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Prehepatic portal hypertension worsens the enterohepatic redox balance in thioacetamide-cirrhotic rats

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Abstract

Background: Oxidative stress has been reported as a key pathogenic factor in many human liver diseases and in experimental models of cirrhosis related to hepatotoxin administration. The aim of this study was to verify the hypothesis that prehepatic portal hypertension aggravates the enterohepatic redox imbalance in thioacetamide-cirrhotic rats. **Materials and methods:** Wistar male rats were used: Control ($n = 9$); rats with prehepatic portal hypertension by triple partial portal vein ligation (TPVL; $n = 9$); thioacetamide-cirrhotic rats (TAA; $n = 9$) and TPVL-rats associated to TAA administration (TPVL + TAA; $n = 9$). Three months after the operation, portal pressure (PP), mesenteric venous vasculopathy (MVV) and portosystemic collateral circulation were studied. Liver and ileal levels of malondialdehyde (MDA), as a lipid peroxidation marker, and catalase (CAT), glutathione peroxidase (GSH-Px), glutathione transferase (GSH-t) and cytosolic and mitochondrial superoxide dismutases (cSOD and mSOD), as antioxidative enzymatic mechanisms, were measured. **Results:** Liver and ileal MDA increased in all the experimental groups, although the higher increase occurred in the ileum of rats with portal hypertension. CAT levels decreased in the liver and the ileum in the three experimental groups. The decrease in liver and ileal GSH-Px and GSH-t was greater in rats with portal hypertension, alone or associated with TAA. mSOD activation was demonstrated in the liver when portal hypertension was added to TAA. On the contrary, this compensatory response was not activated in the ileum, where mSOD was significantly decreased. **Conclusion:** Prehepatic portal hypertension by triple partial portal vein ligation impaired the enterohepatic antioxidative activity and aggravated the intestinal oxidative stress in thioacetamide-cirrhotic rats.

Keywords: Liver cirrhosis; Portal hypertension; Partial portal vein ligation; Thioacetamide; Oxidative stress; Catalase; Glutathione peroxidase; Superoxide dismutase; Rat

1. Introduction

The most frequently used experimental model of prehepatic portal hypertension is achieved by partial portal vein ligation in the rat [1]. This experimental model is usually chosen, since it causes the less hepatic impairment. However, partial portal vein ligation produces some hepatocellular dysfunction related to the portal blood deprivation [2]. We have previously demonstrated that prehepatic portal hypertension in the rat causes steatosis and hepatic lipid metabolism changes which are similar to those found in the human Metabolic Syndrome [3–5]. Prehepatic portal hypertension also induces deleterious consequences in the intestine, i.e. "hypertensive portal intestinal vasculopathy" [6]. We have shown in rats with triple partial portal vein ligation that it is characterized by inflammatory changes [7]. Particularly, hyperdynamic splanchnic circulation, bacterial translocation, mast cell infiltration, angiogenesis, villous atrophy and goblet cell hyperplasia stand out in the experimental portal hypertensive enteropathy [8,9].

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Portal hypertension induces two inflammatory conditions: hepatic steatosis and portal hypertensive enteropathy which are related, among other etiopathogenic factors, to alterations in the redox balance [10,11].

Oxidative stress occurs in all respiring cells and arises when there is marked imbalance between the production of reactive oxygen molecules and their removal by antioxidants. Oxidative stress has a key role in the inflammatory processes [12]. Reactive oxygen species (ROS) are highly reactive molecules due to unpaired electrons. Abnormal synthesis of ROS leads to damage of lipids, proteins and nucleic acids. The oxidation of molecular oxygen produces superoxide radicals ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) that initiate the peroxidation of unsaturated fatty acids in the membranes. Both, H_2O_2 and $O_2^{\bullet -}$ produce the highly reactive hydroxyl radical (OH^-) through the Haber-Weiss reaction. And OH^- can, in turn, induce lipid peroxidation, which is a free radical chain leading to the loss of membrane structure function [13].

To antagonize the destructive effects of ROS there is an intricate system in tissues. Nonenzymatic antioxidants (vitamins C and E, minerals, glutathione (GSH), uric acid and ubiquinol) collaborate with enzymatic ROS scavengers, superoxide dismutases (SODs), catalase (CAT) and glutathione peroxidase (GSH-Px) the main enzymatic antioxidants [14].

We can hypothesize that if prehepatic portal hypertension produces inflammatory impairments in the liver and the intestine, it could be a factor that would worsen chronic liver diseases.

The most studied experimental models of cirrhosis in the rat are extrahepatic cholestasis [1,15] and those related to hepatotoxin administration, like carbon tetrachloride [1,16] and thioacetamide [17].

The aim of this study was to test the hypothesis that prehepatic portal hypertension aggravates chronic liver diseases. Thus, we associated prehepatic portal hypertension by triple partial portal vein ligation (TPVL) with cirrhosis by thioacetamide (TAA) administration in the rat and measured the liver and intestinal levels of malondialdehyde (MDA), as a marker of lipid peroxidation and an indirect index of oxidative stress [13] and CAT, glutathione transferase (GSH-t), GSH-Px, cytosolic superoxide dismutase (cSOD) and mitochondrial superoxide dismutase (mSOD).

2. Materials and methods

2.1. Animals and experimental design

Male Wistar rats with a mean body weight of 220 ± 30 g, from the *Vivarium* of Complutense University of Madrid, were used. The animals were fed a standard laboratory rodent diet (rat/mouse A04 maintenance diet, Panlab, Spain) and water *ad libitum*. They were housed in a light/dark-controlled room, with an average temperature (22 ± 2 °C) and humidity (65–70%) in groups of three to four animals.

All the studies were approved by the Complutense University Ethical Committee and adhered to the guidelines of Commission Directive 86/609/EEC (The Council Directive of the European Community) concerning the protection of animals used for experimental and other scientific purposes. The National legislation, in agreement with this Directive, is defined in Royal Decree no. 1201/2005.

The animals were randomly divided in four groups of nine rats each: Control group, in which the animals did not undergo any operative intervention or hepatotoxic drug administration; Portal hypertension group, in which prehepatic portal hypertension by TPVL was carried out; TAA group, in which TAA (Sigma, Germany) was administered in drinking water for 12 weeks, according to the method described by Li et al. [17]. In brief, the rats received an initial concentration of 0.03% TAA and then, this dose was weekly modified depending of the animal's weight changes; TPVL + TAA group, in which TPVL-rats 12 days after the operation were administered TAA according to the method described for Group 3. We have previously shown that this method of TAA administration is a valid model of cirrhosis in the rat [18].

All the animals were sacrificed by anesthesia overdose at 3 months of evolution and body, liver, spleen and testes weights were recorded. Portal pressure (PP) was registered and the existence of mesenteric venous vasculopathy (MVV) and portosystemic collateral circulation was also verified. Distal ileum and liver samples (always from the middle lobe) were taken and homogenized on ice in 5–10 ml of cold buffer (50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA) per gram tissue. Then, they were centrifuged at $10{,}000 \times g$ for 15 min at 4 °C and the supernatants were frozen and stored at −80 °C until assayed.

2.2. Surgical techniques

2.2.1. Portal hypertension by triple partial portal vein ligation (TPVL) technique

The animals were anesthetized by i.m. injection of Ketamine (100 mg/kg) and Xylazine (12 mg/kg). A midline abdominal incision was made and part of the intestinal loops were gently shifted towards the left side and covered with moistened gauze.

The surgical procedure used to produce portal hypertension by TPVL has been described previously [19]. In brief, the portal vein was isolated and three ligatures were performed in its superior, middle and inferior portions. The stenoses were calibrated by a simultaneous ligature (4-0 silk) around the portal vein and a 20-gauge needle. The midline abdominal incision was closed in two layers with an absorbable suture (polyglycolic acid) and 3-0 silk. Analgesia was maintained during the first 24 h of p.o. with Buprenorphine (0.05 mg/(kg 8 h) s.c.)