Influence of Porcine Strain, Age, and pH of the Isolation Medium on Porcine Pancreatic Islet Isolation Success

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INCREASING interest in clinical pancreatic islet transplantation for curing type I diabetes and the worldwide human donor shortage motivate a search for alternative donor strategies. Because of its unlimited availability, physiological similarity, and comparable organ size, the pig may serve as a suitable donor species for xenotransplantation of pancreatic islets. The first serious barrier on the way to clinical xenotransplantation is the isolation of viable and functioning islets from the porcine pancreas. When the program for isolating porcine islets was started, it was decided to establish the semiautomated digestion technique developed by Ricordi et al.[1] To date, our laboratory has performed 103 islet isolations from porcine pancreata: 58 isolations were done to develop our standard technique and to train the team of technicians so that they are capable of high-quality isolation, 45 further isolations were carried out to analyze three parameters in greater detail, (ie, the pig race, the age of the pancreas donor, and the pH of the isolation medium), and to evaluate their influence on islet yield and viability. This report provides evidence that these parameters may exert a greater influence on islet yield and viability than previously supposed.

MATERIALS AND METHODS

Animals and Organ Procurement

Pancreata were harvested from brain-dead female pigs, either from local slaughterhouses, (ie, namely the hybrid pigs, which are crossbreeds of three to five races, that are commonly used for meat production) or from commercial breeders (eg, the purebred German Landrace, Pietrain, and Munich minipigs "Troll"). Donor pigs were either young individuals (≤ 14 months) or adults (2 to 3 years old, so-called "retired breeders"). Since the hygiene laws in the European Community forbid harvesting organs from pigs which are used for meat production thereafter, a warm ischemia time of 25 minutes was unavoidable. Only the splenic lobe of the pancreas was prepared. The pancreatic duct was cannulated with a polyethylene tube (∅ 0.96 mm) and the gland was transported in cold Eurocollins solution. Cold ischemia time ranged from 30 to 240 minutes.

Islet Isolation

Isolation of porcine islets was performed with the basic technique described by Ricordi et al[1] and subsequently with our so-called standard technique, which included some modifications. Fat, blood vessels, and connective tissue were dissected from the pancreas before collagenase solution was injected (HBSS with 2.5 PZ-U collagenase/mL). Collagenase was obtained from Serva, Heidelberg, Germany (cat. no. 17448, 1.9 PZ-U/mg). After the organ had been loaded into the digestion chamber, collagenase solution, heated to 37°C by a heating circuit, was pumped through the system. The chamber was gently shaken by hand for 10 seconds every minute. To monitor the digestion process, a tissue sample was taken every other minute, stained with dithizone and microscopically screened. When the first well-digested islets were observed, recirculation was interrupted and the islets were eluted. In most cases, this took place 10 to 20 minutes after digestion had started. HBSS, supplemented with 5% FCS and cooled to 4°C, served as elution solution. During the elution phase, the chamber was shaken by hand and monitoring was continued. Approximately 30 minutes after the elution phase had started the last islets could be detected and the elution was terminated. To separate the tissue from the collagenase solution, the digest was centrifuged (270*g*, for 4 minutes at 4°C, 2x). After sedimentation, islet samples were taken, stained with dithizone, counted, and the number of islets per gram of organ was calculated. Islet viability was determined by costaining with fluorescein diacetate and propidium iodide (FDA/PI).

RESULTS

Preliminary Results Obtained With the Basic Technique

Of 103 isolations, 58 were performed to establish our standard technique. Of the 58, 16 were necessary to improve the technical equipment (digestion chamber, heating circuit, pump, cooling device) and islet monitoring (digestion endpoint). Isolation results (islets/g organ) were not evaluated during this experimental phase. The next 42 isolations with the basic technique were carried out to vary parameters like warm and cold ischemia time, collagenase batches, and concentrations and media. The results obtained were 475 ± 624 (range: 0 to 2813) islets/g organ with varying islet viability. On the basis of these experiences, which were highly unsatisfactory as to islet yield, viability, and reliability, we established our standard technique.

Results Obtained With Our Standard Technique: Influence of the Donor Pig Strain

To analyze the influence of the genetic background of the pigs on islet yield and viability, 39 isolations with hybrid pigs (crossbreed) were compared with results obtained with three purebred races, German Landrace (n = 5), Pietrain (n = 5) and Minipig "Troll" (n = 5). The results of

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This work was supported by the Deutsche Forschungsgemeinschaft, DFG grant no. UL67/1-1.

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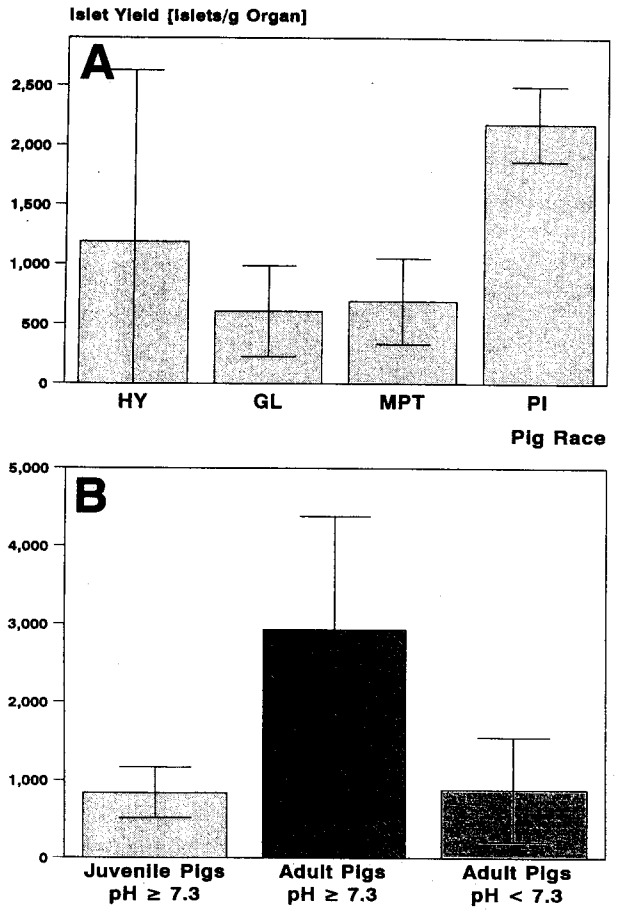

Fig 1. **(A)** The influence of the donor pig race on the islet yield. Results of 54 isolations performed with various pig races: HY-Hybrid pigs (n = 39), GL-German Landrace (n = 5), MPT-Minipig "Troll" (n = 5) and PI-Pietrain (n = 5). **(B).** The influence of donor age and pH of the collagenase solution. Results of isolations performed with juvenile (n = 7) and adult hybrid pigs (n = 9), final pH of the collagenase solution of < 7.3, and of isolations performed with adult hybrid pigs (n = 8) at a pH of < 7.3.

this study are documented in Fig 1A and may be summarized as follows:

1. The 39 isolations using adult hybrid pigs as organ donors (left column) resulted in a mean islet yield of 1190 islets per gram of organ with the large standard deviation of 1440. Islet viability remained variable as tested with FDA/PI staining.
2. Of the three purebred races (columns 2 to 4), Pietrain gave the best results with 2180 ± 317. German Landrace (606 ± 379) and minipigs Troll (691 ± 357) resulted in significantly lower islet yields. Thus, so far the best and most reproducible islet yield was obtained with purebred Pietrain pigs. Improved yields also resulted in improved islet viability (data not shown).

Influence of Donor Age

To investigate the influence of donor age on islet yield and viability, isolations were performed in juvenile (n=7) and adult hybrid pigs (n=9). Unfortunately, Pietrain pigs were not available, when this study was started. Figure 1B (left and middle columns) shows the first results of this investigation:

1. The islet yield (islets/g organ) is significantly lower in juvenile pigs (837 ± 326) than in adult individuals (2930 ± 1450).
2. Microscopic screening of isolated islets showed that the preparations from juvenile porcine pancreata contained larger amounts of fragmented islets than in adult individuals, where islets retained their "potato-like morphology" to a greater extent.

Influence of the pH of the Collagenase Solution

To test the influence of the pH of the collagenase solution on islet yield, reproducibility, and viability, isolations were performed using adult hybrid pigs and collagenase solutions with pH < 7.3 (n = 8) or ≥ 7.3 (n = 9). Figure 1B (middle and right columns) shows the result of this study: it is evident that pH < 7.3 gives significantly lower islet yields (879 ± 668) than isolations at pH ≥ 7.3 (2930 ± 1450). Islet viability (FDA/PI) improved with increasing pH.

DISCUSSION

The data presented here, and particularly those obtained with the basic isolation technique on 42 pigs, demonstrate two difficulties: (1) they are one more example of the difficulties encountered when establishing a new method in one's own laboratory, (2) and they show that it is unusually difficult to isolate well-preserved and viable islets from the porcine pancreas, in spite of first achievements in this field.[1,2]

To overcome these problems, two strategies were followed: (1) a modification of the basic technique, and (2) a histological analysis of the porcine pancreas to obtain detailed data on the pancreatic morphology and to correlate these data with islet isolation parameters. While the histological findings are reported elsewhere in this issue,[3] the modifications of the basic technique are the subject of this paper.

The hypothesis that the strong variation of islet yield in isolations obtained from hybrid pigs may be caused by the genetic variation of these pigs, which are crossbreeds of three to five purebred races, was confirmed to some extent by the results obtained with Pietrain pigs. Isolations from these pigs not only produced higher islet yields, but were more reproducible, as the lower standard deviation shows. In addition, high islet yields in Pietrain and low yields in minipigs tally quite well with the finding that islet volume density or total islet mass is good in Pietrain and poor in Minipigs.[3] The observation that German Landrace pigs gave poor islet yields conflicts with the observation of

good islet volume density.[3] Further studies are necessary to elucidate this important point.

With regard to the correlation of genetics and islet yield, it may be necessary to analyze more purebred races or even to develop special pig strains in order to obtain appropriate conditions for satisfactory islet isolations.

It is quite obvious from our results that, beside the genetic aspect, donor age plays a major role, since adult individuals provide better islet yields than young individuals, an observation which is in good concordance with the only previous report.[4] However, the donor age may create severe logistic problems because of the increased weight of the animals, hence, minipigs may provide an answer to the problem.

It has been suggested that a pH of 8 may be favorable for islet isolation.[5,6] However, the only other report analyzing the influence of the pH systematically was performed in rats. It showed that a variation of the pH from 6.2 to 8.0 had no influence on islet number and volume.[7] Our data indicate that this parameter may play an important role when the donor species is changed to pig. Preliminary further studies in our laboratory suggest an optimum pH of $\geq$ 7.7 for porcine islet isolation.

To provide the clinician with an optimal islet xenograft, it appears necessary to analyze still more of these technical parameters that guide and control the digestion process before we can effectively focus our view on the immunobiology of the xenograft.

ACKNOWLEDGMENT

The authors would like to thank C. Gier for her excellent technical assistance and the Deutsche Forschungsgemeinschaft for its generous support of this work.

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