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Hamano, Mitsuaki
Kuroda, Yoshikazu
Saitoh, Yoichi

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PANCREAS TRANSPLANTATION USING NON-SUTURE CUFF TECHNIQUE IN THE NECK

Mitsuaki HAMANO, Yoshikazu KURODA, and Yoichi SAITOH

First Division, Department of Surgery,
Kobe University School of Medicine

INDEXING WORDS

heterotopic pancreas transplantation; cuff technique; diabetes mellitus

SYNOPSIS

To get a successful pancreas transplantation in a rat model, we performed heterotopic pancreas transplantation with non-suture cuff technique to neck vessels. As the graft was placed in the neck, we could avoid the clamping of systemic circulation which is essential but detriment to the rat during vascular anastomosis in intraabdominal grafting. The diameter of the portal vein was enlarged to prevent outflow block after transplantation. The cuff was fixed by fat clay during anastomosis to perform vascular anastomosis easily. As a result the success rates was 16/20, 80%. 16 rats survived more than 30 days and maintained serum glucose level within 200mg/dl throughout the experiment. The heterotopic pancreas transplantation using non-suture cuff technique to the neck vessels is safe, reliable and recommended as the experimental model using the rat.

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Authors' names in Japanese : 浜野光章, 黒田嘉和, 齋藤洋一

INTRODUCTION

The establishment of an animal experimental model is essential for solving clinical problems. Healthy adult rats of pure breed are ideal for the study of immunology of organ transplantation because the major histocompatibility (MHC) is investigated in detail in rats. However, smaller animals require microsurgical techniques for vascular anastomosis and this has provided the major difficulties with lengthy training, postoperative complications, fairly long warm ischemic time and clamping the systemic circulation for long periods of time. To get survival cases as for pancreas transplantation using microsurgical techniques in the abdomen in rats, the complete anastomosis of both the arterial and venous connections and reducing the clamping time of systemic circulation are required. However, in the present state of microvascular anastomosis of less than 2 mm, the satisfactory results were not obtained because of the stricture of anastomosis, thrombosis or the formation of granuloma. In 1984 Olausson et al. reported non-suture organ grafting using cuff technique to the neck vessels[12]. The cuff technique is faster than suturing, decreasing warm ischemic time, and there is no bleeding after revascularization. On the other hand, it is not easy to get a constant survival rate after pancreas transplantation in the abdomen even if the technique is no problem because intraabdominal grafting is invasive. We also demonstrated that the pancreas transplantation in the neck was safer and less invasive compared with intraabdominal grafting in a canine model[7]. For the study of experimental pancreas grafting using small animals, the achievement of a safe and rapid technique is essential. Based on these facts, we performed non-suture cuff technique to the neck vessels for pancreas transplantation in a rat model.

MATERIALS AND METHODS

Animals : Inbred male Lewis rats weighing 250-300g were used as donors and recipients. Diabetes mellitus (DM) was induced by a single intravenous injection of streptozotocin (55mg/kg) with phosphoric buffer solution (PH = 6.0) from V. pudenda externa. Within three days, 90% of rats developed DM. The random plasma glucose of all the DM rats exceeded 400mg/dl before transplantation. The animals were obtained as specific pathogen-free stock from Charles River. All animals were maintained in a temperature and humidity controlled clean room at the institute for Laboratory Animal Research, Kobe University School of Medicine.

Operation procedures : (Graft harvesting-Fig. 1) Pancreas transplantations were performed using microsurgical techniques with a microscope of 4 magnifications. Under the ether anesthesia, the abdomen was entered via a midline incision. Operation

area always had to be kept wet, so that operator had to drop a little saline in it occasionally.

A 7-0 silk suture was usually used but exceptions. After the stomach was lifted and retracted forward and upward, a suture could be placed at the middle of spleen. No-touch technique was performed by handling this suture and cotton buds. Tributaries in the vicinity of the pancreas had to be carefully ligated to prevent bleeding from the pancreas after transplantation. The right gastroepiploic artery and vein were ligated. At this time care had to be taken that the pancreaticoduodenal artery was not injured. A silk tie was placed on the duodenum 0.5cm below the pylorus, and the duodenum was divided between the stomach and the ligature. Free stomach was lifted and retracted forward and upward so that the gastric artery could be doubly ligated. The esophagus and the short gastric artery were ligated and divided, and the stomach was removed.

Rectum was ligated and divided, oral side of this was retracted to the right under the duodenum and intestinum tenue and downward and so Treitz ligament was developed to the right. Blunt dissection was used to separate the duodenum and pancreas from the ascending and transverse colon. Then the superior mesenteric artery and vein were exposed. The intestinum tenue was ligated and divided at the level of the Treitz ligament. At last the superior mesenteric artery and vein were ligated and divided at the extremity inferior of the pancreas, Large intestine and intestinum tenue were removed.

Ligation and division of the bile duct and the hepatic artery were accomplished close to the liver separately.

After the left renal artery and vein were ligated and divided, the left kidney was removed. From left side, blunt dissection of the aorta, including the celiac and superior mesenteric arteries, was made, and other aortic tributaries were ligated and divided.

Afterwards the right renal artery and vein were ligated and divided.

The duodenum was removed from the pancreas by dividing the attached pancreas as closely as possible to the duodenum using 7-0 fine silk sutures. At the same time pancreas duct was doubly ligated.

Diaphragma was resected from the attached aorta and the aorta was free.

A right angle clamp was placed on the aorta 1cm above the celiac artery. The portal vein was divided at the liver hilum. About 5ml of cold heparinized normal saline was perfused through the aorta. Then care had to be taken that aortic segment was not pierced and pressure was not so high.

As the perfusion was terminated, the aorta was ligated below the superior mesenteric artery. An aortic segment, with the celiac and superior mesenteric artery attached, was obtained by sharp division of the aorta immediately below the clamp

and below the ligature. And duct-ligated total pancreas without the duodenum was removed and placed in cold saline.

Bridging tissue between right sinister and right dexter of portal vein was resected so that connected area of the portal vein could be enlarged (Fig. 5a).

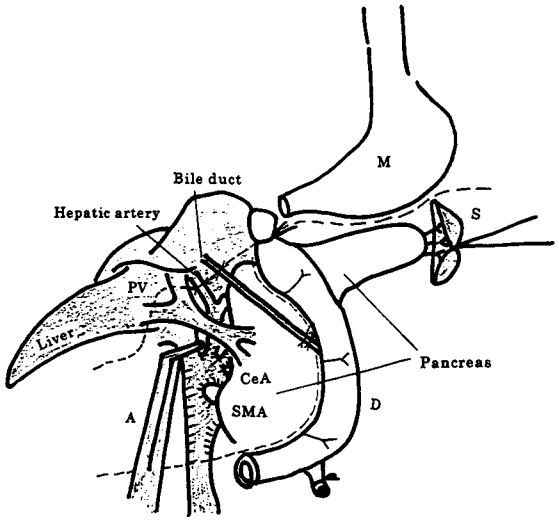

Figure 1. Graft harvesting.
Duct ligated total pancreas without the duodenum was harvested from the donor rat with an attached segment of donor aorta including the celiac and superior mesenteric arteries as arterial inflow and the portal vein was maintained for venous outflow.

(Engraftment-Fig. 3)

In the recipient rat, a skin incision was made on the right anterolateral aspect of the neck from the base of the jaw to the clavicula. The jugular vein was carefully cleaned of all connective tissue by sharp dissection as long as possible. The jugular vein was clamped caudally with a small, soft vascular clamp and was ligated, dissected free in the cranial portion and was perfused with heparinized saline.

Free space of operative area was filled with saline so that vessels could be floated and easily handled. The vessel was then severed and a suture 6-0 silk was placed in the lateral wall (Fig. 2a). Cuff was fixed by fat clay and clamp (Fig. 2b). The suture and the vein were then passed through cuff (Fig. 2c). A suture was **retracted by clamp**. The vessel wall could easily be everted over the cuff without the need of an assistant (Fig. 2d). A ligature was tied around the vessel and cuff (Fig. 2e).

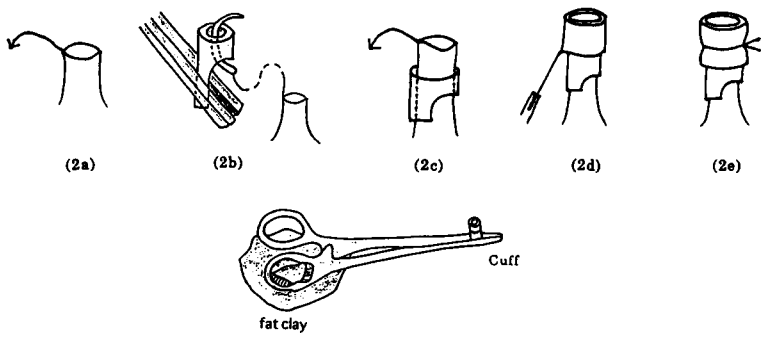

Figure 2.
The vessel was then severed and a suture 6-0 silk was placed in the lateral wall (Fig. 2a). Cuff was fixed by fat clay and clamp (Fig. 2b). The suture and the vein were then passed through cuff (Fig. 2c). A suture was retracted by clamp. The vessel wall could easily be everted over the cuff without the need of an assistant (Fig. 2d). A ligature was tied around the vessel and cuff (Fig. 2e).

The sternocleidomastoid muscle was ligated and dissected so that the right common carotid artery could be exposed. The common carotid artery was then similarly prepared, a suture 9-0 silk was placed in the lateral wall, cuff (3FG, 1.02mm × 3.60mm) being of the same length but with inner diameter approximately half that of the vein cuff (5FG, 1.65mm × 3.60mm).

The jugular vein cuff and carotid artery cuff were fixed by fat clay and clamp. No-touch technique was performed by wrapping pancreas with cold wet gauzes. The donor aorta was connected to the carotid artery cuff with a ligature around the donor vessel and the cuff. Bilateral suture of the portal vein were retracted by clamps so that operation could be easy. The donor portal vein was connented and secured to the jugular vein cuff.

After that, the vein and aorta clamps were released in the order. After revascularization the spleen was removed. Bleeding were carefully controlled by gentle compression of the connection, local application of a hemostatic agent (Avitene) and with electrocautery.Transplanted pancreas was fixed in the neck. At this time care had to be taken that vessels were not kninked. Skin suture was performed by 3-0 silk.

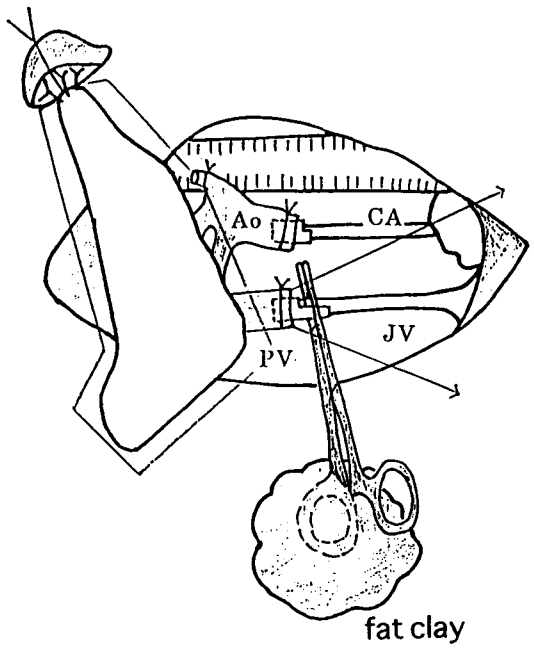

Figure 3.
The jugular vein cuff and carotid artery cuff were fixed by fat clay and clamp. No-touch technique was performed by wrapping pancreas with cold wet gauzes. The donor aorta was connected to the carotid artery cuff with a ligature around the donor vessel and the cuff. Bilateral suture of the portal vein were retracted by clamps so that operation could be easy. The donor portal vein was connected and secured to the jugular vein cuff.

Experimental protocol : Pancreas transplantations (n = 20) were performed from Lewis rats (n = 20) to DM Lewis rats (n = 20).
Parameters : Blood glucose was measured consecutively on days 1-10 post-transplant, and then at 20 and 30 days post-transplant. Graft survival was judged when a rat had normoglycemia (below 200mg/dl). Graft failure was judged when a rat had hyperglycemia above 200mg/dl for at least 2 consecutive days.

RESULTS

The successful rate was 80% (16/20). 4 grafts were lost. Two rats died of venous outflow obstruction of the graft and one rat died of acute hemorrhagic pancreatitis. One rat died of pneumonia due to the long-term anesthesia in the diabetic recipient. 16 grafts were survived more than 30 days and their blood

glucose were all within 200mg/dl at 30th. Normoglycemia was established within 8hrs of whole pancreas transplantation (Fig. 4). The blood glucose levels rose up immediately after transplantation and started to decline within 2hrs and reached values below 100mg/dl between 5 and 8hrs.

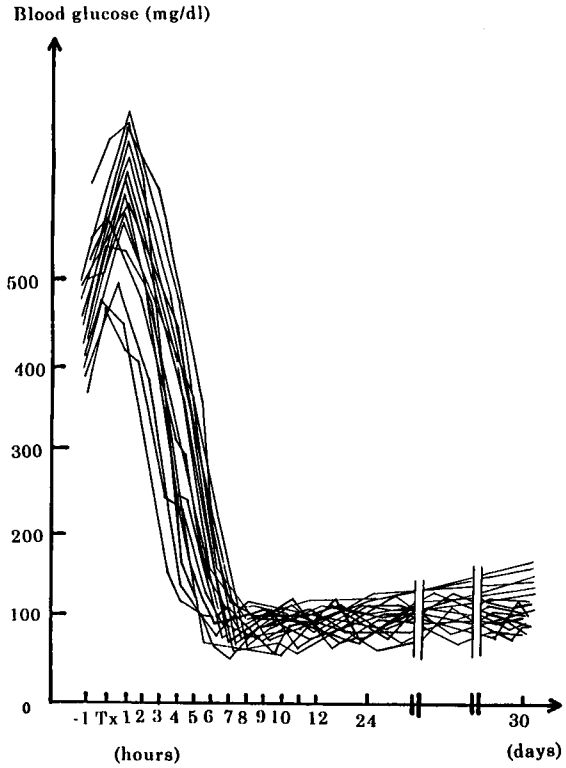

Figure 4.
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DISCUSSION

Rats are ideal for the animal experiments of organ transplantation because the major histocompatibility (MHC) is investigated at detail in the various breed of rats as compared that the major histocompatibility (MHC) of large animals is unclear[4]. We performed pancreas transplantation in rats as the animal experimental model, but pancreas transplantations in rats were quite difficult. In the first place, the difficulty was accompanied by the preparation and breeding of DM rats, and control of ether anesthesia for DM rats. With overuse of streptozotocin, blood glucose became high (more than 500) soon and the condition of the whole body became feeble. As DM rats became polyuria and feeble, DM rats had to be supplied with more than enough water. Streptozotocin must be mixed with phosphoric buffer solution (PH = 6.0) lest rats shoud be thirsty. Atropin sulfate was injected intraperitoneally to prevent the suppression of breathing during operation under ether anesthesia. Operator must shorten the operation time as short as possible for the successful transplantation in recipient operation. This was performed to prevent the death because of pneumonia after long-term operation under deep ether anesthesia. In addition, pancreas transplantations were performed from the fourth day to the seventh day after the injection of streptozotocin. At the day of transplantation, blood glucose was ideally between 400 and 500mg/dl at random.

In the second place, the difficulty was accompanied by procedures of the pancreas transplantation. The presence of the duodenum in most of these grafts has been associated with complications related to duodenal stump disruption or duodenal necrosis[9]. A lethal GVHR (Graft-versus-host reaction) is not induced after liver or pancreas grafting alone, but is always induced when a longer length of intestinal graft and spleen is implanted especially as for allograft[2, 15]. Based on these results we removed the duodenum from pancreas. Acute pancreatitis and pancreatic thrombosis are major two complications after pancreas transplantation that are associated with both an increased patient morbidity and a decrease in pancreas graft survival rates[5]. Sun lee et al. reported that the major complications, acute hemorrhagic pancreatitis could be partially prevented by carefully ligating tributaries in the vicinity of the pancreas, reducing the warm ischemic time, perfusing with cold saline, and using a no-touch technique[16]. Moreover one of the most important point in pancreas transplantation is that the portal vein as venous connections must be enlarged to prevent congestion of blood. So the jugular vein was carefully cleaned of all connective tissue by sharp dissection as long as possible in case of using cuff technique. In our cases using microsurgical technique, two methods were used (Fig. 5a, 5b).

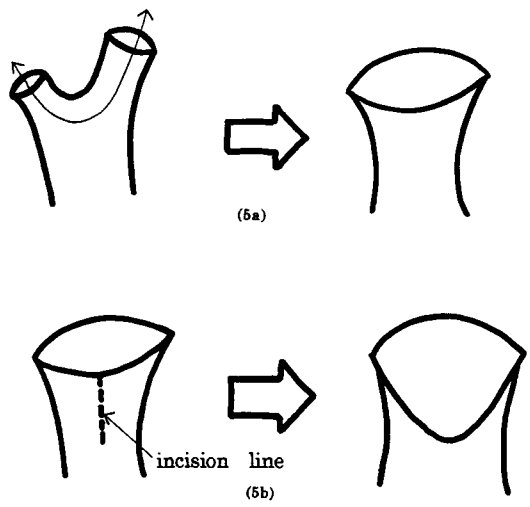

Figure 5.
One of the most important point in pancreas transplantation is that the portal vein as venous connections must be enlarged to prevent congestion of blood.

As to prevent thrombosis, we used in situ perfusing, which was reported to be effective in pancreas transplantations by Belzer et al[1, 10, 11]. As the pancreas became edematous when the pressure of perfusion was high, we setted the pressure of perfusion (about 150cmH_2O).

The pancreas is a composite organ consisting of two functionally and morphologically distinct components, the exocrine and the endocrine parts. In pancreas transplantation, the management of pancreatic juice become a subject of discussion. Now urinary bladder drainage method or enteric drainage method or duct obstruction by retrograde injection of prolamine are mainly performed in clinical settings. The intestinal drainage method, which is recommended by Groth, is physiological if the risk of suture failure and graft infection are overcame[3]. The fatal infection, such as peritonitis, is occurred by the leakage of pancreas juice which is occurred by suture failure. Urinary bladder drainage method, which is recommended by Sollinger, make possible to diagnose a rejective reaction in the early stage by amylase and PH in the urine, or cytology[14]. In general a lot of duodenum juice is lost, when the length of duodenum is too long, and the disturbance of electrolyte (acidosis)

is occurred. In North Europe, duct obstruction by retrograde injection of prolamine is performed because of its simplicity[8]. However, it causes fibrosis and atrophy of exocrine tissue, with subsequent fibrosis of endocrine tissue.

Reemtsma et al. showed that previous ligation of the pancreatic duct facilitated subsequent pancreas transplantation in the rat[13]. However it is known that pancreatic duct ligation causes changes in the pancreatic β cells at least in the dog. Simple duct-ligated method often causes acute pancreatitis or fistule of pancreatic juice and is not recommended in clinical settings. But it has been proved that pancreatic duct ligation, as for limited days, did not incur serious complication in rat[6], we used duct ligation technique.

In the third place, the difficulty was accompanied by microvascular anastomosis with a microscope of 4 magnifications. In the experiments of pancreas transplantation in rats, the complete anastomosis of both the arterial and venous connections was required to get the survival cases. In the pancreas grafting using a microsurgical technique in the abdomen, various devices, such as enlargement of portal vein or the incision obliquely of aortic segment or adventitectomy or use of hemostatics, were performed. But the successful rate of pancreas grafting using microsurgical technique in the abdomen was not good. In addition, the aorta and vena cava have to be clamped together with bulldog clamp and so systemic circulation is disturbed. This is one of the main causes of death if the periods of time is long. However in the pancreas grafting to the neck vessels in rats, the clamping of systemic circulation is avoided because cervical vessels are situated bilateraly. Cuff technique also makes it possible the complete anastomosis of both the arterial and venous connections. As a result, successful rate of pancreas transplantations using a non-suture cuff technique to neck vessels was higher in comparison with that using a microsurgical technique in the abdomen. In conclusion, heterotopic pancreas transplantation using non-suture cuff technique to neck vessels is safe, reliable and recommended as the experimental model using the rat.

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