ORIGINAL ARTICLE

Surgical removal of the pancreas with one-step autotransplantation of isolated Langerhans islets into the hepatic portal system in the pig*

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KEY WORDS

autogenic graft, chronic pancreatitis, pentoxifylline, porcine pancreatic islets

ABSTRACT

INTRODUCTION Autotransplantation of isolated Langerhans islets is regarded as the only way to prevent iatrogenic diabetes in patients who had been scheduled for pancreatectomy due to painful chronic pancreatitis. A sufficient number of Langerhans islets capable of secretory activity need to be transplanted to maintain normoglycemia after the surgical procedure. In order to optimize all stages, including collection, storage, isolation and transplantation of pancreatic islets, a reproducible animal-based experimental model should be developed before a new method is introduced into clinical practice.

OBJECTIVES The aim of the present study was to develop a reproducible autogenic model-based method for collection, conservation and isolation of porcine pancreas so that transplantation of isolated pancreatic islets could be performed and postoperative normoglycemia achieved.

MATERIAL AND METHODS Pigs were subjected to total pancreatectomy with simultaneous splenectomy and without removal of the duodenum. The collected pancreas was stored in the University of Wisconsin solution with the addition of pentoxifylline (PTX) until the isolation procedure (<4 hours). Efficacy of isolation was evaluated based on the number, quality and viability of obtained islets. Following autotransplantation into the liver, secretory activity of the islets was assessed intravitally by serum glucose monitoring.

RESULTS The islet yield per gram of pancreas was 1452 (standard deviation [SD] ±125) for the PTX group and 384 (SD ±115) for the control non-PTX group ($p < 0.01$). Viability of islets for individual isolations did not reveal any statistically significant differences between groups and was estimated at 85–93%. Three out of five animals demonstrated normoglycemia with features of neoangiogenesis in the islets transplanted into the liver, which was confirmed by histological examination. One animal developed hyperglycemia up to 430 mg/dl, and histological image showed intensive apoptosis and degranulation in the transplanted islets.

CONCLUSIONS Efficacy of the isolation method was confirmed by achieving normoglycemia after autotransplantation of pancreatic islets into the liver, while histological examination showed hepatic vascularization to be the most appropriate location for an autogenic graft. PTX presence in the preserving solution for the pancreas storage produced the cytoprotective effect, which directly correlated with the islet yield.

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* The study is dedicated to the memory of Professor Tadeusz Orłowski.

INTRODUCTION

Pancreatectomy involves complete removal of the pancreas and may lead to postoperative full-symptom, iatrogenic insulin-dependent diabetes. While insulin therapy protects the patient from hyperglycemia, effective prevention of hypoglycemia is a serious, life-threatening, clinical problem. Therefore, one-step autotransplantation of pancreatic islets collected from the removed organ is recommended in patients who had undergone removal of the pancreas to protect them from serious complications caused by type 1 diabetes mellitus.

In clinical practice, autotransplantation of isolated pancreatic islets is performed in patients who have been qualified for surgical removal of the pancreas due to chronic pancreatitis accompanied by pharmacotherapy-resistant pain. After warm and cold ischemia and the subsequent storage under hypothermic conditions, the retrieved pancreas is subjected to isolation and cleaning, and the final product in the form of islet suspension is introduced into the hepatic portal vein of a recipient. This method protects patients against postoperative hypoglycemia and insulin-dependent diabetes.

Autotransplantation of pancreatic islets causes a number of clinical difficulties, including early decomposition and degranulation of cells destroyed during the isolation procedure and problems determining the number of pancreatic islets required to prevent postoperative hypoglycemia.

Our study aimed to develop a repeatable method for isolation of porcine pancreatic islets, determine the number of isolated islets required to achieve normoglycemia, and evaluate the quality of isolated islets using pentoxifylline (PTX) as an inhibitor of perioperative inflammation. In order to eliminate immunological factors, which could affect the post-transplantation survival and activity of pancreatic islets, experiments were conducted on a Polish-breed pig model.

MATERIAL AND METHODS

The experimental study was conducted on adult male, Polish-breed pigs with body weight of 38–42 kg. Animals for experimental purposes were bought at the Experimental Medicine and Animal Husbandry Research Unit, Medical University of Warsaw.

The experiments were conducted in animal quarters at the Central Clinical Hospital, Medical University of Warsaw and the Pancreatic Laboratory of General and Transplant Surgery Clinic at the Transplantology Institute, Medical University of Warsaw following approval of the Local Bioethics Committee of the Medical University of Warsaw.

Prior to the experiment, animals had been placed in cages under standard conditions, including constant humidity (approx. 75%) and air temperature of about 75°F. Animals were given *ad libitum* access to standardized food and water and were exposed to the so-called "artificial day-night" 12 h/24 h cycle. During the experiment, animals stayed in separate cages under standard conditions. In the control group, pancreases for isolation were obtained from the pig abattoir in Pruszków.

Protocol for the experiment

Anesthesia and analgesia Surgical procedures were carried out under general anesthesia using halothane and ketamine. Intramuscular analgesia was administered to pigs postoperatively.

Surgical procedure Medial laparotomy was performed. After the pancreas had been dissected, total pancreatectomy with splenectomy and without removal of the duodenum was carried out. The collected pancreas was perfused with University of Wisconsin (UW) solution at the temperature of 39°F. The pancreas was then stored until the isolation procedure in 250 ml of UW solution with PTX, at the concentration of 1 mg/g of the pancreatic tissue, and the temperature of 39°F. The control group comprised porcine pancreases, which were stored in UW solution without PTX and were collected at the abattoir and subjected to isolation without transplantation.

Pancreatic islets isolation Standard isolation of pancreatic islets was performed at the Pancreatic Laboratory of the Transplantology Institute. After the cannula had been inserted into the pancreatic duct, collagenase solution was injected (Collagenase P). Islet isolation was conducted in the Ricordi chamber filled with digestion solution. Next, the material was rinsed twice using Hanks Balanced Salt Solution. Prior to transplantation, isolated islets had been suspended in 250 ml of Ringer solution.

Transplantation of porcine pancreatic islets A catheter, 4F in diameter, was introduced into the porcine hepatic portal system; next, the suspension of pancreatic islets was administered gravitationally. As soon as the transplantation of pancreatic islets had finished, the catheter insertion site in the portal vein was ligated with the vascular suture and subsequent lamellar sewing up of the abdominal integument was carried out.

Evaluation of isolated pancreatic islets Evaluation of the isolation efficacy was based on the islet yield. Viability of pancreatic islets was assessed by a direct dithizone-staining.

Evaluation of transplanted pancreatic islets activity Daily measurements of serum glucose, which were performed for 10 days, was used to determine the insulin secretion index for transplanted islets. After 10 days, autopsy was performed and porcine livers were collected to conduct histological examination (specimens were stained using hematoxylin-eosin [HE]).

RESULTS

During the experiment, the duration of pancreas storage in UW solution with PTX was short (<4 hours). All 5 isolations produced similar

TABLE 1 Results of pancreatic islet isolation performed using PTX conserving solution containing Collagenase P and PTX

Examined groups	Mean time of warm ischemia (min)	Mean time of cold ischemia (min)	Mean weight of the isolated pancreas (g)	Mean number of obtained islets per gram of the pancreas
control without PTX n = 4	11 SD ±2.7	114 SD ±13.8 p = NS	35 SD ±11.79 p = NS	384 SD ±115 p <0.01
with PTX n = 5	8.75 SD ±1.25	91 SD ±14.7	36 SD ±11	1452 SD ±125

Abbreviations: NS – non significant, PTX – pentoxifylline, SD – standard deviation

TABLE 2 Serum glucose levels following autotransplantation of isolated pancreatic islets on consecutive days (d) after the surgical procedure.

Ordinal number of the experiment	Total number of transplanted islets	Total number of transplanted islets per gram of the pancreas	Serum glucose level before the surgical procedure (mg/dl)	Serum glucose level immediately after the surgical procedure (mg/dl)	1d	2d	3d	4d	5d	6d	7d	10d
1	78600	1310	50	60	30	60	100	170	75	75	70	70
2	33000	1650	50	30	40	150	145	150	85	130	85	85
3	76450	1390	65	90	50							
4	73000	1460	45	30	45	50	75	75	150	220	430	330
5	58000	1450	40	30	45	45	45	100	60	55	65	65

results; the mean number of the isolated islets was 1452 (standard deviation [SD] ±125). In the control group, in which pancreases were taken from the abattoir and the PTX conserving solution was not applied during storage, the mean number of the isolated islets was 384 (SD ±115) and, from the statistical point of view, it was significantly much lower compared with the examined group ($p < 0.01$). There were no significant differences between the groups in terms of islet viability, which was estimated at 85–93%. Overall results are shown in TABLE 1.

Five porcine recipients underwent transplantation of their own pancreatic islets, which had been subjected to isolation using Collagenase P solution and PTX. From 33000 to 78600 pancreatic islets per each recipient were transplanted.

One pig had to be sacrificed on the 1st day due to perioperative complications. In all other cases, total normoglycemia was achieved on the 1st day after the surgical procedure. In one case, worsening of the glycemia control was observed on the 5th day (the maximum serum glucose level was 430 mg/dl). TABLE 2 presents the glucose level on consecutive postoperative days in individual recipients and shows relationships between clinical outcomes and the number of pancreatic islets transplanted into the hepatic portal vein.

Histological specimens stained with HE, collected from porcine livers with the normal glucose level, revealed a microscopic image of normal pancreatic islets in the hepatic tissue, in which progressive neovascularization and visible single epithelial cells were observed. Islets did not show any features of degranulation or cell apoptosis (FIGURE 1).

In hyperglycemic pig no. 3, histology image of the liver revealed apoptosis of pancreatic islet cells accompanied by degranulation, necrosis and leukocyte infiltration (FIGURE 2).

Microscopic examination showed no features of neovascularization and no endothelial cells in the pancreatic islets.

DISCUSSION In recent decades, transplantation of isolated pancreatic islets has advanced from the stage of a single clinical experiment to the status of clinical standard. At the same time, reference centers have been able to collect long-term outcomes even in a few hundred patients.[1-3]

Experiments of each center which participated in the clinical trial for islet transplantation, were based on the knowledge from previous studies conducted using experimental animal models.

The use of an animal-based model eliminates donor-dependent factors and ensures similar experimental conditions for all examined groups. The issue of extensiveness with respect to total pancreatic resection and the risk of postoperative complications should be also taken into consideration. In our experimental material, one case ended up with a fatal complication, which occurred in an animal during very early postoperative period, but the incident was not directly related to islet cell transplant.

Pancreatic islet autotransplantation permits elimination of other factors that may influence results of the experiment such as an immunological response of a recipient to the presence of allogenic tissue as well as the type and toxicity of immunosuppressants.

The experience of our center involves a number of studies based on a rat model. Introduction of the method for transplantation of isolated pancreatic islets in humans had been preceded by experiments involving isolation and

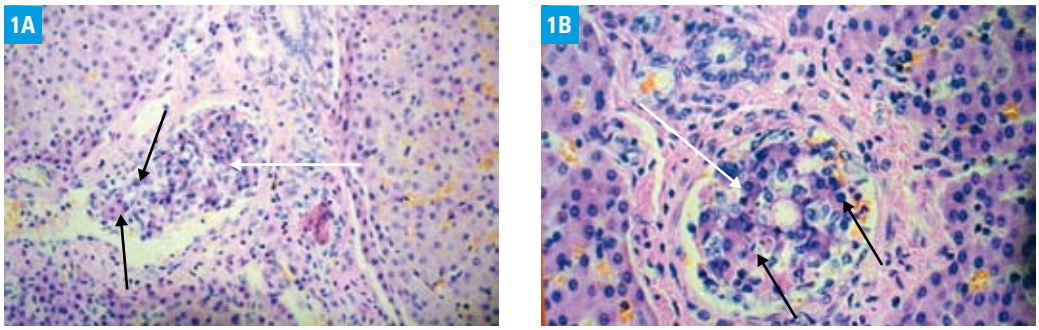

FIGURE 1 Hematoxylin-eosin staining, **1A** – magnification of 40 ×, **1B** – magnification of 80 ×; the black arrow – endothelial cells, the white arrow – pancreatic islet in the hepatic tissue

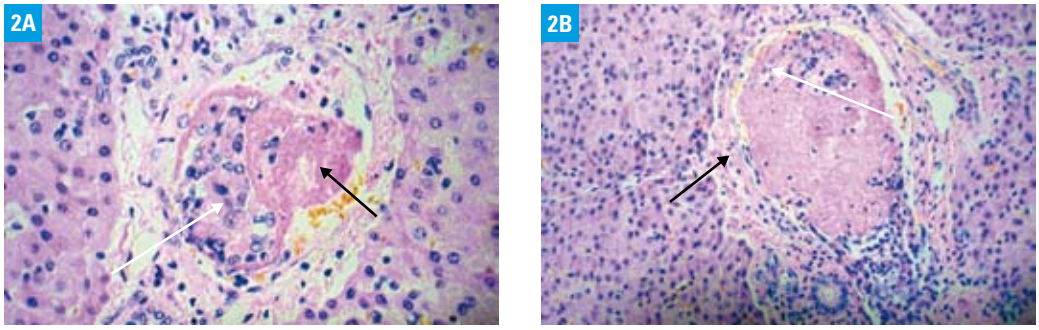

FIGURE 2 Hematoxylin-eosin staining, **2A** – magnification of 80 ×, **2B** – magnification of 40 ×; the black arrow – degranulation and necrosis of pancreatic islet, the white arrow – infiltration of lymphocytes into the pancreatic islet

transplantation of pancreatic islets in the pig. At the same time, many studies have been conducted in order to determine standards for the islet isolation from the human pancreases along with evaluation of the quantity, quality and viability of isolated islets.[4]

The key issue of the isolation process is to isolate enough islets to obtain normal post-transplantation insulin secretion. Fabia et al. pointed out that the application of PTX for hepatic conservation had a positive effect on ischemic damage of the organ caused by reperfusion.[5] In our study, addition of PTX into perfusion solution produced a significantly higher islet yield in the experimental group. In the control group, pancreases were taken from the abattoir, which could affect the condition of a pancreas donor despite the fact that there were no significant differences in storage duration. This finding is an indirect evidence for the importance of a donor's condition for islet isolation outcome.

The loss of islets assessed on the basis of experimental findings, which occurs in the immediate post-transplantation period is approximately 40–60%. Reduction in the number of islets takes place during the first 10–14 days following transplantation, when the process of neovascularization occurs. Our choice of a 10-day post-transplantation follow-up of the animals allowed us to show islet neovascularization, which began on the 3rd day after the procedure and to evaluate the actual activity of the islets.[6,7]

It is assumed that approximately 10,000 islet equivalent/kg body weight of pancreatic islets is necessary to achieve insulin independency in a recipient after islet allotransplantation.[8,9] In our experiment, efficacy following autotransplantation of pancreatic islets was estimated at 75% (FIGURE 3). As a result, a normal glycemic control in the immediate postoperative period was achieved. The number of transplanted islets corresponded to the minimal number of islets sufficient to invert iatrogenic diabetes.

The experimental model developed at our center was the basis for an introduction of the clinical program. Our own method for autotransplantation of porcine islets proved fully effective and allowed us to be the first in Poland to have performed human islet transplantation (Fiedor P. unpublished data).

The quantity and quality of Langerhans islets obtained through isolation of porcine pancreas, collected during surgical procedure, enables effective autotransplantation into the hepatic portal system, which prevents iatrogenic diabetes in a recipient.

PTX has a cytoprotective effect on the isolated islets, which results in benefits in terms of the quantity and quality of the islet cells obtained for transplantation purposes.

Normoglycemia maintained for 10 consecutive days after autotransplantation and the features of neoangiogenesis in pancreatic islets

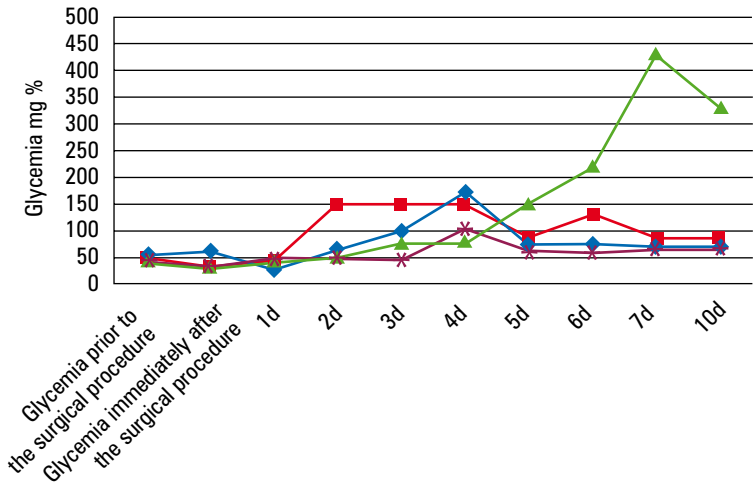

FIGURE 3 Glycemia prior to and after autotransplantation of pancreatic islets

transplanted into the porcine liver (confirmed by histological examination) indicates that autotransplantion is an effective approach to prevent postoperative diabetes in patients with total resection of the pancreas.

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ARTYKUŁ ORYGINALNY

Chirurgiczne usunięcie trzustki z jednoczasową autotransplantacją izolowanych wysp Langerhansa do układu wrotnego wątroby świni*

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SŁOWA KLUCZOWE

pentoksyfilina, przeszczep autogeniczny, przewlekłe zapalenie trzustki, wieprzowe wyspy trzustkowe

STRESZCZENIE

WPROWADZENIE Przeszczepienie autogenicznych izolowanych wysp trzustkowych stanowi jedyny sposób zapobiegania jatrogennej cukrzycy u pacjentów zakwalifikowanych do pankreatektomii z powodu bólowej postaci przewlekłego zapalenia trzustki. Warunkiem utrzymania normoglikemi po zabiegu jest przeszczepienie odpowiedniej liczby wysp Langerhansa zdolnych do podjęcia czynności wydzielniczej. Optymalizacja wszystkich etapów procesu pobrania, przechowywania, izolacji i przeszczepienia wysp trzustkowych wymaga opracowania powtarzalnego modelu na zwierzętach doświadczalnych przed wdrożeniem nowej metody do praktyki klinicznej.

CELE Opracowanie powtarzalnej metody pobrania, konserwacji i izolowania trzustki wieprzowej w celu przeszczepienia izolowanych wysp trzustkowych i uzyskania normoglikemii pooperacyjnej na modelu autogenicznym.

MATERIAŁ I METODY Świnie poddawano zabiegowi całkowitej pankreatektomii z jednoczesną splenektomią i pozostawieniem dwunastnicy. Pobraną trzustkę przechowywano w płynie UW (University of Wisconsin) z dodatkiem pentoksyfiliny (*pentoxifyline* – PTX) do czasu rozpoczęcia izolacji (<4 godzin). Skuteczność izolacji oceniano poprzez liczbę uzyskanych wysp, ich jakość oraz żywotność. Czynność wydzielniczą wysp po autogenicznym przeszczepieniu do wątroby oceniano przyżyciowo, monitorując poziom glukozy w surowicy krwi.

WYNIKI W procesie izolacji wysp trzustkowych uzyskano 1452 ±125 (odchylenie standardowe – *standard deviation* [SD]) wyspy/g narządu w grupie z PTX oraz 384 ±115 (SD) wyspy/g trzustki w grupie kontrolnej bez PTX ($p < 0,01$). Żywotność wysp w poszczególnych izolacjach nie różniła się istotnie pomiędzy grupami i wynosiła 85–93%. Normoglikemię udało się utrzymać u 3 z 5 zwierząt, u których badaniem histologicznym potwierdzono neoangiogenezę w wyspach osadzonych w wątrobie. U jednego zwierzęcia wystąpiła hiperglikemia do 430 mg/dl, a w obrazie histologicznym stwierdzono nasiloną apoptozę i degranulację przeszczepionych wysp.

WNIOSKI Uzyskanie normoglikemii po transplantacji własnych wysp trzustkowych do wątroby potwierdziło skuteczność metody izolacji, a badanie histologiczne wykazało ich unaczynienie w wątrobie jako najlepszym miejscu dla autoprzeszczepu. Dodanie PTX do płynu konserwującego podczas przechowywania trzustek miało efekt cytoprotekcyjny, co bezpośrednio przekłada się na liczbę wysp otrzymanych podczas izolacji.

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