Transpl Int (1994) 7 [Suppl 1]: S436–S440

Yoshikazu Kuroda
Kunihiko Hiraoka
Yasuki Tanioka
Shin-ichi Matsumoto
Yasuhiro Fujino
Akio Morita
Yasuyuki Suzuki
Yonson Ku
Yoichi Saitoh

Metabolic intervention to affect canine pancreas recovery following ischemia during preservation by the two-layer method

Y. Kuroda (✉) · K. Hiraoka · Y. Tanioka
S. Matsumoto · Y. Fujino · A. Morita
Y. Suzuki · Y. Ku · Y. Saitoh
First Department of Surgery,
Kobe University School of Medicine,
7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650,
Japan

Abstract We have demonstrated that a high adenosine triphosphate (ATP) level in a canine pancreas during preservation by the two-layer method is an important determinant for the ultimate success of pancreatic transplantation. In this study, we investigated (a) the effect of factors that seemed to have an influence on energy metabolism in the canine pancreas at the tissue ATP level and (b) graft viability during preservation by the two-layer method. ATP tissue concentration was determined by high-performance liquid chromatography and graft viability was assessed on the basis of survival rate following autotransplantation. First, the pancreas was harvested from either 72-h-fasted ($n = 5$) or fed dogs ($n = 5$) and preserved by the two-layer Euro-Collins solution (EC)/perfluorochemical (PFC) method for 24 h. All the pancreatic grafts were viable in both fed and fasted groups. There was also no significant difference in ATP tissue concentration between the two groups (7.48 ± 0.55 vs. 7.03 ± 0.74 μmol/g dry weight, NS). Second, the pancreatic grafts subjected to 60 min of warm ischemia were preserved by either the two-layer (EC/PFC) or (EC + adenosine/PFC) method for 24 h. Without adenosine, ATP tissue concentration did not recover (1.62 ± 0.26 after warm ischemia vs. 1.56 ± 0.40 μmol/g dry weight after preservation, NS) and all the pancreatic grafts failed. However, provision of adenosine led to restoration of ATP tissue levels (1.90 ± 0.53 vs. 7.23 ± 2.17 μmol/g dry weight, $P < 0.01$) and four of five grafts functioned immediately and maintained normoglycemia after transplantation. These results clearly demonstrated that the nutritional state of the pancreatic graft before procurement had no influence on ATP tissue level as well as graft viability during 24-h preservation by the two-layer method. On the other hand, provision of adenosine during 24-h preservation enhanced ATP synthesis of the pancreatic tissue, thereby improving viability of the ischemically damaged pancreas.

Key words Pancreatic transplantation · Preservation of canine pancreas · Two-layer cold storage method · ATP · Adenosine

Introduction

Oxygenation of a canine pancreas during preservation by the two-layer method [8] results in continued adenosine triphosphate (ATP) production [9, 11]. Graft function after transplantation correlates well with ATP tissue concentration, indicating that tissue ATP levels are an important determinant for graft quality in the two-layer method [10]. ATP synthesis seems to be profoundly affected by the resources of its energy substrate, since substrate deficiency invariably results in the disturbance of ATP regeneration. In this regard, several investigators have focused on the nutritional state of a donor before procurement and the extent of ATP degradation and loss of its degradates during procurement and preservation [3, 6] (Fig. 1). In liver preservation, Palombo et al. have demonstrated that effective support of adenine nucleotides by glycolysis in flushed-preserved rat liver is independent of the presence of exogenous glucose but dependent on the nutritional status of the donor prior to procurement [15]. Boudjema et al. have also reported that the nutritional status of the donor affects the outcome of pig liver flushed preservation and transplantation [2]. On the other hand, addition of inosine [4] or hypoxanthine [16] to the perfusate restores adenine nucleotides in ischemically damaged kidneys during perfusion. In addition, retrograde oxygen persufflation during hypothermic preservation leads to continued ATP production [17] and improves the quality of ischemically damaged kidney [5, 18, 19].

In the present study, we examined (a) the effect of factors that seemed to have an influence on energy metabolism in the canine pancreas at the ATP tissue level and (b) graft viability during preservation by the two-layer method.

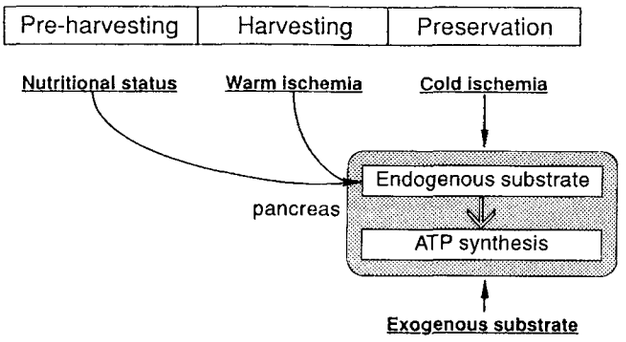

Fig. 1 Factors that seem to have an influence on the energy metabolism of the canine pancreas during preservation by the two-layer method

Materials and methods

Mongrel dogs of both sexes, weighing 12–20 kg, were used in this study. Perfluorodecaline, which is one of perfluorochemicals (PFC) and Euro-Collins solution (EC) were a kind gifts from Dr. K. Yokoyama (The Green Cross Corporation, Osaka, Japan). University of Wisconsin solution (UW) was generously provided by DuPont Critical Care (Waukegan, Ill.). 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 [7], excising the remainder of the pancreas at the time of autotransplantation. After surgery, the dogs received saline with 10% glucose (30 ml/kg weight) and parenteral penicillin (25 mg/kg weight) for 3 days. After 3 days, standard kennel diets were given.

Assessment of graft viability

Fasting blood glucose was measured daily. Graft viability was judged by graft survival following autotransplantation. Normoglycemia during at least 5 days after autotransplantation was assessed as graft survival [1].

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 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 precipitated 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 from fed or 72-h-fasted dogs were preserved by the two-layer [EC/perfluorochemical (PFC)] method for 24 h.

Experiment 2 Pancreatic grafts with or without 60 min of warm ischemia were preserved by the two-layer (EC/PFC) method for 24 h.

Experiment 3 Pancreatic grafts with or without 60 min of warm ischemia were preserved by the two-layer method using EC or EC with adenosine for 24 h.

Statistical analysis

All values are expressed as mean ± SD. Statistical analysis was performed using Student's *t*-test.

Results

Experiment 1. Effect of fasting on ATP tissue concentrations and viability of canine pancreases during 24-h preservation by the two-layer (EC/PFC) method (Table 1)

ATP tissue concentrations in fed and fasting groups were 7.48 ± 0.55 and 7.03 ± 0.74 μmol/g dry weight, respectively, and all the pancreatic grafts in both groups were viable (4/4, 100% and 3/3, 100%, respectively). It was clear that fasting did not influence ATP tissue levels and viability of the grafts.

Table 1 Effect of fasting on ATP tissue concentrations and viability of canine pancreas during 24-h preservation by the two-layer [Euro-Collins (EC)/PFC) perfluorochemical] method

Nutritional state before procurement	ATP tissue concentration (μmol/g dry weight)	Graft survival rate (%)
Fed	7.48 ± 0.55	4/4 (100)
Fasted	7.03 ± 0.74	4/4 (100)

Experiment 2. Effect of warm ischemia on ATP tissue levels and viability of canine pancreases during 24 h preservation by the two-layer (EC/PFC) method (Table 2)

Without 60 min of warm ischemia, all graft were viable (4/4, 100%) and ATP tissue levels were 4.44 ± 0.49 and 7.59 ± 0.97 μmol/g dry weight before and after preservation, respectively. However, ATP tissue levels were significantly decreased during 60 min of warm ischemia (4.44 ± 0.49 vs. 1.62 ± 0.26 μmol/g dry weight before and after warm ischemia, respectively, $P < 0.05$) and did not recover after preservation by the two-layer (EC/PFC) method (1.62 ± 0.26 vs. 1.56 ± 0.40 μmol/g dry weight before and after preservation, respectively, NS). No grafts were viable (0/3, 0%).

Experiment 3. Effect of exogenous adenosine on ATP tissue concentrations and viability of canine pancreases with or without 60 min of warm ischemia during 24-h preservation by the two-layer (EC/PFC) method (Table 3)

Without 60 min of warm ischemia, the addition of adenosine led to increased ATP tissue levels during preservation by the two-layer (EC/PFC) method (4.44 ± 0.47 vs. 7.59 ± 0.97 μmol/g dry weight without and with adenosine, respectively) but had no influence on graft viability (4/4, 100% and 4/4, 100% without and with adenosine, respectively). However, the addition of adenosine led to a significant increase in ATP tissue levels in the grafts subjected to 60 min of warm ischemia (7.23 ± 2.17 vs. 1.90 ± 0.53 μmol/g dry weight after and before preservation, respectively, $P < 0.01$) and made it possible to preserve them for 24 h (4/5, 80%).

Table 2 Effect of warm ischemia on ATP tissue concentration and viability of canine pancreas during 24-h preservation by the two-layer (EC/PFC) method

Warm ischemia (min)	ATP tissue concentration (μmol/g dry weight)		Graft survival rate (%)
	Before preservation	After preservation	
0	4.44 ± 0.49	7.59 ± 0.97	4/4 (100)
60	1.62 ± 0.26*	1.56 ± 0.40**	0/3 (0)

* $P < 0.05$; ** $P < 0.01$

Table 3 Effect of exogenous adenosine on ATP tissue concentration and viability of canine pancreas without or with 60 min of warm ischemia during 24-h preservation by the two-layer (EC/PFC) method

Warm ischemia (min)	Adenosine	ATP tissue concentration (μmol/g dry weight)		Graft survival rate (%)
		Before preservation	After preservation	
0	–	4.44 ± 0.47	7.59 ± 0.97	4/4 (100)
	+		10.32 ± 1.13	4/4 (100)
60	–	1.62 ± 0.26	1.56 ± 0.40	0/3 (0)
	+	1.90 ± 0.53*	7.23 ± 2.17*	4/4 (80)

* $P \geq 0.01$

Discussion

During simple cold storage, anaerobic glycolysis plays an important role in ATP synthesis within the anoxic organ [13]. In comparison, ATP is synthesized mainly by oxidative phosphorylation via oxidation of carbohydrate, amino acids, and fatty acids [13], and partly via "salvage pathway" of purines and their nucleosides [17–19] within the aerobic organ during hypothermic perfusion. The two-layer method supplies oxygen to the pancreas and allows continued ATP production [9], which is essential for the metabolic process in the maintenance of cellular integrity [11]. Based on a good correlation between high ATP tissue concentrations and good posttransplant outcome [10], we examined (a) the effect of factors that seemed to have an influence on energy metabolism in the pancreas at the ATP tissue level and (b) viability of the pancreatic graft during preservation by the two-layer method. As the nutritional status of the donor influences the outcome of rat or pig liver preservation and transplantation [2, 15], we first examined the effect of the nutritional status on ATP tissue concentrations and graft viability using pancreas harvested from either fed or 72-h-fasted dogs. In canine pancreas, the nutritional status of the pancreas before procurement had no influence on ATP levels and viability during 24-h preservation by the two-layer method. As ATP was rapidly degraded and salvageable nucleotide was degrated and lost during procurement (warm ischemia) and preservation (cold ischemia) [3, 6, 14], the pancreas subjected to 60 min of warm ischemia was preserved by the two-layer (EC + adenosine/PFC) method for 24 h. Without adenosine, the pancreas did not recover ATP tissue levels and viability. However, provision of adenosine to the preservation solution made the restoration of ATP tissue levels possible and improved the viability of the pancreatic graft subjected to 60 min of warm ischemia. Recently, we have reported that the two-layer (UW/PFC) method can improve the quality of the pancreatic graft subjected to significant warm ischemia [12]. As UW contains the same concentration (5 m*M*) of adenosine as the solution used in these experiments, it seems reasonable to think that adenosine in UW was used for the ATP synthesis necessary to repair the ischemically damaged cells. However, the mechanism responsible for the effectiveness of adenosine during preservation by the two-layer method in restoration of function of the ischemically damaged pancreas remains unclear and is under investigation.

Acknowledgement We thank Sachiko Nishiyama for skillful manuscript preparation.

References

1. Abouna GM, Heil JE, Sutherland DER, Najarian JS (1985) Factors necessary for successful 48-hour preservation of pancreas grafts. Transplantation 45:270
2. Boudjema K, Lindel SL, Southard JH, Belzer FO (1990) The effects of fasting on the quality of liver preservation by simple cold storage. Transplantation 50:943
3. Farkouh EF, Daniel AM, Beaudoin JG et al (1971) Predictive value of liver biochemistry in acute hepatic ischemia. Surg Gynecol Obstet 132:832
4. Fernando AR, Armstrong DMG, Griffirths JR et al (1977) Protective effect of inosine on the canine kidney during ischemia at 37 °C. Transplantation 23:504
5. Fischer JM, Czerniak A, Hauer U, Isselhard W (1978) A new simple method for optimal storage of ischemically damaged kidneys. Transplantation 25:43
6. Kamiike W, Watanabe F, Hashimoto T et al (1982) Changes in cellular level of ATP and its catabolites in ischemic rat liver. J Biochem 91:1349
7. Kuroda Y, Orbita K, Iwagaki S et al (1987) New technique of pancreatic exocrine diversion to esophagus in canine segmental pancreatic autotransplantation. Transplantation 44:583
8. Kuroda Y, Kawamura T, Suzuki Y, Fujiwara H, Yamamoto K, Saitoh Y (1988) A new simple method for cold storage of pancreas using perfluorochemical. Transplantation 46:457
9. Kuroda Y, Fujino Y, Kawamura T, Suzuki Y, Fujiwara H, Saitoh Y (1990) Mechanism of oxygenation of pancreas during preservation by two layer cold storage method. Transplantation 49:694
10. Kuroda Y, Fujino Y, Morita A, Ku Y, Saitoh Y (1991) Correlation between high adenosine triphosphate tissue concentration and good posttransplant outcome for the canine pancreas graft after preservation by the two-layer cold storage method. Transplantation 52:989
11. Kuroda Y, Fujino Y, Morita A, Tanioka Y, Ku Y, Saitoh Y (1992) The mechanism of action of a two-layer (Euro-Collins' solution/perfluorochemical) cold storage method in canine pancreas preservation. The effect of 2,4 dinitrophenol on graft viability and ATP tissue concentration. Transplantation 53:992
12. Kuroda Y, Morita A, Fujino Y, Tanioka Y, Ku Y, Saitoh Y (1993) Restoration of pancreas graft function by a two-layer (University of Wisconsin solution/perfluorochemical) cold storage method after significant warm ischemia. Transplantation 55:227

13. Lehninger AL (1970) Catabolism and the generation of phosphate-bond energy. In: Lehninger AL (ed) Biochemistry. Worth Publishers Inc, New York 265
14. Palombo JD, Hirschberg Y, Pomposelli JJ et al (1988) Decreased loss of liver ATP during hypothermic preservation in rat pretreated with glucose: implication for organ donor management. Gastroenterology 95:1043
15. Palombo JD, Pomposelli JJ, Hirschberg Y, Blackburn GL, Bistrian BR (1989) Glycolytic support of adenine nucleotides in rat liver flushed-preserved with UW or Collins' II. Transplantation 48:901
16. Pegg DE, Wustman MC, Foreman J (1981) Metabolism of normal and ischemically injured rabbit kidneys during perfusion for 48 hours at 10 °C. Transplantation 32:437
17. Pegg DE, Foreman J, Hunt CJ, Diaper MP (1989) The mechanism of action of retrograde oxygen persufflation in renal preservation. Transplantation 48:210
18. Rolles K, Foreman J, Pegg DE (1984) Preservation of ischemically injured canine kidneys by retrograde oxygen persufflation. Transplantation 38:102
19. Ross H, Escott M (1979) Gaseous oxygen perfusion on the renal vessels as an adjunct in kidney preservation. Transplantation 28:362