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Molecular hydrogen attenuates hypoxia/reoxygenation injury of intrahepatic cholangiocytes by activating Nrf2 expression

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Abstract

Hypoxia/reoxygenation (H/R) injury of cholangiocytes causes serious biliary complications during hepatobiliary surgeries. Molecular hydrogen (H_2) has been shown to be effective in protecting various cells and organs against oxidative stress injury. Human liver cholangiocytes were used to determine the potential protective effects of hydrogen against cholangiocyte H/R injury and explore the underlying mechanisms. We found that H_2 ameliorated H/R-induced cholangiocytes apoptosis. Our study revealed that H_2 activated NF-E2-related factor 2 (Nrf2) and downstream cytoprotective protein expression. However, the protective function of H_2 was abolished when Nrf2 was silenced. Apoptosis in cholangiocytes isolated from a rat model of liver ischemia/reperfusion injury indicated that H_2 significantly attenuates ischemia/reperfusion cholangiocyte injury *in vivo*. In conclusion, our study shows that H_2 protects intrahepatic cholangiocytes from hypoxia/reoxygenation-induced apoptosis *in vitro* or *in vivo*, and this phenomenon may depend on activating Nrf2 expression.

Introduction

Ischemia/reperfusion (I/R) injury is a severe deleterious postsurgical complication in liver transplantation, partial hepatic resection and trauma settings (Liu et al., 1996). Hypoxia/reoxygenation (H/R) is the root of I/R injury and has been regarded as an important model to study the pathophysiology of I/R injury *in vitro* (Monno et al., 2010, Pardo and Tirosh, 2009). During hepatobiliary surgeries, hepatic I/R injury or H/R injury is caused by interruption of hepatic blood inflow, which result in massive liver parenchyma injury (Jaeschke, 2003). Several studies have demonstrated that generation of reactive oxygen species (ROS),

proinflammatory cytokines and oxidants are associated with hepatic I/R or H/R injury (Abu-Amara et al., 2010, Ren et al., 2011, Jaeschke, 1991, Jaeschke, 1998). Regulation of ROS and proinflammatory cytokines is suggested to be an important therapeutic strategy for hepatic H/R injury.

Cholangiocytes, also known as biliary epithelial cells, are an important cell type in the liver parenchyma, in addition to hepatocytes. As a kind of important but delicate cells, cholangiocytes are also sensitive to oxidative stress and involved in the process of cholangiopathies (Mizuguchi et al., 2014, Brain et al., 2013). Although an increasing number of studies have investigated intracellular mechanisms and therapeutic targets to prevent H/R-induced hepatocyte injury (Pardo and Tirosh, 2009, Chen et al., 2012, Spencer et al., 2013), only a few have focus on H/R-induced cholangiocyte injury. It's worth noting that H/R injury also induces cholangiocytes necrosis or apoptosis and results in serious complications, such as biliary obstruction or stricture (Sanchez-Urdazpal et al., 1992, Xu et al., 2004).

Several studies have demonstrated that molecular hydrogen (H_2) is an important gaseous signaling molecule with antioxidant and anti-inflammatory protective effects on cells and organs (Huang et al., 2010). H_2 can act as a scavenger to selectively alleviate ROS and activate potent cellular protective effects. Recent research has revealed that H_2 protected tissue from oxidative stress injury via activating NF-E2-related factor 2 (Nrf2) expression (Kawamura et al., 2013). H_2 has been shown to be effective in protecting various cells, including hepatocytes, and organs against I/R injury (Ohsawa et al., 2007, Fukuda et al., 2007, Nakao et al., 2010), but its exact effect on cholangiocytes with H/R injury is still unclear.

To avoid the influence from other cells and to accurately investigate underlying mechanisms, our study explored the potential function of H_2 on intrahepatic cholangiocytes *in vitro* and *in vivo*.

Section snippets

Isolation of cholangiocytes

After informed consent was obtained from patients and the tissue acquisition protocol was approved by the Zhejiang University Institutional Review Board, liver explant tissue from a female patient with hepatic hemangioma without other hepatic diseases was obtained. Cholangiocytes were isolated from the normal liver tissue samples with minor modifications to previously described protocols (Tabibian et al., 2014, Coots et al., 2012). Briefly, about 5g of liver tissue was cut into small pieces…

Isolated cholangiocytes express cholangiocyte-specific markers and are highly purified

Following cholangiocyte-specific magnetic bead-purification of cells isolated from liver, cholangiocyte marker expression was re-examined to confirm the presence of cholangiocytes. Immunofluorescence staining revealed that the isolated cells were CK19 positive, indicating a highly purified population of intrahepatic cholangiocytes (Fig. 1A). Similar to the gall bladder epithelium, isolated cells expressed higher cholangiocyte marker or cell adhesion molecule mRNA expression, including CK7, GGT, …

Discussion

Liver I/R injury cannot be completely avoided during many hepatobiliary surgeries, such as liver transplantation and hepatectomy. Previous studies have released that cholangiocytes produce high levels of ROS during the H/R process (Noack et al., 1993). I/R injury of the bile duct induces serious postoperative complications, such as nonanastomotic biliary strictures and liver allograft failure, after hepatobiliary surgeries (Demetris et al., 2006). Although cholangiocytes are more resistant to…

Conflict of interest

The authors declare that there are no conflicts of interest.…

Acknowledgment

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K.J. Livak *et al.*
Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method
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Amelioration of rat cardiac cold ischemia/reperfusion injury with inhaled hydrogen or carbon monoxide, or both

J. Heart Lung Transplant. (2010)

I. Ohsawa *et al.*

Consumption of hydrogen water prevents atherosclerosis in apolipoprotein E knockout mice

Biochem. Biophys. Res. Commun. (2008)

J.H. Tabibian *et al.*

Characterization of cultured cholangiocytes isolated from livers of patients with primary sclerosing cholangitis

Lab. Invest. (2014)

K. Takei *et al.*

Cynaropicrin attenuates UVB-induced oxidative stress via the AhR-Nrf2-Nqo1 pathway

Toxicol Lett. (2015)

P. Yu *et al.*

Hydrogen-rich medium protects human skin fibroblasts from high glucose or mannitol induced oxidative damage

Biochem. Biophys. Res. Commun. (2011)

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Mechanism of hepatobiliary toxicity of the LPA<inf>1</inf> antagonist BMS-986020 developed to treat idiopathic pulmonary fibrosis: Contrasts with BMS-986234 and BMS-986278

2022, Toxicology and Applied Pharmacology

Citation Excerpt :

...Assays were repeated 3 times for HepG2 cells and 2 times for hBEC. BMS-986020 was evaluated at 10, 30, and 100 μM for 2 h, using dichlorofluorescein fluorescence as described previously (van de Wier et al., 2013; Yu et al., 2015). Furosemide (1 mM) and H2O2 (3 mM) were used as a positive controls....

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Molecular Hydrogen Protects Human Melanocytes from Oxidative Stress by Activating Nrf2 Signaling

2020, Journal of Investigative Dermatology

Citation Excerpt :

...The protective effects of H2 were abolished by Nrf2 silencing, indicating that Nrf2 signaling was essential for the survival and function of melanocytes under oxidative stress. Our findings are highly consistent with previous reports showing that Nrf2 signaling is required for the protective effect of H2 in various cells and tissues in response to different stressors (Kawamura et al., 2013; Murakami et al., 2017; Tamaki et al., 2016; Yu et al., 2015). We previously showed that

H2 could suppress the formation of pressure ulcers due to cutaneous ischemia and reperfusion by activating Nrf2 expression (Fang et al., 2018)....

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Recovery of the Cholangiocytes After Ischemia and Reperfusion Injury: Ultra-Structural, Hystological and Molecular Assessment in Rats

2018, Journal of Clinical and Experimental Hepatology

Citation Excerpt :

...For decades, innumerable substances have been employed to ameliorate hepatic I/R, mostly focused on the hepatocyte.33,34 Few studies proposed treatment for cholangiocyte damage caused by I/R.9,35,36 We suggest that BAX inhibitor drugs might prevent apoptosis in this setting and could be tested as a treatment for I/R. Caution in this setting is needed since over induction of apoptosis might deregulate the cell cycle and therefore increase the cancer risk.37 In summary, we have found that I/R injury to cholangiocytes occurs from 6 to 24-h after reperfusion and can not be clearly identified by regular optic microscopy....

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Linarin could protect myocardial tissue from the injury of Ischemia-reperfusion through activating Nrf-2

2017, Biomedicine and Pharmacotherapy

Citation Excerpt :

...Meanwhile, oxidative stress also could aggravate cell apoptosis, calcium overload and inflammatory cytokines secretion which could promote the progression of I/R injury. At present, drug treatment for reducing oxidative stress was deemed to be an ideal method to avoid or reduce the injury of I/R [17–19]. Nuclear factor erythroid2-related factor 2(Nrf-2) is an important regulator for anti-oxidation....

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Septin 2 accelerates the progression of biliary tract cancer and is negatively regulated by mir-140-5p

2016, Gene

Citation Excerpt :

...HCCC-9810, SSP25, RBE and GBC-SD cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS), 100 IU/ml penicillin and 100 μg/ml streptomycin. Human normal cholangiocytes were isolated from the normal liver tissue samples using magnetic bead-purification (Miltenyi Biotec, Germany) and were confirmed cholangiocytes phenotype as previously described (Yu et al., 2015). The normal cholangiocytes were cultured in DMEM/F12 medium (Invitrogen) with 10% FBS, 100 mg/ml penicillin–streptomycin, 2 mM glutamine, 10 ng/ml epidermal growth factor (Peprotech, Rocky Hill, NJ, USA), 20 ng/ml HGF (Peprotech), 2 mg/ml hydrocortisone, 10 ng/ml choleratoxin, 2 nM tri-iodothyronine (all from Sigma, St. Louis, MO, USA), and 1 × ITS (Insulin, Transferrin, Selenium Solution, Invitrogen)....

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Developmental Biology, Volume 396, Issue 2, 2014, pp. 201-213

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1 These authors contributed equally to this work.

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