ELSEVIER

Relationship Between Ischemia/Reperfusion Injury and Acute Rejection of Allogeneic Liver Transplant in Rats

Y. Wang, J. Wu, B. Jiang, J. Wang, C. Liu, C. Peng, and B. Tian

ABSTRACT

Objective. This study aimed to investigate the relationship between the severity of ischemia/reperfusion (I/R) injury and the acute rejection (AR) of allogeneic liver transplants in rats.

Methods. The experimental rats were divided in different groups: normal control group (sham group, group I); syngeneic liver transplant control group (similar gene group, group II); and allogeneic liver transplant groups (groups III to VI). The rats were humanely killed at 1, 3, 5, and 7 days after transplantation or sham operation to determine the severity of I/R injury, rejection classification, and hepatocyte apoptosis. Messenger RNA (mRNA) and protein expression levels of Fas, perforin, and granzyme B were assessed in the liver tissues using real-time polymerase chain reaction and immunohistochemistry, respectively.

Results. The rejection scores of the transplanted liver tissues gradually increased until these scores were proportional to the severity of I/R injury in groups III, IV, and V. The maximum scores were reached at 7 days after transplantation as the duration of transplantation was extended. The mRNA and protein expression levels of Fas, perforin, and granzyme B were significantly increased at 1, 3, 3, 5, and 7 days after liver reperfusion in groups III, IV, and V compared with those in groups I, II, and VI ($P < .05$).

Conclusion. The occurrence of AR after allogeneic liver transplantation in rats was positively correlated with the severity of I/R injury. Given that I/R injury caused serious damage to the transplanted liver, the occurrence of AR consequently decreased.

THE INCIDENCE rate of acute rejection (AR) after liver transplantation is approximately 40% to 80%. AR causes functional losses affecting the survival of liver transplant recipients. In China, liver transplantation mostly involves non–heart-beating donors. In addition to the innate characteristics of donor livers, the primary factors influencing the quality of donor livers are the severity of warm ischemia, cold ischemia, and ischemia/reperfusion (I/R) injury. I/R injury is an important cause of AR and chronic rejection [1,2]. This type of injury affects specific immune processes after liver transplantation and an early functional loss of the transplanted liver. New sources of donor livers are currently developed, although existing donor liver resources are maximized. New solutions have been proposed to solve the shortage of donor liver cells. For instance, the quality of donor livers should be improved to reduce the injury of donor livers in vitro or after recovery from perfusion and prevent the onset of various complications after liver transplantation, particularly the incidence of AR. Therefore, the reduction of the severity of I/R injury after liver transplantation to prevent and control AR is clinically important. In this study, an animal model of AR was established after the liver was transplanted in rats to study the effect of different degrees of I/R injuries and hepatocyte apoptosis during AR after liver transplantation. This animal model was also used to determine the expression levels of Fas, perforin, and granzyme B. Our preliminary findings revealed the relationship between the severities of I/R injury and AR after liver transplantation. The mechanisms involved were also determined.

From the Department of Hepatobiliary Surgery, Hunan Provincial People's Hospital, Changsha, Hunan Province, China.

Address reprint requests to Jin-shu Wu, Department of Hepatobiliary Surgery, Hunan Provincial People's Hospital, Changsha 410005, Hunan Province, China. E-mail: ygbzcn@126.com

0041-1345/13/$–see front matter
http://dx.doi.org/10.1016/j.transproceed.2013.06.019

MATERIALS AND METHODS

Animals

The donors were male and female Sprague-Dawley rats. The recipients were 2- to 3-week-old male Lewis rats, weighing 220 g to 280 g (Experimental Animal Department of Central South University), with clean feeding. The donors were provided with food or drink before the experiment. By contrast, the recipients were not provided with food for 6 hours prior to transplantation. The number of recipients was slightly higher than the number of donors. The experimental rats were divided into 6 groups: group I, syngeneic sham group; group II, syngeneic liver transplantation group subjected to warm ischemia and cold ischemia for 2 minutes (1.42 ± 0.64 minutes) and 80 minutes, respectively; and groups III to VI, allogeneic liver transplantation groups. The rats in group III were subjected to warm ischemia and cold ischemia for 2 minutes (1.42 ± 0.64 minutes) and 80 minutes, respectively. The rats in group IV were subjected to warm ischemia and cold ischemia for 2 minutes (1.42 ± 0.64 minutes) and 10 hours, respectively. The rats in group V were subjected to warm ischemia and cold ischemia for 15 minutes (13.56 ± 1.78 minutes) and 80 minutes, respectively. The rats in group VI were subjected to warm ischemia and cold ischemia for 15 minutes (13.56 ± 1.78 minutes) and 10 hours, respectively. Each group was further divided into 4 subgroups and then humanely killed at 1, 3, 5, or 7 days after transplantation, respectively ($n = 3$; Table 1). The transplanted livers were harvested on the respective days after transplantation. Blood was then withdrawn from the inferior vena cava. This study was performed in strict compliance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol on animal use was reviewed and approved by the Institutional Animal Care and Use Committee of the Hunan Provincial People's Hospital, China.

Table 1. The Experimental Rats Were Divided Into 6 Groups, Ischemia Time ($\bar{x} \pm s$)

Group	Ischemia Time	
	Warm Ischemia Time	Cold Ischemia Time
I: syngeneic sham group	0	0
II: syngeneic liver transplantation group	1.42 min ± 0.64 min	80 min
III: allogeneic liver transplantation group	1.42 min ± 0.64 min	80 min
IV: allogeneic liver transplantation group	1.42 min ± 0.64 min	10 h
V: allogeneic liver transplantation group	13.56 min ± 1.78 min	80 min
VI: allogeneic liver transplantation group	13.56 min ± 1.78 min	10 h

METHODS

Liver transplantation was performed according to an improved version of Kamada's two-cuff technique. In brief, the subhepatic inferior vena cava and the portal vein were connected based on the cuff technique. The suprahepatic vena cava was then sutured. Stenting was performed for bile ducts. The hepatic artery was reconstructed by the bracket method. The suprahepatic inferior vena cava was reconstructed with an improved continuous suture. Liver perfusion was controlled using an automatic vein infusion pump. The perfusion rate was set at 2 mL/min with a total perfusion volume between 25 and 30 mL to ensure an even perfusion. The durations of warm and cold ischemia of the donor livers in each group were consistent with those of the experimental groups. The average time of the anhepatic phase ranged from 20 minutes to 24 minutes (21.78 ± 1.68 minutes). The experimental rats were randomly grouped. The harvested liver tissues were fixed with 10% formaldehyde, embedded in paraffin, serially sliced in 4-μm thick sections, and stained with hematoxylin and eosin. The liver tissues were observed under an ordinary optical microscope, and the expression of donor liver cell apoptosis was detected using a terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method. Apoptosis kits (Wuhan Boster, China) were used. TdT enzyme-free reaction buffer (PBS) was used as a negative control sample. Tissue sections treated with DNase I were used as a positive control sample. The cells with a buffy-stained nucleus viewed under an optical microscope were considered apoptotic. The morphology of an apoptotic cell should meet the following requirements: a single cell; no inflammatory reactions or apoptosis around the cell; cell membrane shrinkage; and a densely stained nucleus presenting buffy-stained particles or fragments. Five visual fields (400×) were selected for each section and the apoptotic cells were counted. The apoptotic index (AI) was calculated using the following formula: AI = (the number of apoptotic cells/the total number of cells) × 100%.

The protein expression levels of Fas, perforin, and granzyme B were detected in paraffin-embedded liver tissues using immunohistochemical staining. Immunohistochemistry was performed according to the manufacturer's instructions. Ten visual fields (400×) viewed under a light microscope were selected randomly for each section. An HPIAS-1000 automatic pathological image analysis system was used to determine the mean absorbance of the buffy-stained granules in the hepatic cells.

The messenger RNA (mRNA) expression levels of their respective genes were detected using real-time polymerase chain reaction (PCR).

Total RNA was extracted from the hepatic tissue using one-step extraction with a Trizol kit (Canada Bio Basic Inc.). Ultraviolet spectrophotometry was performed to determine the density and the purity of the total RNA. Agarose gel electrophoresis was performed to detect the integrity of the total RNA. The obtained substance was reverse transcribed to complementary DNA (cDNA) for PCR. The optical densities of the target genes (Fas mRNA, perforin mRNA, and granzyme B mRNA) and β-actin were determined using the SYNGENE gel imaging system. The relative expression level of a target gene was presented as the ratio between the optical densities of the target gene and β-actin.

The Fas, perforin, and granzyme B gene sequences of the rats were obtained from http://www.ncbi.nlm.nih.gov/nuccore/ (GenBank Accession Nos.: X66539, NM016993, BC063166). The primer sequences of the Fas, perforin,