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ORIGINAL ARTICLE

Preoperative erythropoietin treatment improves survival following major hepatic resection in a cirrhotic rat model

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KEYWORDS
Liver cirrhosis;
Erythropoietin;
Surgical resection;
Protective effect;
Liver regeneration

Summary
Aim: Major hepatic resection of a cirrhotic liver may result in a fatal clinical course. Preoperative erythropoietin (EPO) treatment has been shown to have protective properties and to stimulate liver regeneration. This study aims to investigate the effect of preoperative EPO on survival following major hepatic resection in a cirrhotic rat model.
Methods: Cirrhotic liver was induced by intraperitoneal injection of thioacetamide (200 mg/kg/mL) in 72 Lewis rats. Each 36 rats received EPO (1 IU/g, every second day, 5 times preoperatively) or saline (control) and major hepatectomy (removal of the left and half of the median lobe) was performed. Biochemical and immunohistochemical parameters, cytokines and overall survival were compared following surgery.
Results: Rats that received preoperative EPO had decreased hepatic aspartate aminotransferase, alanine aminotransferase and interleukin (IL)-1β expression, 48 hours following surgery. They had increased hepatocyte growth factor and vascular endothelial growth factor expression at 1 hour, increased IL-6 expression at 24, 48 and 120 hours and increased Ki-67, 120 hours following surgery. Overall, survival was significantly improved among EPO-treated rats ($P = 0.034$).
Conclusion: Preoperative EPO treatment has a protective effect and stimulates liver regeneration, leading to improved overall survival following major hepatectomy in a cirrhotic rat model.

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Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths worldwide [1]. Surgical resection is the only curative treatment to date among various locoregional therapies for HCC [2]. However, most patients with HCC have chronic liver diseases such as chronic hepatitis B or C and alcoholic liver disease, with severities ranging from fibrosis to severe cirrhosis, that limit the possibility of surgical resection [3]. The cirrhotic liver cannot tolerate acute tissue loss induced by surgical resection due to its impaired liver function and decreased ability to regenerate. Tissue loss during resection can lead to posthepatectomy liver failure (PHF), a major cause of death [4]. Therefore, it is imperative to enhance the cirrhotic liver's capacities for cell protection and regeneration following hepatic resection, as this could increase the number of candidates for surgical resection and improve survival outcomes for those who undergo surgery.

Erythropoietin (EPO) is a low molecular weight glycoprotein hormone stimulator of erythropoiesis that is most commonly delivered to patients on dialysis or with tumor-induced anemia [5]. There is increasing evidence proving biological roles of EPO in tissues outside of the hematopoietic system provoking significant experimental interest. In the liver, EPO has been shown to increase the capacity for cell protection and reduce apoptosis [6—8]. Rats treated with EPO have significantly decreased ischemia-reperfusion injury following liver transplantation of fatty livers [9]. This effect is mediated by the Jak-2 pathway and an Akt-dependent intracellular cascade downregulating the expression of pro-apoptotic mediators and molecules [10,11]. EPO treatment has also been shown by numerous studies to have a positive effect on liver regeneration following hepatectomy [12,13]. This regenerative process was related to cytokines such as interleukin (IL)-6 and growth factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) [9].

Because EPO has various biological roles but it has not been studied in the context of cirrhotic liver, we hypothesized that EPO has a protective effect and induces liver regeneration following hepatic resection in cirrhotic livers. We investigated the potential therapeutic role of EPO in a rat model of cirrhotic liver following major hepatic resection.

Materials and methods

Animals

Seventy-two male Lewis rats (body weight 250—300 g; Charles River Laboratories, Sulzfeld, Germany) were used in the experiments. All animal study protocols were approved by our institution's animal care and use committee (IRB No. 2013-00062). To induce liver cirrhosis, intraperitoneal (IP) injection of thioacetamide (200 mg/kg/mL, 1 mL/kg; Sigma-Aldrich, USA) was performed every second day for 8 weeks. The rats were then randomly divided into two groups (group A and group B; $n = 36$ each). Rats in group A received an IP injection of 1000 U/kg EPO (Eporon, Dong-A ST, Seoul, Korea) daily for 5 days prior to surgical resection, while those in Group B received IP saline at the same dose and regimen.

Surgical procedure

Anesthesia was performed using isoflurane inhalation with 40% oxygen. The abdomen was opened through a midline incision. A partial (50%) hepatectomy resecting the left and half of the median lobe of the liver was then performed according to Higgins and Anderson's method using a Harmonic scarpel and Bovie coagulation [14]. The total liver weight was calculated as 3.4% of the total rat weight and the excised liver specimens were weighed and additional resection was performed, such that a 50% hepatectomy was achieved. The abdomen was then closed with a single-layer running suture (4-0 Vicryl). All surgical operations were performed under sterile conditions. Animals were sacrificed 1 hour, 24 hours, 48 hours, 5 days, 7 days and 28 days following surgical resection ($n = 6$ each) and serum and liver tissue were collected. The group sacrificed at 28 days was used to assess overall survival.

Biochemical parameters

Blood was collected via vena cava puncture and centrifuged at 3500 rpm for 5 min; the derived serum was stored at −80 °C. Serum EPO concentration was measured using an immunoluminometric assay. Serum was analyzed for total bilirubin aspartate aminotransferase (AST), alanine aminotransferase (ALT) and C-reactive protein (CRP) as described.

Immunohistochemical parameters

Resected liver samples were fixed in 10% neural-buffered formaldehyde and embedded in paraffin. Paraffin-embedded blocks were sectioned at 4-μm for hematoxylin and eosin staining and immunohistochemistry. To analyze hepatocyte proliferation, immunohistochemistry for Ki-67 (MIB-2, 1:50; Dako, Glostrup, Denmark) was performed using the Ventana BechMark XT Autostainer (Ventana, Tucson, AZ) according to the manufacturer's recommendations. Ki-67 index was obtained by averaging the proportion of positive cells within the four hot-spot areas. Analysis for histopathology and immunohistochemistry was conducted by two board-certified pathologists.

Cytokines analysis by total RNA extraction and quantitative RT-PCR

Frozen tissue samples were homogenized on ice in 1 mL STAT-60 total RNA/mRNA isolation reagent (Tel-Test, Friendswood, TX, USA) and incubated for 5 min at room temperature. 0.2 mL of chloroform was added to the supernatant and the mixture was vortexed and incubated for 15 min at room temperature. Samples were then centrifuged at 13,000 rpm for 20 min at 4 °C. Following centrifugation, the supernatant was transferred to a new tube and 0.5 mL of isopropanol was added to induce precipitation. The pellet

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