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EVALUATION OF THE VIABILITY AND ENERGY METABOLISM OF ISCHEMICALLY DAMAGED CANINE PANCREAS DURING PRESERVATION BY THE TWO-LAYER (UNIVERSITY OF WISCONSIN SOLUTION/PERFLUOROCHEMICAL) COLD STORAGE METHOD

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INDEXING WORDS

pancreas transplantation ; two-layer cold storage method ; warm ischemia ; adenine nucleotides

SYNOPSIS

We evaluated the viability and energy metabolism of ischemically damaged pancreas during preservation by the two-layer (University of Wisconsin solution (UW) / perfluorochemical (PFC)) cold storage method. The pancreas grafts subjected to 60～120 min warm ischemia were preserved by the two-layer (UW /PFC) cold storage method for 24 hours (group 3), a simple cold storage in UW for 24 hours (group 2) or without preservation (control) (group 1). The tissue concentration of adenine nucleotides (ANs) were determined using high performance liquid chromatography (HPLC) and the viability of the pancreas graft was tested in the canine model of segmental pancreas

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Abbreviations :
ATs, adenine nucleotides ; TAN, total adenine nucleotides ; ATP, adenosine triphosphate ; ADP, adenosine diphosphate ; AMP, adenosine monophospate

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autotransplantation. The functional success rates of pancreas grafts of groups 1, 2 and 3 after 60 min of warm ischemia were 5/5 (100%), 4/5 (80%) and 5/5 (100%). After 90 min warm ischemia, the success rates of groups 1, 2 and 3 were 0/5 (0%), 0/5 (0%) and 5/5 (100%) respectively. Only the two-layer method (group 3) was effective for functional recovery of the pancreas suffered 90 min warm ischemia. However, after more than 105 min, the result was 0/5 (0%) in group 3. In groups 1 and 2, there was no correlation between the posttransplant viability and tissue concentration of ANs and energy charge potential (ECP) at the end of preservation. However, in group 3, there was an excellent correlation between the posttransplant viability and tissue concentration of adenosine triphosphate (ATP) and total adenine nucleotides (TAN) at the end of peservation. We conclude that tissue concentration of ATP and TAN at the end of 24 hour preservation by the two-layer (UW/PFC) method will predict the posttransplant outcome of pancreas graft subjected to significant warm ischemia.

INTRODUCTION

As warm ischemic injury of pancreas graft before and during procurement strongly influences results of the pancreas transplantation, it is important to predict the viability of the ischemically damaged pancreas graft before transplantation.

Recently we developed a two-layer cold storage method[10] that continuously supplied sufficient oxygen to a pancreas during preservation[11], and demonstrated that the two-layer method reduced cold ischemic injury of the pancreas graft during preservation[6,8]. We have also demonstrated that correlation between high ATP tissue concentration and good posttransplant outcome of a canine pancreas graft after preservation by a two-layer cold storage method[12]. In addition, we have demonstrated that the two-layer (UW/PFC) method could improve the quality of pancreas graft subjected to 60 min warm ischemia and prolong the allowable period of warm ischemia up to 90 min[13].

However, there are some qualitative differences between warm and cold ischemic injuries[7]. 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 two-layer method were excellent markers to predict posttransplant outcome.

MATERIALS AND METHODS

Mongrel dogs of both sexes, weighing 12-18kg were used for the experiments. Perfluorodecaline, which is one of PFCs, was a kind gift of Dr.K.Yokoyama (The Green Cross Corpo -ration, Osaka,Japan). UW was generously provided by DuPont Critical Care (Waukegan,IL). Chemicals were from Sigma Co., Ltd.

Operation procedures: Anesthesia was induced and maintained with sodium pentobarbiturate (25mg/kg weight). 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 unflushed and left in situ for 60-120 min of ischemia. After warm ischemia the pancreas graft was flushed with 20ml of heparinized cold saline (400units / 20ml saline) and autotransplanted in the neck[9] or the graft was flushed with 20ml of cold UW, preserved for 24 hours and then autotransplanted following flushing with 20ml of heparinized cold saline. The remainder of the pancreas was excised at the time of autotransplantation. Vascular anastomosis time was 25-30 min. After operation, the dogs received saline with 10% glucose (30ml/kg weight) and parenteral penicillin (25mg/kg weight) for three days. After three days, standard kennel diets were given.

Experimental protocol: There were eight experimental groups in which all dogs received segmental autograft that were immediately (group1) or stored at 4℃ by the simple cold storage in UW (group 2) for 24 hours or the two-layer (UW/PFC) cold storage method (group 3) for 24 hours after warm ischemic time of 60, 90, 105 and 120 (subgroups A,B,C and D).

Functional studies: Blood glucose concentration was determined daily during the first postoperative week after autotransplantation and biweekly thereafter. An intravenous glucose tolerance test (IVGTT) was performed one week and two weeks after transplantation. Maintenance of normoglycemia for at least five days after transplantation[1] or a K value of IVGTT more than 1.0 two weeks after transplantation was considered a functional success of pancreas graft[10]

Tissue extraction method for adenine nucleotides: After warm ischemia and at the end of preservation, a part of pancreas was rapidly frozen with bronze tongs in liquid nitrogen and kept-70℃ until analysis. Lyophilized tissues were ground to a powder using a mortar and pestle. Dry tissue powder (200mg) was then homogenized in 3-ml ice cold 0.5N perchloric acid. The precipitated protein were removed by centrifugation, and 500 μl of supernatant was neutralized by the additions of 50 μl 1.0N $KHCO_3$ and 50N Tris. Following centrifugation, 10 μ-l of supernatant was injected into HPLC for analysis.

Measurement of adenine nucleotides: HPLC on a reverse-phase colum of Shim-pack CLC-ODS (6 × 150mm), equilibrated with 0.1M phosphate buffer (PH 6.0) containing 1% methanol was employed to separate and quantitate ANs.

Caluculation and statistical analysis: TAN was caluculated as the sum of ATP + adenosine diphosphate (ADP) + adenosine monophosphate (AMP). Energy charge potential (ECP) was defined as (ATP +1/2 ADP)/TAN[2]. All values are expressed as mean ± SD. Statistical analysis was done using the Student's t test.

RESULT

Table 1 shows the viability of canine pancreas autografts after significant warm ischemia and cold preservation. After 60 min warm ischemia, the functional success rates of group 1A, 2A and 3A were 5/5,(100%), 4/5 (80%) and 5/5 (100%), respectively. However, after 90 min warm ischemia, the functional success rates of group 1B, 2B and 3B were

0/5 (0%),0/5 (0%) and 5/5 (100%), respectively. Only the two-layer method (group 3B) was effective for functional recovery of the pancreas. But after 105 (group 3C) and 120 (group 3D) min warm ischemia, the results were 0/5 (0%) and 0/5 (0%) respectively.

Table 2 shows tissue concentration of ANs and ECP after significant warm ischemia and cold preservation. In group 2, there was no changes of ATP and TAN levels and ECP during preservation. However, in group 3, ATP and TAN levels and ECP were increased during 24 — hour preservation. Tissue concentration of ATP after preservation in group 3C (3.26 ± 1.19 μ mol/g dry weight) was significantly lower than in groups 3A and 3B ($P < 0.01$).Tissue concentration of TAN after preservation in group 3C (5.88 ± 1.36 μ mol/g dry weight) were significanthy lower than in group 3B ($P < 0.01$). Tissue concentrations of ATP and TAN after preservation in group 3D (2.93 ± 0.62 μ mol/g dry weight and 4.37 ± 0.45 μ mol /g dry weight) were significantly lower than in groups 3A and 3B ($P < 0.01$).

Table 3 shows relationship between the posttransplant viability and tissue concentration of ANs, TAN and ECP. In group 2, there was no relationship between the viability and tissue ANs level, TAN level and ECP. However, in group 3, the viable grafts ($n = 10$) had a significant higher ATP and TAN levels compared to nonviable grafts ($n = 10$) (8.52 ± 2.59 VS 3.09 ± 0.91 μ mol/g dry weight, $P < 0.01$), and 11.58 ± 3.51 VS 5.12 ± 1.24 μ mol/g dry weight ($P < 0.01$), respectively. It was clear that ATP and TAN had statisticaly significant difference between viable grafts and nonviable grafts after warm ischemia and the two-layer method.

Figure 1 shows the individual tissue concentration of ATP and TAN for each graft after significant warm ischemia and preservation by the two- layer method. There was no overlap between the lowest ATP (6.03 μ mol/g dry weight) in the viable grafts and the highest ATP (4.71 μ mol/g dry weight) in the nonviable grafts. If ATP level of 6.0 μ mol /g dry weight was determined as a critical value for the viability following transplantation, specificity, sensitivity predictive value and efficacy were 100, 100, 100 and 100%. And there was

also no overlap between the lowest TAN (7.79 μ mol/g dry weight) in the viable grafts and the highest TAN (7.39 μ mol /g dry weight) in the nonviable grafts. If TAN level of 7. 7 μ mol /g dry weight was determined as a critical value for the viability following transplantation, specificity, sensitivity, predictive value and efficacy were 100, 100, 100 and 100 % . Both ATP and TAN were reliable markers for determining the transplantation.

DISCUSSION

Restoration of cellular function of the pancreas graft subjected to significant warm ischemia by the two-layer method will make it possible to use pancreas grafts from cadaver with cardiac arrest, wait safely the excision of the pancreas until last in heart-beating donors of multiple organs and enlarge the donor pool. Cerra et al.[4] reported that the canine pancreatic allografts tolerated warm ischemia time up to one hour and Florack et al.[5] demonstrated that the canine pancreatic autografts tolerated warm ischemia time up to 60 min. Recently we have demonstrated that the two-layer (UW/PFC) method could improve the quality of pancreas graft subjected to 60 min warm ischemia and prolong the allowable period of warm ischemia up to 90 min[13].

On the other hand, the assessment of a pancreas graft viability before transplantation is very important to prevent to transplantation of a nonfunctioning allograft especially after significant warm ischemia because there is progressive depletion of ANs during warm ischemia, ultimately leading to ischemic damage[3]. But the relationship between the tissue concentration of ANs before transplantation and organ viability after transplantation is controversial. In human liver preservation, Lanir et al. demonstrated a direct correlation between a high ATP concentration and good posttransplant outcome[14]. 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[7].

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 a two-layer cold storage method [12]

This study clearly have demonstrated that tissue concentration of ATP and TAN at the end of 24 hour preservation by the two-layer (UW/PFC) method will also predict the posttransplant outcome of pancreas graft subjected to significant warm ischemia. But the mechanism responsible for the effectiveness of the two-layer (UW/PFC) cold storage method in restoration of function of the pancreas graft subjected to significant warm ischemia remains to be unclear and is under investigation.

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Table 1. Functional success rates of canine pancreas autografts after warm ischemia and cold preservation

Group	Warm ischemic time (min)	Preservation method	Preservation time (hr)	Functional grafts / No. transplants	Success rate (%)
1 A	60	–	–	5/5	100
B	90	–	–	0/5	0
2 A	60	UW	24	4/5	80
B	90	UW	24	0/5	0
3 A	60	UW/PFC	24	5/5	100
B	90	UW/PFC	24	5/5	100
C	105	UW/PFC	24	0/5	0
D	120	UW/PFC	24	0/5	0

Table 2. Tissue concentrations of adenine nucleotides, TAN and ECP after warm ischemia and cold preservation

Group	ATP (μmol/g dry weight)		ADP (μmol/g dry weight)		AMP (μmol/g dry weight)		TAN (μmol/g dry weight)		ECP	
	after warm ischemia	after preservation	after warm ischemia	after preservation	after warm ischemia	after preservation	after warm ischemia	after preservation	after warm ischemia	after preservation
1 A	1.30 ± 0.34	–	1.02 ± 0.34	–	2.46 ± 0.54	–	4.78 ± 0.83	–	0.38 ± 0.03	–
B	1.34 ± 0.44	–	0.45 ± 0.27	–	1.55 ± 0.45	–	3.33 ± 0.38	–	0.46 ± 0.13	–
2 A	1.79 ± 0.72	1.83 ± 0.58	0.95 ± 0.45	1.31 ± 0.70	1.93 ± 0.95	1.89 ± 0.84	4.67 ± 0.95	5.04 ± 1.07	0.49 ± 0.15	0.49 ± 0.11
B	1.75 ± 0.31	1.40 ± 0.46	1.52 ± 0.38	1.00 ± 0.46	1.38 ± 0.18	1.81 ± 1.06	4.64 ± 0.61	4.02 ± 0.63	0.54 ± 0.02	0.49 ± 0.18
3 A	1.55 ± 0.55	10.60 ± 2.03 *	1.05 ± 0.14	2.20 ± 1.46	1.66 ± 0.63	1.12 ± 0.51	4.26 ± 0.37	13.91 ± 3.57 *	0.49 ± 0.13	0.85 ± 0.04
B	1.21 ± 0.35	6.45 ± 0.46 *	0.51 ± 0.29	1.72 ± 0.47	1.54 ± 0.95	1.07 ± 0.71	3.27 ± 1.28	9.25 ± 1.17 *	0.49 ± 0.13	0.80 ± 0.08
C	1.19 ± 0.27	3.26 ± 1.19	0.61 ± 0.13	1.51 ± 0.48	1.24 ± 0.37	1.11 ± 0.71	3.04 ± 0.60	5.88 ± 1.36	0.50 ± 0.06	0.68 ± 0.14
D	1.40 ± 0.41	2.93 ± 0.62 *	0.55 ± 0.36	0.98 ± 0.35	0.78 ± 0.37	046 ± 0.09	2.73 ± 0.55	4.37 ± 0.45 *	0.62 ± 0.10	0.78 ± 0.06

* $P < 0.01$

Table 3. Relationship between posttransplant viability and tissue concentrations of adenine nucleotides, TAN and ECP

	ATP (μmol/g dry weight)		ADP (μmol/g dry weight)		AMP (μmol/g dry weight)		TAN (μmol/g dry weight)		ECP	
	after warm ischemia	after preservation	after warm ischemia	after preservation	after warm ischemia	after preservation	after warm ischemia	after preservation	after warm ischemia	after preservation
Group 1										
viable grafts (n = 5)	1.30 ± 0.34	–	1.02 ± 0.34	–	2.46 ± 0.54	–	4.78 ± 0.83	–	0.38 ± 0.03	–
non-viable grafts (n = 5)	1.34 ± 0.44	–	0.45 ± 0.27	–	1.55 ± 0.45	–	3.33 ± 0.38	–	0.46 ± 0.13	–
Group 2										
viable grafts (n = 4)	1.78 ± 0.83	1.89 ± 0.66	1.08 ± 0.38	1.44 ± 0.74	1.68 ± 0.88	1.83 ± 0.96	4.54 ± 1.05	5.15 ± 1.20	0.51 ± 0.16	0.50 ± 0.12
non-viable grafts (n = 6)	1.85 ± 0.24	1.48 ± 0.50	1.56 ± 0.43	1.10 ± 0.47	1.41 ± 0.19	1.61 ± 1.10	4.82 ± 0.55	3.93 ± 0.69	0.55 ± 0.01	0.53 ± 0.18
Group 3										
viable grafts (n = 10)	1.40 ± 0.45	8.52 ± 2.59 *	0.76 ± 0.36	1.96 ± 1.05	1.60 ± 0.76	1.09 ± 0.58	3.76 ± 1.03	11.58 ± 3.51 *	0.49 ± 0.12	0.82 ± 0.06
non-viable grafts (n = 10)	1.30 ± 0.34	3.09 ± 0.91 *	0.58 ± 0.26	1.24 ± 0.48	1.01 ± 0.42	079 ± 0.58	2.89 ± 0.57	5.12 ± 1.24 *	0.56 ± 0.10	0.73 ± 0.11

* $P < 0.01$

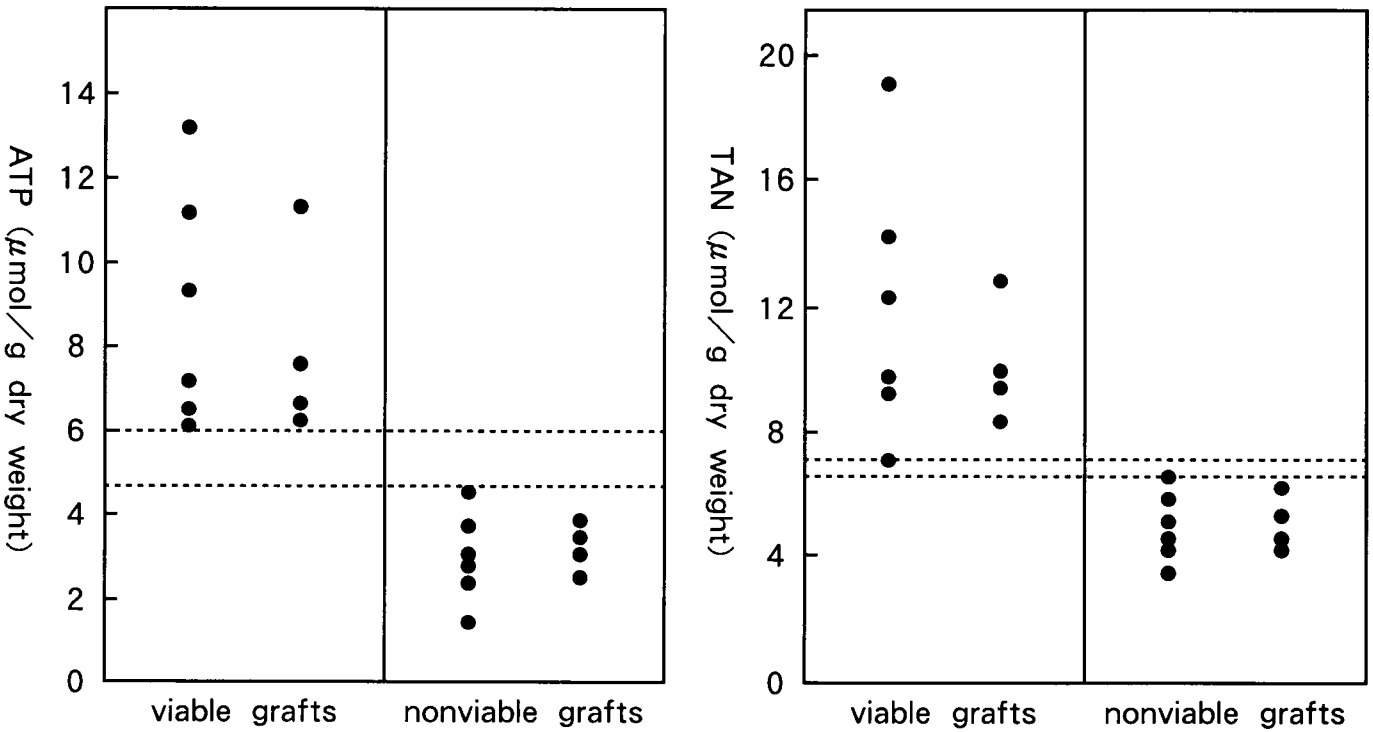

Figure 1. Relationship between tissue concentrations of ATP and TAN and posttransplant viability of ischemically damaged pancreas graft during preservation by the two-layer method