Novel Method to Remove Bilirubin from the Blood of Jaundiced Rats: A Preliminary Study

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Research Article

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Abstract

BACKGROUND

The removal of bilirubin from serum is the primary method for treating hyperbilirubinemia. However, currently used treatment methods have several limitations.

AIM

To introduce a novel method to remove bilirubin from the blood of jaundiced rats.

METHODS

This novel therapy involved cross-circulation of blood between a rat liver preserved in vitro and a rat with hyperbilirubinemia. The liver was perfused with blood from the model animal, resulting in the clearance of serum bilirubin. Twenty rats with jaundice caused by acute liver failure induced using D-galactosamine were treated. All model animals were randomly divided into two groups based on whether they received the novel therapy. Serum samples were collected before modelling, at the beginning of treatment, and 2 hours after treatment. The levels of serum transaminase and bilirubin were detected and compared between different time points. Histological examination of the liver in vitro was also performed after the treatment. Long-term survival was compared by Kaplan-Meier analysis between rats who did and did not receive the novel treatment.

RESULTS

In vitro, the liver could be perfused with the blood from the model animal through the portal vein. The bile produced by the liver after 1 hour of therapy was darker than the bile produced while harvesting. Across different hyperbilirubinemia models, serum total bilirubin level was significantly improved (24.8 ± 1.2 vs. 17.4 ± 1.2 μmol/L, $P<0.05$), despite a rise serum transaminase levels after treatment (AST: 4612 ± 382 vs. 5144 ± 390 U/L, $P>0.05$; ALT: 5051 ± 722 vs. 5488 ± 707 U/L, $P>0.05$). No necrosis was found in the preserved liver tissue after treatment, and the hepatic lobule structure was normal. Hepatocyte necrosis was not found on histological examination. This novel treatment significantly raised the long-term survival rates of jaundiced rats ($P<0.05$).

CONCLUSION

This novel method could safely and effectively help eliminate bilirubin from the blood of jaundiced rats.

Core Tip

This study shows that bilirubin content from the blood of a jaundiced rat could be lowered by establishing cross-circulation between an in vitro liver model and a rat with hyperbilirubinemia. After treatment, total bilirubin levels improved significantly, the hepatic lobule structure of the liver model in

vitro was normal, and hepatocyte necrosis was absent. Our study contributes significantly to the field in that unlike other therapies for removing bilirubin from serum, which emulate only some of the liver's physiological functions, our novel therapy may compensate for all functions and so improves the prognosis of jaundiced rats.

Introduction

Jaundice is the primary symptom of severe liver disease that affects the ability of the liver to metabolise and secrete bilirubin[1,2]. Bilirubin is a cytotoxic substance that can cause mitochondrial dysfunction, lead to organ system dysfunction, and even endanger life[3–6]. Currently, there are several limitations to jaundice treatment, especially for jaundice caused by liver failure, as existing liver replacement therapy cannot emulate the full physiological function of the liver[7–9]. In recent years, it has been proven that the liver can be preserved using machine perfusion to maintain a physiological milieu. Therefore, this study was designed to verify whether a novel therapy involving cross-circulation between the liver preserved in vitro and the jaundiced rat can be effective.

Materials And Methods

Novel therapy procedure

This novel therapy is designed to stimulate cross-circulation between a liver preserved in vitro and a jaundiced rat (Figure 1). First, the jaundiced model rat underwent carotid and jugular vein catheterisation using an anticoagulant catheter after anaesthesia. Next, a donor's liver was harvested after 30% partial hepatectomy, and superior-inferior vena cava ligation and biliary intubation were performed. The donor's liver was then preserved in normal saline at 4 ℃. Third, blood flow was restored by connecting the carotid artery and jugular vein cannulas to the portal vein and the infrahepatic inferior vena cava of the donor's liver (Figure 2).

Animals

Forty 6-week-old Sprague-Dawley rats, weighing 230–250 g, housed under a 12-h daylight/darkness cycle and in an air-conditioned animal room with 50% humidity, were obtained from the Experimental Animal Center of Xi'an Jiaotong University. These rats were jaundiced due to acute liver failure induced by D-galactosamine. They were randomised into two groups according to the therapy administered: group T (therapy, n = 20), rats in this group received the novel therapy; group C (control, n = 20), rats in this group did not receive any therapy, but underwent carotid artery and jugular vein cannulation. Another 20 rats were used as donors and underwent partial 50% hepatectomy before their livers were harvested. Buprenorphine (0.05 mg/kg) was injected subcutaneously as an analgesic 1 hour before the operation. All the rats were anaesthetised, with 5 % vol isoflurane at a flow rate of 1.5 L/min in a Plexiglas box used to induce anaesthesia and 2 % vol isoflurane at a flow rate of 0.6–0.8 L/min in a cone mask for the

maintenance of anaesthesia. All rats were heparinised by injecting 20 U of heparin diluted in 2 ml of normal saline solution through the dorsal vein of the penis before treatment. This study was performed according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences. The study design was approved by the Committee on the Ethics of Animal Experiments of Xi'an Jiaotong University (Permit Number 2010-105).

Animal model

All the rats in group T and group C received an intraperitoneal injection of 1.1 g/kg of D(+)-galactosamine hydrochloride (Sigma Chemical Corporation, St Louis, Missouri, USA) as a 100 mg/ml solution (pH 6.8) in normal saline[10] 48 hours before receiving the novel therapy.

Liver function tests

Liver function tests, including serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (TBIL) levels were performed before modelling, at the beginning of treatment in group T (ending of cannulation in group C), and 2 hours after treatment in group T (2 hours after cannulation in group C). Rats used for survival analysis were observed for 48 hours after treatment.

Histological analysis

After treatment, in vitro liver tissue was obtained and fixed in 10% buffered formalin overnight before embedding in paraffin. Liver sections were cut to 4-μm thick and stained with haematoxylin and eosin.

Statistical analysis

Numerical data were expressed as a mean ± SEM (Standard Error of Mean) and compared using the Student's *t*-test or Mann–Whitney U test. All data were analysed using Prism 6 (GraphPad Software Inc., CA, USA). Overall survival was estimated by the Kaplan-Meier method, and differences between groups were compared via the log-rank test, using IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA). A *P*-value less than 0.05 was considered statistically significant. A biomedical statistician reviewed the study data for statistical correctness.

Results

Animal model

A drug-induced liver failure model in rats was successfully established in this study. In group T, serum AST, ALT, and bilirubin levels increased significantly 24 hours after modelling (AST: 97 ± 2 vs. 4612 ± 382 U/L, *P*□0.05; ALT: 96 ± 3 vs. 5051 ± 722 U/L, *P*□0.05; TBIL: 9.7 ± 0.4 vs. 24.8 ± 1.2 mmol/L, *P*□0.05; see Figure 4A-C). The trend of serum bilirubin level change in group C was similar to that in group T (AST: 98 ± 3 vs. 4747 ± 527 U/L, *P*□0.05; ALT: 96 ± 3 vs. 4369 ± 390 U/L, *P*□0.05; TBIL: 10.0 ± 0.3 vs. 24.9 ± 1.3 mmol/L, *P*□0.05; see Figure 4D-F).

Therapy

All the jaundiced rats were treated successfully, and no rats died during the treatment. The donor liver was perfused well after restoring blood flow, and no ischaemic lesions were found (Figure 3A). Catheter obstruction was not observed during the experiment. The donor liver produced bile during the treatment, and the colour of bile during treatment was darker than that during harvesting (Figure 3B).

Liver function test and histological findings

Serum bilirubin concentration in group T decreased significantly after the treatment (24.8 ± 1.2 vs. 17.4 ± 1.2 μmol/L, P 0.05, see Figure 4C). However, serum AST and ALT levels were elevated after treatment (AST: 4612 ± 382 vs. 5144 ± 390 U/L, P 0.05; ALT: 5051 ± 722 vs. 5488 ± 707 U/L, P 0.05, see Figure 4A-B). The serum AST, ALT, and bilirubin levels of rats in group T continued to rise after cannulation (TBIL: 24.9 ± 1.3 vs. 26.0 ± 1.3 mmol/L, P 0.05; AST: 4747 ± 527 vs. 5466 ± 591 U/L, P 0.05; ALT: 4369 ± 390 vs. 5093 ± 380 U/L, P 0.05, see Figure 4D-F). After treatment, the hepatic lobule structure of the liver in vitro was normal, and hepatocyte necrosis was not observed on histological examination (Figure 5).

Survival

The survival curves for both groups are shown in Figure 6. Overall, the survival rate of rats in group T was higher than that in group C within the 48 hours after therapy, and there was a significant statistical difference between the two groups (P = 0.012).

Discussion

This study details a novel therapeutic strategy for bilirubin elimination from the blood of animal models. This therapy may help improve the prognosis of patients with jaundice.

There are several theoretical pathophysiological approaches to filter serum bilirubin in cases of liver failure. One approach would be to increase liver regeneration or perform liver transplantation. Unfortunately, the current supply of acceptable donor livers is not adequate to meet the high demands of listed patients awaiting transplantation, resulting in thousands of deaths each year. An extracorporeal system is another promising feasible approach to liver support. However, evidence suggests that the sole use of liver support systems to remove bilirubin is not adequate to improve survival in patients with liver failure[7,11–13] because these systems can only partially replace the physiological function of the liver.

In this study, we proposed a novel therapy to eliminate bilirubin from serum. A liver preserved in vitro was perfused with the blood of a jaundiced rat through cross-circulation. As the carotid artery pressure is higher than that of the jugular and portal veins, studies suggested that if the liver was perfused with the blood at a higher pressure, it might disturb the microcirculation and increase the rate of hepatocellular

apoptosis[14–16]. However, no obvious hepatocyte apoptosis was detected on histological examination of the liver in vitro after treatment. This might have been because the pressure of the arterial blood significantly decreased after passing through the catheter. In addition, the liver was well perfused during the treatment and was able to produce bile, indicating that the liver can play a role in detoxification and secretion in vitro.

Cross-circulation between two humans has been found to be effective for treating uraemia, treatment of liver failure, provision of large numbers of leukocytes, and cardiopulmonary bypass during cardiac surgery[17–21]. However, it appears that chance selection of a donor and recipient of divergent histocompatibility might result in severe and even potentially fatal outcomes, such as immunologic complications, disease transmission, and compromised donor safety. In this study, cross-circulation was developed between the organ preserved in vitro and the recipient, which might have helped avoid the above problems. In addition to a significant decrease in serum bilirubin, our method also helped reduce 48h survival of the jaundiced rats. Unlike other therapies for removing bilirubin from serum, this therapy could compensate for all liver functions; this might be the main reason for improved 48h prognosis in model rats.

Although the results of this study are encouraging, several limitations should also be considered. First, this study only proved that this novel treatment could reduce the serum bilirubin level of an individual with hyperbilirubinemia and improve the long-term survival in an animal model. The effect of this treatment on coagulation function and other toxic substances in serum remains unclear. In addition, this study only verified the feasibility and effectiveness of the novel treatment method in the rat; thus, further exploration is needed in larger mammals.

Declarations

ACKNOWLEDGEMENTS

None

Institutional review board statement:

The study design was reviewed and approved by the Committee on the Ethics of Animal Experiments of Xi'an Jiaotong University (Permit Number 2010-105).

Institutional animal care and use committee statement:

All animal experiments conformed to the internationally accepted principles listed in the Guide for the Care and Use of Laboratory Animals, published by the National Academy of Sciences.

Conflict-of-interest statement:

None

Data sharing statement:

The data provided in the study are accessible to the public.

ARRIVE guidelines statement:

The authors have read the ARRIVE

guidelines and the manuscript was prepared and revised according to the

ARRIVE guidelines.

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Figures

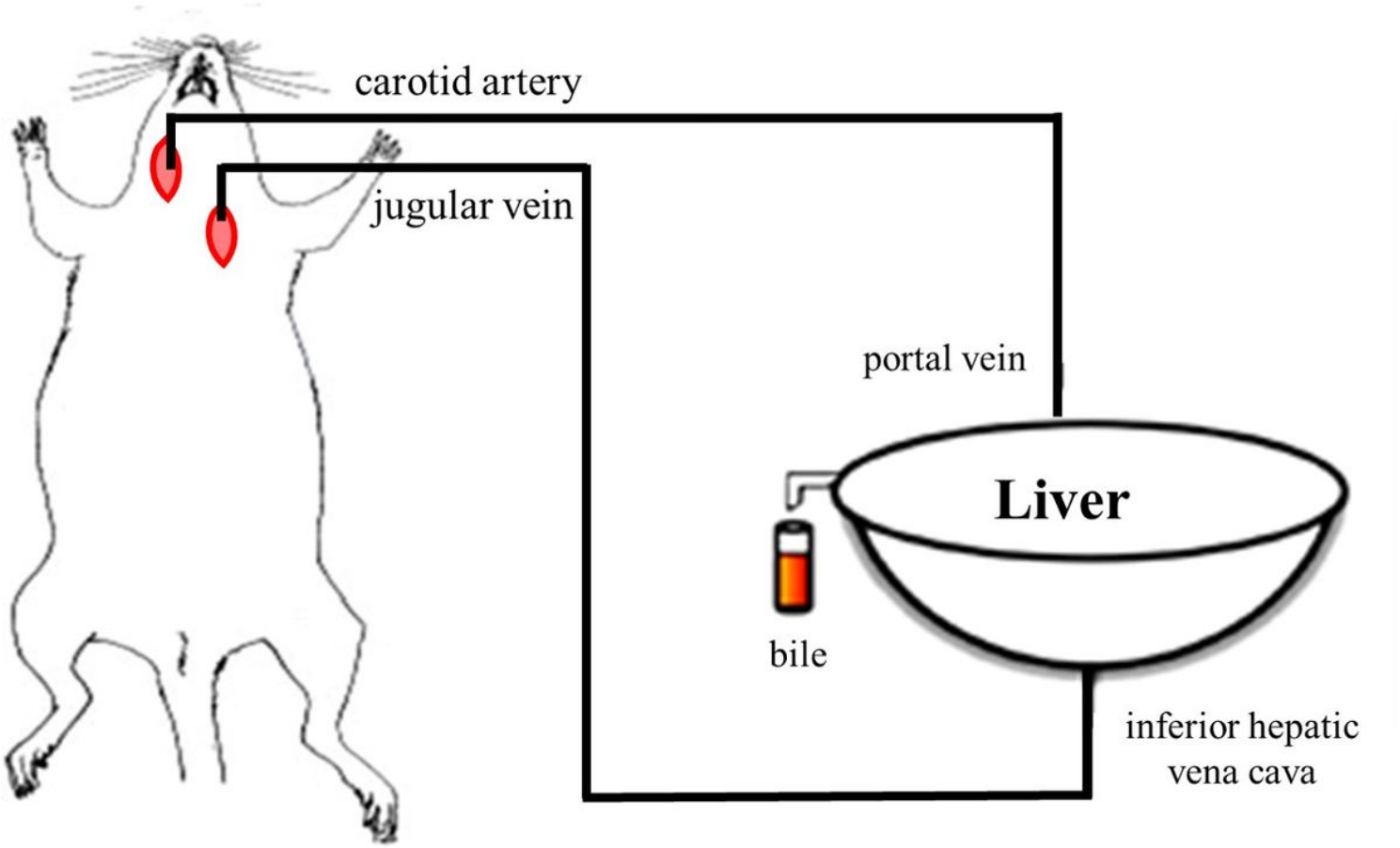

Figure 1

Schematic diagram of the novel treatment method.

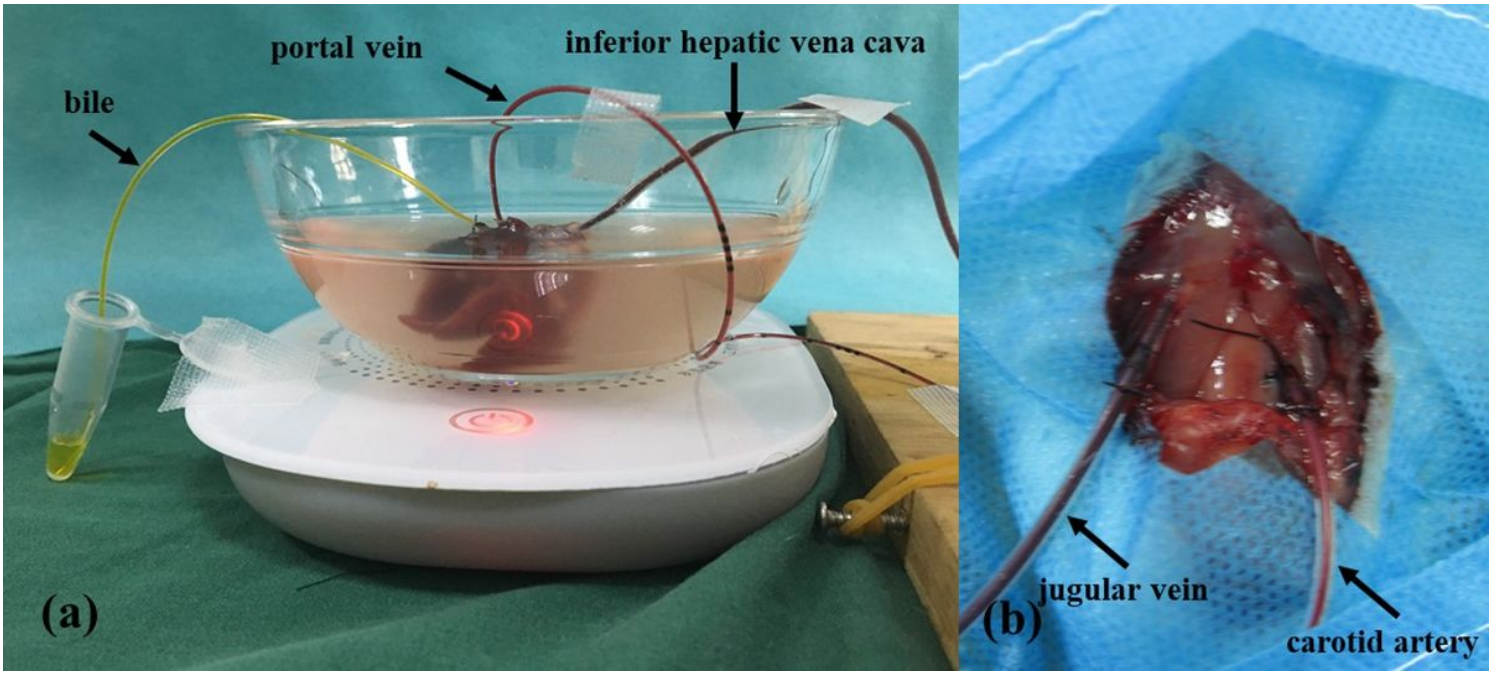

Figure 2

The novel method for the treatment of hyperbilirubinemia. A: The liver was perfused with arterial blood from model animals via the portal vein. The blood then flowed back to the jugular vein of the model animal through the inferior vena cava. The liver was well perfused and produced bile; B: Carotid and jugular vein catheterization was performed using an anticoagulant catheter.

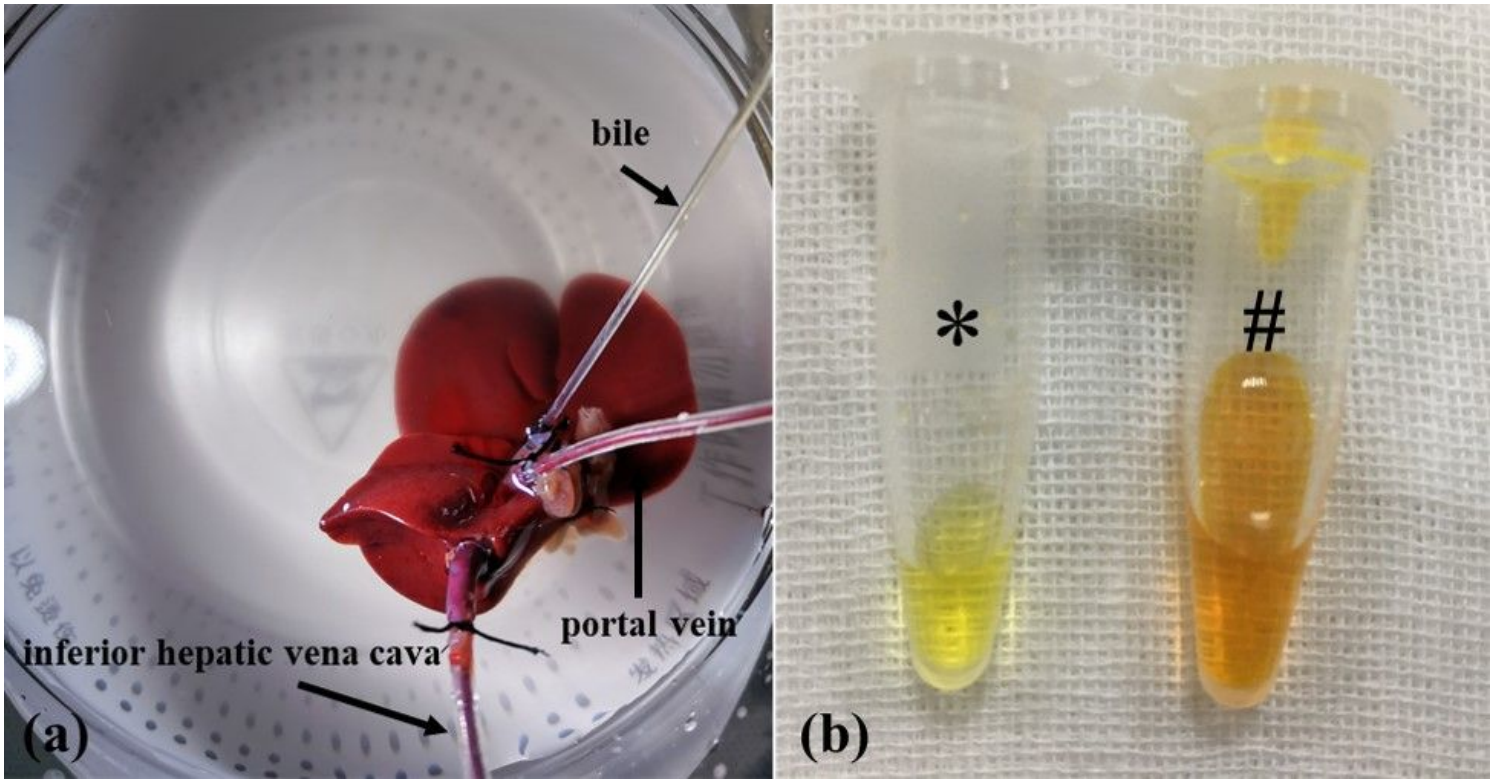

Figure 3

A: The liver preserved in vitro; B: The bile produced during harvesting (*) and treatment (#).

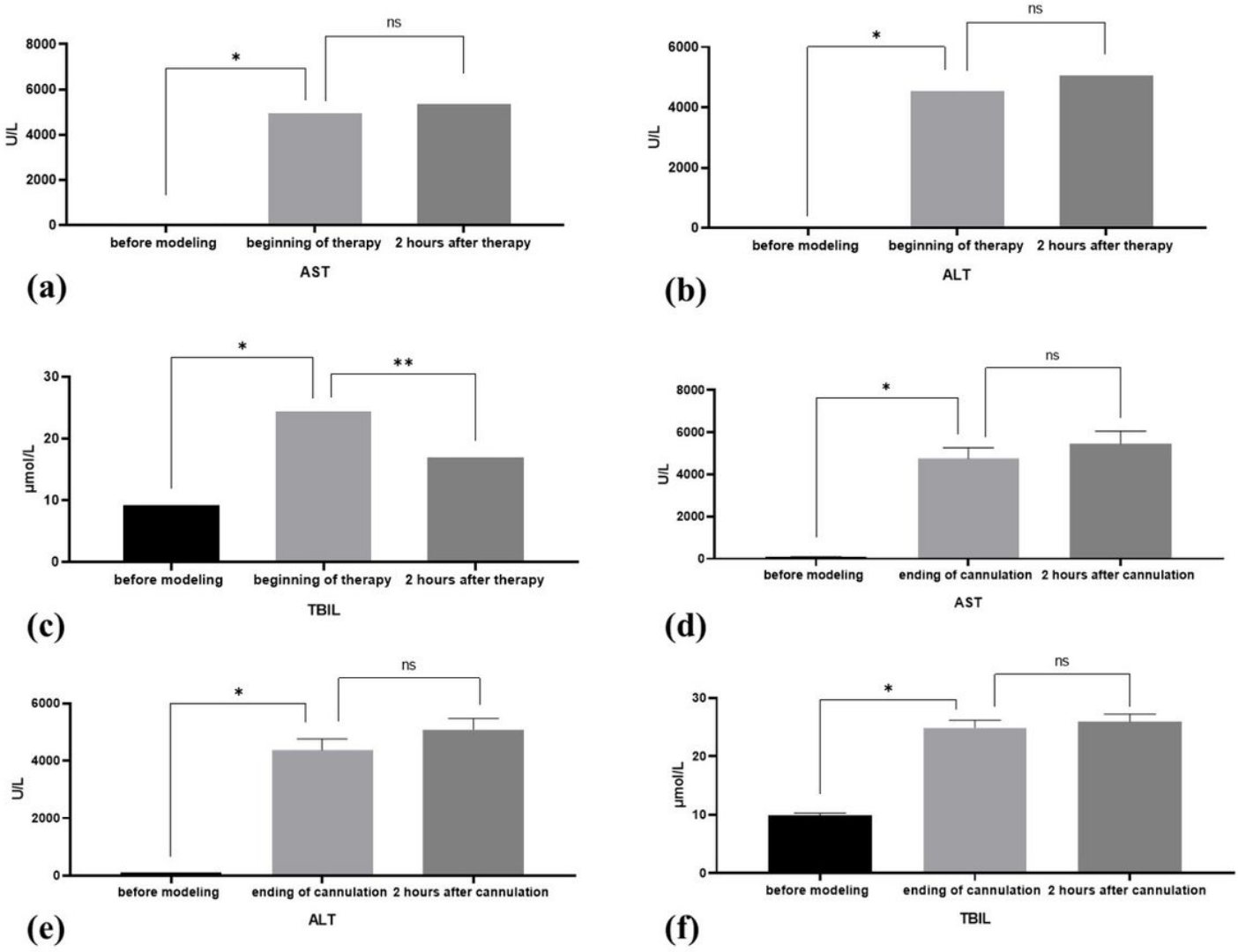

Figure 4

Changes of serum AST, ALT, and TBIL levels in the model animal.

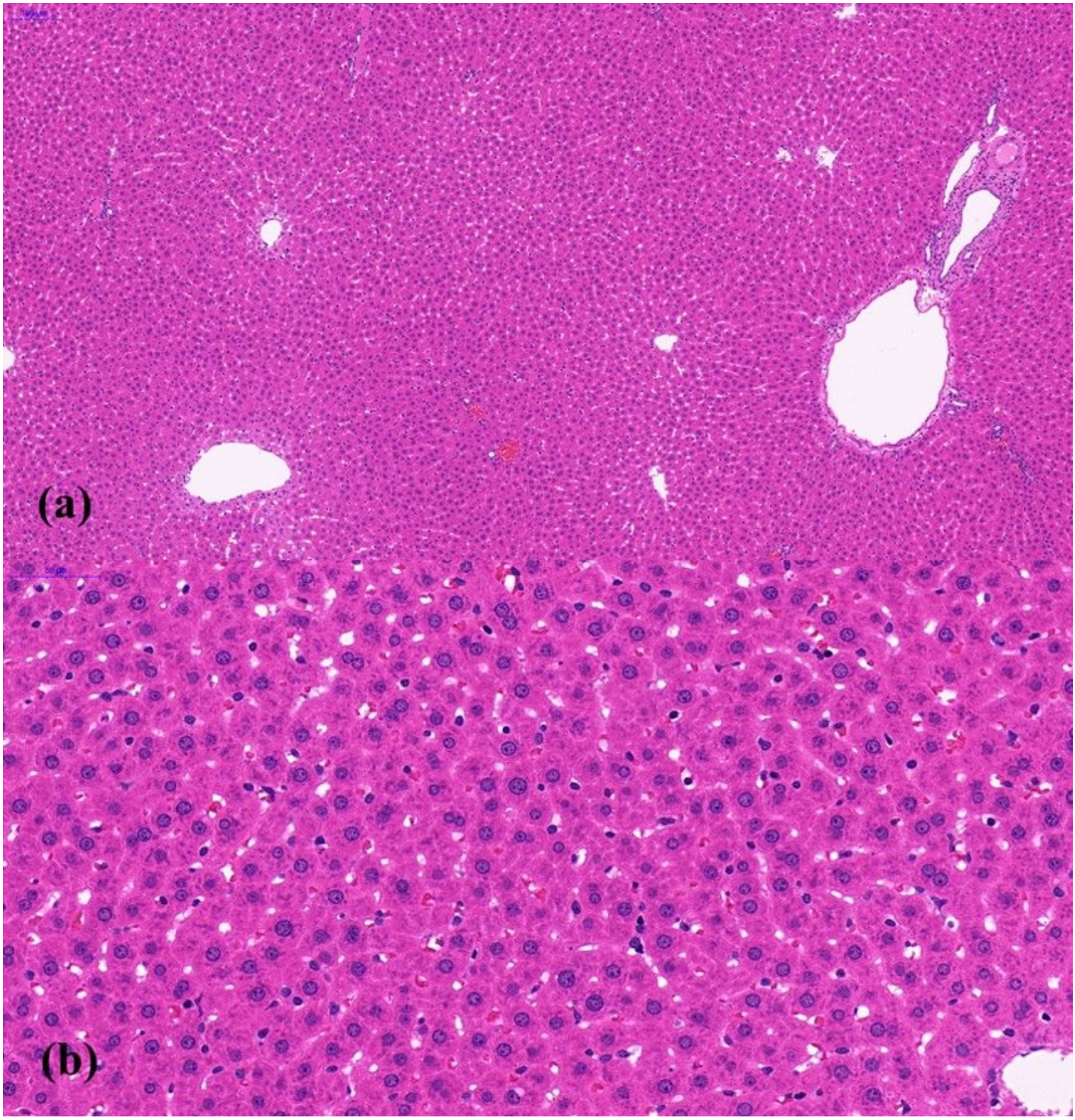

Figure 5

Histological examination of the liver in vitro after treatment.

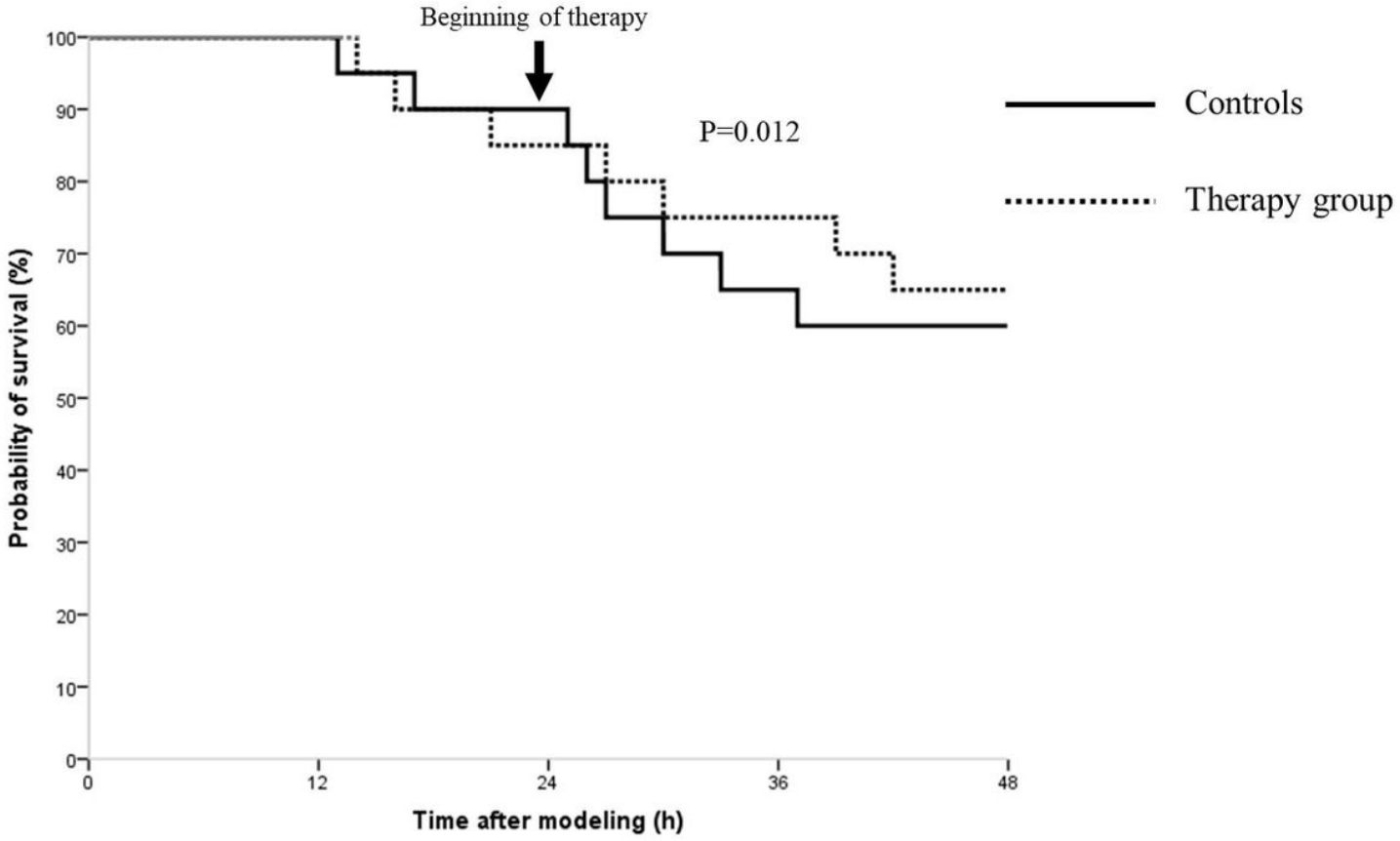

Figure 6

48-hour survival of rats in different groups