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Hydrogen-rich saline attenuates neuronal ischemia–reperfusion injury by protecting mitochondrial function in rats

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ABSTRACT

Background: Hydrogen, a popular antioxidant gas, can selectively reduce cytotoxic oxygen radicals and has been found to protect against ischemia–reperfusion (I/R) injury of multiple organs. Acute neuronal death during I/R has been attributed to loss of mitochondrial permeability transition coupled with mitochondrial dysfunction. This study was designed to investigate the potential therapeutic effect of hydrogen-rich saline on neuronal mitochondrial injury from global cerebral I/R in rats.

Materials and methods: We used a four-vessel occlusion model of global cerebral ischemia and reperfusion, with Sprague–Dawley rats. The rats were divided randomly into six groups ($n = 90$): sham (group S), I/R (group I/R), normal saline (group NS), atractyloside (group A), hydrogen-rich saline (group H), and hydrogen-rich saline + atractyloside (group HA). In groups H and HA, intraperitoneal hydrogen-rich saline (5 mL/kg) was injected immediately after reperfusion, whereas the equal volume of NS was injected in the other four groups. In groups A and HA, atractyloside (15 μL) was intracerebroventricularly injected 10 min before reperfusion, whereas groups NS and H received equal NS. The mitochondrial permeability transition pore opening and mitochondrial membrane potential were measured by spectrophotometry. Cytochrome c protein expression in the mitochondria and cytoplasm was detected by western blot. The hippocampus mitochondria ultrastructure was examined with transmission electron microscope. The histologic damage in hippocampus was assessed by hematoxylin and eosin staining.

Results: Hydrogen-rich saline treatment significantly improved the amount of surviving cells ($P < 0.05$). Furthermore, hydrogen-rich saline not only reduced tissue damage, the degree of mitochondrial swelling, and the loss of mitochondrial membrane potential but also preserved the mitochondrial cytochrome c content ($P < 0.05$).

Conclusions: Our study showed that hydrogen-rich saline was able to attenuate neuronal I/R injury, probably by protecting mitochondrial function in rats.

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1. Introduction

Transient global cerebral ischemia–reperfusion (I/R) injury is one of the major complications that occurred during the perioperative period of cardiac arrest and resuscitation [1]. Cerebral I/R triggers a complex cascade of biochemical events including excitotoxicity, ionic imbalance, oxidative stress, and apoptotic-like cell death mechanisms that lead to total breakdown of cellular integrity and eventually cell death [2]. For patients undergoing craniotomy surgery, pharmacologic interventions that can protect the brain against cerebral I/R injury would be very beneficial. Over the last two decades, several neuroprotective agents have been investigated in animal models of cerebral ischemia [3,4]. Although many of these agents have been found to be neuroprotective in animal models, they have failed to be translated from the bench to the bedside [5]. Thus, there is a huge unmet medical need to develop novel therapies for acute cerebral I/R injury.

Hydrogen gas (H_2) is a new medical gas that exerts organ-protective effects through regulating oxidative stress, inflammation, and apoptosis [6–8]. H_2 reacts only with strong oxidants [9] and is often too mild to disturb metabolic oxidation–reduction reactions or to disrupt reactive oxygen species involved in cell signaling [6]. Recent data show that H_2 is beneficial to cerebral I/R injury [10,11]. Some studies have found that hydrogen-rich saline (normal saline [NS] containing a therapeutic dose of hydrogen) can ameliorate the damage of organ including lung, intestine, and brain through reducing oxidative stress [12–15]. Actually, application of hydrogen-rich saline represents an alternative mode of molecular hydrogen. The primary advantage of hydrogen-rich saline is that it is a portable, easily administered, and safe means of delivering H_2 [16]. In contrast to H_2, hydrogen-rich saline may be more suitable for clinical application. All these findings make hydrogen-rich saline a very suitable candidate to provide the protective effect on the brain against cerebral I/R injury.

The aim of the present study was to explore whether hydrogen-rich saline has protective effects on mitochondrial dysfunction in an *in vivo* model of global cerebral I/R. We investigated the effects of hydrogen-rich saline on mitochondrial dysfunction by analyzing the occurrence of mitochondrial swelling, changes in mitochondrial membrane potential, and release of proapoptotic factor cytochrome c after I/R. Our results show that hydrogen-rich saline reduces apoptotic incidence after global cerebral I/R, probably by reducing mitochondrial dysfunction.

2. Material and methods

2.1. Animal care and specifications

Male Sprague–Dawley rats from the Animal Central of Jinling Hospital were maintained at an ambient temperature of 22°C–24°C under the 12 h light–dark cycle and free access to standard rodent chow and tap water. We included only male rats in the present study because it is now well established that estrogens exert profound protective effects in animal models of focal and global ischemia [17]. The animals were fasted for 12 h before operation. The animal care and experiment were approved by the Ethics Committee of Jinling Hospital, and were performed according to the Guide for the Care and Use of Laboratory Animals approved by the National Institutes of Health.

2.2. Production of hydrogen-rich saline

Hydrogen was dissolved in NS 6 h under high pressure (0.4 MPa) to a supersaturated level with equipment provided by the Department of Diving Medicine, the Second Military Medical University, Shanghai, China. Hydrogen-rich saline (pH 6.9 ± 0.1) was prepared weekly to ensure a concentration of >0.6 mmol/L by gas chromatography as described previously [6]. Then it was stored under atmospheric pressure at 4 °C in an aluminum bag with no dead volume and was sterilized by γ radiation. The dose and time points of hydrogen-rich saline administration were selected based on our own previous study and our preliminary data [18,19].

2.3. Atractyloside

Atractyloside (Sigma–Aldrich, Beijing, China) is a natural compound that functions as a specific inhibitor of the adenine nucleotide translocase (ANT), which is a mitochondrial adenosine diphosphate or adenosine triphosphate carrier. Atractyloside is a proapoptotic ligand of ANT that induces pore formation by ANT, and results in permeabilization of the mitochondria membrane [20]. The dose and the way of atractyloside administration were from previous studies [21,22].

2.4. Induction of cerebral I/R

Transient global cerebral I/R was produced using the four-vessel occlusion (4-VO) method [23,24]. Briefly, rats weighing 280–300 g were anaesthetized (10% chloral hydrate, 400 mg/kg, intraperitoneally (i.p), and placed on a stereotaxic frame. The rat vertebral arteries were irreversibly occluded by coagulation between the first and the second cervical vertebra. Then bilateral common carotid arteries were isolated, encircled with No. 10 suture and drawn out of the back of the cervical vertebra. On the second day, while the animal was awake, the suture was tightened to block the blood flow of bilateral common carotid artery for 15 min. After that, the suture was removed and the blood flow of bilateral common carotid artery was resumed. The criteria of successful model were that rats presented unconscious, bilateral dilation of pupils, and loss of spontaneous voluntary movements and the righting reflex throughout the ischemia and initial reperfusion periods [25]. In total, 105 rats were used. Three rats died during the operation. Out of 102 rats that survived after operation, five rats showed seizure, four rats displayed respiratory failure, and three rats showed pulmonary edema after I/R (Fig. 1). In all the experiments, the rectal temperature was maintained at 37 ± 0.5 °C using a homeothermic blanket. Sham-operated animals underwent vertebral artery occlusion without the common carotid arteries occlusion.