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Neuroprotection and reduced gliosis by pre- and post-treatments of hydroquinone in a gerbil model of transient cerebral ischemia

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ABSTRACT

Hydroquinone (HQ), a major metabolite of benzene, exists in many plant-derived food and products. Although many studies have addressed biological properties of HQ including the regulation of immune responses and antioxidant activity, neuroprotective effects of HQ following ischemic insults have not yet been considered. Therefore, in this study, we examined neuroprotective effects of HQ against ischemic damage in the gerbil hippocampal cornu ammonis 1 (CA1) region following 5 min of transient cerebral ischemia. We found that pre- and post-treatments with 50 and 100 mg/kg of HQ protected CA1 pyramidal neurons from ischemic insult. Especially, pre- and post-treatments with 100 mg/kg of HQ showed strong neuroprotective effects against ischemic damage. In addition, pre- and post-treatments with 100 mg/kg of HQ significantly attenuated activations of astrocytes and microglia in the ischemic CA1 region compared to the vehicle-treated-ischemia-operated group. Briefly, these results show that pre- and post-treatments with HQ can protect neurons from transient cerebral ischemia and strongly attenuate ischemia-induced glial activation in the hippocampal CA1 region, and indicate that HQ can be used for both prevention and therapy of ischemic injury.

1. Introduction

Transient cerebral ischemia causes extensive neuronal death in some brain areas including the hippocampus [1–3]. In particular, the cornu ammonis 1 (CA1) region in the hippocampus has been well known as one of regions most vulnerable to transient cerebral ischemia and selective neuronal death in this region occurs typically some days after initial ischemic insult, which is referred to as "delayed neuronal death" [3,4]. Many possible mechanisms regarding the delayed neuronal death have been suggested, such as glutamate excitotoxicity, oxidative stress and neuroinflammatory

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response [5–7]. Nevertheless, exact mechanism and/or explanation of the delayed neuronal death induced by transient cerebral ischemia have not been fully clear.

Hydroquinone (HQ), an aromatic organic compound of phenol type, is naturally found in many plant-derived food products such as wheat, coffee, tea and red wine, and it is used in naturopathic remedy as an antioxidant [8,9]. Although tumorigenic effects and toxicity of HQ have been addressed by many researches, some have reported HQ-mediated beneficial effects such as anti-inflammatory and immunosuppressive properties [10,11]. It has been suggested that HQ influences immune cell responses, namely HQ increases allergic immune response through increasing interleukin (IL)-4 production in $CD4^+$ T cells and enhancing immunoglobulin E levels in antigen-primed mice [12]. Furthermore, HQ inhibits inflammatory response mediated by functional activations of monocytes, macrophages and lymphocytes [13,14]. HQ also exhibits potent antioxidant activity against peroxynitrite generation, which is a potent cytotoxic oxidant formed by the reaction of nitric oxide and superoxide radical in activated macrophages induced by lipopolysaccharides (LPS) [15].

Although many investigators have focused on biological effects of HQ including regulation of immune responses and antioxidant activity, few studies regarding neuroprotective effects of HQ against transient cerebral ischemia have been demonstrated. Therefore, in this study, we examined the neuroprotective effects of HQ in the hippocampal CA1 region following 5 min of transient cerebral ischemia in gerbils, which are well known as a suitable model for the evaluation of neuroprotective agents against transient cerebral ischemia [16–19].

2. Materials and methods

2.1. Treatment with HQ

Male Mongolian gerbils (*Meriones unguiculatus*) at 6 months of age (B.W., 65–75 g) were used according to the guidelines that are in compliance with the current international laws and policies (Guide for the Care and Use of Laboratory Animals, The National Academies Press, 8th Ed., 2011) and approved by the Institutional Animal Care and Use Committee (IACUC) at Kangwon University (Chuncheon, South Korea; approval no. KW-130424-1).

To elucidate the neuroprotective effects of HQ against ischemic damage, the gerbils were divided into 8 groups ($n = 7$ in each group), as follows: 1) vehicle-treated sham-operated group (vehicle-sham-group), 2) vehicle-treated ischemia-operated groups (vehicle-ischemia-group), 3) and 4) 50 and 100 mg/kg HQ-pre-treated sham-operated groups (pre-HQ-sham-groups), 5) and 6) 50 and 100 mg/kg HQ-post-treated sham-operated groups (post-HQ-sham-groups) 7 and 8) 50 and 100 mg/kg HQ-pretreated ischemia-operated groups (pre-HQ-ischemia-groups), 9 and 10) 50 and 100 mg/kg HQ-post-treated ischemia-operated groups (post-HQ-ischemia-groups). HQ was purchased from Sigma (St. Louis, MO, USA) and dissolved in saline. For pre- and post-treatment, HQ or saline was intraperitoneally administered once at 30 min before or after ischemic surgery, respectively. Sham-operated animals were subjected to the same surgical procedures except that the common carotid arteries were not occluded.

2.2. Hematoxylin and eosin (H&E) staining

To examine potential toxic effects of HQ in the brain of the vehicle- and HQ-sham group ($n = 7$ in each group), H&E staining was performed according to a published procedure [2]. Briefly, the animals were perfused with 4% paraformaldehyde 5 days after HQ treatment. The brains were serially sectioned into 30 μm coronal sections in a cryostat (Leica, Wetzlar, Germany) and stained with H&E. Images were obtained using a light microscope (BX53, Olympus, Hamburg, Germany) equipped with a digital camera (DP72, Olympus) connected to a PC monitor.

2.3. Induction of transient cerebral ischemia

According to our published procedure [20], the surgical procedure for transient cerebral ischemia was performed. In brief, the animals were anesthetized with a mixture of 2.5% isoflurane in 33% oxygen and 67% nitrous oxide. The complete interruption of blood flow through the 5 min-occlusion of bilateral common carotid arteries was confirmed by observing the central artery in retinae under an ophthalmoscope. Normothermic (37 ± 0.5 °C) condition was maintained before, during and after the surgery until the animals completely recovered from anesthesia. Sham-operated animals were subjected to the same surgical procedures except that the common carotid arteries were not occluded.

2.4. Spontaneous motor activity

For the effect of HQ on ischemia-induced hyperactivity, spontaneous motor activity (SMA) was measured 1 day after ischemia-reperfusion according to published procedure [21]. Briefly, the locomotor activity of gerbils ($n = 7$ in each group) was recorded with the Photobeam Activity System-Home Cage (San Diego Instruments, San Diego, CA). Movement was detected by the interruption of an array of 32 infrared beams produced by photocells. The spontaneous motor activity was monitored during 60 min, and the locomotor activity data were acquired by an AMB analyser (IPC Instruments, Berks, U.K.). The results were evaluated in terms of entire distance (meters) traveled for 60 min test period.

2.5. Cresyl violet (CV) staining

According to our published procedure [22], in brief, animals were perfused transcardially with 4% paraformaldehyde and their brain tissues were serially cut into 30-μm coronal sections. To examine cellular distribution and damage of neurons, CV staining was performed by our published procedure [20]. In brief, cresyl violet acetate (Sigma, MO, USA) was dissolved at 1.0% (w/v), and glacial acetic acid was added at 0.28% in this solution. The sections were stained and dehydrated by immersing in serial ethanol baths.

2.6. NeuN immunohistochemistry and Fluoro-Jade B staining

To investigate the neuronal damage/death after ischemia-reperfusion, neuronal nuclear antigen (NeuN, a marker for neurons) immunohistochemistry and Fluoro-Jade B (F-J B, a high affinity fluorescent marker for the localization of neuronal degeneration) histofluorescence staining were done according to the previously published procedures [22,23]. In brief, the sections were incubated with diluted mouse *anti*-NeuN (1:1000, Chemicon, Temecula, CA) and subsequently exposed to biotinylated goat anti-mouse IgG and streptavidin peroxidase complex (1:200, Vector, Burlingame, CA). And they were visualized by staining with 3,3'-diaminobenzidine (Sigma). For F-J B staining, the sections were first immersed in a solution containing 1% sodium hydroxide and then transferred to a solution of 0.06% potassium permanganate, transferred to a 0.0004% Fluoro-Jade B (Histochem, Jefferson, AR) solution. After washing, the sections were placed on a slide warmer (approximately 50 °C), and examined using an epifluorescent microscope (Carl Zeiss, Göttingen, Germany) with blue (450–490 nm) excitation light and a barrier filter. In order to quantitatively analyze NeuN immunoreactive neurons and F-J B positive cells,

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