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Tanioka, Y

Kuroda, Y

Saitoh, Y

(Citation)
The Kobe journal of the medical sciences,40(5-6):175-189

(Issue Date)
1994

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0034037

Kobe J. Med. Sci. 40, 175/189, December 1994

AMELIORATION OF REWARMING ISCHEMIC INJURY OF THE PANCREAS GRAFT DURING VASCULAR ANASTOMOSIS BY INCREASING TISSUE ATP CONTENTS DURING PRESERVATION BY THE TWO-LAYER COLD STORAGE METHOD*

Yasuki TANIOKA, Yoshikazu KURODA and Yoichi SAITOH

First Division, Department of Surgery,
Kobe University School of Medicine

INDEXING WORDS

pancreas transplantation ; two-layer cold storage method ; rewarming ischemic injury ; adenine nucleotides

SYNOPSIS

Rewarming ischemic injury during vascular anastomosis severely compromises posttransplant pancreas graft survival because the graft has already been subjected to warm and cold ischemia before vascular

* This article is the dissertation submitted by Yasuki Tanioka, Kobe University School of Medicine for the requirement of Doctor of Medical Sciences.

Abbreviations : ANs, adenine nucleotides; TAN, total adenine nucleotides; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; PFC, perfluorochemical; UW, University of Wisconsin solution.

Received for publication : December 20, 1994

Authors' names in Japanese : 谷岡康喜, 黒田嘉和, 斎藤洋一

anastomosis. We examined whether preservation of the pancreas graft by the two-layer method ameliorates rewarming ischemic injury of the graft during vascular anastomosis and also investigated the energy metabolism of the pancreas graft before, during and after rewarming ischemic period. After flushing with cold University of Wisconsin solution (UW), the pancreas grafts were preserved by the two-layer (UW/perfluorochemical [PFC]) method (group 1) or simple cold storage in UW (group 2) for 24-hr and then autotransplanted. In control, the pancreas grafts were flushed out with cold UW and immediately autotransplanted without preservation (group 3). After completion of vascular anastomosis, vascular clamp was not released until 90, 120, or 150 min of rewarming ischemia, including anastomosis time, has elapsed. After 90 min of rewarming ischemia, graft survival rates were 5/5, 100%, 5/5, 100%, and 5/5, 100% in groups 1, 2 and 3, respectively. After 120 min, all the grafts in groups 2 and 3 failed (0/5, 0%, and 0/5, 0%, respectively), however, all the grafts in group 1 survived (5/5, 100%). Even after 150 min, 1 of 3 grafts in group 1 survived (1/3, 33%). After 24 hr preservation, tissue adenosine triphosphate (ATP) and total adenine nucleotides (TAN) levels of the grafts in group 1 were about 2-fold the reference values before harvesting and significantly higher compared with group 2($p<0.05$; $p<0.05$). After 120 min of rewarming ischemia, tissue ATP levels in group 1 were 84 % of the reference values and significantly higher compared with group 2($p<0.05$). TAN levels of group 1 were also significantly higher compared with group 2($p<0.05$). Two hours after reperfusion, ATP and TAN levels in group 1 were significantly higher than group 2($p<0.05$). There were no remarkable difference between group 1 and group 2 concerning adenosine diphosphate (ADP), adenosine monophosphate (AMP) levels. We conclude that the two-layer (UW/PFC) method ameliorates rewarming ischemic injury of the pancreas graft during vascular anastomosis by increasing tissue ATP concentration and TAN levels during preservation and maintaining tissue ATP and TAN levels during vascular anastomosis. Consequently,

ATP levels are rapidly recovered after reperfusion and the graft survives.

INTRODUCTION

Table I shows sequential process of ischemic injury during transplantation. Rewarming ischemia of a pancreas graft during vascular anastomosis severely compromises posttransplant viability because the grafts has already been subjected to warm and cold ischemia before vascular anastomosis. In liver transplantation, it was suggested that any strategies aimed at increasing the energy state of the grafts before vascular anastomosis or preventing adenosine triphosphate (ATP) degradation before implantation might be particularly useful in protecting the graft.[2,3] Recently, we have developed the two-layer cold storage method for supplying enough oxygen to the pancreas during preservation[7] and have clarified that the oxygenation of the graft during preservation by this method allows ATP production and ATP is an essential factor for maintaining pancreas viability during preservation.[8,13] In this study, we examined whether preservation of the pancreas graft by the two-layer method ameliorated rewarming ischemic injury of the graft during implantation and also investigated adenine nucleotides (ANs) levels before, during and after rewarming ischemic periods.

MATERIALS AND METHODS

Mongrel dogs of both sexes, weighing 12-18 kg were used for the experiments. Perfluorodecaline, which is one of perfluorochemicals (PFCs), was a kind gift of Dr. K.Yokoyama (The Green Cross Corporation, Osaka, Japan). University of Wisconsin solution (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 pancreas with splenic artery and vein attached was meticulously performed, followed by splenectomy. The segmental pancreas graft was flushed through the splenic artery with cold UW, preserved by the two-layer (UW/PFC) method (group 1) for 24 hours or simple cold storage in UW (group 2) for 24 hours or no preservation (group 3) and autotransplanted in the neck. [9] End to end anastomosis between the splenic artery and end to side anastomosis of the splenic vein and the right external jugular vein were completed with continuous 6−0 nylon sutures. The remainder of the pancreas was excised at the time of autotransplantation. After completion of anastomosis, vascular clamp was not released until the designated amount of time was elapsed. When indicated rewarming ischemic time was elapsed, the clamp was removed and the pancreas was perfused. After operation, the dogs received saline with 10% glucose (30 ml/kg weight) and parenteral penicillin (25 mg/kg weight) for three days. After three days, standard kennel diets were given.

Experimental protocol : There were seven experimental groups. Segmental autografts were preserved by the two-layer (UW/PFC) method (group 1) or simple cold storage in UW (group 2) for 24 hours. In group 3, segmental autografts were not preserved. Rewarming ischemic time was 90, 120 and 150 min.

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 or a K value of IVGTT more than 1.0 two weeks after transplantation was

considered a functional success of pancreas graft.[6]

Tissue extraction method for adenine nucleotides : Before harvesting and after preservation, rewarming ischemia, and 30 min and 2 hr period of reperfusion a part of pancreas was rapidly frozen with bronze tongs in liquid nitrogen, lyophilized overnight, and kept at −80 ℃ until analysis. The dry tissue powder was weighed (200mg) and homogenized in 3 mℓ ice cold 0.5 N perchloric acid. The precipitated protein was removed by centrifugation, and 500 μ ℓ of supernatant was neutralized by the additions of 50 μ ℓ 1.0 N KOH and 50 μ ℓ Tris. Following centrifugation, 10 μ ℓ of supernatant was injected into HPLC for analysis.

Measurement of adenine nucleotides : High-performance liquid chromatography (HPLC) on a reverse-phase colum of Shim-pack, CLC-ODS (6 x 150 mm) purchased from Shimazu manufacturing Co., Ltd, which was equilibrated with 100mM sodium phosphate buffer (pH 6.0) containing 1.0% methanol, was employed to separate and quantitate ANs according to the method of Wynants et al. .[18]

Measurement of core temperature of the graft : Core temperature of the graft was measured during the period of rewarming ischemia using a digital thermometer.

Calculation and statistical analysis : TAN was calculated as the sum of ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP). All values are expressed as mean ± SD. Statistical analysis was done using the Student's t test. A p value of < 0.05 was considered significant.

RESULTS

As shown in Table Ⅱ , after 90 min of rewarming ischemia graft

survivals were 5/5, 100%, and 5/5, 100%, and 5/5, 100% in groups 1, 2 and 3 respectively. K values two weeks after transplantation were 2.12 ± 0.70, 1.95± 0.09, and 1.98±0.48 in groups 1, 2 and 3 respectively. There were no differences in K value between three groups. After 120 min of rewarming ischemia, all the grafts in groups 2 and 3 failed (0/5, 0% and 0/5, 0% respectively), however all the grafts in group 1 were viable, (5/5, 100%) and K values were 1.83± 0.46. Even after 150 min of rewarming ischemia, one of three grafts in group 1 survived, 1/3, 33% but a K value was 1.05. Core temperatures of the grafts in three groups rapidly increased during implantation, reached to 30℃ first five minutes and 32.6± 0.9, 32.2± 1.2 and 32.4± 0.8 in groups 1, 2, and 3 respectively at the end of 120 min of rewarming ischemia. There were no differences in core temperature of the grafts during the period of rewarming ischemia between the groups (Figure 1). Table Ⅲ shows tissue ANs and TAN levels of each groups after preservation, rewarming ischemia and reperfusion. After 24 hr preservation, tissue ATP levels of the grafts in group 1 were about two fold of the reference values before harvesting (8.23±0.72 vs. 4.44±0.49 μ mol/g dry weight, $p<0.05$) and significantly higher compared with group 2 (8.23±0.72 vs. 1.76±0.52 μ mol/g dry weight, $p<0.05$). There were no significant differences in ADP and AMP levels of the grafts between two groups. TAN levels of the grafts in group 1 were 12.43± 2.98 and significantly higher compared with group 2 (12.43±2.98 vs. 6.18±2.04 μ mol/g dry weight, $p<0.05$). After 120 min of rewarming ischemia, tissue ATP levels in group 1 were 84% of the reference values and significantly higher compared with group 2 (3.75±0.25 vs. 1.57±0.48 μ mol/g dry weight, $p<0.05$). TAN levels in group 1 were also significantly higher compared with group 2 (6.63±0.89 vs. 4.14± 1.20 μ mol/g dry weight, $p<0.05$). After 30 min and 2 hr of reperfusion, tissue ATP levels in group 1 were 1.65±0.14 and 1.86±0.36 μ mol/g dry weight, respectively, and higher compared with group 2(0.81±0.21 and 1.03±0.18 μ mol/g dry weight, respectively;$p< 0.05$ and $p< 0.05$). There were no significant differences in ADP and AMP levels between two groups. After 2 hr of

reperfusion, TAN levels of the grafts in group 1 were 7.01 ± 0.67 and significantly higher compared with group 2 (7.01 ± 0.67 vs. 2.18 ± 0.26 μ mol/g dry weight, $p < 0.05$) .

DISCUSSION

As every pancreas graft is damaged to some extent during transplantation process, it is very important to minimize these damages for successful pancreas transplantation. We demonstrated that the two-layer method reduced cold ischemic injury of the pancreas graft [4,6,7] and restored the function of the graft subjected to warm ischemia during preservation. [10] This time, we have designed these experiments to simulate the reimplantation stage of transplantation and have examined whether preservation of the pancreas by the two-layer method increases its tolerance to rewarming ischemia during vascular anastomosis. This study clearly demonstrated that preservation of the pancreas graft by the two-layer (UW/PFC) method increased its tolerance to rewarming ischemia. A new term, "rewarming ischemia" , was used to describe the ischemia during anastomosis because the temperature increased but the graft was not actually exposed to warm ischemia in the classical sense during anastomosis. [2] In fact, the temperatures increased during anastomosis time and periods following. As there were no differences in temperature between 3 pancreases in the 3 groups during vascular anastomosis, it seemed unlikely that the results were simply due to the temperature difference among the 3 groups during the rewarming ischemic period. As Strasberg et al. demonstrated that this stage was particularly sensitive to the preexisting energy state of the liver graft[3], suggesting that any strategies aimed at increasing the energy state of the graft before vascular anastomosis might be particularly useful in protecting the graft, and two-layer method allowed ATP production within a viable pancreas graft, we examined tissue ANs before harvesting and after preservation, rewarming ischemia, and reperfusion.

After preservation by the two-layer (UW/PFC) method, tissue ATP levels were about 2-fold the reference values before harvesting and significantly higher compared with the values in UW alone. There were no difference of ADP and AMP levels between group 1 and group 2. TAN levels of group 1 were also significantly higher compared with the values in UW alone. The rate of ATP depletion during rewarming ischemia was dependent upon the amount of ATP available at the start of period and ATP levels in the grafts preserved by the two-layer (UW/PFC) method. The two-layer method, which increases the energy state of the pancreas graft before vascular anastomosis and consequently maintains ATP and TAN levels during vascular anastomosis, may be useful in protecting the graft against rewarming ischemia. On the other hand, simple cold storage in UW does not allow to maintain such ATP and TAN levels during preservation that protects rewarming ischemic injury. In liver and kidney transplantations, a number of workers have indicated that the ability of the graft to regenerate ATP after reperfusion correlates well with viability.[1,5,11,12,15,16,17] After 2-hr perfusion, recovery of tissue ATP and TAN levels in the grafts preserved by the two-layer method was not complete, although ATP and TAN levels were higher than the values in UW alone. As it has been suggested that metabolic changes and reperfusion time necessary to reach the basal metabolic state after transplantation are proportional to the duration of ischemia[3], it seems to be necessary to examine ANs after longer periods of restoration of blood flow. In conclusion, the two-layer (UW/PFC) method ameliorates rewarming ischemic injury of the canine pancreas graft during vascular anastomosis by increasing tissue ATP and TAN contents during preservation and maintaining tissue ATP and TAN level during vascular anastomosis. Consequently, tissue ATP levels are rapidly recovered after reperfusion and the graft survives.

ACKNOWLEDGEMENT

The author is indebted to Dr. Akio Morita and Shinichi Matsumoto, First Division, Department of Surgery, for their technical support in this work.

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Table I.

Sequential process of ischemic injuries during transplantation.

Pre-harvesting ►	Harvesting ►	Preservation ►	Implantation (vascular anastomosis) ►	Reperfusion
Warm ischemic injury	Warm ischemic injury	Cold ischemic injury	Rewarming ischemic injury	Reperfusion injury

Table Ⅱ.

The effect of preservation of canine pancreas by the two-layer (UW/PFC) method on rewarming ischemic injury during implantation.

Group	Preservation method	Rewarming ischemic time (min)	Functional grafts No. transplant	Success rate (%)
1	UW/PFC	90	5/5	100
		120	5/5	100
		150	1/3	33
2	UW	90	5/5	100
		120	0/5	0
3	No preservation	90	5/5	100
		120	0/5	0

Table Ⅲ.

Tissue ANs and TAN levels after preservation, rewarming ischemia, and reperfusion.

Preservation method	Time course	ATP	ADP	AMP	TAN
		μ mol/g dry weight			
UW/PFC	after preservation (24 hr)	8.23±0.72 [a]	3.01±1.80	1.52±0.81	12.43±2.98 [b]
	after rewarming ischemia (120 min)	3.75±0.25 [c]	1.08±0.47	2.30±1.02	6.63±0.89 [d]
	after (30 min) reperfusion (120 min)	1.65±0.14 [e]	0.40±0.11	1.55±1.13	3.60±1.15
		1.86±0.36 [f]	1.55±0.25	3.60±0.47	7.01±0.67 [g]
UW	after preservation (24 hr)	1.76±0.52	0.82±0.25	3.63±1.33	6.18±2.04
	after rewarming ischemia (120 min)	1.57±0.48	0.37±0.17	2.29±0.65	4.14±1.20
	after (30 min) reperfusion (120 min)	0.81±0.21	0.15±0.10	1.04±0.09	2.00±0.39
		1.03±0.18	0.60±0.07	0.56±0.05	2.18±0.26

UW/PFC vs. UW, $p<0.05$ (a, b, c, d, e, f, g)

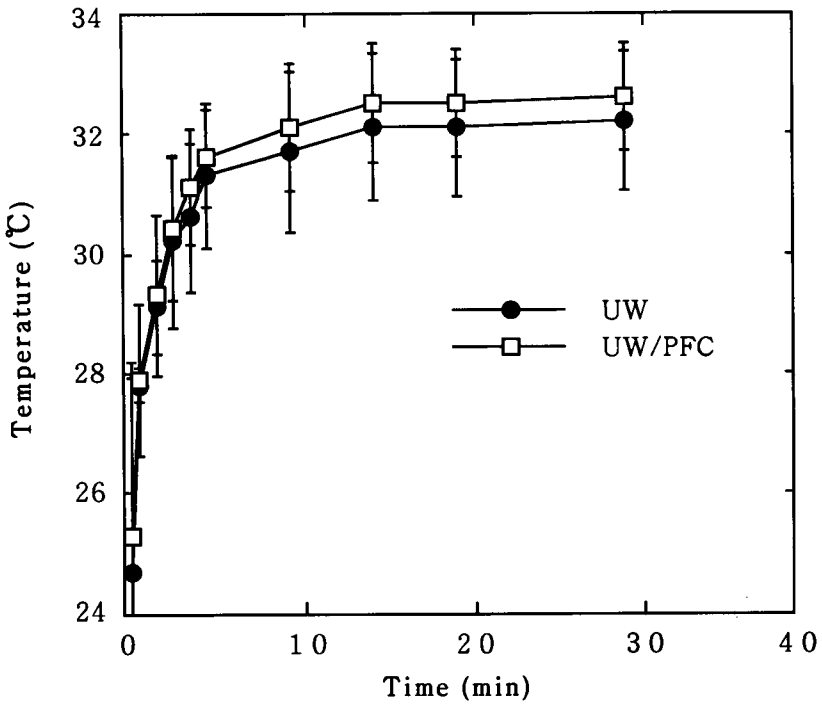

Figure 1.

Time course of core temperature of the grafts preserved by the two-layer(UW/PFC) method or simple cold storage in UW.