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Difference in energy metabolism between fresh and warm ischemic canine pancreases during preservation by the two-layer method

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Abstract We have demonstrated that adenosine triphosphate (ATP) is synthesized within a canine pancreas during preservation by the two-layer method and there is a direct correlation between a high ATP tissue level and good post-transplant outcome. The purpose of this study was to examine the difference in energy metabolism between fresh and warm ischemic pancreases during preservation by this method. First, fresh pancreases were preserved with simple cold storage in Euro-Collins solution (EC; group 1A), or by the two-layer method using EC (group 1B), EC with 2,4 dinitrophenol (DNP; group 1C), an uncoupler of oxidative phosphorylation, or modified EC (ECM; group 1d), which contained mannitol in place of glucose for 48 h. ATP tissue concentrations in group 1B were significantly higher than in group 1A (7.91 ± 1.21 vs. 1.21 ± 0.31 μmol/g dry weight, $P < 0.01$) but almost equal to group 1d (7.91 ± 1.21 vs. 7.59 ± 0.97 μmol/g dry weight, NS). DNP (group 1C) caused a significant decrease in tissue ATP levels in group 1A (0.61 ± 0.07 vs. 7.91 ± 1.21 μmol/g dry weight, $P < 0.01$). Second, pancreases subjected to 60 min of warm ischemia were preserved by simple cold storage with EC (group 2A) or the two-layer method using EC (group 2B) or EC with adenosine (group 2C) for 24 h. ATP tissue levels in groups 2A and 2B after preservation were 1.40 ± 0.46 and 1.56 ± 0.40 μmol/g dry weight and graft survival rates were 0/5 (0%) and 0/3 (0%), respectively. However, tissue ATP levels in group 2C after preservation were significantly higher compared with the value before preservation (7.23 ± 2.17 vs. 1.90 ± 0.53/g dry weight, $P < 0.01$) and graft survival rate was 4/5, 80%. Other nucleosides, hypoxanthine, inosine, and adenine did not substitute for adenosine. In addition, studies with [2–3 H] adenosine demonstrated that almost all of the adenosine was converted to adenine nucleotides. This study clearly demonstrated that fresh grafts synthesize ATP mainly via mitochondrial oxidative phosphorylation using endogenous substrates. However, after significant warm ischemia, pancreases produce ATP mainly via direct phosphorylation of exogenous adenosine during preservation by the two-layer method.

Key words Pancreatic preservation · Two-layer cold storage method · Warm ischemia ATP · Oxidative phosphorylation of adenosine

Introduction

The extent of clinical pancreatic transplantation is limited by the availability of donor organs. It is obvious that the number of pancreases available for transplantation would be significantly increased if it were possible to use pancreases from cardiac-arrested donors. Recently, we have demonstrated that a canine pancreas is oxygenated and ATP is synthesized within a viable graft during preservation by the two-layer method [9, 11]. In addition, there is a direct correlation between tissue ATP level and posttransplant outcome [10, 14].

To clarify the possibility of pancreatic transplantation using pancreases from cardiac-arrested donors, we examined the difference in energy metabolism between fresh and warm ischemic canine pancreases during preservation by the two-layer method.

Materials and methods

Mongrel dogs of both sexes, weighing 12–20 kg were used for the experiment. Perfluorodecaline (PFD), one of the perfluorochemicals (PFCs), was a kind gifts from Dr. K. Yokoyama (The Green Cross Corportation, Osaka, Japan). A Shim-pack was purchased from Shimazu Manufacturing Co., Ltd. Chemicals were purchased from Wako Co., Ltd.

Operation procedures

Anesthesia was induced and maintained with sodium pentobarbiturate (25 mg/kg weight). After laparotomy, a left lobectomy of the pancreas with the splenic artery and vein attached was meticulously performed, followed by splenectomy. The segmental pancreatic graft was flushed out with 50 ml cold heparinized preservation solution (1000 units/50 ml solution) through the splenic artery and preserved according to the experimental protocol immediately or after the pancreatic graft was left unflushed in the abdominal cavity for 60 min at body temperature. After preservation, the pancreatic graft was autotransplanted in the neck, as described previously [8] excising the remainder of the pancreas at the time of autotransplantation. After surgery, the dogs received saline with 10% glucose (30 ml/lg weight) and parenteral penicillin (25 mg/kg weight) for 3 days. After 3 days, standard kennel diets were given.

Assessment of graft function

Fasting blood glucose was measured daily. Normoglycemia during at least 5 days after autotransplantation was assessed as graft survival [9].

Measurement of adenine nucleotides

High-performance liquid chromatography (HPLC) on a reversed column, CLC-ODS (6 × 150 mm) purchased from Shimazu Manufacturing Co., Ltd, which was equilibrated with 100 m*M* sodium phosphate buffer, (pH 6.0, containing 1.0% methanol, was employed to separate and quantitate adenosine triphosphate (ATP).

Preparation of tissue extracts

At the end of preservation, part of the pancreas was rapidly frozen with bronze tongs in liquid nitrogen, lyophilized overnight, and kept at −80 °C until analyzed. 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 percipitated protein was removed by centrifugation, and 500 µl of supernatant was neutralized by the addition of 50 µl 1.0 *N* KOH and 0.5 *N* Tris. Following centrifugation, 10 µl of supernatant was injected into HPLC for analysis.

Experimental protocol

Experiment 1 Pancreatic grafts were preserved with simple cold storage in Euro-Collins solution (EC; group 1) and by the two-layer method using EC (group 2), EC + 2,4 dinitrophenol (DNP; group 3), and modified EC (ECM; group 4), which contained mannitol in place of glucose, for 48 h.

Experiments 2 and 3 Pancreatic grafts subjected to 60 min of warm ischemia were preserved by the two-layer (EC/PFC) method or the two-layer method using EC with 5 m*M* adenosine, 5 m*M* hypoxanthine, 5 m*M* inosine, or 5 m*M* adenine for 24 h.

Experiment 4 Pancreatic graft subjected to 60 min of warm ischemia was preserved by the two-layer method using EC with 5 m*M* [2-^{3}H]adenosine (specific activity 2.6×10^{11} cpm/mmol) for 48 h. Adenine nucleotides and nucleosides were measured by HPLC and radioactivity in adenine nucleotides and nucleosides were measured by a Beckmann scintillation counter.

Statistical analysis

All values are expressed as mean ± SD. Statistical analysis was performed using Student's *t*-test. A P value of less than 0.05 was considered significant.

Results

Experiment 1. Energy metabolism of fresh canine pancreatic graft during preservation by the two-layer method (Table 1)

After 48-h preservation with simple cold storage in EC (group 1), pancreatic grafts did not survive (0/4, 0%). However, the two-layer (EC/PFC) method (group 2) made it possible to preserve them for 48 h (4/4, 100%) and tissue ATP levels were significantly higher compared with group 1 (7.91 ± 1.21 vs. 1.21 ± 0.31 µmol/g dry weight, $P < 0.01$). EC contains glucose as a source of energy if needed during preservation but when the pancreatic graft was preserved by the two-layer method using ECM (group 4), which contained mannitol in place of glucose, tissue ATP levels were not changed compared

with group 2 (7.59 ± 0.97 vs. 7.91 ± 1.21 μmol/g dry weight, NS). It was clear that ATP was synthesized using endogenous substrates within the fresh pancreatic grafts because mannitol was not metabolized. Exogenous glucose did not act as a substrate for ATP synthesis during preservation by the two-layer method. DNP, an uncoupler of oxidative phosphorylation, inhibited ATP synthesis (0.61 ± 0.07 vs. 7.91 ± 1.21 μmol/g dry weight in groups 4 and 2, respectively, $P < 0.01$) and caused loss of graft viability (0/3, 0%), suggesting that the two-layer (EC/PFC) method clearly protected pancreatic viability and ATP was synthesized within the fresh pancreatic graft via mitochondrial oxidative phosphorylation.

Experiment 2. Energy metabolism of ischemically damaged canine pancreas during preservation by the two-layer method (Table 2)

When the pancreatic graft subjected to 60 min of warm ischemia was preserved by the two-layer (EC/PFC) method, the graft survival rate was 0/3 (0%) and tissue ATP levels were not increased during preservation compared to the value before preservation (1.56 ± 0.40 vs. 1.62 ± 0.26 μmol/g dry weight, NS). We have reported that the two-layer method using University of Wisconsin (UW) solution makes the resuscitation and preservation of ischemically damaged pancreas possible for 24 h [11]. As UW contains 5 m*M* adenosine [2], the pancreatic graft subjected to 60 min of warm ischemia was preserved by the two-layer method using EC with adenosine. Tissue ATP levels were significantly increased compared with the value before preservation (7.23 ± 2.17 vs. 1.90 ± 0.53 μmol/g dry weight, $P < 0.01$) and significantly higher than in the two layer (EC/PFC) method (7.23 ± 2.17 vs. 1.56 ± 0.40 μmol/g dry weight, $P < 0.01$) and graft survival rate was 4/5, 100%.

Experiment 3. ATP synthesis from various precursors in the pancreatic graft subjected to 60 min of warm ischemia during preservation by the two-layer (EC/PFC) method (Table 3)

Inosine, hypoxanthine, and adenine were tested at 5 m*M*. None of the three substrates tested approached the effectiveness of adenosine as precursors of ATP.

Experiment 4. Distribution of radioactive nucleotides and nucleosides in a pancreatic graft exposed to 60 min of warm ischemia during preservation by the two-layer method using EC + [2-^{3}H]adenosine (Fig. 1)

After 48-h preservation, the radioactivity found in the acid-soluble extract accounted for 79.4% of the total activity taken up by the pancreas. Of the radioactivity in acid-soluble extract, 53.2% was found in the adenine nucleotide fraction (ATP 42.2%, ADP 10.0%, AMP 1.0%). Much less radioactivity was taken up into inosine (6.8%), hypoxanthine (12.7%), and adenine (1.5%). A small amount of the total activity was associated with the unresolved fraction.

Table 1 Energy metabolism of fresh pancreatic graft during 48-h preservation (*EC* Euro-collins solution, *PFC* perfluorochemical, *DNP* 2,4 dinitrophenol, *ECM* modified EC)

Group	Preservation method	Graft survival rate (%)	Tissue ATP concentration (μmol/g dry weight)
1	EC	0/4 (0)	1.21 ± 0.31
2	EC/PFC	4/4 (100)	7.91 ± 1.21 *
3	EC + DNP/PFC	0/3 (0)	0.61 ± 0.07
4	ECM/PFC	4/4 (100)	7.59 ± 0.97 *

* $P < 0.01$ vs. groups 1 and 3

Table 3 ATP synthesis from various precursors in ischemically damaged pancreas during preservation by the two-layer (EC/PFC) method

Precursor (5 m*M*)	Tissue ATP concentration (μmol/g dry weight)
–	1.56 ± 0.40
Adenosine	7.23 ± 2.17 *
Hypoxanthine	1.80 ± 1.08
Inosine	2.42 ± 0.98
Adenine	2.31 ± 1.12

* $P < 0.01$ vs. other precursors

Table 2 Energy metabolism of ischemically damaged pancreatic graft during 24-h preservation

Group	Preservation method	Tissu ATP (μmol/g dry weight)		Graft survival rate (%)
		After warm ischemia	After preservation	
1	EC	1.55 ± 0.55	1.40 ± 0.46	0/5 (0)
2	EC/PFC	1.62 ± 0.26	1.56 ± 0.40	0/3 (0)
3	EC + adenosine/PFC	1.90 ± 0.53	7.23 ± 2.17 *	4/5 (80)

* $P < 0.01$ vs. groups 2

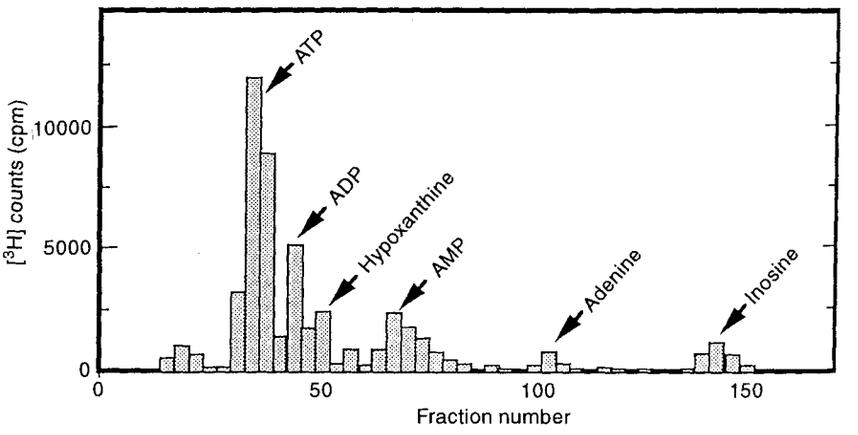

Fig. 1 Distribution of radioactive nucleotides and nucleosides obtained with high-performance liquid chromatography. The pancreatic graft subjected to 60 min of warm ischemia was preserved by the two-layer method using Euro-Collins solution with 5 m*M* [2-^{3}H]adenosine (specific activity 2.6×10^{11} cpm/mmol) for 48 h. *Arrows* represent the elution position of designated substances

Discussion

During preservation by the two-layer method, ATP was produced in fresh pancreatic grafts that were not subjected to significant warm ischemia using endogenous substrates mainly via oxidative phosphorylation (Table 1); ATP was synthesized in ischemically damaged pancreatic grafts via direct phosphorylation of exogenous adenosine (Tables 2 and 3, Fig. 1), suggesting that there is a difference in energy metabolism between fresh and warm ischemic pancreatic grafts.

As endogenous substrates for ATP synthesis are depleted during procurement and preservation [5, 6] and adenine nucleotide levels during preservation influence posttransplant outcome [7, 12], several investigators suggest that nutritional support of donor liver may result in improved posttransplant outcome [1, 3, 13, 15]. In pancreases, however, we have demonstrated that the nutritional condition of the donor prior to procurement has no influence on ATP levels and viability of the graft during 24-h preservation, probably due to a lower capacity to store glycogen in the pancreas [4]. Therefore, metabolic intervention to promote ATP synthesis during preservation is important to maintain the viability of the pancreatic graft, particularly following significant ischemia.

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