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Research Paper

Establishment of simultaneous pancreas and kidney transplantation (SPK) model with cuff technique and portal venous drainage in rats☆

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Abstract

Objective: To establish a simultaneous pancreas and kidney transplantation (SPK) model in the rat. **Methods**: SD rats served as donors and recipients. The donor portal vein and the recipient superior mesenteric vein were anastomosed and the donor renal veins and recipient renal veins were anastomosed by cuff method. Arterial reconstruction was carried out by end to side anastomosis of the donor abdominal aorta to the recipient abdominal aorta. Enteric drainage was performed by side to side anastomosis between donors' duodenum and recipients' jejunum. The donor ureter -bladder valve was anastomosed to the bladder of recipients. **Results**: Out of 30 cases of SPK transplantation, 24 had normal serum glucose and serum creatinine after operation. The successful rate was 80 %. **Conclusion**: This model of SPK in rats is stable and reliable, which could be applied for further scientific research.

Keywords: simultaneous pancreas and kidney transplantation; rat; model

INTRODUCTION

simultaneous pancreas and kidney transplantation (SPK) is regarded as the most effective method for diabetes complicated with uremia. But the basic study about SPK can not catch up with the development of the clinic. At present, the animal model of pancreas transplantation was mostly established according to the method of systemic venous drainage[1, 2]. The method indicated that the venous drainage of the donor pancreas was reconstructed by end to side anastomosis between the donor portal vein and the recipient cava [3]. However, the normal way of insulin delivery was changed in this method that might result in some complications, such as changes in glucose tolerance and insulin sensitivity, hyperinsulinism, insulin resistance, and so on [4]. The purpose of this study was to establish a stable SPK model in rat, which was in accordance with physiology.

MATERIALS AND METHODS

Animals

The male closed flock SD rats served as donors and recipients, which were offered by the Experimental Animal Center of Jiangsu Province. The donors (220-250 g) and the recipients (250-280 g) were matched under the condition that the weights of the recipients were about 30 g heavier than those of the donors.

Model of the diabetes mellitus

Diabetes was induced by a single intravenous injection of streptozocin (STZ; Sigma, USA) at a dose of 60 mg/kg weight. The nonfasting blood glucose and urine glucose of the rats were detected before the inducement of DM and on alternate day after the STZ injection. Only rats with nonfasting blood glucose more than 16.8 mmol/L and strong positive reaction of urine glucose were selected as recipients.

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The procedure of operation

They were anesthetized with an intraperitoneal injection of 1 % pentobarbital sodium solution (40 mg/kg weight).

Donor operation

After the portal vein and pancreas were separated by the methods introduced by ZHU [5]. The abdominal aorta was ligated about 2 cm below the left renal artery. After that, the left ureter was dissociated and ureter-bladder valve about 5 mm×5 mm was made. Abdominal aorta was dissociated when the right renal artery and several lumbar artery were cut. Following the whole separation of left renal and left adrenal gland vessel, abdominal aorta was clamped near the diaphragma, and the portal vein and renal vein were cut immediately. The graft was perfused by cold (4℃) saline containing heparin at the concentration of 25 U/ml with low pressure via the aorta until no blood flowed from the vein. Then the pancreas-kidney was saved in cold saline for cannula anastomosis.

The preparation for renal vein cuff

The whole procedure was operated in the 4℃ saline. A polyethylene tute about 0.5 cm was adopted for cuff, whose external diameter was 2.0 mm and inner diameter was 1.5 mm. The renal vein was drafted by 3 nylon symmetrically, and then the retention suture were passed through the cuff. Finally, the vein was fixed on the cuff evertedly by 5-0 nyl on.

Recipient operation

About 2 cm of left renal vein was reserved after the removal of left kidney and the ligation of renal vein at crotch. After heparinization, the superior mesenteric vein was exposed along the middle colic vessels and a 1. 5-cm-long segment of the vein was mobilized. The recipient aorta was clamped, and a hole was made at the front wall with a diameter similar to that of the donor aorta. The anastomosis was performed with running suture, using 9-0 nylon. The back and front walls were both sutured from outside. Then, the vein anastomosis was done as follows: the anastomosis of the back wall was made firstly from the tail to the head part of the vein inside, and that of the front wall was made from the head to the tail part of the vein outside with running suture. At last the renal veins were anastomosed by cuff methods. The donor kidney was placed in left renal hole, and the vein was braced by 3 nylon symmetrically. Then the cuff was put into the vessel and fixed with 5-0 nylon. The vein and artery were reopened one after another. The color of the graft recovered quickly. Finally bladder and duodenum were anastomosed between the donors and recipients respectively, and the right kidney was removed. At last, the whole peritoneal cavity was rinsed with warm saline solution and the abdomen wall was closed.

Postoperative treatment

After the operation, 100 000 U Penicillin was injected muscularly to prevent infection. The room temperature was kept between 25-28℃. The blood glucose was measured alternate day. The value of the blood glucose less than 11.2 mmol/L was regarded as normal standard, and the value more than 11.2 mmol/L on 2 sequential tests showed the pancreas graft loss.

RESULTS

Of the 30 recipients, 24 survived more than 5 d with normal blood glucose. The successful rate of transplantation was 80 %. The donor operation took 50.90 ± 3.13 min and the recipient operation taking 73.03 ± 3.85 min. The mean time for artery anastomosis was about 10 min, and that of vein anastomosis was about 10 min,too. The mean cold ischemic time was 45 min. The mean value of blood glucose preoperative, 1,3,5 d after transplantation was 18.0 ± 0.54,4.76 ± 0.12,5.18 ± 0.10,5.69 ± 0.12 mmol/L. The causes of death were cuff thrombosis(1 case), abdominal cavity infection (1 case), narrowing of portal vein anastomotic stoma (2 cases), renal failure due to obstruction of ureter (1 case) and 1 case of unknown cause.

DISCUSSION

Former rat models of pancreas transplantation were mostly established according to the methods of systemic vein drainage introduced by Lee[5],Which might result in some complications such as hyperinsulinemia and insulin resistance. In this article a new endocrine drainage through portal vein was selected, which was in accordance with the clinic and physiology. And the methods were documented in our previous research [6-8]. The side-to-side anastomosis between duodenums was adopted. It could solve the problem of ischemia or necrosis of intestine and avoid the complication caused by bladder drainage[9]. The method of portal vein drainage and enteric drainage has been getting more and more recognition because it is more physiological [10]. In addition, the potential immunological benefits of portal vein de-

livery of donor antigens attract much attention in organ transplantation [11].

At present the model of SPK in rats adopt three - vascular anastomosis [12]. But the method required proficiency, and longer time to block the vessels which may lead to more effect to the blood circulation. In this article a cuff-anastomosis was used to avoid the problems, which was first used in orthotopic liver transplantation by Kamada [13]. In our country, the method was first used by PENG in pancreas transplantation[14]. The operation is very simple and could be finished within 2 min, which may avoid the bowel necrosis caused by long blockage of portal vein system [15] . And the anastomosis is firm and may lead to less complication such as stenosis, errhysis, leakage of the stoma. As to cuff method, there are several styles. Both renal vein to superior mesenteric vein and portal vein to upper renal vein produce a common problem-tortuosity of vein. In present study, the kidney was placed in the former renal bed, which decreased the incidence of tortuosity.

The establishment of SPK is difficult, and there are several experiences worth introducing: a. heparinization; b. prevention of hypovolemic shock; c. warm-keeping; d. no direct touch of pancreas with hands and instruments; e. prevention of tortuosity.

In conclusion, this study demonstrates that SPK transplantation in rats is stable and reliable, which is in accordance with the clinical pancreas transplantation and can be used for further research.

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