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Original article

The influence of porcine pancreas digestion parameters and islet histomorphology on islet isolation outcome

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Abstract

Transplantation of the pig islets of Langerhans is considered as the future treatment for patients suffering from type I *diabetes mellitus*. Despite the adaptation of modified Ricordi method and highly purified collagenase, the results of pancreas digestions are precarious. Selection of proper donor and optimal digestion procedure are fundamental. The aim of this study was to assess the impact of pancreas procuring parameters on pig islets yield. The pancreata were harvested from 69 market sows weighting over 150 kg. After intraductal injection of cold collagenase solution pancreata were transported in UW solution or under conditions of two layer method (TLM). In laboratory pancreata were digested at 37°C according to Ricordi isolation method or stationary in the bottle. The particular parameters of isolation procedure were considered as substantial. Pig weight, volume of infused collagenase solution, TLM application and pancreas dividing before digestion positively affected islet yield. Additionally, the influence of pancreatic islet tissue histomorphology on isolation outcome was studied. Proper donor selection as well as adequate digestion parameters could improve pig islet recovery during islet isolation.

Key words: pig islets, isolation parameters, islet histomorphology

Introduction

Xenotransplantation of microencapsulated pig islets of Langerhans is considered to be a future treatment method in patients suffering from type I diabetes mellitus (Tibell et al. 1994, Calafiore 2003). Exogenous insulin replacement therapy provides significant benefit but is not sufficient to provide the control to extent required to maintain the constant state of euglycemia. Xenotransplantation of isolated pig islets may be a solution for the shortage of human cadaveric pancreata donors thanks to the structural

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similarity between human and porcine insulin and unlimited source of pig pancreatic islets. It should be mentioned that the islets isolation outcome are unforeseen due to elusive character of pig islets resulting from the lack of connective tissue surrounding the islets. Therefore the selection of the proper donor and optimal pancreas digestion procedure are fundamental. There are a lot of reports favouring strain, age and weight of the pigs as the most affecting donor parameters (Socci et al. 1990, Toso et al. 2000, Kim et al. 2007). Our experience showed that the best pig donors are the market-weight sows weighting over 150 kg (Sabat et al. 2003). Additionally the variability of the parameters of particular steps of the digestive processes, in slaughterhouse and in the laboratory are precarious. Warm and cold ischemic times (WIT and CIT), type of collagenase and selection of isolation method (static or automatic) had influence on the isolation outcome (Socci et al. 1990, O'Neil et al. 2001, Krickhahn et al. 2002). In this paper we attempt to assess values of respective parameters and their impact on islet isolation results. Moreover, the analysis of differences in pancreatic islet histomorphology, resulting from pig weight (100 kg vs. 200 kg) was carried out.

Materials and Methods

Pancreas digestion

69 pancreata, as the waste material from market weight pigs (sows weighting over 150 kg) were processed. The pancreata were dissected after pigs exsanguinations, hot water bathing (64°C) and shaving (warm ischemia time – WIT: 14.1 ± 0.9 minutes). Subsequently, pancreata were flushed intraductally with cold collagenase solution (Roche P, 2465.6 ± 819.9 U/mL Hanks' Balanced Salt Solution) supplemented with 10% porcine serum. Instantly after distention (2.7 ± 1.6 ml per gram of pancreas), pancreata were dissected and transported to the laboratory using University of Wisconsin (UW) preservation solution (ViaSpan, DuPont, USA) or using two layer method (TLM) – oxygenated perfluorocarbon and UW solution as a long preservation (3 hours). In the laboratory, islets were isolated using semi-automated Ricordi method (Socci et al. 1990) or simply in a digestion bottle (Sabat et al. 2003) with or without minimal shaking at 37°C. The progress of digestion was monitored under the microscope by analysing collected suspension samples stained with dithizone every 2 minutes. The digestion was ceased when more than 50% of free islets were detected in the visual area (time of digestion – 39.5 ± 19.0 min). The isolations were classified as successful when number of islets was over 1000 per gram (49% of performed isolation).

Histomorphological examination

Small biopsies of the pancreatic tissue were taken from pigs weighted 100 kg (n=8) or 200 kg (n=10) and were fixed in 4% formaldehyde. For histomorphological analysis of islets, biopsies were dehydrated using alcohol gradients, embedded in paraffin, sectioned, deparaffinized and stained using primary antibodies against insulin (DAKO, Denmark), and secondary antibodies conjugated with horseradish peroxidase (DAKO, Denmark). After immunohistochemical staining, samples were analyzed using Olympus microscope (IX71, Japan) equipped with CCD camera (DP70, Olympus, Japan) and software for image capture. The islets were measured, calculated and analyzed using Cell P software (Olympus, Japan).

Statistical analysis

Comparison of the isolation data was performed using Mann-Whitney and Chi-square tests. Histomorphological data were compared using t-test.

Results

Statistical analysis proved significant association between the isolation success (number of islets over 1000 per gram of pancereas) and: pig weight (successful 262.8 ± 6.9 kg vs. failed 233.4 ± 7.4 kg, p=0.005) or volume of intraductally infused collagenase solution (successful isolation 3.2 ± 0.3 ml/g vs. failed 2.3 ± 0.2 ml/g, p=0.026) or pancreas digestion time (successful 33.6 ± 1.6 min. vs. failed 45.2 ± 4.0 min., p=0.01). The pancreas dividing before digestion (successful 63% vs. whole pancreas – successful 35%, p=0.04), TLM application (successful 69% vs. UW preservation – successful 45%) and pig shaving without hot water bathing (successful 60% vs. with bathing – successful 47%) exhibited the influence on the isolation outcome.

Comparison of pancreas digestion methods indicated that digestion without shaking (successful 60% vs. with shaking – successful 46%) and adaptation of the bottle method (successful 51% vs. Ricordi method – successful 43%) had beneficial influence on islet yield. Particular parameters applied in the experiments, such as warm ischemia time (14.1 ± 0.9 min., p>0.05), type of catheter, pefablock application or collagenase concentration (2465.6 ± 819.9 U/ml; p>0.05) had no significant effect on number of isolated islets. Failed isolations were connected with slaughterhouse procedures, pancreas procurement and technical errors.

Results of histological analysis are presented and summarized in Table 1. The number of islets/cm^2

Table 1. Results of histological analysis of pancreatic biopsies from pigs weighting 100 kg and 200 kg (p>0.05).

Pig groups	100 kg (n=8)	200 kg (n=10)
Number of islets/cm^2 pancreatic tissue (mean ± SD)	394.78 ± 149.24	322.70 ± 103.52
Number of islets >100 µm (%)	11.3	16.6
Islet surface area [µm^2] (mean ± SD)	2674.14 ± 820.52	3719.91+1448.50
Islet size [µm] (mean ± SD)	56.91 ± 6.86	65.75 ± 12.37

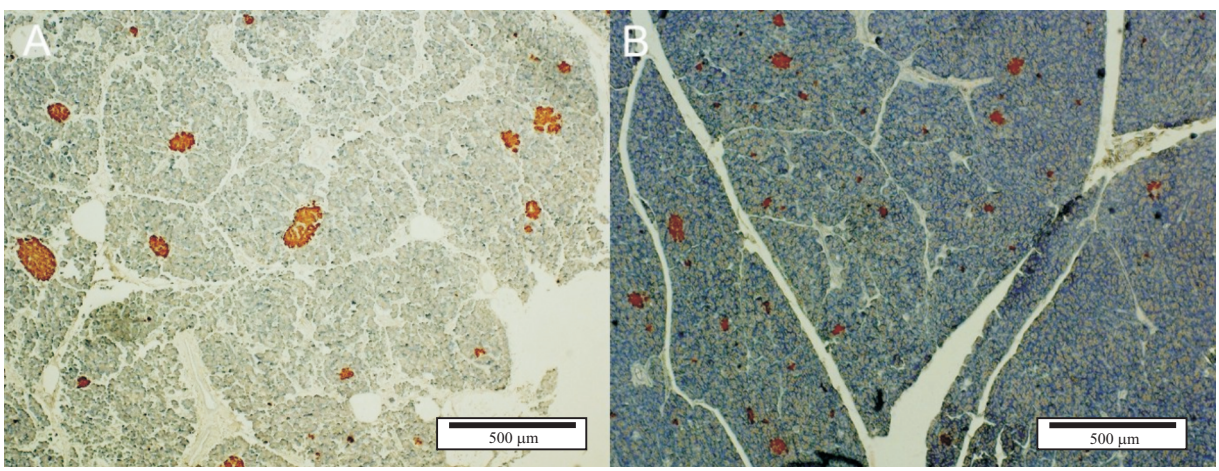

Fig. 1. Photomicrograph of pig pancreatic tissue stained section. A – Large islets from pig weighting 200 kg and B – small islets from pig weighting 100 kg (bar = 500 µm).

is higher in group of pigs weighting 100 kg than in group weighting 200 kg although pigs weighting 200 kg showed the highest percentage yield of large islets (16.6%). The differences in islets surface area were observed. The group of pigs weighting 200 kg revealed relatively larger surface of islets than weighted 100 kg (3719.9 µm^2 ± 1448.5 vs. 2674.1 µm^2 ± 820.5, p>0.05). The total surface area and size of islets increased with increasing pig weight (Fig. 1).

Discussion

Insulin-dependent diabetes mellitus is a metabolic disease caused by destruction of pancreatic beta cells by own immune system. In spite of human islets transplantation (Merani and Shapiro 2006, Khan and Harlan 2009), by now, the only one successful treatment method is pancreas transplantation – complicated surgical procedure. However minimum islet transplant administered to one patient is 10000 islet equivalents/kg of body weight, which means that we require three or four pancreata from human cadaveric donors (Shapiro et al. 2000). The shortage of human pancreas donors demands looking for alternative organ sources. The similarity of insulin molecule and carbohydrate stimulation of beta cells in human or pigs, give a promise of the important and unlimited porcine islets source (Toso et at. 2000). Application of immunoisolation allows to avoid immunosuppressive drugs used for protection of the transplant from the recipient immune system (Lim and Sun 1980, De Vos et al. 1997). Unlimited availability of pigs and possibility of repeated transplantation procedures gives the hope for introduction of this method to numerous diabetic patients. The current problem is the method of isolation of the islets from the pig pancreas as well as its yield. The difficulty with a pig islet recovery is the result of islet morphology, their variability and the lack of the connective tissue capsule making them extremely fragile (Socci et al. 1990). In the present study it was found that the islet number decreased as the weight of the pig increased although the islet surface area increases according to the weight of the pig. It corresponds with Ulrich et al. (1994) who reported results that the individual islet may grow but it does not divide. Furthermore, islet size depends on the pig weight (Dufrane et al. 2005). As it is shown above, the time of pig islet digestion with collagenase should be shortened to avoid destruction of the islets which get easily broken. Many other factors, including strain of the donor (Sabat et al. 2003, Kim et al. 2007), affects the success in islet isolation. This study demonstrated difference in processing of particular steps.

Conclusions

As it was shown, an adequate donor selection, pancreas preservation and digestion method had impact on isolation outcome. Therefore predictable yield of porcine free islets is still difficult to obtain. Performed analysis proved statistically significant positive effect of pig weight, increased volume of intraductally infused collagenase solution, shortened pancreas digestion time on isolation success. In conclusion, use of extremely selected pancreas donors and following exact the isolation procedure should improve pig islet recovery during isolation process.

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