Preconditioning Donor Livers With Cromolyn or Compound 48/80 Prolongs Recipient Survival in a Rat Orthotopic Liver Transplantation Model

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ABSTRACT

Background. Acute rejection (AR) remains a challenge in organ transplantation. Preconditioning donor organs can reduce AR and prolong survival. Whether preconditioning with cromolyn (CRM), a mast cell (MC) stabilizer, or compound 48/80 (CMP 48/80), a MC degranulator, can alleviate AR and prolong survival has not been studied.

Methods. We used the male-DA-to-female-Lewis-rat orthotopic liver transplantation (OLT) model. Donors were preconditioned with CRM in a MC stabilizing way (CRM group) or CMP 48/80 in a MC depleting way (CMP 48/80 group). Rats preconditioned with phosphate-buffered saline were used as controls (PBS group). After preconditioning, OLT surgeries were carried out. OLT male-Lewis-to-female-Lewis-rats were used as the syngeneic group (syngeneic group).

Results. Rats in the PBS group developed AR rapidly and died at 7.40 ± 1.14 days. Rats in the CRM and CMP 48/80 groups had significantly slower rejections and died at day 17.40 ± 1.67 or 14.20 ± 2.28, respectively ($P < .05$). Rats in the syngeneic group survived more than 60 days. Rejection activity indexes (RAIs) and liver functions were all alleviated through CRM or CMP 48/80 preconditioning. Interferon-γ messenger RNA (mRNA) expressions were reduced and interleukin-10 mRNA levels were higher in allografts in the CRM and CMP 48/80 groups, compared with the PBS group. These were confirmed by testing serum interferon-γ and interlerkin-10.

Conclusion. Preconditioning donor livers with CRM or CMP 48/80 can reduce AR and prolong survival of recipients after OLT.

LIVER transplantation is the most effective treatment for end-stage liver disease. Important concerns in liver transplantation include preserving organ function and prolonging recipient survival. Research has been directed at achieving such goals through donor preconditioning, preservation solution, and recipient optimization. Preconditioning donors with hypoxia [1], up-regulating hemooxygenase 1 (HO-1) [2], among others, can alleviate ischemia/reperfusion (IR) injury. IR injury affects both early- and long-term allograft function. Accumulating evidence suggests that the severity of IR injury to the allograft determines its immunogenicity. The mechanisms of promoting allograft immunogenicity are multifactorial and complex. The innate immune response, such as complement activation and up-regulation of multiple proinflammatory genes, is stimulated by IR injury. This inflammatory response, in the early stages after transplantation, is amplified by a subsequent adaptive response [3,4]. Therefore, IR injury promotes acute rejection (AR) [4,5].

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Mast cells (MCs) participate in IR injury in many organs, such as the heart [6] and intestine. The role of MCs in liver IR injury and the effect of preconditioning by modulating MC in liver transplantation have not been well characterized. MCs reside in the sinusoids of the liver [8] in many species (humans [9], rats [10], and canines [11]). MCs take effect mainly through releasing chemicals stored within the MC granules, which include histamine [12], interleukin [6,13], and MC tryptase [14]. There are some methods to modulate MCs. A MC stabilizer, cromolyn (CRM), and a MC degranulator, compound 48/80 (CMP 48/80; chemical formula of the monomer: C11H15NO), have previously been used to study MC function in vivo [7,15]. We demonstrated that CRM can stabilize hepatic MCs to prevent degranulation and that CMP 48/80 can deplete hepatic MCs through degranulating most of the stored granules by repeated injections [16] (data not shown).

Therefore, we aimed to investigate whether donor liver preconditioning with CRM in an MC stabilizing way or CMP 48/80 in MC depleting way would reduce AR and prolong recipient survival in a rat orthotopic liver transplantation (OLT) model.

METHODS

Ethics Statement

All animal handling and surgical procedures were approved by the Animal Care and Use Committee of the Shanghai Jiao Tong University School of Medicine.

Animals and Reagents

All animals were purchased from Sino-British Sippr/Bk Laboratory Animal Ltd. (Shanghai, China) and maintained in standard conditions with access to food and water *ad libitum*. Animals were fasted for the 12 hours prior to surgery. CRM and CMP 48/80 were purchased from Sigma (St. Louis, Mo, United States).

Rat OLT Surgery

Inbred male Dark Agouti (DA, $RT1^a$) and female Lewis (LEW, $RT1^l$) rats, aged 12–14 weeks, served as donors and recipients, respectively. Donors weighed 210–240 g, and recipients 180–210 g. Syngeneic transplantation was performed in 210–240 g male Lewis (6–8 weeks) to 180–210 g female Lewis (12–14 weeks) rats as controls. All surgical procedures were performed with clean but nonsterile instruments. Donors were anesthetized with pentobarbital sodium and recipients with ether. A nonarterialized OLT was performed according to Kamada's technique [17] with a small modification. Briefly, the right adrenal, right renal, gastroduodenal, left phrenic, and splenic veins, and the hepatic artery were ligated. Prior to donor liver explantation, the livers were perfused via the portal vein with 20 mL of 4°C heparinized normal saline (50 U/mL).

The explanted livers were immersed in 4°C cold saline. Cuffs were mounted on the portal vein (PV) and infrahepatic vena cava (IHVC). The cuffs were made from the cannulas of Cordis sheath introducer system (Johnson & Johnson Medical Shanghai Ltd., Shanghai, China). PV cuffs were made from 4 French cannulas (inner diameter 1.35 mm; outer diameter 2.0 mm) and IHVC cuffs were made from 6 French cannulas (inner diameter 2.0 mm; outer diameter 2.7 mm). Thirty minutes prior to recipient anesthesia, 10 mg/kg of atropine and 100,000 U penicillin were injected intramuscularly into the hind leg.

For the recipient procedure, an abdominal midline incision was made. The hepatic artery was doubly ligated and divided. The bile duct was divided at the hepatic duct bifurcation. The PV and IHVC were cross-clamped. The suprahepatic vena cava (SHVC), including part of the diaphragm, was clamped with a pediatric Satinsky's clamp. The recipient liver was removed, and the donor liver placed orthotopically. The SHVC anastomosis was performed in an end-to-end fashion with continuous 7-0 nylon suture (Jinhuan Medical Products Co., Ltd., Shanghai, China). PV and IHVC anastomoses were performed using the cuff technique. The bile duct was reconstructed with an indwelling Teflon stent (inner diameter 0.6 mm; outer diameter 0.8 mm; length 8 mm).

Recipients were administered 2 mL saline via the femoral vein and 2 mL into the peritoneum intraoperatively to compensate for fluid loss, and 100,000 U penicillin intramuscularly into the hind leg. Post-transplantation, animals were placed under a heating lamp for 2 hours with free access to food and water. There were no significant differences in duration of cold preservation (<40 minutes) and portal venous clamping time (16–18 minutes) between groups. Immunosuppressive therapy was not used. Rats that died within 3 days of transplantation were excluded from the study. Five rats in each group were kept for survival assessment.

Donor Liver Preconditioning With CRM and CMP 48/80

CRM group. CRM (100 mg/kg, intraperitoneally) was administered to DA rats 16 hours and 40 minutes prior to donor surgery as a MC stabilizing way before [18]. And it can stabilize hepatic MCs in rat liver.

CMP 48/80 group. CMP 48/80 was used to precondition DA rats, by an MC depletion method described by Wei et al [16]. We have verified that this method can deplete MCs in the rat liver as well. Briefly, a 0.1% (weight/volume) solution of CMP 48/80 in phosphate buffered saline (PBS) was administered intraperitoneally twice a day for 8 doses (0.6 mg/kg for the first 6 doses, 1.2 mg/kg for the last 2 doses) beginning with the evening dose. Donor surgery was performed 5–6 hours after the last dose.

PBS group. PBS in the same volumes as the CMP 48/80 group was given intraperitoneally as the control.

Syngeneic group. OLT male-Lewis-to-female-Lewis rats without any treatment were used as the negative control.

Sample Collection

Five transplanted rats in each group were used for sample collection. Three days after transplantation, blood samples were obtained from the vena cava after euthanasia. After coagulation, samples were centrifuged to isolate the sera, which were stored at −80°C until use. The median liver lobes were harvested and fixed in 4% paraformaldehyde in PBS for histological analysis. The left lateral segments were harvested, snap-frozen in liquid nitrogen, and stored at −80°C for RNA extraction.

Liver Enzyme Measurement

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using a Hitachi 7600-120 automatic biochemical analyzer (Tokyo, Japan). Results were expressed in international units per liter.