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Research Report

Neuroprotective effects of gallic acid against hypoxia/reoxygenation-induced mitochondrial dysfunctions in vitro and cerebral ischemia/reperfusion injury in vivo

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ABSTRACT

Oxidative stress and mitochondrial dysfunction are frequently implicated in the pathology of secondary neuronal damage following cerebral ischemia/reperfusion. Recent evidence suggests that gallic acid (GA) reverses oxidative stress in rat model of streptozotocin-induced dementia, but the roles and mechanisms of GA on cerebral ischemia/reperfusion injury remain unknown. Here we investigated the potential roles and mechanisms of GA in hypoxia/reoxygenation induced by sodium hydrosulfite ($Na_2S_2O_4$) in vitro and cerebral ischemia/reperfusion induced by middle cerebral artery occlusion (MCAO) in vivo. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, 5, 5′, 6, 6′-tetrachloro-1, 1′, 3, 3′-tetraethylbenzimidazol carbocyanine iodide (JC-1), Dichlorofluorescin diacetate (DCF-DA) and MitoSOX fluorescent assay, Clark-type oxygen electrode, firefly luciferase assay, and calcium-induced mitochondrial swelling were conducted to detect cell death, mitochondrial membrane potential (MMP), intracellular and mitochondrial reactive oxygen species (ROS), oxygen consumption, ATP level, and mitochondrial permeability transition pore (MPTP) viability. We firstly find that modulation of the mitochondrial dysfunction is an important mechanism by GA attenuating hypoxia/reoxygenation insult. To further

Abbreviations: ANT, adenine nucleotide translocator; BCA, bicinchoninic acid; CsA, cyclosporin A; CypD, Cyclophilin D; Cyt C, Cytochrome C; DAPI, 4', 6-diamidino-2-phenylindole; DCF-DA, dichlorofluorescin diacetate; DMEM, dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; EB, ethidium bromide; EBSS, Earle's balanced salt solution; FBS, fetal bovine serum; GA, gallic acid; H & E, haematoxylin and eosin; JC-1, 5, 5′, 6, 6′-tetrachloro-1, 1′, 3, 3′-tetraethylbenzimidazol carbocyanine iodide; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; MMP, mitochondrial membrane potential; MPTP, mitochondrial permeability transition pore; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; NADPH, nicotinamide adenine dinucleotide phosphate; NOX, NADPH oxidases; ROS, reactive oxidative species; rt-PA, recombinant tissue plasminogen activator; TTC, 2, 3, 5-triphenyl-tetrazolium chloride; TUNEL, dUTP nick-end labeling; VDAC, voltage-dependent anion channel.

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assess the effects of GA on cerebral ischemia/reperfusion injury, 2, 3, 5-triphenyltetrazolium chloride (TTC) staining, dUTP nick-end labeling (TUNEL) assay, and Cytochrome C (Cyt C) release were performed in MCAO rats. The results support that GA is useful against cerebral ischemia/reperfusion injury as a potential protective agent.

1. Introduction

Ischemic stroke is an acute cerebrovascular event associated with brain tissue damage due to significant deprivation of oxygen and glucose caused by a reduction or complete blockade of artery supplying blood to the brain (Chen et al., 2011). Already five minutes after the onset of ischemia neurons begin to die (Radermacher et al., 2013). Therefore, early restoration of cerebral blood flow by using recombinant tissue plasminogen activator (rt-PA) is crucial for sustaining neuronal viability (Radermacher et al., 2013). Unfortunately, reperfusion is believed to contribute to delayed secondary brain injury because the freshly arriving oxygen will serve as a substrate for excessive reactive oxidative species (ROS) production (Chen et al., 2011; Radermacher et al., 2013; Schaller and Graf, 2004).

Mitochondria have long been known to play a critical role in the pathogenesis of cerebral ischemia/reperfusion injury, via ROS generation, mitochondrial dysfunction, and mitochondrial (type II) apoptosis (Christophe and Nicolas, 2006; Turrens, 2003). Mitochondria are abundant in cerebral tissue, and mitochondrial complex I is a major source of cerebral intracellular ROS (Turrens, 2003). Cerebral ischemia/reperfusion injury promotes secondary mitochondrial dysfunction, which is characterized by reduction of mitochondrial membrane potential (MMP), depletion of ATP synthesis, and inducing a sudden increase in permeability of the mitochondrial permeability transition pore (MPTP). Finally, excessive amounts of ROS are released (Heo et al., 2005; Sanderson et al., 2013; Siesjo et al., 1999). Imbalance between generation and degradation of ROS collectively leads to oxidative stress (Radermacher et al., 2013). Much evidence suggests that oxidative stress is a fundamental mechanism of cerebral ischemia/reperfusion injury (Chen et al., 2011; Schaller and Graf, 2004). Therefore, improving mitochondrial dysfunction and inhibiting oxidative stress are beneficial in the treatment of cerebral ischemia/reperfusion injury.

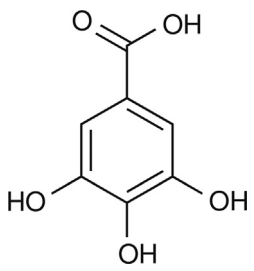

Fig. 1 – Chemical structure of Gallic acid (GA), 3, 4, 5-trihydroxybenzoic acid.

MPTP is considered to be one of the most important targets for modulating mitochondrial dysfunction following cerebral ischemic injury (Schinzel et al., 2005; Tsujimoto et al., 2006; Vaseva et al., 2012). MPTP is formed at contact sites between the inner and outer mitochondrial membranes and consists of the voltage-dependent anion channel (VDAC), the adenine nucleotide translocator (ANT), and Cyclophilin D (CypD), the mitochondrial isoform of the peptidylprolyl cis-trans isomerase cyclophilin chaperone family located on the inner membrane of mitochondria (Elrod and Molkentin, 2013). According to Halestrap, MPTP is closed during the ischemic period and opening has been shown to occur during reperfusion (Halestrap, 2009). In response to opening of MPTP, MMP is dissipated and the death effector proteins such as Cytochrome C (Cyt C) are released from the inter-membrane space, which initiate mitochondrial (type II) apoptosis (Gómez-Crisóstomo et al., 2013). In addition, the neuroprotective effect of ischemic post-conditioning or treatment with cyclosporin A (CsA) in cerebral ischemia/reperfusion injury contributes to inhibition of MPTP opening (Cho et al., 2013; Siesjo et al., 1999; Sun et al., 2012). However, as an immunosuppressant drug, the serious adverse reaction of the immune system limits its clinical application (Rezzani, 2006).

Gallic acid (GA, 3, 4, 5-trihydroxybenzoic acid, Fig. 1) is one of the most important plant polyphenolic compounds, which can be abundantly found in natural plants, tea, and red wines (Shahrzad et al., 2001). Recent research find that GA attenuates streptozotocin-induced memory deficits by inhibiting oxidative stress and activating related enzyme-dependent signaling systems (Kade and Rocha, 2013; Mansouri et al., 2013). In addition, as the impact of GA on the regulation of systems energy metabolism in rat, the relationship between GA and mitochondria has become an active topic of exploration (Shi et al., 2013). However, no study has tested the effects of GA on cerebral ischemia/reperfusion injury, and the mechanisms are also barely known. In the current study, we have investigated the potential roles and mechanisms of GA in hypoxia/reoxygenation induced by sodium hydrosulfite ($Na_2S_2O_4$) in vitro and cerebral ischemia/reperfusion induced by middle cerebral artery occlusion (MCAO) in vivo.

2. Results

2.1. *Protective effects of GA against hypoxia/reoxygenation-induced cytotoxicity in SH-SY5Y cells*

It is generally accepted that $Na_2S_2O_4$-induced hypoxia/reoxygenation elicited remarkable neurons injury (Wei et al., 2013; Zhang and Eyzaguirre, 1999). The MTT assay revealed that