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Inhalation of high concentrations of hydrogen ameliorates liver ischemia/reperfusion injury through A_{2A} receptor mediated PI3K-Akt pathway

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Abstract

Background and aims

This study explored the hepatoprotection of high concentrations of hydrogen (HCH) inhalation in a mouse hepatic ischemia/reperfusion (I/R) injury model and the potential mechanism.

Methods

To explore the role of the PI3K-Akt pathway in the hepatoprotection of HCH, C57BL/6 mice were randomly divided into five groups: Sham, I/R, I/R+HCH, LY294002 (PI3K inhibitor)+I/R+HCH, and LY+I/R groups. Mice received inhalation of 66.7% hydrogen and 33.3% oxygen for 1 h immediately after surgery. LY294002 was intravenously injected at 10 mol/kg. To explore whether PI3K-Akt pathway activation was mediated by the A_{2A} receptor, additional four groups were included: ZM241385 (A_{2A} receptor antagonist)+I/R+HCH, ZM241385+I/R, bpv(HOpic) (PTEN inhibitor)+I/R, and ZM241385+bpv+I/R+HCH. Six hours after I/R, serum biochemistry, histological examination, Western blotting, and immunohistochemistry were performed to evaluate the hepatoprotection of HCH and the role of the PI3K-Akt pathway and A_{2A} receptor in this protection.

Results

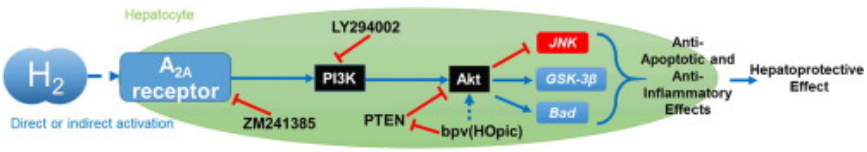
Liver dysfunction, hepatic pathological injury, infiltration of inflammatory cytokines, and hepatocyte apoptosis were observed after hepatic I/R, accompanied by inhibition of the PI3K-Akt pathway. HCH

significantly improved liver function, reduced serum inflammatory cytokines, and inhibited hepatocyte apoptosis, and also induced the PI3K-Akt pathway activation. In the presence of LY294002 or ZM241385, the protective effects of HCH were markedly attenuated, but the effects of ZM241385 were reversed by bpv(HOpic).

Conclusion

Our findings indicate that HCH may protect the liver against I/R injury through the A_{2A} dependent PI3K-Akt pathway.

Graphical abstract



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Introduction

Hepatic ischemia and reperfusion (I/R) injury is a phenomenon in which cellular damage is induced by hypoxia following the restoration of blood flow and oxygen delivery after transplantation surgery, tissue resections, and hemorrhagic shock [1]. Pathologically, liver I/R injury may cause hepatocyte swelling, hepatocyte vacuolization, endothelial cell disruption, neutrophil infiltration, and hepatocyte necrosis and apoptosis [2]. Hepatic I/R injury may significantly compromise graft survival and postoperative liver function, resulting in a high mortality. It affects liver function and significantly increases the risk to the circulatory system and respiratory system [3]. To date, numerous studies have explored the treatment and prevention of liver I/R injury [4], [5], [6], but no effective strategies have been developed. Therefore, liver I/R injury is an important clinical problem that requires further study.

In the pathogenesis of hepatic I/R injury, oxidative stress and inflammation are the two major mechanisms, and some strategies targeting reactive oxygen species (ROS) and inflammation are used for the treatment of hepatic I/R injury [7], [8].

Hydrogen is the simplest molecule in nature. It not only exists in nature but also can be generated in the human intestine. Traditionally, it is believed to function as an inert gas at body temperature in mammalian cells because it cannot react with biological compounds, including oxygen (O_2) gas, in the absence of catalysts at body temperature. Thus, hydrogen has been used during deep diving for the prevention of

nitrogen narcosis [9]. In recent years, hydrogen has been found to protect against I/R injury to the brain [10], heart [11], kidney [12], liver [13], [14], and retina [15], mainly by scavenging hydroxyl radicals, inhibiting inflammation, and suppressing cell apoptosis. However, in most studies, 2% or 4% hydrogen gas was used [10]. Recently, our group treated diseases with high concentrations of hydrogen (HCH) gas (67% H_2, 33% O_2) in animal models [15], [16]. The mixed gas is produced using an AMS-H-01 hydrogen oxygen nebulizer (Asclepius, Shanghai, China), which can produce H_2 and O_2 by electrolyzing water [15], [16]. Whether HCH is also protective towards hepatic I/R injury and, if so, the mechanism underlying the hepatoprotection of HCH remains unclear.

The phosphatidylinositol-3-kinase (PI3K)/Akt pathway is important for cell survival, and activation of the PI3K/Akt pathway has been found to protect cells against injury. Previous studies have shown that PI3K/Akt pathway activation is important for protection against I/R injury [17], [18]. In addition, studies have shown that A_{2A} receptor is involved in hepatic protective effect [19], and the PI3K/Akt pathway can be regulated by A_{2A} receptor [20].

This study was performed to explore the protective effects of HCH on hepatic I/R injury and to examine the role of the A_{2A} receptor and PI3K/Akt pathway in the protective effects of HCH.

Section snippets

Animals

A total of 96 male C57BL/6 wild-type (WT) mice aged 8–10 weeks and weighing 20–25 g were purchased from the Experimental Animal Center of the Second Military Medical University, Shanghai, China. Mice were housed in a specific pathogen free environment with a 12-h/12-h light/dark cycle and given *ad libitum* access to food and water. All procedures were performed according to the recommendations of the Committee of the Care and Use of Laboratory Animals at the Second Military Medical University.…

Inhibition of the PI3K/Akt pathway activation attenuates the hepatoprotective effects of HCH on hepatic I/R injury

Serum ALT in the I/R group (8351.583±1310.280 IU/L) increased significantly compared with the Sham group (25.833±8.747 IU/L) as shown in Fig. 2A. HCH significantly decreased I/R induced increases in serum ALT (1739.583±419.826 IU/L). LY294002, an inhibitor of PI3K, significantly increased serum ALT after HCH treatment (7099.417±973.170 IU/L). Moreover, there was a significant difference in the serum ALT between I/R group and LY+I/R+HCH group. However, there was no significant difference in serum…

Discussion

In this study, we investigated the hepatoprotective effects of HCH in a mouse model. Our results showed that, after liver I/R injury, inhalation of hydrogen at a high concentration (66.7% H_2) improved the liver pathology and liver function, reduced the oxidative stress and inflammation in the liver, and inhibited the apoptosis of hepatocytes after I/R injury, which were, at least partially, related to the activation of A_{2A} receptor mediated PI3K/Akt pathway because inhibition of this pathway…

Conflict of interest

There is no conflict of interest in this paper.…

Author contribution statement

Li H., Che O. and Liu W. wrote the main manuscript. Ye Z. prepared Fig. 1, Fig. 2. Li H. finished Fig. 5, Fig. 6. Sun X. designed all figures. Zhang R. revised the whole manuscript. Zhang N. finished Fig. 3, Fig. 4. Huang J. designed the supplements 1.…

Acknowledgements

This study was supported by the National Natural Science Foundation of China – China (No. 81371316). The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/4TMRNL ↗…

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2022, Respiratory Physiology and Neurobiology

Citation Excerpt :

...Anti-sirt1, anti-NF-κB and anti-CAT antibodies were obtained from Abcam (MA, USA), and the secondary antibodies conjugated with HRP were obtained from Rockland (PA, USA). A mixture of 66.7 % hydrogen (H2) and 33.3 % oxygen (O2) was produced through water electrolysis in the hydrogen oxygen nebulizer (AMS-H01, Asclepius, Shanghai, China) as previously described (Cui et al., 2016; Li et al., 2017). The critical role of sirt1 in the process of ALI has been well demonstrated (Fu et al., 2019)....

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2021, Life Sciences

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> ...When NRF2 pathway is activated, NRF2 dissociates from Kelch-like ECH-associating protein-1 (KEAP1) and into the nucleus, interacts with small Maf and Jun dimers and promotes downstream antioxidant gene transcription, finally inhibits the expression of Caspase-3 [36]. Mild hypothermia pretreatment and inhaling high concentration hydrogen really act to ameliorate HIRI through phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway, demonstrated by Xiao et al. [37] and Li et al. [38]. The major mechanisms included [39]: ① inhibiting forkhead in rhabdomyosarcoma (FKHR), NF-κB and Yes-associated protein (YAP) and promoting the activity of transcription factors such as cAMP-response element binding protein (CREB) and murine double minute gene-2 (MDM2); ② inhibiting BAD phosphorylation directly; ③ inhibiting Caspase-9 phosphorylation directly....

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2019, International Immunopharmacology

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> ...Recently, a dose-response effect of hydrogen gas inhalation was observed and higher concentrations of hydrogen (22% and 41.6%) showed a better outcome in preventing cigarette smoke-induced lung injury [8]. And the inhaled 66.7% hydrogen and 33.3% oxygen produced by the hydrogen/oxygen nebulizer are aerosol mixtures, which may avoid the risk of explosion for hydrogen at this high concentration [20,21]. Our result showed that water electrolysis-derived hydrogen, the high concentration of hydrogen, is a potential new resource for prevention and treatment of PM2.5-induced lung injury....

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> ...It is well known that phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt) signaling pathway regulates multiple cellular activities including survival, proliferation and metabolism [57]. A growing body of literature verified that activation of the PI3K/Akt signaling pathway significantly attenuated I/R-induce apoptosis [58–60]. Yang et al. showed that terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive cells were considerably increased after MCAO, which was significantly attenuated by berberine [19]....

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1 Both contributed to this paper equally.

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