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Successful 96-hour preservation of the canine pancreas

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Abstract. We tested the preservation of the pancreas for 96 h by a modified two-layer (UW solution/perfluorochemical) cold storage method (group 1) in the canine model of pancreas autotransplantation and compared this with an original two-layer (Euro-Collins' solution/perfluorochemical) cold storage method (group 2) and simple cold storage method with UW solution (group 3). A graft was considered functioning if the dog had a normal blood glucose for at least 5 days after transplantation. The functional success rates after preservation for 72 h were 100%, 100% and 80% for groups 1, 2 and 3 respectively. On the other hand, the functional success rates for groups 1, 2 and 3 after preservation for 96 h were 75%, 0% and 0% respectively. The mean K value of 96-hour preserved grafts for group 1 at 2 weeks after transplantation was 1.52 ± 0.30 compared with 1.98 ± 0.48 before preservation. Biopsies of grafts from group 1 showed almost normal pancreatic architecture even after preservation for 96 h. In addition, biopsies of grafts preserved for 96 h in group 1 at 4 weeks after transplantation showed almost normal endocrine tissue with mild fibrosis of the exocrine tissue. This study demonstrated the possibility of preserving the pancreas for 96 h prior to transplantation.

Key words: Pancreatic transplantation – University of Wisconsin solution – Perfluorochemical – Preservation for 96 h

To reduce ischemic cell injury and tissue edema during simple cold storage of the pancreas, we have developed a two-layer [Euro-Collins' solution (EC)/perfluorochemical (PFC)] cold storage method [1], that continuously supplies sufficient oxygen to the pancreas during preservation [2], and extends preservation time of the canine pancreas with EC for up to 72 h [3]. On the other hand, a new flush-out and preservation solution, University of Wisconsin solution (UW solution), has been developed to reduce cell swelling during preservation [4, 5] and also extends the preservation time of the canine pancreas for up to 72 h [6].

Although the exact mechanism of action of UW solution and the two-layer method in pancreatic preservation is not yet clear, we used UW solution as a flush-out and preservation solution in place of EC in the two-layer cold storage method and examined the possibility of extending the preservation time of canine pancreas for more than 72 h. In this paper, we report the success of 96-hour preservation of the canine pancreas by a modified two-layer (UW solution/PFC) cold storage method.

Materials and methods

Mongrel dogs of both sexes, weighing 12–18 kg were used for the experiments. Perfluorodecaline, which is one of the PFCs, was a kind gift of Dr. K. Yokoyama (The Green Cross Corporation, Osaka, Japan). UW solution was purchased from E. I. Du Pont de Nemours, Waukegan, Ill., USA.

Operation procedures. Anesthesia was induced and maintained with sodium pentobarbiturate (25 mg/kg). After the abdomen was opened, a left lobectomy of the pancreas with the splenic artery and vein attached was meticulously performed, followed by splenectomy. The segmental pancreatic graft was washed with 50 ml of cold heparinized EC (1000 units/50 ml EC or UW solution) through the splenic artery and autotransplanted in the neck after preservation, as has been described previously [7], excising the remainder of the pancreas at the time of autotransplantation. After the operation, the dogs received saline with 10% glucose (30 ml/kg) and parenteral penicillin (25 mg/kg) for 3 days. After 3 days, standard kennel diets were given.

Preservation method. The two-layer cold storage method was performed as has been described previously by our group [1, 3]. The pancreatic graft was floated on the PFC, covered with EC or UW solution in a styrofoam box packed with ice and oxygenated throughout the storage period (Fig. 1).

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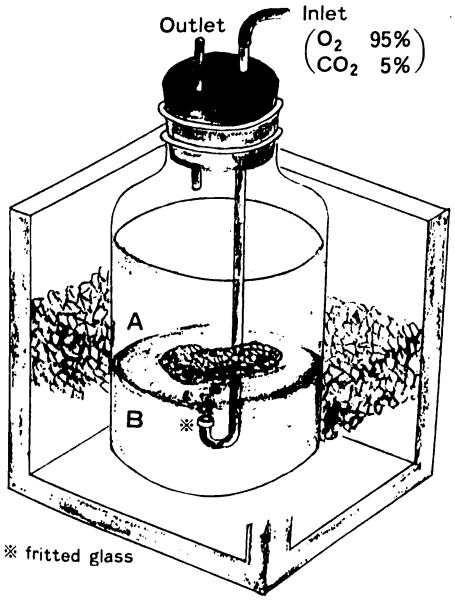

Fig. 1. The two-layer cold storage method. Oxygenation was continued through the fritted glass to PFC throughout the storage period, the pancreatic graft being surrounded by cold UW solution or EC and PFC in a styrofoam box packed with ice. *A*, UW solution or EC; *B*, perfluorochemical

Functional studies. Blood glucose concentration was determined daily during the 1st postoperative week after autotransplantation and biweekly thereafter. Intravenous glucose tolerance tests (IVGTT) were performed at 2 weeks after transplantation. Maintenance of normoglycemia for at least 5 days after transplantation was considered successful preservation [8].

Histological studies. Biopsies of the pancreatic grafts were taken at the time of the original operation, after 72 or 96 h preservation, at 4 weeks after transplantation and at autopsy. The tissue was fixed in Zamboni's solution, paraffin-embedded and stained with hematoxylin and eosin.

Experimental protocol. Four groups of dogs were studied. There were three experimental groups in which all dogs received segmental autografts that were stored at 4°C by the modified two layer (UW/PFC) method (group 1), the original two-layer (EC/PFC) method (group 2) and the simple cold storage method with UW solution (group 3) for 72 or 96 h. The groups were subdivided depending on the storage time: 1A, modified two-layer method for 72 h ($n = 5$); 1B, modified two layer method for 96 h ($n = 8$); 2A, original two layer method for 72 h ($n = 5$); 2B, original two layer method for 96 h ($n = 8$); 3A, simple cold storage method with UW solution for 72 h ($n = 5$); 3B, simple cold storage method with UW solution for 96 h ($n = 8$).

Table 1. Functional success rate in canine segmental pancreatic autografts after preservation at 4°C

Group	Preservation time (h)	Preservation method	Functional grafts No. transplants	Success[a] rate (%)
1A	72	UW/PEC	5/5	100
2A	72	EC/PFC	5/5	100
3A	72	UW	4/5	80
1B	96	UW/PFC	6/8	75
2B	96	EC/PFC	0/8	0
3B	96	UW	0/8	0

[a] Maintenance of normoglycemia for at least 5 days after transplantation was considered successful preservation [8]

Results

After preservation for 72 h, the functional success rate of groups 1A, 2A and 3A were 100% (5/5), 100% (5/5) and 80% (4/5), respectively. But after 96 h preservation, only group 1B was successful, with a success rate of 75% (6/8), and groups 2B and 3B were unsuccessful, 0% (0/8) and 0% (0/8) respectively (Table 1). One of eight dogs in group 1B died of thrombosis 5 days after transplantation and one died of a cause unrelated to the graft. The mean K values of group 1B at 2 weeks after transplantation was 1.52 ± 0.30 compared with 1.98 ± 0.42 before preservation. Biopsies of grafts from group 1B showed almost normal pancreatic architecture. In addition, biopsies of grafts from group 1B at 4 weeks after transplantation showed almost normal pancreatic architecture with minimal fibrotic changes in the exocrine tissue.

Discussion

In order suppress cell swelling or tissue edema, a new flush and preservation solution, UW solution, has been developed using a high molecular weight anion (lactobionate) and a high molecular weight saccharide (raffinose) as impermeants [4, 5] and this extends the preservation time of the canine pancreas for up to 72 h [6]. On the other hand, we have developed a two-layer (EC/PFC) cold storage method to reduce ischemic cell injury and maintain cellular integrity [1] and this also extends preservation time for up to 72 h in the canine pancreas [3]. Since preservation of the canine pancreas for 96 h by either the simple cold storage method with UW solution or the original two-layer (EC/PFC) cold storage method was unsuccessful but a combination of UW solution and the two-layer method made 96-hour preservation possible, it seemed reasonable to suppose that the mechanism of action of UW solution and the two-layer method in preservation of the canine pancreas are different and additive although the precise mechanisms of action of UW solution and the two-layer method are not clear. Recently, we have established that the two-layer method maintains high ATP tissue concentrations in the canine pancreas during and at the end of the cold storage period [2] and we have also established that there is a correlation between high ATP tissue concentrations and good post-transplant results after preservation by the two-layer method [9]. In addition, Pegg et al. [10] have clearly demonstrated that the provision of sufficient oxygen by retrograde oxygen persufflation to rabbit kidney allows the continued production of ATP in sufficient quantity to permit improved maintenance of cellular function. It is not clear whether maintenance of a high ATP tissue concentration during preservation by the two-layer method is a primary determinant of successful preservation or merely reflects the preservation of mitochondrial function during cold storage. The mechanism of action of the two-layer method in canine pancreatic preservation is currently being investigated by our group.

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