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Hydrogen-rich water protects against ischemic brain injury in rats by regulating calcium buffering proteins

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ABSTRACT

Hydrogen-rich water (HRW) has anti-oxidant activities, and it exerts neuroprotective effects during ischemia-reperfusion brain injury. Parvalbumin and hippocalcin are two calcium buffering proteins, which are involved in neuronal differentiation, maturation and apoptosis. The aim of this study was to investigate whether HRW could moderate parvalbumin and hippocalcin expression during ischemic brain injury and glutamate toxicity-induced neuronal cell death. Focal brain ischemia was induced in male Sprague–Dawley rats by middle cerebral artery occlusion (MCAO). Rats were treated with H_2O or HRW (6 ml/kg per rat) before and after MCAO, and cerebral cortical tissues were collected 1, 7 and 14 days after MCAO. Based on our results, HRW treatment was able to reduce brain infarct volume and improve neurological function following ischemic brain injury. In addition, HRW prevented the ischemia-induced reduction of parvalbumin and hippocalcin levels in vivo and also reduced the glutamate toxicity-induced death of neurons, including the dose-dependent reduction of glutamate toxicity-associated proteins in vitro. Moreover, HRW attenuated the glutamate toxicity-induced elevate in intracellular Ca^{2+} levels. All

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these results suggest that HRW could protect against ischemic brain injury and that the maintenance of parvalbumin and hippocalcin levels by HRW during ischemic brain injury might contribute to the neuroprotective effects against neuron damage.

1. Introduction

Brain ischemia can induce serious neuron damage and lead to a loss of neuronal function. Brain ischemia induces neuron death through the production of reactive oxygen species (ROS) and increased intracellular Ca^{2+}, which leads to neurodegeneration (Shepherd, 2001; Lyden et al., 2006). Previous studies have shown crosstalk and reciprocal interactions between Ca^{2+} and ROS, and there is considerable evidence that ROS and the reduction–oxidation state can significantly modulate Ca^{2+} signaling (Feissner et al., 2009; Peng and Jou, 2010).

Ohsawa et al. (2007) have reported that molecular hydrogen acts as a novel antioxidant and can selectively reduce •OH and ONOO– without affecting physiological ROS. Other previous studies have shown that hydrogen-rich water (HRW) can attenuate the overproduction of ROS and protect neuron against oxidative stress (Fu et al., 2009; Gu et al., 2010; Li et al., 2010; Hou et al., 2012). Hydrogen-rich water also shows a neuroprotective effect during focal cerebral ischemia-reperfusion injury induced by middle cerebral artery occlusion (MCAO) and decreases excitotoxic agent-induced damage (Liu et al., 2011). Subsequent studies have confirmed that the consumption of hydrogen reduces oxidative stress in a diverse range of disorders and organ systems including the digestive (Chen et al., 2011; Zheng et al., 2009), cardiovascular (Hayashida et al., 2008; Nakao et al., 2010) and respiratory systems (Mao et al., 2009). These studies strongly suggest the potential for molecular hydrogen to be an effective therapeutic and preventive antioxidant.

Rosenthal et al. (1987) have shown that an increase in the influx of intracellular Ca^{2+} can induce neuron death, whereas the blockade of Ca^{2+} preserves neurons against ischemic injury. The regulation of intracellular Ca^{2+} is key to determining the fate of neuronal cells (Heizmann and Hunziker, 1991; Baimbridge et al., 1992), and calcium-binding proteins play important roles in physiological processes, including cell cycle regulation, the organization of microtubules, and muscle contraction (Heizmann and Hunziker, 1991; Baimbridge et al., 1992). Parvalbumin is one type of calcium-buffering protein and is structurally related to trophonin C and calmodulin (Cates et al., 2002; Heizmann and Berchtold, 1987). Hippocalcin, which is also involved in calcium signaling, is a critical member of the neuronal calcium sensor family and has four separate calcium-binding sites (Lindholm et al., 2002). Parvalbumin and hippocalcin can bind to Ca^{2+} released into the cytoplasm via high affinity Ca^{2+}-binding domains. Parvalbumin and hippocalcin play important roles in the maintenance of Ca^{2+} homeostasis under physiological and pathological conditions; thus, these proteins are regard as Ca^{2+} buffer proteins (Cates et al., 2002; Heizmann and Berchtold, 1987; Lindholm et al., 2002). Calcium overloading can induce neuron damage, and an increase in intracellular Ca^{2+} levels is a feature of several brain disorders (Bleakman et al., 1993; Manev et al., 1989). Taking into consideration the crosstalk and reciprocal interactions between Ca^{2+} and ROS, we hypothesized that there could be a relationship between the neuroprotective effects of HRW against ischemic brain injury and the regulation of the expression of calcium buffering proteins. However, little is known about the effects of hydrogen-rich water on parvalbumin and hippocalcin expression in the brain and in neurons following ischemic injury. Thus, we investigated the neuroprotective effects of HRW and the expression of calcium buffering proteins in the presence of hydrogen-rich water during MCAO-induced ischemic brain injury and during glutamate toxicity-induced neuron damage.

2. Results

2.1. *HRW treatment reduces infarct volume and improves neurological score*

To confirm the neuroprotective effects of HRW against ischemic cerebral injury, we analyzed the infarct volume at 1 d following ischemic brain injury. HRW treatment significantly reduced the infarct volume compared with that of the H_2O-treated animals in the MCAO groups (Fig. 1A). The ischemic lesion volume was $99.15 \pm 8.45\ mm^3$ and $60.22 \pm 7.27\ mm^3$ in the MCAO+H_2O and MCAO+HRW animals, respectively (Fig. 1B). The neurological scores of rats receiving MCAO improved following HRW treatment. The neurological scores were significantly reduced in the MCAO+H_2O group ($p < 0.05$ vs. sham), and HRW treatment at 1, 7 and 14 d after brain injury significantly improved neurological function ($p < 0.01$ vs. MCAO+H_2O group), although neurological dysfunction was still observed ($p < 0.05$ vs. sham group) (Fig. 1C).

2.2. *HRW prevented the injury-induced decrease of parvalbumin and hippocalcin expression after ischemic brain injury in vivo*

Changes in parvalbumin and hippocalcin levels in the H_2O- and HRW-treated animals were analyzed by Western blot and immunohistochemical methods. From our Western blot analysis, at 1, 7 and 14 d following ischemic brain injury, the expression levels of parvalbumin in the cerebral cortices of the H_2O-treated animals were lower than the HRW-treated animals (Fig. 2A). At the same time point, the hippocalcin protein expression levels in the hipocampus of the H_2O-treated animals were also lower than the HRW-treated animals (Fig. 2B). In addition, immunohistochemical staining showed that the numbers of parvalbumin- and hippocalcin-positive cells were significantly decreased in the cerebral cortices of the MCAO+H_2O animals compared to those in the sham+H_2O group. HRW treatment prevented the injury-induced decrease in the numbers of parvalbumin- and