. After brain trauma induction, the animal was immediately connected to an animal respiratory pump (Ugo Basile, Italy) and as soon as it was spontaneously breathing, it was disconnected from the ventilator and returned to the cage to be cared for [30].

Passive-avoidance test

A step-through latency test in a shuttle-box was performed to evaluate the effects of subchronic EA pretreatment on avoidance memory in rats. The shuttle-box apparatus (Borj sanat, Tehran, Iran) consisted of two equally sized (200 × 250 × 200 mm) compartments, a lighted one and a dark one with two independent grid floors. The compartments were separated by a guillotine door. As an accommodation session, the animal was placed in the lighted chamber while the guillotine door was opened and allowed to explore both compartments for 10 min and then removed. After 10 min the animal was again placed in the lighted compartment facing away from the closed guillotine door and 10 s later the door was elevated and the entering delay of rat into the dark compartment was recorded as initial latency (IL). Immediately after entering the dark chamber, the guillotine door was closed and an unavoidable foot-shock (75 V, 0.2 mA, 50 Hz for 3 s) was delivered using a shock generator. Twenty-four hours after the initial session, the retention test was carried out. In this session the animal was again placed in the lighted compartment and the step-through latency (STL) was measured. The max latency was recorded as 300 s [34,38].

Electrophysiological studies

Surgical procedure

Forty-eight hours after TBI induction, the animals were prepared for electrophysiological recordings. They underwent an appropriate level of ketamine/xylazine (50/5 mg/kg, IP) anesthesia and their heads were mounted on a stereotaxic device for surgery (electrode implantation and EPSP recording) [60]. The animal's body temperature was maintained at 36.5 ± 0.5 °C using a heating pad. The animal's skull was drilled and small holes were made to implant the electrodes. A pair of stimulating metal wire microelectrode (stainless steel, 100 μm in diameter, tip separation 500 μm, CFW, USA) and a pair of recording metal wire microelectrodes (tungsten, 50 μm in diameter, tip separation 1 mm, CFW, USA) were implanted into the perforant pathway (PP) at AP = −7.5 mm from bregma; ML = −4 mm; DV = −3.9 mm from dura and granular cells of DG with stereotaxic coordination of AP = −3.8 mm from bregma; ML = −2.3 mm; DV = −3.5 mm from dura, respectively [49]. In order to decrease brain tissue damage, both electrodes were lowered very slowly (0.1 mm/30 s) [34].

Electrophysiological recordings and LTP induction

Following the stimulation of PP, the field potential recordings were obtained in DG granular cells. The PP was stimulated every 30 s. The