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ORIGINAL ARTICLE

Hydrogen-rich saline attenuates postoperative liver failure after major hepatectomy in rats

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Summary
Background/Aims: A major hepatectomy occasionally lead to acute liver failure and death. We demonstrated the anti-oxidative and anti-inflammatory effects and functional mechanisms of hydrogen-rich saline (HS), a novel antioxidant, on an experimental model of rats after a partial hepatectomy (PH).
Methods: The rats underwent a 90% hepatectomy. HS was given intraperitoneally after the operation and every 8 hours after.
Results: HS markedly improved the survival rate of two experimental groups after the massive hepatectomy and inhibited increases in serum levels of TBIL, DBIL, ALT and AST. The histopathological analysis demonstrated that HS attenuated inflammatory changes in the liver. HS administration markedly lowered the massive hepatectomy induced elevation of the serum hyaluronic acid (HA) concentrations. HS inhibited the formation of one of the markers of oxidative damage, malondialdehyde (MDA), and increased the activities of superoxide dismutase

Abbreviations: PH, partial hepatectomy; HS, hydrogen-rich saline; ROS, reactive oxygen species; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; HA, Hyaluronic Acid; HE, hematoxylin and eosin; MDA, malondialdehyde; SOD, superoxide dismutase; TNF-a, tumor necrosis factor-a; IL-6, interleukin-6; HMGB-1, high-mobility group box-1; NF-κB p65, nuclear factor kappa B p65.

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(SOD) in liver tissue. In the HS-treated group, increases in inflammatory cytokines, such as TNF-α, IL-6 and HMGB-1, were inhibited in the liver tissue. The NF-κB p65 staining revealed that HS inhibited the activation of the transcription factor nuclear factor kappa B (NF-kB).
Conclusions: HS attenuates the massive hepatectomy induced liver injury not only by attenuating oxidative damage, but also by reducing the production of inflammatory cytokines, such as TNF-α, IL-6 and HMGB-1, in part through the inhibition of NF-kB activation.

Introduction

Liver failure is a serious and often fatal complication of patients with an extended hepatectomy. After a massive hepatectomy, reactive oxygen species (ROS) are involved directly and indirectly [1] in the pathophysiology, and oxidative stress has been regarded as a major contributor to the development of postoperative liver failure [2,3]. Previous studies have shown that antioxidants can effectively prevent hepatic damage by inhibiting free radical generation or scavenging for free radicals generated by other biochemical reactions [4—6].

Molecular hydrogen (H2) possesses anti-oxidative and anti-inflammatory properties by selectively neutralizing cytotoxic reactive oxygen species (ROS), such as •OH and ONOO— [7]. As a novel antioxidant, H2 has a number of advantages. H2 reacts with only the strongest oxidants, which means that it is mild enough not to disturb metabolic oxidation-reduction reactions or disrupt the ROS involved in cell signaling [8]. Moreover, H2 has favorable distribution characteristics and can penetrate biomembranes and diffuse into the cytosol, mitochondria and nucleus [9]. It has been reported that inhaled hydrogen gas can protect the brain, liver and heart against ischemia-reperfusion (I/R) injury via its antioxidant effect [7,10,11]. Hydrogen also suppresses the inflammation induced in tissue-destructive diseases, such as hepatitis, colitis and intestinal graft injury [12—14]. These findings indicate that the beneficial effects of H2 could be used for injuries in the liver or other organs.

H2 could be a very effective antioxidant. However, in clinical applications, H2 gas inhalation is not convenient and is dangerous because of its flammable and explosive properties. In contrast to H2 gas, HS (H2 gas dissolved in physiologic saline at a supersaturated level; HS) is safe and easy to use in a clinical environment. The aim of this study is to examine the potential application of HS therapy to prevent liver failure and death after a massive hepatectomy.

Materials and methods

Animals

Adult male Sprague-Dawley rats weighing 220—250 g were obtained from the Experimental Animal Center of the Second Military Medical University. These rats were maintained under controlled conditions (25 °C, 55% humidity and a 12-h day/night rhythm) and fed standard laboratory food. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Second Military Medical University (Shanghai, China).

Rat model of the major hepatectomy

Rats were fasted for 12 hours with water ad libitum before the operation. Each rat was weighed and underwent general anesthesia with sevoflurane in ether gas. Following a midline incision, the liver was exposed, and the hepatic ligaments were cut, according to the methods as described by Martins [15]. In the 90% hepatectomy, the right lobe (RL), medial lobe (ML) and left lateral lobe (LLL) are resected, and only the anterior caudate lobe (AC) and the posterior caudate lobe (PC) remain. After the major hepatectomy, the investigators checked for abdominal bleeding, washed the abdominal cavity and closed the abdominal muscles and peritoneum with 5.0-nylon sutures in a simple continuous manner. The warming pads were used to maintain the body temperature of the animals post-surgery. To recorded survival of the animals, we monitoring the animals every 3 hours with noting their breath and heartbeats. The rats with no breath or heartbeats were considered to be death and their post-surgery survival time (hours) were recorded. To determine the degree of liver damage, blood and liver tissue were collected when rats were sacrificed by cervical dislocation with anesthetized by sevoflurane at indicated time (2 hours and 24 hours) after surgery.

Hydrogen-rich saline production

Hydrogen was dissolved in physiologic saline for 6 hours under high pressure (0.4 MPa) to a supersaturated level using a hydrogen-rich saline-producing apparatus produced by our department. The concentration of the saturated hydrogen saline was 0.8 mmol/L, and HS was freshly prepared every week to ensure that a concentration of more than 0.6 mmol/L was maintained. The saturated hydrogen saline was stored under atmospheric pressure at 4 °C in an aluminum bag with no dead volume. Hydrogen-rich saline was sterilized by gamma radiation. Gas chromatography was used to confirm the content of hydrogen by the method described by Ohsawa et al. [7].

Experimental protocol

SD rats were randomly divided into the following three groups: group A ($n = 60$), group B ($n = 40$) and group C ($n = 40$). Group A were used to observe a five-day survival, whereas the animals of group B and group C were sacrificed 2 hours and 24 hours after the operation, respectively. Based on different treatments, the rats in group A, group B and group C were further divided into the following 4 subgroups: group A1 ($n = 15$), group B1 ($n = 10$) and group C1 ($n = 10$) (sham);