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

Chun-Hui Yuan, Gui-Chen Li, He Zhang, Ying Cheng, Ning Zhao, Yong-Feng Liu

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 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.

Abstract

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\cite{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 amylase in the pancreatic juice of the first and the seventh days were determined.

Tissue extraction method for adenosine nucleotides: After preservation, a part of pancreas was rapidly frozen in liquid nitrogen, lyophilized overnight, and kept at -80°C 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/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.

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\(^{\text{a}}\) | 1.76±0.17\(^{\text{b}}\) | 53.4±7.1\(^{\text{b}}\) | 8.6±1.3\(^{\text{b}}\) |
| 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                   |

\(^{\text{a}}F=36.9, P<0.05; {^{\text{b}}F}=32.9, P<0.05; {^{\text{b}}F}=7.38, P<0.05; {^{\text{a}}F}=38.2, P<0.05; {^{\text{b}}F}=15.9, P<0.01; {^{\text{b}}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 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-------|---|---|---|---|---|---|---|---|
| 2     | 5 | 27±7\(^{\text{a}}\) | 70±11\(^{\text{a}}\) | 22±17\(^{\text{a}}\) | 294±37\(^{\text{a}}\) | 136±26\(^{\text{a}}\) | 81±21\(^{\text{a}}\) | 48±14\(^{\text{a}}\) |
| 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 |

\(^{\text{a}}F=9.87, P<0.05; {^{\text{a}}F}=25.9, P<0.05; {^{\text{a}}F}=67.4, P<0.05; {^{\text{a}}F}=48.2, P<0.05; {^{\text{a}}F}=45.2, P<0.05; {^{\text{a}}F}=15.9, P<0.01; {^{\text{a}}F}=13.2, P<0.01; {^{\text{a}}SNK test: between group 2, 3a, 3b, P>0.05; between group 3c, 3d, P>0.05; {^{\text{a}}SNK test: between group 2, 3a, 3b, P>0.05.}

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 (Table 1). The plasma insulin levels in splenic and peripheral veins in groups 1, 2, 3a, and 3b were much more 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, 3a, 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, 3a 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 (Table 3). 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
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±85           | 359±27                    |
| 3a      | 6  | 183±48           | 355±37                    |
| 3b      | 6  | 180±42           | 327±37                    |
| 3c      | 6  | 83±24            | 29±11                     |
| 3d      | 4  | 77±30            | 28±10                     |

If =10.3, P <0.05; \( F =205.5, P <0.05; \) SNK test: between group 2, 3a, 3b, P <0.05; \( F =205.5, P <0.05; \) SNK test: between group 2, 3a, 3b, P >0.05; between group 3c, 3d, P >0.05. *compare with Group 2, P <0.05.

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

| Group   | n  | ATP    | ADP    | AMP    | TAN    |
|---------|----|--------|--------|--------|--------|
| 1       | 1(n=3)| 7.26±0.55| 3.33±0.20 | 1.49±0.34 | 11.43±1.37 |
| Group 2 | n=6 | 5.80±0.52 | 1.01±0.21 | 1.51±0.26 | 7.93±1.30 |
| Group 3a| 6   | 5.28±0.37 | 1.31±0.35 | 1.55±0.35 | 8.02±0.78 |
| Group 3b| 6   | 4.74±0.41 | 2.01±0.31 | 1.04±0.24 | 7.36±0.78 |
| Group 3c| 6   | 2.18±0.21 | 0.83±0.19 | 0.81±0.23 | 4.04±0.51 |
| Group 3d| 6   | 2.11±0.17 | 0.86±0.21 | 1.04±0.25 | 3.33±0.27 |

If =17.0, P <0.05; \( F =23.9, P <0.05; \) 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. Therefore, resuscitation of an ischemically damaged pancreas is essential to enlarge the donor pool using the pancreas graft from the cardiac arrest donor. 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 °C 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, wait safely for the excision of the pancreas and enlarge the donor pool. Cerra reported that the canine pancreatic allografts tolerated warm ischemia up to one hour. Florack et al. 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, ultimately leading to ischemic damage. But the relationship between the tissue concentration of ANs before transplantation and organ viability after transplantation is controversial. In human liver preservation, Lanit et al. 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.

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. 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. 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.

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.

REFERENCES

1. PI F, Badosa F, Sola A, Rosello Catafau J, Xaus C, Prats N, Gelí E, Hotter G. Effects of adenosine on ischaemia-reperfusion injury associated with rat pancreas transplantation. Br J Surg 2002; 89: 1366-1375
2. Uhlmann D, Aarmann B, Ludwig S, Escher E, Pietsch UC, Tannapfel A, Teupser D, Hauss J, Witzigmann H. Comparison of Celsior and UW solution in experimental pancreas preservation. J Surg Res 2002; 105: 173-180
3. Wakai A. Effect of adenosine on ischaemia-reperfusion injury associated with rat pancreas transplantation. Br J Surg 2002; 89: 494
4. Thayer SP, Fernandez-del Castillo C, Balcom JH, Warshaw AL. Complete dorsal pancreatectomy with preservation of the ventral pancreas: a new surgical technique. Surgery 2002; 131: 577-580
5. Fujita H, Kuroda Y, Saitoh Y. The mechanism of action of the two-layer cold storage method in canine pancreas preservation—protection of pancreatic microvascular endothelium. Kobe J Med Sci 1995; 41: 47-61
6. Matsumoto S, Kandaswamy R, Sutherland DE, Hassoun AA, Hiraoka K, Sageshima J, Shibata S, Tanioka Y, Kuroda Y. Clinical application of the two-layer (University of Wisconsin solution/perfluorochemical plus O2) method of pancreas preservation before transplantation. Transplantation 2000; 70: 771-774
7. Sun B, Jiang HC, Piao DX, Qiao HQ, Zhang L. Effects of cold preservation and warm reperfusion on rat fatty liver. World J Gastroenterol 2002; 6: 271-274
8. Kuroda Y, Tanioka Y, Matsumoto S, Hiraoka K, Morita A, Fujino Y, Suzuki Y, Ku Y, Saitoh Y. Difference in energy metabolism between fresh and warm ischemic canine pancreases during preservation by the two-layer method. Transpl Int 1994; 7(Suppl 1): S441-445
Randhawa P. Allograft biopsies in management of pancreas transplant recipients. J Postgrad Med 2002; 48: 56-63

Kim Y, Kuroda Y, Tanioka Y, Matsumoto S, Fujita H, Sakai T, Hamano M, Suzuki Y, Ku Y, Saitoh Y. Recovery of pancreatic tissue perfusion and ATP tissue level after reperfusion in canine pancreas grafts preserved by the two-layer method. Pancreas 1997; 14: 285-289

Matsumoto S, Kuroda Y, Fujita H, Tanioka Y, Sakai T, Hamano M, Kim Y, Suzuki Y, Ku Y, Saitoh Y. Extended margin of safety of preservation period for reisusciation of ischchemically damaged pancreas during preservation using the two-layer (University of Wisconsin solution/perfluorochemical) method at 20 degrees C with thromboxane A2 synthesis inhibitor OKY-046. Transplantation 1996; 62: 879-883

Obermaler R, Benz S, Kortmann B, Benthues A, Ansnge N, Hopt UT. Ischemia/ reperfusion-induced pancreatic in rats: a new model of complete normothermic in situ ischemia of a pancreatic tail-segment. Clin Exp Med 2001; 1: 51-59

Matsumoto S, Kuroda Y, Fujita H, Tanioka Y, Kim Y, Sakai T, Hamano M, Suzuki Y, Ku Y, Saitoh Y. Resuscitation of ischemically damaged pancreas by the two-layer (University of Wisconsin solution/perfluorochemical) mild hypothermic storage method. World J Surg 1996; 20: 1030-1034

Tanioka Y, Kuroda Y, Kim Y, Matsumoto S, Suzuki Y, Ku Y, Fujita H, Saitoh Y. The effect of ouabain (inhibitor of an ATP-dependent Na+/K+ pump) on the pancreas graft during preservation by the two-layer method. Transplantation 1996; 62: 1730-1734

Moorhouse JA, Granhamme GR, Rosen NJ. Relationship between intravenous glucose tolerance and the fasting blood glucose level in healthy and diabetic subjects. J Clin Endocrinol Metab 1964; 24: 145-159

Troisi R, Meester D, Regaert B, van den Broecke C, Cuvelier C, Hesse UJ. Tolerance of the porcine pancreas to warm and cold ischemia: comparison between University of Wisconsin and histidine-tryptophan-ketoglutarate solution. Transplantation Proc 2002; 34: 820-822

Fujino Y, Suzuki Y, Tsujimura T, Takahashi T, Tanioka Y, Tominaga M, Ku Y, Kuroda Y. Possible role of heat shock protein 60 in reducing ischemic-reperfusion injury in canine pancreas grafts after preservation by the two-layer method. Pancreas 2003; 23: 393-398

Ludwig S, Armborn B, Escher E, Gabel G, Teupser D, Tannapfel A, Piganelli JD. Preservation of human islet cell functional recovery by the two-layer method after reperfusion. Artif Organs 2001; 25: 420-424

Ott M, Okiohichi N, Satomi S, Taguchi Y, Mori S, Miura I. Assessment of liver graft function after cold preservation using 31P and 23Na magnetic resonance spectroscopy. Transplantation 1992; 53: 730-734

Ullmann D, Armborn B, Ludwigs S, Escher E, Pietsch UC, Tannapfel A, Teupser D, Hauss J, Witzigmann H. Comparison of Celsior and UW solution in experimental pancreas preservation. J Surg Res 2002; 105: 173-180

Lakey JR, Raftere RV, Warnock GL, Kncteman NM. Cold ischemic tolerance of human pancreas: assessment of islet recovery and in vitro function. Transplant Proc 1994; 26: 3416

Shi L, Liu J, Zhang QZ, Lu YQ, Shu YG, Chen GL, Xin ZH, Xu JY. Alterations of ATPase activity and erythrocyte oxygen consumption in patients with liver-blood deficiency syndrome. China J Nat J New Gastroenterol 1997; 3: 180-181

Humar A, Kandaswamy R, Drangstvet MB, Parr R, Gruessner AG, Sutherland DE. Prolonged preservation increases surgical complications after pancreas transplants. Surgery 2000; 127: 545-551

Bottino R, Balamarugam AN, Bertera S, Pietropaolo M, Trucco M, Piganelli JD. Preservation of human islet cell functional mass by anti-oxidative action of a novel SOD mimick compound. Diabetes 2002; 51: 2561-2567

Kim Y, Kuroda Y, Tanioka Y, Matsumoto S, Fujita H, Sakai T, Hamano M, Suzuki Y, Ku Y, Saitoh Y. Recovery of pancreatic tissue perfusion and ATP tissue level after reperfusion in canine pancreas grafts preserved by the two-layer method. Pancreas 1997; 14: 285-289

Pi F, Hotter G, Closa D, Prats N, Fernandez-Cruez L, Badosa F, Gelpi E, Rosello-Catalafau J. Differential effect of nitric oxide inhibition as a function of preservation period in pancreas transplantation. Dig Dis Sci 1997; 42: 962-971

Matsumoto S, Suzuki Y, Fujino Y, Tanioka Y, Deai T, Iwana Y, Mitsuutsuji M, Iwakasi T, Tominaga M, Ku Y, Kuroda Y. Ultrastructural analyses of pancreatic grafts preserved by the two-layer cold-storage method and by simple cold storage in University of Wisconsin solution. Transpl Int 2002; 15: 425-430

Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation 1988; 45: 673-676

Kim Y, Kuroda Y, Tanioka Y, Matsumoto S, Fujita H, Sakai T, Hamano M, Suzuki Y, Ku Y, Saitoh Y. Recovery of adenosine triphosphate tissue levels of grafts preserved by the two-layer method after reperfusion. Artif Organs 1996; 20: 1120-1124

Lanir A, Jenkins RL, Caldwell C, Lee RG, Khettry U. Clouse ME. Hepatic transplantation survival: correlation with adenine nucleotide level in donor liver. Hepatology 1988; 8: 471-475

Tsujimura T, Suzuki Y, Takahashi T, Yoshida I, Fujino Y, Tanioka Y, Li S, Ku Y, Kuroda Y. Successful 24-h preservation of canine small bowel using the cavitary two-layer (University of Wisconsin solution/perfluorochemical) cold storage method. Am J Transplant 2002; 2: 420-424