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Primary Culture of Porcine Pancreatic Acinar Cells

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ABSTRACT

Objective To develop a method for the primary culture of porcine pancreatic acinar cells.

Interventions Dispersed pancreatic acinar cells available utilizing RPMI-1640 medium containing collagenase III. After purification, the isolated acinar cells were cultured in RPMI-1640 medium with the addition of 2.5% fetal bovine serum.

Main outcome measures The morphological characteristics of acinar cells were described. ^{3}H-thymidine incorporation of acinar cells and the activity of amylase or lipase were determined during the culture process.

Results There were no remarkable morphological changes in the pancreatic acinar cells during the 20 days' culture. The acini showed a tendency to gather but did not attach to the walls of the culture disks. A good ^{3}H-thymidine incorporation of acinar cells in the primary culture was maintained. The secretion of amylase or lipase from the acini decreased with the length of time of the culture.

Discussion The primary culture of acinar cells from a porcine pancreas which was carried out in this study maintained the normal morphology of the acinar cells and their ability to grow but not their secretion of amylase or lipase. The method would benefit by the further experiments on acini of porcine pancreas.

INTRODUCTION

With the progress made as a result of research in the field of pancreatic physiology and pharmacology, the methodology of acinar cell culture has become of primary importance. Although pancreatic duct cell culture has been well established, little is known about acinar cell culture [1, 2]. In most experiments, the cultured rodent pancreatic acinar cells generally tended to transform into ductal epithelial cells in their morphological characteristics, and membrane marker, not over a week [3]. Furthermore, porcine acinar cell culture has never been described.

Accumulated data demonstrate that the expression of receptors for gut peptides in the pancreatic acinar cells of porcines and humans is very different from that of receptors in rodents [4, 5, 6]. However, the expression of gut peptide receptors or other biological characteristics of porcine acini may be very close to that of humans [7, 8]. Therefore, the information obtained from studies involving porcine acini culture may be more beneficial in understanding human pancreatic biology.

This study developed a handy procedure for the preparation of dispersed porcine acini and established primary culture as a valid procedure for culturing porcine acinar cells.

METHODS

Preparation of Dispersed Pancreatic Acini

Seven pigs (3 months old, weighing 15-20 kg) from the Experimental Animal Center of Chongqing University of Medical Sciences, China, were utilized. After an overnight fast, the pigs were killed by bleeding. A piece of the pancreas was removed and the fat was trimmed. The trimmed gland weighing 1 g was repeatedly injected with RPMI-1640 medium (Gibco Corp, Logan, Utah, USA) containing 200 U/mL collagenase type III (Sigma Corp, St. Louis, MO, USA). Before being used, 10 mM Hepes, $NaHCO_3$ 2.0 g/L, fetal bovine serum (FBS) 2.5%, penicillin 100 U/mL, streptomycin 50 μg/mL, and soybean trypsin inhibitor (Sigma Corp, St. Louis, MO, USA) 0.2 mg/mL (for preparation of dispersed acinar cells) or 0.1 mg/mL (for primary culture) were added to the RPMI-1640 medium. The distended pancreas and enzyme solution was transferred to a sterile flask, and incubated at 37 °C with reciprocal shaking at 130 cycles/min. During digestion, the flask was vigorously swirled by hand for 10 minutes to enhance tissue dissociation. After 40 minutes, the tissue was pipetted several times with a slender tip pipette. Larger cell clumps were removed from the suspension by filtration through a 200 μm mesh nylon gauze. The filtered acini were washed three times with RPMI-1640 medium by resuspension and centrifugation (30 seconds, 500 rpm). The acinar cells were cultured with RPMI-1640 medium containing 10% FBS at 37°C, 5% CO_2 (vol/vol) and were suspended in RPMI-1640 medium at a cellular density of 0.30±0.03 mg acinar protein/mL for thymidine incorporation assay or digestive enzyme activity assay.

Preincubation Thymidine Incorporation Assay

^{3}H-thymidine (Chinese Academy of Atomic Energy, Bejing, China) was added to the culture producing a final concentration of 0.2 μCi for 24 h. Subsequently, the culture was washed three times by centrifugation and resuspension. Then, the culture was mixed with 7 mL of liquid scintillation cocktail. The cocktail (100 mL) contains dimethylbenzene (80 mL), triton X-100 (Sigma Corp, St. Louis, MO, USA) (20 mL), 2,5-diphenyloxazole (PPO) (0.40 g) and 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP) (0.01 g) after digestion with nitric acid at 90 °C for 20 min and bleaching with H_2O_2 for 30 min. The radioactivity was measured by a liquid scintillation counter (1217 LKB, Finland).

Digestive Enzyme Activity Assay

Amylase activity was measured by following the product guide of the kit (Kehua-Dongling Corp, Shanghai, China). The acini were thawed and frozen 5 times and mixed with CNPG3 reagent at 37°C; the activity of the amylase was determined by the OD value at 405 nm. Lipase activity was also measured by following the product guide (Randox Laboratories Ltd., Antrim, UK). The substrate solution (triolein) was mixed with the sample (5:2 vol/vol), and absorbance of the mixed solution at 340 nm was measured after 4 and 9 minutes. Lipase activity was determined by absorbance differentiation.

Preparation of Collagen Solution and Gels

Freshly obtained rat tails were irradiated using UV for 6 h. Subsequently, the tails were fractured into small pieces, soaked in 250 mL 1:1000 acetic acid solution and stirred for 48 h in a 4 °C refrigerator. Centrifugation for 2 h at 2300 rpm separated the acetic acid-collagen solution from the remaining solid residue. Floating collagen gel was prepared from the collagen solution (1.7 mL) and neutralized with RPMI-1640 medium (0.26 mL) plus NaOH (0.13 mL) 10 times.

Experimental Design

The dispersed pancreatic acini from pigs were cultured in RPMI-1640 medium (pancreata

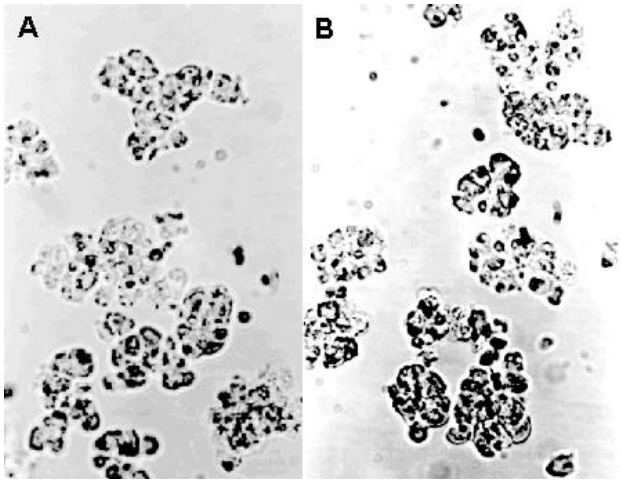

Figure 1. A. Dispersed porcine pancreatic acinar cells. **B.** Porcine pancreatic acinar cells at the 20th day of culture. (×800).

from 4 pigs) or collagen gel (pancreata from 3 pigs). Morphological changes were observed daily throughout the culture. Enzyme assays of the acini were performed daily for the first 8 days of the culture (2 samples from each pig). ^{3}H-thymidine incorporation in acinar cells was measured at the 2nd, 4th, 6th and 8th day after culture (2 samples from each pig).

ETHICS

This study was performed in accord with the Ethics Rules for Experimental Animals approved by the Experimental Animal Center of Chongqing University of Medical Sciences.

STATISTICS

Data were presented as the means ± SD of 3-4 separate experiments in which duplicate determinations were made. The significance of the differences was analyzed using the unpaired Student's t test and linear correlation.

RESULTS

Acini produced by this procedure retained the structural organization found in intact tissue (Figure 1). When the acini were viewed under the microscope, the cell clump grape-like. The viability of the acinar cells was greater than 95% as determined by trypan blue exclusion.

During the 20 days of culture in the liquid medium, the viability of the acini cells remained greater than 95% and no significant morphological change was observed (Figure 1). The cells did not adhere to the wall of the culture disk and tended to gather together. However, the cell clump could be separated again after pipetting. ^{3}H-thymidine incorporation in cultured acinar cells (n=8) at the 2nd (4,800±740 cpm), 4th (6,000±920 cpm), 6th (5,500±760 cpm), and 8th (5,800±1,120 cpm) day was significantly higher than scintillation liquid control (270±15 cpm, n=4; P<0.001) (Figure 2).

When the cells were seeded onto the floating collagen gels, the acini cells anchored onto the surface of the gels on the first day, but the gels quickly decreased in number and, after two days, they were almost completely lost. The morphology of the cells on the gel showed no significant change compared to the cells which were seeded into the liquid medium. No significant difference between gel (4,700±870 cpm, n=6) and liquid (4,950±920 cpm, n=8) medium were found with ^{3}H-thymidine incorporation after 2 days of culture.

A very low level of amylase or lipase was detected in the medium. Freshly dispersed acinar cells contain a high level of amylase and lipase. With the increase of culture time, the

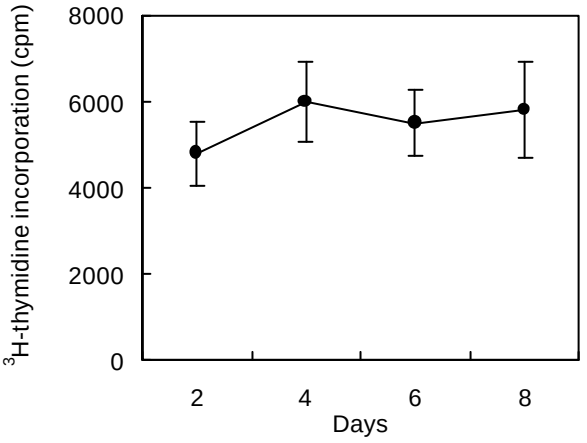

Figure 2. ^{3}H-thymidine incorporation into cultured pancreatic acinar cells (n=8).

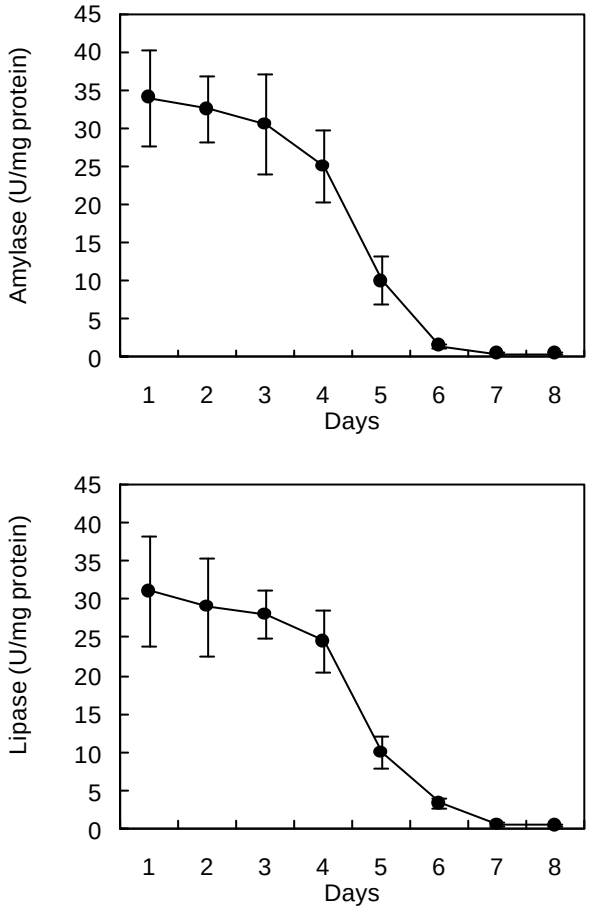

Figure 3. Activities of digestive enzymes in cultured acinar cells (n=8).

enzyme activity from the porcine acini gradually decreased (amylase: r=-0.81, n=8, P<0.01; lipase: r=-0.86, n=8, P<0.01) (Figure 3).

DISCUSSION

To the best of our knowledge, this study describes the primary culture of porcine pancreatic acinar cells for the first time.

The isolation of rodent acini described in the literature is quite complicated [3]. The preparation of the solution for pancreas digestion is also time-consuming. In this study, we modified and simplified the procedure to a great extent. The dispersed porcine acini were well-prepared using commercial RPMI-1640 medium and adding collagenase without oxygen saturation.

It was inevitable that the acini would transform into ductal epithelium which adhered to the wall of the culture disk in the primary culture of rodent acinar cells [3]. Throughout the entire study, the cultured acini maintained the same morphological characteristics as freshly prepared ones. No adhesion of acini to the disk was found although the cultured acinar cells tended to gather. The great viability of acinar cells showed by Trypan blue exclusion and the significant ^{3}H-thymidine incorporation into acinar cells demonstrated that the primary culture of porcine acinar cells developed in this studycan keep acinar cells alive. However, similar to what takes place in cultured rodent acini, the enzyme activity of porcine acini gradually decreased after several days of culture. It is still unclear why the culture technique developed in this study maintains the potency of acinar cell growth but not the ability of enzyme production or secretion. Whether the material or microenvironment was not suitable for enzyme production in the culture merits further study.

Collagen gel has always been used as the substrate in the primary culture of cells [1, 9]. The present results indicated that it is not necessary in the culture of porcine pancreatic acinar cells since both the morphology characteristics and the ^{3}H-thymidine incorporation of acini in the culture showed no significant differences in floating collagen gels and in liquid medium.

In conclusion, the primary culture of acinar cells obtained from the porcine pancreas maintained the normal morphology of the acinar cells and their ability to grow but not to secrete amylase or lipase. The method would be beneficial for further research involving acini of the porcine pancreas.

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Key words Amylases; Cell Culture; Lipase; Pancreas; Swine

Abbreviations FBS: fetal bovine serum; PPO: 2,5-diphenyloxazole; POPOP: 1,4-bis(5-phenyloxazol-2-yl)benzene

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