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Isolation of pig pancreatic islets by a new method with hydraulic shaking: preliminary report*

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The limited availability of human pancreas represents a serious problem in islet transplantation. In the past few years many efforts have been made to isolate pancreatic islets from large mammals in order to achieve valid and reproducible isolation methods [1–4].

For several reasons swine may be considered an ideal source of islet tissue because of the similarity between human and porcine insulin and because of the easy availability of pig pancreata. Some papers have been published recently on this topic with good results [1, 5–7].

However, some problems, such as islet dissociation into single cells after collagenase digestion, are not completely solved. In this article, an automated method involving a hydraulic shaking system is described for islet isolation from the pig pancreas, developed in our laboratory and derived from Ricordi's model.

Key words: Pancreatic islets, pig – Isolation

Materials and methods

Pancreata were obtained from a local slaughterhouse from 6–9-month-old animals weighing on average 120 kg. The warm ischemia time was 24 ± 8.2 min, and the cold ischemia time was 151 ± 39 min. The organs were distended by 2 ml/g pancreatic tissue of collagenase (6 mg/ml) solution (Worthington type IV biochemicals, Freehold, N.J.; 176 U/mg).

Ten splenic pancreatic lobes were processed. After distension, the pancreata were cut into 10-cm^3 pieces and digested in a chamber continuously perfused with HBSS at 37°C. The chamber was derived from Ricordi's digestion chamber with some modifications (material Plexiglas; inner volume 500 ml; peristaltic pump flow rate 100 ml/min). Shaking was performed by a second peristaltic pump providing a recirculation flux (maximum of 520 ml/min) that allowed a controlled and continuous, gentle disruption of pancreatic tissue. The inlet of the recirculation pump was located at the top of the chamber, and the outlet, on the side. Digestion time was checked by observation every 2 min under a microscope. When free islets were detected, the collagenase solution was diluted and collected in 5 l fresh HBSS supplemented with 2% fetal calf serum (FCS) at 4°C. Mean collecting time was 40 ± 6 min. Islet purification was performed by discontinuous Ficoll gradients as described by other authors [5, 7].

Viability and morphological integrity were evaluated by staining with dithizone and a combination of inclusion and exclusion dyes (acridine orange and ethidium bromide) and by light microscopy demonstrating positive aldehyde-fuchsin staining [8].

For determination of the volume, the islets were counted under a microscope equipped with a calibration grid and were divided into 5 diameter classes (50, 100, 150, 200, > 250 μm), considering the islet spheres. Groups of 5 islets were used for insulin secretion as previously described [9]. All results are expressed as mean ± SEM.

Results

The hydraulic shaking system allowed a complete digestion of the pancreatic tissue. Average digestion time was 18.5 ± 1.4 min. After this time the islets were shunted in fresh HBSS; the mean collecting time was 40 ± 6 min. At the end of the procedure only a fibrous network and little pieces of nondistended parenchyma remained in the digestion chamber.

The isolation yielded 7921 ± 1443 islets/g pancreas expressed as EN/g pancreas with a volume of 913 ± 135 mm^3 islet tissue (13.99 mm^3/g pancreas). After the density gradient purification, 44% of the islet tissue was recovered, corresponding to 3557 ± 556 EN/g pancreas with a volume of islet tissue of 410 ± 53 (6.28 ± 0.9 mm^3/g pancreas).

The insulin secretory response to glucose (300 mg/dl) plus theophylline (5 mmol/l) was 7.3 ± 1.5 times the basal rate.

Discussion

In the past few years, some methods for the isolation of islets of Langerhans from the pig pancreas have been published [1, 5–7, 10–11]. Nevertheless, many aspects need to be solved.

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Ricordi reported a marked fragility of swine islets: This represents a serious problem because of a possible rapid dissociation of the pancreas into single cells during the isolation procedure. To overcome this problem, the automated procedure developed for the human pancreas was largely modified by Ricordi's group, increasing the circuit diameters, lowering the temperature of the heating circuit, and reducing the continuous mechanical shaking.

In our isolation method, only one recirculation circuit was used, and the inner chamber volume was enlarged (500 ml). Moreover, the chamber was made of transparent material that allowed continuous observation of the digestion procedure. A hydraulic shaking system replaced the mechanical one of Ricordi's method and represents the most important difference between the two methods.

Hydraulic shaking allows a more gentle disruption of the tissue and may be more tightly controlled. Moreover, the hydraulic shaking may be calibrated in order to obtain the appropriate continuous agitation.

An average digestion of 19 min was necessary to obtain free islets and to start the collecting phase. This time is shorter than that reported from other authors [6, 8]. Only Ricordi reported a lower digestion time [5]. These different results may be explained either by the type and concentration of collagenase used, which was slightly increased in comparison with other reports, or by the shaking system adopted, as it was continuous but more gentle.

The islets obtained showed morphological integrity and a good viability, and all the exocrine tissue was dissociated into single cells. Moreover, the yield was relatively high and the functional response good. These results let us hypothesize that, at least for the fragile pig pancreas, a continuously hydraulic shaking method may represent an elective way to isolate intact islets.

If alternative sources of islet tissue prove to be useful in reversing diabetes and promoting survival after rejection, isolating islets from large mammals may become very important. This method may be transferable to other animals after introducing some modifications and may represent a valid possibility for obtaining intact and viable islets for xenotransplantation.

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