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ADENINE NUCLEOTIDES METABOLISM OF THE CANINE PANCREAS DURING PRESERVATION BY THE TWO-LAYER COLD STORAGE METHOD*

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INDEXING WORDS

pancreas transplantation; two-layer cold storage method; adenine nucleotides; adenosine triphosphate

SYNOPSIS

The relationship between tissue concentrations of adenine nucleotides (ANs) at the end of cold preservation period and organ viability after transplantation is controversial. The purpose of this study is to examine energy metabolism of the pancreas graft preserved by a two-layer cold storage method and the relationship with the viability of the pancreas graft following transplantation. After preservation by simple cold storage with Euro-Collins' solution (EC) (group 1) or University of Wisconsin solution (UW) (group 2), an original two-layer (EC / perfluorochemical(PFC)) method (group 3) and a modified two-layer (UW / PFC) method (group 4) for 24, 48, 72, 96 and 120 hours, tissue concentrations of 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 autotransplantation. While EC alone (group 1) was available only for 24-hour preservation, UW alone (group 2) and the original two-layer method (group 3) were effective up to 72-hour preservation and the modified two-layer method (group 4) was successful up to 96-hour preservation. In simple cold storage with EC or UW, there was no significant difference between tissue concentrations of adenosine triphosphate (ATP) of viable grafts and nonviable grafts. But in groups 3 and 4, there was an excellent correlation between the posttransplant viability and ATP tissue concentration at the end of

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Abbreviations: ANs, adenine nucleotides; TAN, total adenine nucleotides; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophpsphate

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preservation, and ATP was useful to predict the viability of the canine pancreas during preservation by the two-layer method before transplantation.

INTRODUCTION

In clinical pancreas transplantation, it is important to predict the viability of the pancreas graft before transplantation for avoiding transplantation of nonfunctioning allograft because we cannot exactly assess how injured the pancreas graft is during procurement and preservation. So it is essential to develop the method which allows specific assessment of pancreas function correlated with posttransplant outcome without the damage to the tissue for a successful pancreas transplantation.

Recently we developed a two-layer (EC / PFC) cold storage method,[9] which continuously supplied sufficient oxygen to the pancreas graft during preservation[10] and extended the preservation time of the canine pancreas up to 72 hours.[7] In addition, we succeeded in 96 -hour preservation of canine pancreas by a modified two-layer (UW / PFC) cold storage method.[5] We have also demonstrated that the oxygenation of the pancreas during preservation by the two-layer method produces ATP within the pancreas and maintains tissue level of ATP due to the mitochondrial oxidative phosphorylation.[10,11]

In this study we examined energy metabolism and the viability of the pancreas graft preserved by the simple cold storage and the two-layer method, and found ATP tissue level after preservation by the two-layer method was an excellent marker to predict posttransplant viability

MATERIALS AND METHODS

Mongrel dogs of both sexes, weighing 12-20 kg were used in this study. Perfluododecaline, which is one of PFCs, was a kind of Dr. K. Yokoyama (The Green Cross Corporation, 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 (25 mg/kg weight). After laparotomy, a left lobectomy of the pancraes with the splenic artery and vein attached was meticulously performed, followed by splenectomy. The segmental pancreas graft was flushed out with about 50 ml cold heparinized EC or UW (1000 units / 50 ml EC or UW) through the splenic artery and preserved. After preserevation, pancreas graft was autotransplanted in the neck, as described previously,[8] excising the remainder of the pancreas at the time of autotransplantation. At the end of preservation, tissue concentrations of ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were measured.

Preservation protocol : The pancreas grafts were preserved by simple cold storage with EC (group 1) for 24 hrs (n=3) and 48 hrs (n=3) (1A and 1B, respectively), and with UW (group 2) for 24 hrs (n=4), 48 hrs (n=3), 72 hrs(n=3) and 96 hrs (n=3) (2A, 2B, 2C and 2D, respectively). The pancreas grafts were also preserved by an original two-layer method for 24 hrs (n=5) (3A), 48 hrs (n=4) (3B), 72 hrs (n=4) (3C), 96 hrs (n=4) (3D), and by the modified two-layer method for 24 hrs (n=4) (4A), 48 hrs (n=4) (4B), 72 hrs (n=4) (4C), 96 hrs (n=7) (4D) and 120 hrs (n=3) (4E).

Assessment of the graft function : Fasting blood glucose was measured daily. Normoglycemia during at least five days after autotransplantation was assessed as a functional success.[1)]

Preparation of tissue extracts : At the end of preservation a part of pancreas was rapidly frozen with bronze tongs in liquid nitrogen, lyophilizated overnight, and kept at -80 ℃ until analysis. The dry tissue was ground to a powder using a mortar and pestle. The dry tissue powder was weighted (200 mg) and homogenized in 3 ml ice cold 0.5 N perchloric acid. The precipitated protein was removed by centrifugation, and 500 µl of supernatant was neutralized by the additions of 50 µl 1.0 N KHCO3 and 0.5 N Tris-HCL. After the cold storage in ice water for 15 min, it was centrifugated. 10 µl of supernatant was injected into HPLC for analysis.

Measurement of adenine nucleotides : HPLC on a reversed column, CLC-ODS (6 ϕ x 15mm) purchased from Shimazu manufacturing Co., Ltd, which was equilibrated with 100 mM sodium phosphate buffer (pH 6.0) containing 1.0 % methanol, was employed to separate and quantitate adenine nucleotides.

Caluculation and statistical analysis : Total adenine nucleotides (TAN) was caluculated as the sum of ATP + ADP + AMP. Energy charge potential (ECP) was defined as (ATP + 1/2 ADP) / TAN.[2)] All values are expressed as mean ± SD. Statistical analysis was performed by student's t test.

RESULTS

Table 1 shows the results of the viability of canine pancreas autografts and tissue concentrations of ANs after simple cold storage with EC or UW. The functional success rates of groups 1A and 1B were 3/3 (100%) and 0/3 (0%), but in groups 2A, 2B, 2C and 2D, the results were 4/4 (100%), 3/3 (100%), 3/3 (100%) and 0/3 (0%), respectively. Tissue concentrations of AMP and TAN in group 1A (1.49 µmol / g dry weight and 3.48 µmol / g dry weight) were lower than in group 1B (4.27 µmol / g dry weight and 6.27µmol / g dry

Table 1. Viability of canine pancreas autografts and tissue concentrations of ATP, ADP, AMP and TAN, and ECP after simple cold storage with EC or UW

Group	Preservation method	Preservation time (hr)	Functional grafts No. transplants	Success rate (%)	ATP (μmol / g dry weight)	ADP (μmol / g dry weight)	AMP (μmol / g dry weight)	TAN (μmol / g dry weight)	ECP
1A	EC	24	3/3	100	1.04±0.19	0.95±0.76	1.49±0.24	3.48±0.72	0.47±0.04
B	EC	48	0/3	0	1.26±0.26	0.79±0.08	4.27±0.60 *	6.27±0.38 *	0.27±0.05 *
2A	UW	24	4/4	100	1.29±0.61	0.83±0.19	4.94±1.31	7.06±1.68	0.24±0.05
B	UW	48	3/3	100	1.23±0.36	1.26±0.19	2.56±0.17	5.05±0.17	0.37±0.05
C	UW	72	3/3	100	1.42±0.50	1.03±0.09	2.71±0.77	5.16±1.31	0.37±0.02 *
D	UW	96	0/3	0	1.02±0.15	0.97±0.56	2.12±0.47	4.11±0.91	0.37±0.02

* P<0.01

Table 2. Viability of canine pancreas autografts and tissue concentrations of ATP, ADP, AMP and TAN, and ECP after preservation by the two-layer method

Group	Preservation method	Preservation time (hr)	Functional grafts No. transplants	Success rate (%)	ATP (μmol / g dry weight)	ADP (μmol / g dry weight)	AMP (μmol / g dry weight)	TAN (μmol / g dry weight)	ECP
3A	EC+PFC+O_2	24	5/5	100	7.47±0.47	1.67±0.75	1.47±1.38	11.02±2.74	0.78±0.14
B	EC+PFC+O_2	48	4/4	100	7.91±1.24	1.41±0.89	1.22±1.25	10.54±2.33	0.83±0.11
C	EC+PFC+O_2	72	4/4	100	8.29±0.21	1.55±0.39	0.85±0.40	10.68±0.97	0.85±0.04
D	EC+PFC+O_2	96	0/4	0	4.83±1.25 [a]	1.08±0.22	0.93±0.34	6.83±1.67 [c]	0.79±0.01
4A	UW+PFC+O_2	24	4/4	100	7.90±1.73	3.01±1.80	1.52±0.81	12.43±2.98	0.77±0.11
B	UW+PFC+O_2	48	4/4	100	13.22±5.20	2.66±1.96	1.01±0.59	16.90±3.80	0.85±0.11
C	UW+PFC+O_2	72	4/4	100	14.28±1.94	2.04±0.60	0.87±0.14	17.19±1.53	0.89±0.03
D	UW+PFC+O_2	96	5/7	71					
				viable grafts	11.70±2.61	3.48±1.23	1.37±0.64	16.55±3.57	0.81±0.06
				nonviable grafts	3.51±0.81	2.24±1.02	4.67±4.77	10.41±4.98	0.51±0.27
E	UW+PFC+O_2	120	0/3	0	3.98±1.34 [b]	2.06±1.56	3.31±2.03	9.34±1.98 [d]	0.53±0.20 [e]

a Significantly different from group 3A (P<0.01), 3B (P<0.01), and 3C (P<0.01)

b Significantly different from groups 4A (P<0.01), 4B (P<0.01), 4C (P<0.01) and viable grafts in group 4D (P<0.01)

c Significantly different from group 3C (P<0.01)

d Significantly different from group 4C (P<0.01)

e Significantly different from group 4C (P<0.01)

Table 3. Relationship between viability and tissue concentrations of ATP, ADP, AMP and TAN, and ECP of canine pancreas autografts

	ATP (μmol / g dry weight)	ADP (μmol / g dry weight)	AMP (μmol / g dry weight)	TAN (μmol / g dry weight)	ECP
Group 1 : EC					
viable grafts	1.04 ± 0.19	0.95 ± 0.76	1.49 ± 0.24 *	3.48 ± 0.72 *	0.47 ± 0.04 *
nonviable grafts	1.28 ± 0.26	0.79 ± 0.08	4.27 ± 0.60 *	6.27 ± 0.38 *	0.27 ± 0.05 *
Group 2 : UW					
viable grafts	1.31 ± 0.46	1.02 ± 0.24	3.56 ± 1.46	5.89 ± 1.61	0.32 ± 0.08
nonviable grafts	1.02 ± 0.15	0.97 ± 0.56	2.12 ± 0.47	4.11 ± 0.91	0.37 ± 0.02
Group 3 : EC+PFC+O2					
viable grafts	7.86 ± 0.77 *	1.55 ± 0.66	1.20 ± 1.07	10.77 ± 2.04 *	0.82 ± 0.10
nonviable grafts	4.83 ± 1.26 *	1.08 ± 0.22	0.93 ± 0.34	6.83 ± 1.67 *	0.79 ± 0.01
Group 4 : UW+PFC+O2					
viable grafts	11.77 ± 3.73 *	2.84 ± 1.44	1.20 ± 0.66 *	15.82 ± 3.44 *	0.83 ± 0.09 *
nonviable grafts	3.79 ± 1.06 *	2.13 ± 1.22	3.85 ± 2.88 *	9.77 ± 2.92 *	0.52 ± 0.20 *

* P<0.01

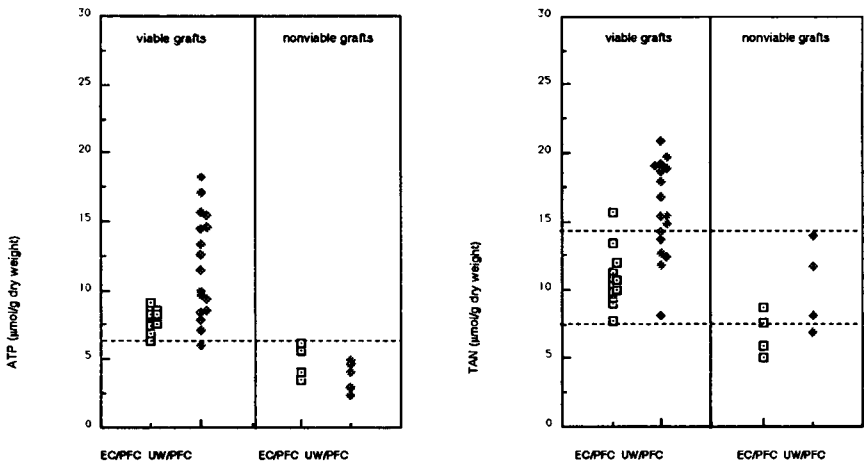

Figure 1. Tissue concentrations of ATP and TAN of each graft preserved by the two-layer method according to the viability

weight), while ECP in group 1A (0.47) was higher than in group 1B (0.27). But concerning to ATP, there was not significant difference between each subgroup irrespective of viability. Table 2 also shows the results of the viability of canine pancreas autografts and tissue concentrations of ANs after preservation by the two-layer method. The functional success rates of groups 3A, 3B, 3C and 3D were 5/5 (100%), 4/4 (100%), 4/4 (100%) and 0/4 (0%), respectively. The original two-layer method was effective for the canine pancreas up to 72-hour preservation but not effective for 96-hour preservation. Tissue concentrations of ATP and TAN in group 3D (4.83 μmol / g dry weight and 6.83 μmol / g dry weight, respectively) were significantly different from in groups 3A, 3B and 3C ($P<0.01$). The functional success rates of groups 4A, 4B ,4C, 4D and 4E were 4/4 (100%), 4/4 (100%), 4/4 (100%), 5/7 (71%) and 0/3 (0%), respectively. Two of seven dogs in group 2D died of causes related to the grafts. One of them was hemorrhagic pancreatitis and the other was venous thrombosis. Tissue concentrations of ATP and ECP in group 4E (3.98 μmol / g dry weight and 0.53, respectively) were significantly lower than in groups 4A, 4B, 4C and viable grafts in group 4D ($P<0.01$).

Table 3 summarized the tissue concentrations of ANs and ECP according to the viability. Pancreas grafts in groups 1A, 2A, 2B and 2C were viable, although the grafts in groups 1B and 2D were nonviable. ANs or ECP were not useful to predict the viability in both EC and UW (groups 1 and 2). In group 3, pancreas grafts of groups 3A, 3B and 3C were viable but those of group 3D were not viable. In group 4, all pancreas grafts of groups 4A, 4B and 4C, and two of group 4D functioned, while the other didn't. In the viable grafts of group 3, the tissue concentrations of ATP, ADP, AMP and TAN, and the value of ECP were 7.86 ± 0.77 μmol / g dry weight, 1.55 ± 0.66 μmol / g dry weight, 1.20 ± 1.07 μmol / g dry weight, 10.77 ± 2.04 μmol / g dry weight and 0.81 ± 0.10, respectively. In the nonviable grafts of group 3, ATP, ADP, AMP, TAN and the value of ECP were 4.83 ± 1.26 μmol / g dry weight, 1.08 ± 0.22 μmol / g dry weight, 0.93 ± 0.34 μmol / g dry weight, 6.83 ± 1.67 μmol / g dry weight and 0.79 ± 0.01, respectively. Tissue concentrations of ATP and TAN of viable grafts were significantly higher compared to nonviable grafts ($P<0.01$). On the other hand, in viable grafts of group 4 the tissue concentrations of ATP, ADP, AMP and TAN, and the value of ECP were 11.77 ± 3.73 μmol / g dry weight, 2.84 ± 1.44 μmol / g dry weight, 1.20 ± 0.66 μmol / g dry weight, 15.82 ± 3.44 μmol / g dry weight and 0.83 ± 0.09, respectively, while in nonviable grafts of group 4 the tissue concentrations of ATP, ADP, AMP and TAN, and the value of ECP were 3.79 ± 1.06 μmol / g dry weight, 2.13 ± 1.22 μmol / g dry weight, 3.85 ± 2.88 μmol / g dry weight, 9.77 ± 2.92 μmol / g dry weight and 0.52 ± 0.20 respectively. ATP, AMP, TAN and ECP of viable grafts in group 4 were significantly different from nonviable grafts in group 4 ($P<0.01$). Only ATP and TAN had statisticaly significant difference between viable grafts and nonviable grafts by both two-layer methods.

Figure 1 shows in detail the individual tissue concentrations of ATP and TAN for each graft after preservation by the two-layer methods. If ATP level of 6.20 μmol / g dry weight was

determined as a critical value for the viability following transplantation, specificity, sensitivity, predictive value and efficacy were 100, 97, 100 and 97 %. However concerning to TAN, there was overlap between the lowest TAN (7.69 μmol / g dry weight) of the viable grafts and the highest TAN (13.93 μmol / g dry weight) of the nonviable grafts. TAN was not a reliable marker for determining the transplantation.

DISCUSSION

During the cold preservation period, there is progressive depletion of ANs, ultimately leading to ischemic damage.[3,15] It seems like that ANs are in fact critical to the tissue viability. But the relationship between the tissue concentrations of AN and organ viability was controversial. In rat kidney, Calman et al. [4] reported that TAN correlated with the subsequent viability. In the liver, Lanir et al.[12] demonstrated clinically that a direct correlation between high ATP concentration and good posttransplant outcome. On the other hand, Harvey et al.[6] reported that ATP concentration of the rat liver was independent of the length of the cold storage or the viability of the graft.

Recently we developed the two-layer cold storage method that supplied sufficient oxygen to the canine pancreas during preservation[9] and extended preserevation time up to 72 hours with EC[7] and up to 96 hours with UW.[5] We have found that the oxygenation of the pancreas during preservation by the two-layer method produces ATP due to the mitochondrial oxidative phosphorylation,[10,11] which is nessessary to maintain cellular integrity during preservation, and permits prolongation of pancreas preservation. It seems reasonable to think that tissue concentration of ATP of the pancreas graft during preservation by the two-layer method will reflect the viability following transplantation.

This study clearly have demonstrated that ATP tissue concentration at the end of cold preservation by the two-layer method will corelate the viability of the pancreas graft following transplantation, while ATP is not a good index of the viability during simple cold storage with EC or UW. But ECP are not useful although it is proposed as a fundamental metabolic control parameters.[2] In viable grafts the level of ECP was high, but ECP level did not correlate the viability. During preservation and reperfusion, regeneration of ATP is nessesary to maintain cell integrity and prevent reperfusion injury.[14]

The tissue ATP level reflects the total mitochondrial function of acinar cell, islet cell and nonparenchymal cell (endothelial cell et.al.), and at present it is not known which component is most important to maintain cellular integrity during preservation by the two-layer method. It is nessesary to find a marker to predict the viability of each component to understand the mechanism of tolerance of cold and warm ischemic damages.[15]

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