Application of modified two-cuff technique and multiglycosides tripterygium wilfordii in hamster-to-rat liver xenotransplant model

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Abstract

AIM: To modify the hamster-to-rat liver xenotransplant technique to prevent postoperative complications, and to study the inhibiting effect of multiglycosides tripterygium wilfordii (TII) on immune rejection.

METHODS: Female golden hamsters and inbred male Wistar rats were used as donors and recipients, respectively. One hundred and twelve orthotopic liver xenotransplants were performed by Kamada’s cuff technique with modifications. Over 72 hour survival of the animal after operation was considered as a successful operation. When the established surgical model became stable, 30 of the latest 42 cases were divided into untreated control group (n=15) and TII group (n=15) at random. Survival of recipients was observed. Liver specimens were collected at 2 and 72 h from the operated animals and postmortem, respectively, for histological study.

RESULTS: The successfully operative rate of the 30 operations was 80 %, and the survival of the control and TII group was 7.1±0.35 was days and 7.2±0.52 days, respectively (t=0.087, P=0.931). The rate of conjunctival hyperemia in control group (100 %) differed significantly from that (31 %) in TII group (P=0.001). Rejection did not occur in both groups within 2 h postoperatively, but became obvious in control group at 72 h after surgery and mild in TII group. Although rejections were obvious in both groups at death of recipients, it was less severe in TII group than in control group.

CONCLUSION: This modified Kamada’s technique can be used to establish a stable hamster-to-rat liver xenotransplant model. Monotherapy with multiglycosides tripterygium wilfordii (30 mg kg-1 d-1) suppresses the rejection mildly, but fails to prolong survival of recipients.

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MATERIALS AND METHODS

Animals and liver xenotransplantation
Female golden hamsters (weighing 80-130 g) and inbred male Wistar rats (weighing 130-180 g) were used as donors and recipients, respectively. One hundred and twelve orthotopic liver xenotransplants were performed by Kamada’s cuff technique with modifications, which were outlined as follows.

Donor operation

Ligaments and vessels around the liver were partially dissected while the liver was protected by wrapping film. The common bile duct was entered from anterior wall and a Teflon catheter was inserted into the lumen proximally. The proper hepatic artery was dissected but not ligated. The infrahepatic vena cava (VC) was clamped at the level of left renal vein. The liver was perfused through the abdominal aorta with 10-20 ml of cold (4 °C) lactated Ringer’s solution containing 5 U/ml of heparin. Meanwhile the thoracic cavity was opened with the thoracic aorta clamped, and the thoracic inferior VC was opened to release the perfusate. Then, the infrahepatic VC was transected at the upper part of the clamp. So the perfusate could escape from the two ends of VC. Deep ligaments around the liver were dissected while perfusing and the right supraparenal vein was ligated. At the end of perfusion, the proper hepatic artery and portal vein (PV) at the level of splenic vein were ligated and transected. The free liver was stored in lactated Ringer’s solution at 0-4 °C.

Preparation of donor liver

Preparation of the donor liver was done in lactated Ringer’s solution at 0-4 °C. Two cuffs with different diameters were mounted on the PV and infrahepatic VC, respectively. The cystic duct near the common bile duct was ligated and followed by removal of the gallbladder. The suprahepatic VC was trimmed and a stitch was left on each side for suture.

Recipient operation

Vessels, the common bile duct and ligaments around the recipient liver were divided, respectively. The right suprarenal vein behind the papilla lobe was ligated, the recipient liver was removed and covered with fresh film orthotopically. The suprahepatic VC was sutured, the PV was anastomosed with cuff method. The infrahepatic VC was anastomosed as the method for suprahepatic VC. Finally, the distal part of Teflon catheter in donor bile duct was inserted into the recipient bile duct and secured by a silk suture. The greater omentum was wrapped around the bile duct. The abdominal incision was closed with continuous suture.

INTRODUCTION

The hamster-to-rat liver transplant model is useful to study immunology and physiology of liver xenotransplantation (XT)[1-11], especially for the extended host response to long-surviving xenografts[12]. As it is very difficult to establish this surgical model[13], we summarized our surgical experience in producing for performing this model.

The model could serve as a tool to evaluate immunosuppressive drugs[14,15]. Tripterygium wilfordii hook F (TWHF) has been used for more than two thousand years as a traditional Chinese herb. TII is extracted and refined from the root of TWHF. In recent years, TII and other extracts of TWHF have been applied to allotransplantation and cardiac XT as immunosuppressive drugs with successful results[16-27]. However, the effects of TII in liver XT are unknown and deserve study.
Treatment of the animal with T\textsubscript{II}

When the model became stable, 30 recipients of the latest 42 cases were divided into untreated controls (\(n=15\)) and treatment group with T\textsubscript{II} 30 mg·kg\(^{-1}\)·d\(^{-1}\) (\(n=15\)) at random by gavages three days prior to operation till the end of experiment.

Liver specimens were collected at 2 and 72 h after operation or at death of the recipients, respectively, fixed in 10 % formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for light microscopy to determine the grade of rejection.

Statistical methods

The results were analyzed by \(t\) test and Fisher’s exact test, respectively. Statistical significance was defined as a \(P\) value less than 0.05.

RESULTS

Liver xenotransplantation

One hundred and twelve operations were performed from October 2000 to March 2001. The successful rate of the latest 30 operations was 80 % (24/30). The causes of death were anesthesia accident in one case, chronic hemorrhage from the surface of donor liver in 3 cases, thrombosis in infrahepatic VC in one case and suppurate peritonitis in one case. The 6 recipients died within 72 h after operation were excluded from the experiment\(^{14}\). The average survival of 24 cases was 7.2±0.44 days.

Effects of T\textsubscript{II} on survival of recipients

The average survival of the untreated controls and T\textsubscript{II} group was 7.1±0.35 days and 7.2±0.52 days, respectively (\(t=0.087\), \(P=0.931\)). Four cases in controls and 2 in T\textsubscript{II} group died within 72 h after operation were eliminated from statistic study.

Effects of T\textsubscript{II} on conjunctival hyperemia of recipients

The morbidity of conjunctival hyperemia was 100 % (11/11) in controls and 31 % (4/13) in T\textsubscript{II} group (\(P=0.001\)). The commence time of conjunctival hyperemia in controls and T\textsubscript{II} group were 4.73±0.47 days and 5.75±0.5 days, respectively (\(t=3.688\), \(P=0.003\)).

Pus in conjunctiva was obvious in controls with conjunctival hyperemia, and three cases had ablepsia. However, no pus and ablepsia were observed in T\textsubscript{II} group.
Histopathology

Two hours after operation  The structures of hepatic lobule were normal and no inflammatory cells in portal area in both groups were observed (Figures 1 and 2).

Seventy-two hours after operation  In controls, hepatic lobules were normal, numerous inflammatory cells consisting mainly of mononuclear cells and neutrophilic granulocytes infiltrated in portal area, edema could be seen in the tunica intima of interlobular veins and inflammatory cells infiltrated into subintima. However, epithelia of interlobular bile ducts were not injured (Figure 3).

In Tg group, the structures of hepatic lobules and interlobular veins were normal, moderate amounts of inflammatory cells were found in portal area, and epithelium of interlobular bile ducts was intact (Figure 4).

Postmortem specimens on day 7 postoperation  In controls, hepatic lobules were damaged severely, inflammatory cells consisting mainly of mononuclear cells and neutrophilic granulocytes in portal area as well as massive hemorrhage were observed, interlobular vessels were disappeared and damaged epithelia of interlobular bile ducts were observed (Figure 5).

In Tg group, many mixed cells could also be seen in portal area, but most of the interlobular vessels and bile ducts were existed with inflammatory intima, and variable injured epithelia, hepatocytes around portal area were swollen, degenerated and partially necrotic and some liver plates were destroyed (Figure 6).

DISCUSSION

Sun et al.[29] firstly advocated that perfusate should enter the liver via dual vascular systems of the hepatic artery and the PV, then the liver and its structures around were divided. The authors performed 300 liver transplantations in rats, and the successful rate of operation and survival rate in one week were 92.7 % and 88.4 %, respectively. So we adopted this dual perfusion except for perfusion by PV in initial trials of 20 cases. We found that perfusion by PV could often result in incomplete perfusion in some small areas and undesirable pressure. Incomplete perfusion did not occur with dual perfusion, and due to the invariant pressure of intestinal circulation, the liver was not mechanically injured by fluctuant pressure and warm ischemia and mechancial injuries were avoided. If the donor liver was dissected at the end of complete perfusion, the vessels became pale and were difficult to be identified and were easily injured accidentally. After modification of the technique, the quality of the liver in donor was excellent in the trials and the survival time of recipient animals was similar to that in the literature[14,30,31].

We used to ligate the cystic duct, and then to remove the gallbladder and electrocauterize the bed of the gallbladder. However, it usually rendered slow hemorrhage in the bed of the gallbladder and the recipients died in hours after transplantation. So we modified the method as follows: ligating cystic duct near the common bile duct, then ligating cervix of the gallbladder and cutting gallbladder at the distal side of the ligation. No postoperative hemorrhage was observed after the modifications.

For the sake of exposing and ligating suprahepatic VC of the donor and the recipient, papilla lobe and right lobe of the liver were turned over frequently and thus were injured easily, and the recipient could die from mild hemorrhage from the surface of the graft. Sometimes, ligation of the infra-branch of suprarenal vein was mistaken for main stem with left supra-branch unligated, which rendered hemorrhage at recipient liver’s cutting and posttransplantation. Therefore, we ligated the right suprarenal vein at the end of perfusion, the structures around the donor liver were divided completely and the right suprarenal vein was exposed easily, so that liver injury and false ligation were reduced. Loop-ligation behind papilla lobe method was used to ligate the suprarenal vein of the recipient. The right suprarenal vein and part of ligaments behind the recipient liver were ligated with 5-0 silk sutures behind the papilla lobe and near the infrahepatic VC. This ligation was reliable, simple, and shortened the anhepatic phase directly.

Hamster’s liver is more fragile than that of rat. Slight press or attrition on its surface could result in contuse and chronic hemorrhage. Moreover, it has poor coagulating mechanism. Thus, fresh film has been used to protect the donor liver in operation, and the pressure on donor liver could be dispersed, the accident stab to the donor liver by instruments could be relieved to some extent and the donor liver could be kept from drying and crisping. Moreover, careful and delicate operation should not be ignored. Nevertheless, in our 30 trials, 3 of 6 failed cases died due to chronic hemorrhage on the surface of the donor liver.

We found that Tg at a dosage of 30 mg·kg⁻¹·d⁻¹ could not prolong the survival of the operated animal. But the rejection in Tg group was less severe in comparison with that of controls. We speculated that this dosage of Tg had certain limited immunosuppressive effect. Li et al.[32] found that the extracts of TWHF could inhibit human peripheral blood mononuclear cells (PBMC) in a concentration-dependent manner and the best inhibitory rate could be achieved at 72 h. Another research suggested that Tg at a dosage of 30 mg·kg⁻¹·d⁻¹ had no effects on the liver, kidney, heart and the total number of WBC in rat, but could inhibit the transforming of spleen lymphocytes significantly. Tg at this dosage could prolong the survival time of renal allotransplantation in Wistar-to-SD combination significantly. When it was combined with CsA, the survival time could be further prolonged[33]. Nevertheless, in other organ transplantations such as small intestine transplantation, whose rejection was relatively strong, and the monotherapy with Tg was demonstrated to have limited effects[21]. Wang et al.[16] applied PG27 (extract of TWHF) combined with a low dosage of CsA to hamster-to-rat cardiac xenotransplantation and all xenografts survived more than 100 days. However, they found that either PG27 or CsA had no such effects. Therefore, although a high dosage of Tg was given to the recipient 3 days before operation in our trials as monotherapy, the survival time of the recipient was not prolonged due to the strong rejection in this model.

In this study, conjunctival hyperemia occurred in all untreated control recipients on day 5 after operation, and deteriorated rapidly, even resulted in ablespia. However, in the recipients treated with Tg, the sign occurred only in 31 % rats (on day 5-6 after transplantation) and ablespia did not occur. The differences between the two groups were highly significant and the similar report has not been found in literature. We speculated that conjunctival hyperemia could be a local representation of rejection in this model. Relieving the sign with Tg treatment supported the hypothesis, but the exact mechanism of the sign needs to be further studied.

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