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

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Article info

Article history:
Received 1 October 2013
Received in revised form 30 April 2014
Accepted 19 May 2014
Available online 24 May 2014

Keywords:
Global cerebral ischemia–reperfusion
Hydrogen
Mitochondria
Permeability transition
Cytochrome c
Neuroprotection

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 (H₂) is a new medical gas that exerts organ-protective effects through regulating oxidative stress, inflammation, and apoptosis [6–8]. H₂ 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 H₂ 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 H₂ [16]. In contrast to H₂, 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 artery occlusion.
2.5. **Intracerebroventricular injection**

The rats were anesthetized (10% chloral hydrate, 400 mg/kg, i.p.) and placed in a stereotaxic apparatus (Stoelting; Xiruan Company, Shanghai, China) with the incisor bar set at 3.3 mm below the interaural line. Small burr holes were made in the parietal bone to allow the insertion of the injection cannula. Briefly, atractyloside was injected bilaterally into the lateral ventricles through a stainless steel cannula (anteroposterior: −0.8 mm relative to bregma, medial to lateral: −1.4 mm relative to bregma, and dorsal to ventral: −4.0 mm below dura) by means of a Hamilton microsyringe. Body temperature was maintained at 37 ± 0.5 °C. The injection lasted 5 min and the needle with the syringe was left in place for 2 min after the injection for the completion of drug infusion.

2.6. **Experimental groups**

Animals were randomly divided into six groups (n = 15 for each group): (1) group S: sham-operated + NS (5 mL/kg, i.p.); (2) group I/R: cerebral I/R + NS (5 mL/kg, i.p.); (3) group NS: cerebral I/R + NS (5 mL/kg, i.p.) + NS (15 µL, intracerebroventricular [ICV]) injection; (4) group H: cerebral I/R + hydrogen-rich saline (5 mL/kg, i.p.) + NS (15 µL) ICV injection; (5) group A: cerebral I/R + NS (5 mL/kg, i.p.) + atractyloside (2 mmol/L, 15 µL) ICV injection; and (6) group HA: cerebral I/R + hydrogen-rich saline (5 mL/kg, i.p.) + atractyloside (2 mmol/L) + hydrogen-rich saline (5 mL/kg, i.p.) ICV injection. Intraperitoneal injection of NS or hydrogen-rich saline was administrated at the beginning of reperfusion, whereas ICV injection of NS or atractyloside was administrated 20 min before reperfusion. To perform an intraperitoneal injection, the rats must be well restrained and the needle is inserted on the mouse’s right side at about a 30° angle after disinfecting the injection site. Before injection, aspirate to make sure that the needle has not penetrated a blood vessel, the intestines, or the urinary bladder.

2.7. **Histopathologic examination**

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) after 72 h of reperfusion, then perfused transcardially with 50 mL saline. After decapitation, the brains were taken out. Cerebral cortex tissue with hippocampus CA1 was cut into 3 cm³, postfixed in the same fixative at 4°C, dehydrated, and then embedded in paraffin blocks. Coronal sections of 6 mm was cut by microtome (Leica CM3050S, Solms, Germany), and stained with hematoxylin-eosin. Cell counts in stratum pyramidale of hippocampus CA1 were carried out at ×20 magnification using a light microscope (Olympus, Tokyo, Japan). The cell with a round or oval shaped nuclei exhibiting no evidence of shrinkage or edema was scored as undamaged. Cell numbers from hippocampus CA1 were summarized in five different fields of each section by a blinded observer. The mean values from the three sections of one paraffin block were used for statistical analysis.

2.8. **Electron microscopy**

Ultrastructural changes in brain mitochondria were assessed by transmission electron microscopy. Briefly, the hippocampus was fixed with 4% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4) and postfixed with 1% osmium tetroxide. The preparations were dehydrated through an ethanol gradient, processed for Epon 812 embedding, and sectioned at a thickness of 80 nm on a rotary microtome. The ultrathin sections were stained with 4% uranyl acetate-lead citrate and examined with a Tecnai G2 Transmission Electron Microscope (FEI Company, Hillsboro, OR).

2.9. **Isolation of mitochondria**

Mitochondria was isolated according to the procedure described by Clark and Nicklas [26]. Rats were decapitated and the brains were immediately removed and homogenized in an ice-cold isolation buffer (0.25 mol/L sucrose, 1 mmol/L K-EDTA, 10 mmol/L Tris–HCl, pH 7.4) with a Teflon pestle. The homogenate was immediately centrifuged at ×2000 g for 3 min, the supernatant was centrifuged again at ×2000 g for 3 min, and the second supernatant was decanted and centrifuged at ×12,000 g for 8 min. The supernatant was discarded and the pellet was resuspended in isolation buffer without K-EDTA. Then the suspension was centrifuged at ×12,000 g for 10 min. The resulting brown mitochondrial pellet was resuspended in the same buffer. The supernatant represented the cytosolic fraction. All the previously mentioned procedures were carried out at 0°C–4°C. Cytosolic and mitochondrial fractions were stored at −20°C until use.

2.10. **Measurement of mitochondria swelling**

Mitochondrial protein concentration was quantified according to the method of Bradford using 1 mg/mL bovine serum albumin (BSA) as standard [27]. The isolated mitochondria were diluted in swelling buffer (120 mmol/L KCl, 10 mmol/L Tris–HCl, 20 mmol/L MOPS, and 5 mmol/L KH2PO4, pH 7.4) to a final concentration of 0.25 mg protein/mL. Opening of the mitochondrial permeability transition pore (mPTP) was
determined by Ca\(^{2+}\)-induced (200 \(\mu\)mol/L) mitochondrial swelling. The decrease in light scattering closely parallels the percentage of the mitochondrial population undergoing permeability transition [28]. The absorbance at 520 nm (A\(_{520}\)) was measured by spectrophotometer (UV-4802, UNICO, Shanghai, China) and recorded at 30 s intervals for 2 min before CaCl\(_2\) addition, at 15 s intervals for the first 30 s after CaCl\(_2\) addition, and at 30 s intervals for the next 6 min. Relative change in A\(_{520}\) was calculated by dividing each A\(_{520}\) by the initial value. The difference between maxA\(_{520}\) (the first relative A\(_{520}\), 2 min before adding CaCl\(_2\)) and minA\(_{520}\) (the last relative A\(_{520}\), 6 min after CaCl\(_2\) addition) was used for statistical analysis. Ca\(^{2+}\)-induced decrease in light transition (maxA\(_{520}\) - minA\(_{520}\)) closely parallels the percentage of the mitochondrial transmission values.

### 2.11. Measurement of mitochondrial membrane potential

Mitochondrial membrane potential (\(\Delta \Psi\)) was estimated by a fluorescence assay with fluorescent dye Rhodamin 123 [29]. Rhodamin 123 was a lipophilic cation and could be transported into mitochondria by negative mitochondrial membrane potential and concentrated within mitochondria matrix. \(\Delta \Psi\) could then be indirectly quantitated by changes of fluorescence in the reaction system with mitochondria supplemented (150 mM sucrose, 5 mM MgCl\(_2\), 5 mM sodium succinate, 2.7 mM rotenone, 5 mM KH\(_2\)PO\(_4\), 2 0 mM Hepes, pH 7.4). Fluorescence was evaluated at \(\lambda_{503}\) and \(\lambda_{em}527\) following uptake of Rhodamin 123 on spectramax M5 microplate reader (Molecular Device, USA).

### 2.12. Measurement of cytosolic and mitochondrial cytochrome c content

Protein concentration in each sample was determined using the Bradford method. Cytosolic and mitochondrial proteins (10 \(\mu\)g) were resolved on a 12% SDS-polyacrylamide gel at 100 V for 2 h. Proteins were then transferred to an Immobilon-P PVDF membrane (Millipore, Billerica, MA) at 300 mA for 1.5 h. Membranes were blocked using 2% BSA and then incubated with primary rabbit polyclonal antibody against cytochrome c (1:1000, Santa Cruz Biotech, Santa Cruz, CA) overnight at 4°C. Membranes were washed with TBS-T buffer and then incubated with secondary goat anti-rabbit HRP-conjugated antibodies against cytochrome c (1:6000, Bio-Rad, Hercules, CA) in 2% nonfat milk for 45 min. Blots were developed with ECL reagent (Amersham Biosciences, Piscataway, NJ) and exposed. Film was analyzed using NIH-developed software (image version 1.40). Membranes were stripped using mild stripping buffer (0.2 mM glycine, 0.003 mM SDS, 0.01% Tween-20) and blocked with 2% BSA for 2 h. Then, the membranes were probed against \(\beta\)-tubulin using primary rabbit polyclonal antibody (1:800, Santa Cruz Biotech, Santa Cruz, CA) and COX IV using primary goat polyclonal antibody (COX IV, 1:800, Santa Cruz Biotech, Santa Cruz, CA) with secondary goat anti-rabbit (1:3000, Bio-Rad, Hercules, CA) and donkey anti-goat (1:3000, Santa Cruz Biotech, Santa Cruz, CA) HRP-conjugated polyclonal antibodies respectively. The level of cytochrome c in mitochondrial and cytosolic samples in each treatment group was expressed as a ratio to \(\beta\)-tubulin (cytosolic samples) or COX IV (mitochondrial samples). Experiments were repeated two to three times.

### 2.13. Statistical analysis

Numerical data were presented as mean \pm standard deviation (SD). Statistical analysis was performed using (ANOVA) followed by Bonferroni test for individual comparisons between group means. A value \(P < 0.05\) was considered statistically significant.

### 3. Results

#### 3.1. Histological examination of the hippocampus by HE staining

In CA1 region of the S group, the cell outline was clear and the structure was compact, with abundant cytoplasm. In the I/R, NS and A groups, cells were arranged sparsely and the cell outline was fuzzy. There were more cells with euchromatin in the H group than those in the I/R group. The cell counts in the CA1 area of hippocampus in the H group were significantly higher than those in the I/R group (50.3 \pm 8.7 versus 9.2 \pm 2.5, \(P < 0.05\)). Whereas the cell counts in the HA group decreased significantly than those in the H group (32.5 \pm 7.1 versus 50.3 \pm 8.7, \(P < 0.05\)) (Fig. 2).

#### 3.2. Ultrastructural changes in the mitochondria

The mitochondria in the S group appeared to exhibit normal membrane integrity. The cristae showed no signs of swelling or injury. In the I/R group, the mitochondria lost their typical rounded or tubular morphology and exhibited an irregular shape; in addition, the matrix was electron-lucent with fragmented cristae, and extreme dilation of the intracristal space was observed. Similarly to those in the I/R group, the mitochondria in the NS and A groups was severely swollen, with disrupted inner and outer membranes and dilated intracristal space. In contrast to the I/R group, the mitochondria in the H group was less swollen and exhibited membranes integrity. In contrast to the H group, the HA group exhibited a more severe collapse of the mitochondrial membrane structures in both the inner and outer membranes (Fig. 3).

#### 3.3. Changes in mitochondrial swelling induced by Ca\(^{2+}\)

There was no significant decrease in the mitochondrial transmission values in isolated mitochondria without Ca\(^{2+}\) intervention in each group (Group S: 0.01 \pm 0.001; group I/R: 0.009 \pm 0.001; group NS: 0.011 \pm 0.001; group H: 0.01 \pm 0.001; group A: 0.012 \pm 0.00; group HA: 0.011 \pm 0.001). Compared with the S group, 200 \(\mu\)mol/L Ca\(^{2+}\) significantly increased the decrease of the mitochondrial transmission values in other groups (\(P < 0.05\)). In contrast to the I/R group, hydrogen-rich saline significantly attenuated the decrease of the mitochondrial transmission values induced by high Ca\(^{2+}\) (0.0431 \pm 0.005 versus 0.0553 \pm 0.007, \(P < 0.05\)). Compared with the H group, more severe decrease in the mitochondrial transmission
Fig. 2 – Histologic changes in the CA1 region of the hippocampus. Hematoxylin and eosin staining and cell counts per visual field in the slides. \(^aP < 0.05\), versus the S group, \(^bP < 0.05\), versus I/R group, and \(^cP < 0.05\) versus the group H. (Color version of figure is available online.)
values in the HA group (0.0483 ± 0.006 versus 0.0431 ± 0.005, \( P < 0.05 \)) (Fig. 4).

3.4. Effects of hydrogen-rich saline treatment on mitochondrial membrane potential (\( \Delta \Psi \))

In contrast to the S group, \( \Delta \Psi \) in the other groups was significantly decreased (\( P < 0.05 \)). Compared with the I/R group, \( \Delta \Psi \) in the H group was higher (170 ± 7 versus 146 ± 9, \( P < 0.05 \)). In contrast to the H group, \( \Delta \Psi \) in the HA group was significantly decreased (157 ± 10 versus 170 ± 7, \( P < 0.05 \)) (Fig. 5).

3.5. Effects of hydrogen-rich saline treatment on translocation of cytochrome c

Analysis of blot intensity revealed significantly higher levels of cytochrome c in the cytosol of I/R, NS, A, H and HA rats compared to S rats (\( P < 0.05 \)). In contrast to the I/R group, the level of cytochrome c in the cytosol in the H group was lower (0.542 ± 0.042 versus 0.905 ± 0.022, \( P < 0.05 \)). The level of cytosolic cytochrome c was higher in the HA group compared with the H group (0.857 ± 0.025 versus 0.542 ± 0.042, \( P < 0.05 \)) (Fig. 6). In mitochondrial samples, levels of cytochrome c of I/R, NS, A, H and HA rats were...
lower compared to S rats ($P < 0.05$). Compared with the I/R group, the level of cytochrome c was higher in the H group ($0.782 \pm 0.112$ versus $0.402 \pm 0.075$, $P < 0.05$). In contrast to the H group, the level of cytochrome c was lower in the HA group ($0.498 \pm 0.082$ versus $0.782 \pm 0.112$, $P < 0.05$) (Fig. 7).

4. Discussion

This study was designed to address the potential involvement of mitochondrial mediator in the neuroprotection of hydrogen-rich saline against transient cerebral ischemia. We found the following: 1) hydrogen-rich saline protected mitochondrial function from ischemic insult, as indicated by hyperpolarized mitochondrial membrane potential (MMP) during reperfusion; 2) hydrogen-rich saline significantly attenuated mPTP opening in cerebral I/R animals during reperfusion, and likewise, isolated mitochondria demonstrated a reduced sensitivity to $Ca^{2+}$-induced mPTP opening in vitro; and 3) pharmacologically opening of the mPTP with atractyloside abrogated the hydrogen-rich saline-induced attenuation in mPTP opening in vitro and ultimately reduced...
release of pro-apoptotic factor cytochrome c following global cerebral I/R.

Our histological data showed that hydrogen-rich saline significantly increased the number of surviving cells in the CA1 area of hippocampus in the I/R rats. This result was consistent with the findings that inhalation of 2% H₂ reduced the infarct size in the cerebral ischemic/reperfusion rats [6], and findings that 2% H₂ reduced apoptosis in the brain of neonate rats [8].

The mPTP is believed to comprise of three core components: a voltage-dependent anion channel in the outer mitochondrial membrane, an ANT in the inner mitochondrial membrane, and cyclophilin D in the mitochondrial matrix. ANT has two conformational forms: m and c. In m-form, it functions as a nucleotide transporter; while in c-form it functions as the mPTP channel when adenine nucleotide binding is antagonized [30,31]. Cyclophilin D, a peptidyl-prolyl cis-trans isomerase, could alter the conformation of ANT from m-form to c-form in the presence of high Ca²⁺. Mitochondrial respiration generates oxidant by-products that are quickly quenched by endogenous antioxidants. A dramatic increase in oxygen during reperfusion sends the mitochondria into overdrive, tipping the oxidant to antioxidant ratio in favor of the former. Oxidative stress is an important underlying factor in cell death during cerebral I/R [32,33] by producing DNA lesions and interfering with protein function [34]. Changes in gene and protein expression are reported to occur as early as 15 min from the onset of reperfusion [35] and tissue damage occurs within 20 min of reperfusion [36]. Additionally, an increase in Ca²⁺ permeability results in the loss of mitochondrial membrane potential and eventual rupture of the mitochondrial membranes. These events are generally regarded as the mitochondrial permeability transition (MPT) and precede apoptosis and necrosis [37]. We found that hydrogen-rich saline treatment preserved mitochondrial integrity, morphology and function in our rat model.

The mitochondrial morphometric analysis in our study suggested that hydrogen-rich saline prevented mitochondrial swelling during cerebral I/R, and provides evidence for the mitochondrial-protective properties of hydrogen-rich saline at the cellular level. Swollen mitochondria are associated with a decrease in light absorbance [38]. A significant decrease in light absorbance by I/R brains suggests that reperfusion was severely detrimental to mitochondrial integrity. Mitochondrial swelling plays a crucial role in cell injury as it is affected by several processes critical to the I/R cascade including mitochondrial electron transport, production of reactive oxygen species, cytochrome c release, mechanical signaling pathways, and opening of MPT. Mitochondrial rupture occurs in the last phase of mitochondrial swelling [39]. Thus morphometric and spectrophotometric analyses of mitochondrial size allow a quantitative measure of the degree of cell injury sustained during I/R.

The presence of cytochrome c in the cytoplasm and the mitochondria was assayed by Western blotting. Rats in the group H have high capability to preserve cytochrome c in the mitochondria, which contributes to preserve mitochondria function and prevent cytochrome c related apoptosis after I/R. Rats in the group S exhibited high cytochrome c content in the mitochondria with traces of cytochrome c in the cytoplasm. Groups I/R, NS and A exhibited a marked increase in cytosolic cytochrome c content accompanied by a subsequent decrease of cytochrome c in the mitochondria. Group H also had marked increases in cytosolic cytochrome c; however, mitochondrial cytochrome c levels were significantly higher than in I/R rats. In addition, necrotic cell death mechanisms can also target the mitochondria after I/R [40]. This could explain the discrepancy observed between I/R and H of cytosolic cytochrome c expression.

In summary, our study demonstrated that hydrogen-rich saline protects mitochondria from cerebral I/R injury. The neuroprotection appears to be mediated by the inhibition of mPTP opening. Since accumulating evidence has suggested that hydrogen-rich saline exerted protective effects in animal models of organ and tissue ischemia, inflammation, trauma, Alzheimer’s disease and other degenerative diseases of the central nervous system, which might provide an effective treatment for the I/R related diseases. Although the relationship between hydrogen-rich saline and mPTP needs to be clarified in future studies, a focus should be placed on mitochondria that play a major role in the hydrogen-rich saline-induced neuroprotection.

Acknowledgment

This work was supported by National Natural Science Foundation of China (No. 81271216 and No. 81300946).

The authors thank Dr. Xue-jun Sun, from the Department of Diving Medicine, the Second Military Medical University, Shanghai, China, for providing hydrogen-rich saline.

Authors’ contributions: Y.C. and M.D. conceived and designed the experiments. Y.C., M.J., and J.Y. analyzed the data. Y.C. and H.C. contributed reagents/materials/analysis tools. Y.C., H.Z., M.J., and M.D. wrote the paper. All authors reviewed and provided their comments and approved the final version on this article.

Disclosures

The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in the article. The authors have no conflicts of interest to declare.

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