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LONG-TERM PRESERVATION OF CANINE PANCREAS BY A NEW SIMPLE COLD STORAGE METHOD USING PERFLUOROCHEMICAL —THE TWO-LAYER COLD STORAGE METHOD (EURO-COLLINS' SOLUTION/PERFLUOROCHEMICAL)—

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INDEXING WORDS

pancreas preservation; two-layer cold storage method; perfluorochemical (PFC)

SYNOPSIS

Long term preservation of canine pancreas by a new simple cold storage method using perfluorochemical (PFC), two-layer (Eruo-Collins' solution (EC)/PFC) cold storage method, was tested in the canine model of segmental pancreas autotransplantation. The functional recovery of the graft preserved by this method (group 1) was determined by daily fasting blood glucose concentration and intravenous glucose tolerance test at two weeks after autotransplantation and compared with simple cold storage with EC (group 2), cold storage in EC with simple bubbling of oxygen (group 3), and control (no preservation) (group 4). Maintenance of normoglycemia at least 5 days after transplantation was considered a success of preservation. The functional success rates after 24 hour preservation were 4/4 (100%), 4/4 (100%) and 4/5 (80%) for groups 1,2 and 3 respectively, and the functional success rates of groups 1,2 and 3 after 48 hour preservation were 5/6 (86%), 1/5 (20%) and 1/5 (20%) respectively. In addition the functional success rates after 72 hour preservation were 6/6 (100%), 0/6 (0%) and 0/5 (0%) for groups 1, 2, and 3 respectively. It was clear that the two-layer cold storage method had made it possible to preserve the canine pancreas for up to 72 hours although the upper limit of reliable preservation time of groups 2 and 3 was 24 hours. The mean K value of group 1 after 72 hour preservation was 1.78 ± 0.42 compared with 2.05 ± 0.32 of group 4 at two weeks after transplantation. Biopsies of grafts of group 2 and 3 after

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72 hour preservation showed remarkable autolytic changes in exocrine and endocrine tissues. In contrast, biopsies of grafts of group 1 after 72 hour preservation showed almost normal architecture in both tissues. In addition, biopsies of 72 hour preserved grafts of group 1 at 4 weeks after autotransplantation showed almost normal pancreatic architecture with minimal fibrotic changes in the exocrine tissue. This study demonstrated the possibility of long-term preservation of the pancreas for transplantation.

INTRODUCTION

Pancreas transplantation has been applied clinically for treatment of selected patients with diabetes mellitus (1). To accomplish successful pancreas transplantation a simple and reliable method of pancreas preservation is necessary. It is generally accepted that preservation of the pancreas by simple cold storage method is superior to hypothermic perfusion system (2). A number of investigators have succeeded in preserving the canine pancreas by simple cold storage for 48-72 hours using hyperosmolar solutions that prevent cellular swelling during preservation (3-5). On the other hand, the perfusion system has the advantage of supplying oxygen to the pancreas to maintain metabolism during preservation (2). In other words the provision of high oxygen tension is advantageous for preservation of pancreas (6) and kidney (7) due to reduce anoxic cell swelling and maintain cellular integrity. But it is difficult and complex to succeed long-term preservation of the pancreas by the perfusion system (2). Idezuki and associates (6) described a technique for storage of pancreaticoduodenal and segmental grafts in a hypothermic hyperbaric chamber. In spite of favorable results with hyperbaric oxygenation, the procedure was not used often because of the complexity and difficulty of the technique. Recently, Fischer et al. (7) have developed a technique for normobaric oxygenation of the kidney by retrograde oxygen persufflation, and have gotten excellent result. On the basis of these experiments, we have developed a new cold storage method (8, 9) with normobaric oxygenation of the pancreas during simple cold storage in Euro-collins' solution (EC*), using perfluorochemical (PFC), which is a high oxygen carrier (10). In this paper, we report the success of long-term preservation of the canine pancreas by the two-layer cold storage method.

MATERIALS AND METHODS

Mongrel dogs of both sexes, weighing 12-18 kg were used for the experiment

Operation procedures

Anesthesia was induced and maintained with sodium pentobarbiturate (25

mg/kg). After laparotomy, a left lobectomy of the pancreas with splenic artery and vein attached was meticulously performed, followed by splenectomy. The segmental pancreas graft was washed out with 50 ml of cold heparinized EC (1,000 unit 50 ml EC) through the splenic artery and autotransplanted in the neck immediately or after preservation, as described by Kuroda et al (11, 12) (Fig. 1), excising the remainder of the pancreas at the time of autotransplantation. After operation, the dogs received saline with 10% glucose (30 ml/kg) and parenteral penicillin (25 mg/kg) for three days. After 3 days, standard kennel diets were given.

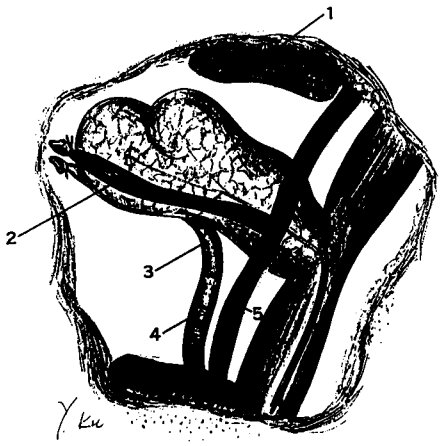


Fig. 1. Segmental pancreatic autotransplantation with pancreatic exocrine diversion to the esophagus. (1), sternocleidomastoid muscle; (2), splenic vein; (3), splenic artery; (4), common carotid artery; (5), external jugular vein.

Preservation method

The two-layer cold storage apparatus is shown in Fig. 2 (8, 9). Because the specific gravity of PFC is 1.95 and PFC is insoluble to water (10) (Fig. 3), PFC is clearly separated from EC. The pancreas graft is on the surface of PFC and covered with EC according to its specific gravity. The gaseous flow rate was between 50-100 ml/min. Oxygenation was continued through the fritted glass to PFC throughout the storage period, the pancreas graft being surrounded by cold EC and PFC in a styrofoam box packed in ice.

Functional studies

Blood glucose concentration was determined daily during fist postoperative week after autotransplantation and biweekly thereafter. Intravenous glucose

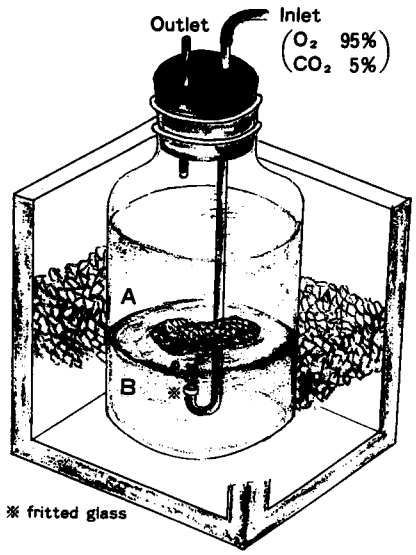
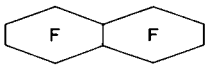


Fig. 2. Two-layer cold storage method. Oxygenation was continued through the fritted glass to PFC throughout the storage period, the pancreas graft being surrounded by cold EC and PFC in a styrofoam box packed in ice: A, Euro-Collins' solution; and B, perfluorochemical.

PERFLUOROCHEMICAL
(PERFLUORODECALIN)

$C_{10}F_{18}$ Mw=462.07



Appearance :
Colorless, dense liquid

Physicochemical properties :

Water insoluble	
Specific gravity (d_{20}^{20})	1.935～1.945
Surface tension at 25℃	15～16 dynes/cm
Refractive index (η_D^{25})	1.3125～1.3135
Boiling point	140 ～ 143 ℃
Vapor pressure at 37℃	13.5 mmHg (measured by Ramsay-Young's method)

Fig. 3. Physiochemical properties of perfluorochemical.

tolerance test (IVGTT) was performed at 2 weeks after transplantation. For the IVGTT, glucose (0.5 g/kg) was injected in a bolus and blood glucose was determined after 2.5, 5, 10, 15, 30, 60, 90 and 120 min. The K value was calculated from the blood

glucose concentration obtained from the 2.5~30 min measurements (13), expressing the percentage of decline/min from peak concentration. Maintenance of normoglycemia (fasting glucose < 140 mg/dl) at least 5 days after transplantation was considered a success of preservation (14).

Histological studies

Biopsies of the pancreas grafts were taken at the time of the original operation, after 24, 48 or 72 hour preservation, at four weeks after transplantation and at autopsy. The tissue was fixed in Zamboni's solution, paraffin-embedded and stained with hematoxylin and eosin.

Experimental protocol

Ten groups of dogs were studies. There were nine experimental groups in which all dogs received segmental autografts that were stored at 4°C by the two-layer method (group 1), in EC (group 2) and in conventionally oxygenated EC (group 3) for 24, 48 or 72 hours: 1a, two-layer method for 24 hours (n=4); 1b, two-layer method for 48 hours (n=6); 1c, two-layer method for 72 hours (n=6); 2a, EC for 24 hours (n=4); 2b, EC for 48 hours (n=5); 2c, EC for 72 hours (n=6); 3a, conventionally oxygenated EC for 24 hours (n=5); 3b, conventionally oxygenated EC for 48 hours (n=5); 3c, conventionally oxygenated EC for 72 hours (n=5). There was control group (group 4) (n=5) in which all dogs received fresh, unpreserved segmental pancreas autografts.

Statistics

Statistical analysis was done using the Student's test.

RESULTS

After 24 hour preservation, the functional success rates of groups 1a, 2a, and 3a were 4/4 (100%), and 4/4 (100%), and 4/5 (80%) respectively and one of five dogs of group 3a and one of six dogs of group 1b died of causes unrelated to the pancreas graft. After 48 hour preservation, the functional success rates of groups 1b, 2b, and 3b were 5/6 (83%), 1/5 (20%), and 1/5 (20%) respectively (Table 1), and the success rate of group 1b was 100% if the dog died of causes unrelated to the pancreas graft was excluded. Four of five dogs of each groups 2b and 3b became hyperglycemic at 2-4 days. After 72 hour preservation, all the grafts of group 2c and 3c failed. In contrast, all the grafts of group 1c maintained normoglycemia for more than 5 days after transplantation (Table 1). The mean K values of groups 1b and 1c were 1.98 ± 0.32 and 1.78 ± 0.42 respectively compared with 2.05 ± 0.32 of group 4 (control) at

two weeks after transplantation (Fig. 4).

Table 1. Functional success rate in canine segmental pancreas autograft after preservation at 4°C

Group	Preservation time (hr.)	Preservation method	Functional graft/ No. transplant	Success rate (%)
1a	24	EC+PFC+O_2	4/4	100
2a	24	EC	4/4	100
3a	24	EC+O_2	4/5	80
1b	48	EC+PFC+O_2	5/6	83
2b	48	EC	1/5	20
3b	48	EC+O_2	1/5	20
1c	72	EC+PFC+O_2	6/6	100
2c	72	EC	0/6	0
3c	72	EC+O_2	0/5	0
4	0	No preservation	5/5	100

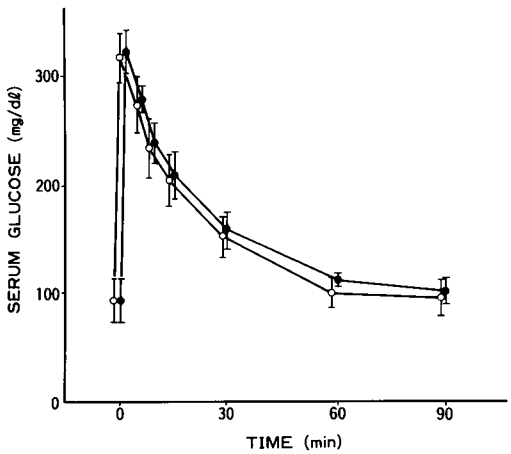


Fig. 4. Intravenous glucose tolerance test of 72-hr preserved pancreas grafts at 2 weeks after autotransplantation. The results are expressed as the mean ± SD of five dogs. (○——○) control (no preservation (n=5); (●——●) 72-hr preserved grafts by the two-layer method (n=5).

Histological studies of group 2c from the biopsies taken after 72 hour preservation showed autolytic and vacuolar changes of exocrine tissues and islet cells (Fig. 5A). In contrast, biopsies of the grafts in group 1c after 72 hour preservation showed almost normal architecture in exocrine and endocrine tissues (Fig. 5B). In addition, biopsies of the grafts in group 1c taken at four weeks after autotransplantation showed almost normal pancreatic architecture with minimal fibrotic changes in the exocrine tissue (Fig. 6).

Fig. 5(A)

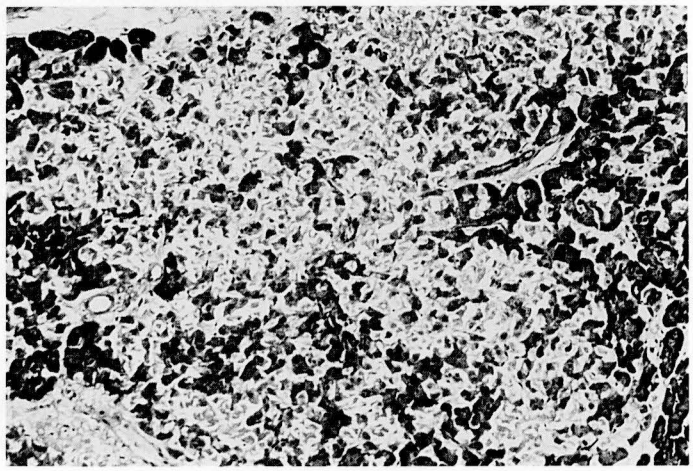


Fig. 5(B)

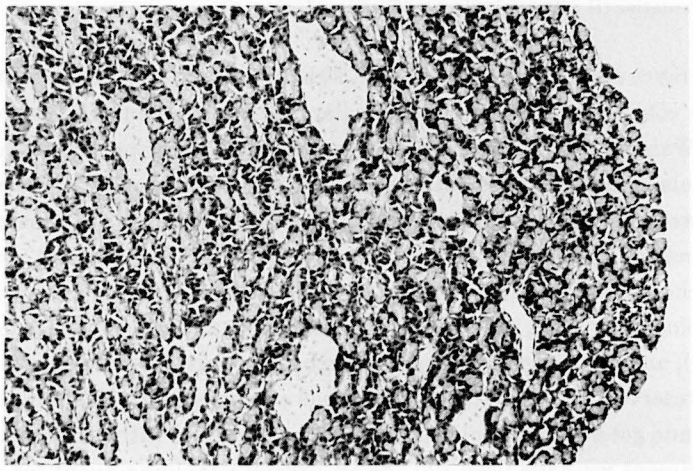


Fig. 5. Photomicrographs of pancreas grafts stored at 4°C: (A) for 72 hr with EC (group 2c); and (B) for 72 hr by the two-layer method (group 1c) (H & E; original magnification x 100).

DISCUSSION

The success of cold storage for organ preservation appears to be related to the factors that prevent cellular swelling and maintain cellular integrity during preservation. In simple cold storage of canine pancreas, 24 hour preservation is reliable with EC (15), Collins' solution (16-19) and Sack's solution (20), but preservation more than 48 hours is not effective with these so-called "intracellular" solutions. However, Florack et al. (4) has succeeded in 48 hour preservation and

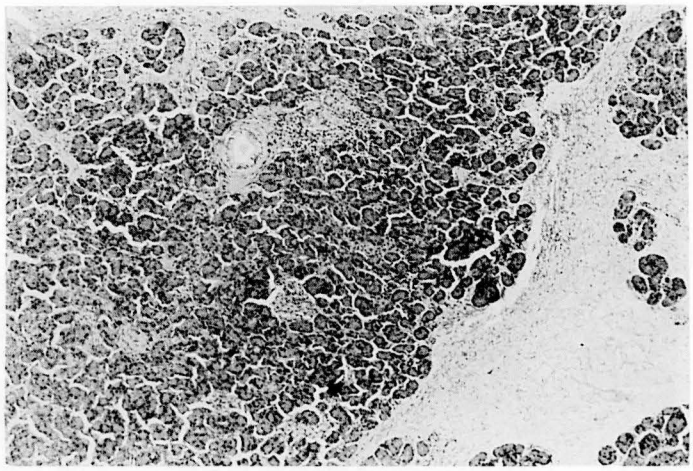


Fig. 6. Biopsy of 72-hr preserved graft by the two-layer method (group 1c) taken at 4 weeks after autotransplantation (H & E; original magnification x 100).

Toledo-Pereyra et al. (3) has succeeded in 72 hour preservation of the canine pancreas by simple cold storage using hyperosmolar and hyperkalemic silica-gel filtrated plasma. Wahlberg et al. (5) has also succeeded in 72 hour preservation using new hyperosmolar solution (UW-solution). These hyperosmolar solutions seem to reduce the cell swelling during cold storage (2). On the other hand, the provision of high oxygen tension is advantageous for the preservation of pancreas and kidney due to reduce ischemic injury and maintain cellular integrity during preservation (6, 7). PFC is biologically inert, dissolves approximately 10 times as much oxygen as water at 4°C (10), and can therefore be used as an oxygen-carrying substance. Kamada et al. (21) preserved rat liver using continuous perfusion with PFC emulsion in the perfusate and got a better result than that of the perfusate without PFC emulsion. In this experiment we have used PFC as an oxygen supplying agent for simple cold storage of the canine pancreas in EC. Without PFC, the preservation of the canine pancreas grafts for 48 hours by simple cold storage in EC or oxygenated EC were not successful (Table 1). However, the two-layer (EC/PFC) cold storage method has made it possible to preserve the canine pancreas in EC for up to 72 hours as tested by subsequent autotransplantation. Recently we clarified that PFC itself had no effect for preservation of the pancreas graft without oxygen bubbling (22). As PFC acts only as an oxygen supplying agent (10), it is likely that the higher oxygen tension could have an essential in extending the preservation time with EC. Recently it was also clarified that the oxygenation of the pancreas was mainly due to direct diffusion of dissolved oxygen in PFC to the pancreas through undersurface of the pancreas

(23). The possibility of extending preservation time by combining other preservation solutions with this method has been investigated (24). Further studies would clarify the metabolism of the pancreas during preservation by this method. This unique two-layer cold storage method is simple and reliable and is recommended for preservation of the pancreas.

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