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• BASIC RESEARCH •

Evaluation of the viability and energy metabolism of canine pancreas graft subjected to significant warm ischemia damage during preservation by UW solution cold storage method

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Abstract

AIM: To evaluate the viability and energy metabolism of long warm ischemically damaged pancreas during preservation by the UW solution cold storage method.

METHODS: The pancreas grafts subjected to 30-120 min warm ischemia were preserved by the UW solution cold storage method for 24 h. The tissue concentrations of adenine nucleotides (AN) and adenosine triphosphate (ATP) and total adenine nucleotides (TAN) were determined by using high performance liquid chromatography (HPLC) and the viability of the pancreas graft was tested in the canine model of segmental pancreas autotransplantation.

RESULTS: The functional success rates of pancreas grafts of groups after 30 min, 60 min, 90 min, 120 min of warm ischemia were 100%, 100%, 67.7%, 0%, respectively. There was an excellent correlation between the posttransplant viability and tissue concentration of ATP and TAN at the end of preservation.

CONCLUSION: The UW solution cold storage method was effective for functional recovery of the pancreas suffering 60-min warm ischemia. The tissue concentration of ATP and TAN at the end of 24 h preservation by the UW solution cold storage method would predict the posttransplant outcome of pancreas graft subjected to significant warm ischemia.

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INTRODUCTION

Preservation is necessary if organs for transplantation are removed from donors prior to preparation of the recipient[1-3]. The methods used for preservation of pancreas grafts for experimental transplantation have produced variable results[4,5]. As warm ischemic injury of pancreas graft before and during procurement strongly influences the results of pancreas transplantation, it is important to predict the viability of the ischemically damaged pancreas graft before transplantation[6,7]. Recently we preserved the segmental pancreas in the UW solution after 30-120 min, and our experimental model was heterotopic segmental (left limb) pancreas autotransplantation in totally pancreatectomized dogs, and demonstrated that the UW solution cold storage method was effective for functional recovery of the pancreas suffering 60-min warm ischemia[8]. There are some qualitative differences between warm and cold ischemic injuries[9-12]. In this study we examined the viability and energy metabolism of the pancreas graft after significant warm ischemia and cold storage, and found tissue concentrations of ATP and TAN after preservation by the UW solution cold storage method were excellent markers to predict the posttransplant outcome[13,14].

MATERIALS AND METHODS

Animals

Mongrel dogs of both sexes, weighing 10-15 kg were used for the experiments. UW solution was from China Pharmaceutical Corporation Guangzhou Branch. Chemicals were from Sigma Co.Ltd.

Operative procedures are as follows. Anesthesia was induced and maintained with sodium pentobarbiturate (25 mg/kg). The pancreas was exposed through a midline abdominal incision, and the left limb (tail) was removed with the splenic vessels in preparation for grafting, followed by splenectomy. The segmental pancreas graft was unflushed and left *in situ* for 30-120 min. After warm ischemia the pancreas was flushed with 30-50 mL heparinized cold UW solution (10 000 units/L heparin) and preserved in 50 mL heparinized cold UW solution for 24 h. A splenectomy was performed. The remainder of the pancreas was excised at the time of transplantation. The pancreatic tail was autotransplanted heterotopically, immediately or after preservation, with anastomosis of the splenic vessels to iliac vessels. A proper delicate tube was inserted into the pancreatic duct to drain the pancreatic juice. After operation , the dogs received saline with 100 g/L glucose (30 mL /kg) and 3.2 Mu penicillin for 3-5 days, then standard kennel diets were given.

Experimental protocol

There were two groups of control dogs: group 1, sham-operated group, abdomen was only opened and closed; group 2, no warm ischemia, pancreatic tail was flushed and preserved immediately after being harvested. The experimental group (group 3) was divided into 4 subgroups according to the warm ischemia time: group 3a, 30 min warm ischemia; group 3b, 60 min warm ischemia; group 3c, 90 min warm ischemia; group 3d, 120 min warm ischemia.

Functional studies

Blood glucose concentration was determined daily during the first postoperative week after autotransplantation. An

intravenous glucose tolerance test (IVGTT) was performed one week after transplantation. Glucose, 0.5 g/kg, was administered as a bolus and blood samples were collected serially over a 2-h period for plasma glucose. IVGTT K values were calculated from the plasma glucose levels at the end of 5 to 60 min[15]. Maintenance of normoglycemia for at least five days after transplantation or a key value of IVGTT more than 1.0 one week after transplantation was considered a functional success of pancreas graft. The plasma insulin levels in splenic and peripheral vein one hour after transplantation were examined. The pancreatic juice was collected every day and amylopsin in the pancreatic juice of the first and the seventh days were determined.

Tissue extraction method for adenine nucleotides: After preservation, a part of pancreas was rapidly frozen in liquid nitrogen, lyophilized overnight, and kept at -80 ℃ until analysis. The dry tissue powder was weighed (200 mg) and homogenized in 3 mL of ice cold 0.5 mol/L perchloric acid. The precipitated protein was removed by centrifugation, and 500 μL of supernatant was neutralized by the additions of 50 μL 1 mol/L KOH and 50 μL Tris. Following centrifugation, 10 μL of supernatant was injected into HPLC for analysis.

Measurement of adenine nucleotides

High-performance liquid chromatography (HPLC, Waters, 510 Pump, 486 Detector) was performed on a reverse-phase column of Shim-pack, CLC-ODS (15 cm×3.96 mm, 4 μm) which was equilibrated with 100 mmol/L sodium phosphate buffer (pH 6.0) according to the method of Wynants *et al*.

TAN was calculated as the sum of ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP).

Histological studies

Biopsies of the pancreas grafts were taken after cold preservation and one hour after transplantation. For light microscopy, the tissues were fixed in 100 ml/L formalin and stained with hematoxylin and eosin. For electron microscopic studies the tissues were prefixed in 25 g/L glutaraldehyde, postfixed in 25 g/L osmium tetroxide, sectioned at 1 μm, and stained with uranyl acetate and lead citrate.

Statistical analysis

All data were expressed as mean±SD. *F* test was used to compare values of different groups, χ^2 test for comparison of viability. A value of $P<0.05$ was considered statistically significant.

RESULTS

Graft function

Pancreatic graft endocrine function Plasma glucose and IVGTT K values in groups 1, 2, 3a and 3b recovered to normal 2-3 days after transplantation, while groups 3c and 3d did not one week after transplantation(Table1). The plasma insulin levels in splenic and peripheral veins in groups 1, 2, 3a, and 3b were much higher than those in groups 3c, and 3d ($P<0.05$, Table 2).

Pancreatic graft exocrine function The pancreatic juice flow over the pancreatic duct 30 min after transplantation increased gradually and came to a climax on the fourth day after transplantation, and then declined gradually. The daily amounts of pancreatic juice of groups 1, 2, 3 a and 3b were much more than those of groups 3c and 3d ($P<0.05$). The amylase activities in the pancreatic juice of the first and seventh days in groups 1, 2, 3a and 3b were much more than those in groups 3c and 3d ($P<0.05$, Tables 2, 3)

Tissue ATP, ADP, AMP and TAN after preservation The tissue concentrations of ATP, ADP, AMP and TAN after 24-hour preservation in groups 1, 2, 3 a and 3b were much higher than those in groups 3c and 3d ($P<0.05$, Table 4).

Viability of canine pancreas autografts after preservation After significant warm and cold preservation, the functional success rates of groups 2, 3a, 3b, 3c and 3d were 5/5(100%), 6/6(100%), 6/6(100%), 4/6(66.7%) and 0/4(0%), respectively (Table3). The UW cold preservation method was effective for functional recovery of the pancreas after 30 to 60-min warm ischemia (Table 3).

Relationship between posttransplantation viability and tissue ATP and TAN There was no overlap between the lowest ATP in the viable grafts and highest ATP in the nonviable grafts. If ATP level of 4.0 μmol/g dry weight was determined as a critical

Table 1 Plasma glucose and IVGTT K value at the first week after transplantation and plasma insulin level in splenic and peripheral vein one hour after transplantation (mean±SD)

Group	*n*	Plasma glucose (mol/L)	IVGTT K value	In splenic vein (mmol/L)	In peripheral vein (mmol/L)
1	3	5.6±0.9[15]	1.78±0.17[25]	53.4±7.1[35]	8.6±1.3[45]
2	5	6.6±0.9	1.58±0.15	51.3±8.2	8.1±1.2
3a	6	6.7±1.1	1.45±0.12	50.6±7.6	7.5±1.1
3b	6	6.8±0.8	1.42±0.18	47.8±7.6	7.5±0.8
3c	6	11.9±1.3	0.87±0.16	35.0±5.2	3.2±0.7
3d	4	12.9±1.8	0.60±0.13	31.4±8.1	2.7±0.5

[1]F=36.9, $P<0.05$; [2]F=32.9, $P<0.05$; [3]F=7.38, $P<0.05$; [4]F=38.2, $P<0.05$; [5]SNK test: between group 1, 2, 3a, 3b, $P>0.05$; between group 3c, 3d, $P>0.05$.

Table 2 Pancreatic juice flow during the first week after transplantation (mean±SD, mL)

Group	*n*	Pancreatic juice flow / d						
		1	2	3	4	5	6	7
2	5	27±7[18]	70±11[28]	221±17[39]	294±37[49]	136±26[59]	81±21[69]	48±14[79]
3a	6	24±7	74±17	216±36	285± 36	138±24	91±19	53±15
3b	6	24±8	63±15	204±33	287±43	142±4	87±22	41±8
3c	6	12±4	20±6	68±19	69±19	83±18	54±12	30±7
3d	4	7±3	15±8	12±6	16±6	8±3	12±5	10±4

[1]F=9.87, $P<0.05$; [2]F=25.9, $P<0.05$; [3]F=67.4, $P<0.05$; [4]F=88.2, $P<0.05$; [5]F=45.2, $P<0.05$; [6]F=15.9, $P<0.01$; [7]F=13.2, $P<0.01$; [8]SNK test: between group 2, 3a, 3b, $P>0.05$; between group 3c, 3d, $P>0.05$; [9]SNK test: between group 2, 3a, 3b, $P>0.05$.

value for the viability following transplantation, the specificity, sensitivity, predictive value and efficacy were all 100%. And there was also no overlap between the lowest TAN in the viable grafts and highest TAN in the nonviable grafts. If TAN level of 7.0 μmol/g dry weight was determined as a critical value for the viability following transplantation, specificity, sensitivity, predictive value and efficacy were all 100%. Both ATP and TAN were reliable markers for determining the transplantation.

Table 3 Amylase in pancreatic juice of the first and the seventh day and viability after significant warm and cold preservation (mean±SD)

Group	*n*	Amylase (μkat/L)		Functioning grafts/rate(%)
		The first day	The seventh day	
2	5	182±45[13]	359±27[24]	5/(100)
3a	6	183±48	355±37	6/(100)
3b	6	180±42	327±37	6/100)
3c	6	83±24	29±11	4/(66.7)[5]
3d	4	77±30	28±10	0/(0)[5]

[1]F=10.3, P<0.05; [2]F=205.5, P<0.05; [3]SNK test: between group 2, 3a, 3b, P>0.05; [4]SNK test: between group 2, 3a, 3b, P>0.05; between group 3c, 3d, P>0.05. [5]compare with Group 2, P<0.05.

Table 4 Tissue concentration of ATP, ADP, AMP and TAN (mean±SD, μmol/L)

	ATP	ADP	AMP	TAN
Group 1 (n=3)	7.26±0.55[13]	3.33±0.20	1.49±0.34	11.43±1.37[23]
Group 2 (n=5)	5.86±0.52	1.01±0.21	1.51±0.26	7.93±1.30
Group 3a (n=6)	5.28±0.37	1.31±0.35	1.55±0.35	8.02±0.78
Group 3b (n=6)	4.74±0.41	2.01±0.31	1.04±0.24	7.36±0.78
Group 3c (n=6)	2.18±0.21	0.83±0.19	0.81±0.23	4.04±0.51
Group 3d (n=4)	2.11±0.17	0.86±0.21	1.04±0.25	3.33±0.27

[1]F=17.0, P<0.05; [2]F=23.9, P<0.05; [3]SNK test: between group 2, 3a, 3b, P>0.05.

Histologic studies

Under light microscope, the pancreas in groups 1 and 2 stored for 24 h showed normal architecture. After 24 h, preservation vacuolization of the acinar cells and interstitial edema were seen in grafts of groups 3a and 3b, and only mild edema of the islets was evident. Grafts of groups 3c and 3d showed severe edema, and after revascularization there was hemorrhage in the interstitial space.

Under electron microscope, the pancreas in groups 1 and 2 stored for 24 h showed well preserved cells. In grafts of groups 3a and 3b, acinar cells showed no nuclear changes, but rough endoplasmic reticulum (RER) cisternae were dilated. In grafts of groups 3c and 3d, irreversible cell damage was seen in most, but not all, specimens.

DISCUSSION

Pancreas graft injury due to warm ischemia strongly affects the posttransplant outcome[16,17]. Therefore, resuscitation of an ischemically damaged pancreas is essential to enlarge the donor pool using the pancreas graft from the cardiac arrest donor[18]. We have demonstrated that canine pancreases subjected to 60 min of warm ischemia can be resuscitated during preservation by the UW solution preservation method at 4 ℃ for 24 h.

Restoration of cellular function of the pancreas graft subjected to significant warm ischemia by the UW solution cold preservation method will make it possible to use pancreas grafts from cadaver with cardiac arrest[19,20], wait safely for the excision of the pancreas and enlarge the donor pool. Cerra[21,22] reported that the canine pancreatic allografts tolerated warm ischemia up to one hour. Florack *et al*.[23,24]demonstrated that the canine pancreatic autografts tolerated warm ischemia up to 60 min.

On the other hand, the assessment of a pancreas graft viability before transplantation is very important to prevent transplantation of a nonfunctioning allograft especially after significant warm ischemia because there is progressive depletion of ANs during warm ischemia[25], ultimately leading to ischemic damage. But the relationship between the tissue concentration of ANs before transplantation and organ viability after transplantation is controversial[26,27]. In human liver preservation, Lanir *et al*.[28,29] demonstrated a direct correlation between a high ATP concentration and good posttransplant outcome. On the contrary, correlation between the ATP level at the end of cold preservation and viability following transplantation was not proved in the rat liver[30,31].

We have also demonstrated that correlation between high ATP tissue concentration, which is necessary to maintain cellular integrity, and good posttransplant outcome of a canine pancreas graft after preservation by the UW solution cold preservation method[32,33]. It is suggested that tissue concentration of ATP and TAN at the end of 24-h preservation by the UW solution cold preservation method will predict the posttransplant outcome of pancreas graft subjected to significant warm ischemia[34,35]. But the mechanism responsible for the effectiveness of the UW solution cold preservation method in restoration of function of the pancreas graft subjected to significant warm ischemia remains unclear and is under investigation[35-38].

We conclude that the tissue concentration of ATP and TAN at the end of 24-h preservation by the UW solution cold storage method will predict the posttransplant outcome of pancreas graft subjected to significant warm ischemia.

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