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Lactulose accelerates liver regeneration in rats by inducing hydrogen

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ABSTRACT

Background: Oxidative stress and inflammation are implicated in the process of liver regeneration. Lactulose orally administered can be bacterially fermented and induces dramatic amounts of endogenous hydrogen. Hydrogen has been confirmed to have antioxidant and anti-inflammatory properties. This study investigated the potential influence of lactulose administration on liver regeneration.

Materials and methods: Antibiotics were used to suppress bacterial fermentation of lactulose, and hydrogen-rich saline was used as a supplementary measure of exogenous hydrogen. The liver regeneration model was produced in Sprague–Dawley rats through 70% partial hepatectomy.

Results: Compared with non–lactulose-treated group, lactulose administration remarkably increased the weights of remnant liver and inhibited increases in serum levels of transaminases more notably. In the lactulose-treated group, increases of markers for regeneration, such as proliferating cell nuclear antigen and cyclin D1, were highly elevated. Biochemically, lactulose administration increased liver superoxide dismutase activity and decreased malondialdehyde content. In the lactulose-treated group, excessive increases in inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α, were inhibited significantly. Increased heme oxygenase-1 and superoxide dismutase 2 expression were also observed after lactulose treatment. The antibiotics suppressed the regeneration-promoting effect of lactulose by reducing hydrogen production, whereas supplementing hydrogen by hydrogen-rich saline would get a similar regeneration-promoting effect as lactulose administration.

Conclusions: Lactulose administration accelerates posthepatectomized liver regeneration in rats by inducing hydrogen, which may result from attenuation of the oxidative stress response and excessive inflammatory response.

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1. Introduction

Hepatectomy is a common surgical therapy for diseases such as hepatocellular carcinoma, cholangiocarcinoma, and liver hemangioma. As a centuried and developing surgical therapy, the morbidity and mortality of hepatectomy have declined significantly in recent years. However, insufficient liver regeneration and postoperative liver failure still restrict its applicability [1]. Oxidative stress, involving reactive oxygen species and antioxidant enzymes, which arises during intraoperative and postoperative process, is an important adverse factor for liver regeneration [2,3]. Reducing reactive oxygen species production and preventing oxidative stress in the remnant liver are regarded as important methods to accelerate liver regeneration [4,5].

Molecular hydrogen (H_2) has been proven effective in antioxidant, anti-inflammatory, and antiapoptotic properties [6]. For mammals, H_2 has been found to protect the liver from a variety of injuries, such as ischemia–reperfusion damage, drug-induced liver injury, obstructive jaundice, and hepatitis [7–9]. Given that the liver-protective function of H_2 has been revealed, it is anticipated that H_2 plays a role in the process of liver regeneration.

Lactulose is always used in the treatment of constipation and hepatic encephalopathy [10,11]. Meanwhile, lactulose orally administered can be bacterially fermented in the large intestine and induces dramatic amounts of endogenous H_2 [12]. Therefore, we hypothesized that lactulose orally administered could accelerate liver regeneration after hepatectomy on the basis of providing additional hydrogen production. To determine our hypothesis and the underlying mechanisms, we carried out this study.

2. Materials and methods

2.1. Animal model

Male Sprague–Dawley rats weighing 200–220 g were purchased from the Zhejiang Province Experimental Animal Center. All procedures were approved by the Ethics Committee of Zhejiang University and conformed to the Care and Use of Laboratory Animals Guide published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Rats were randomly assigned to the following six groups: sham group (sham), hepatectomy (HT) group, lactulose (LAC) group, lactulose plus antibiotics (LAC + Abs) group, lactulose plus antibiotics and H_2 (LAC + Abs + H_2) group, and hydrogen group (H_2). Rats in the LAC, LAC + Abs, and LAC + Abs + H_2 groups received specially made drinking water (contained 0.5% lactulose), whereas other rats received ordinary water. Rats in the LAC + Abs and LAC + Abs + H_2 groups were administrated with metronidazole (30 mg/kg) and gentamicin (40 mg/kg) intragastrically from 3 d before operation (three daily) until sacrificed. Seventy percent partial hepatectomy, including the left lateral and median lobes, was performed in all the other groups, except sham group. In the LAC + Abs + H_2 and H_2 groups, hydrogen-rich saline (HRS) was administered intragastrically from the day of operation (twice daily) until sacrificed.

Rats were sacrificed at different time points after the operation ($n = 6$ per time points). Blood collected from the inferior vena cava was centrifuged, and serum was stored at −80°C before liver biochemical determinations. The total liver was removed, and after being weighed, all the fractions were snap-frozen in liquid nitrogen, and then stored at −80°C as samples.

2.2. Preparation of HRS

HRS was prepared by dissolving hydrogen in physiological saline for 12 h under high pressure (0.4 MPa). The HRS was stored under atmospheric pressure at 4°C in a sealed and lucifugal bag with no dead volume. After irradiation sterilization, the HRS was administered by peritoneal injection in 1 wk.

2.3. Hydrogen concentration measurement

Eighteen rats without hepatectomy were divided into three groups as follows: control group, group A, and group B. The rats in the three groups were kept with ordinary water and diet. Rats in group A were administrated with saline intragastrically. At the same time, rats in group B were administrated with metronidazole and gentamicin intragastrically (three daily). Three days later, all rats in the three groups were fasted overnight. Rats in group A and group B received 0.25 g/kg of lactulose, but rats in the control group only received normal saline. A single rat was placed in an oxygenated and sealed glass chamber of size 55 × 40 × 20 cm for 1 hour after receiving lactulose. One hour later, the gas samples were collected, and the concentration of hydrogen was tested by Gas Chromatograph (Agilent, Santa Clara, CA). Assays were performed three times for every gas sample.

2.4. Evaluation of liver injury

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin were quantitated by an Automated Chemical Analyzer (Dimension RxL Max HM, Siemens, Germany) to evaluate the degree of liver injury. Ammonia levels were assayed with the detection kits (Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Liver regeneration rate

Liver regeneration rate = $\{C - (A - B)\}/A \times 100\%$, in which A is the estimated whole liver weight before the operation, based on the results of a pilot experiment that the whole liver weight in our model was equal to 4.39% of the bodyweight ($4.39 \pm 0.09\%$, $n = 10$); B is the resected liver weight; and C is the remnant liver weight at the time of death. Therefore, liver regeneration rate was used to evaluate liver regeneration after hepatectomy.