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Prevention of antibody-mediated lysis of islets of Langerhans by encapsulation – the effect of capsule composition

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Islet encapsulation prevents xenograft rejection. The ability of 4 types of microcapsules to prevent antibody-mediated islet cell damage has been assessed in vitro. Microcapsules containing porcine islet tissue were formed using alginate high in mannuronic (MALG) or guluronic (GALG) acid units and poly-L-lysine (PLL) or poly-L-ornithine (PLO). Microencapsulated islets and free islets were cultured overnight in human or autologous pig serum. Islet metabolism was assessed using the MTT assay ($n = 6$, 10 replicates/experiment). Values were deemed significant when $p < 0.05$; Student's t-test. In all experiments in which metabolism of encapsulated islets was unaffected by culture in human serum, the increase in metabolic rate observed was 1.8 ± 0.5 (MALG-PLL), 2.15 ± 0.77 (MALG-PLO), 2.21 ± 0.79 (GALG-PLL) and 2.42 ± 1.58 (GALG-PLO) (p-NS; $F > 0.2$ ANOVA). All free islets cultured in human serum showed a decrease in metabolism when compared to islets cultured in pig serum. In four out of six experiments, MALG-PLL, MALG-PLO and GALG-PLL capsules prevented a decrease in metabolism after culture in human serum. GALG-PLO prevented significant decrease in metabolism in all experiments. Islet encapsulation using any of the above combinations can prevent reduction in metabolism due to xenoreactive antibodies. The technique was most efficient when used to form GALG-PLO capsules.

A28

The effect of pig breed on islet isolation

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Pigs could provide an abundant source of islets for clinical xenotransplantation. Islet yields may be influenced by the breed of pig and the breed which yields maximum numbers of viable islets has yet to be determined. We have compared islet yields from Large White/Landrace and Large White/Welsh crossbred pigs (15–50 kg weight).
Pancreata were obtained from a local slaughterhouse and islets isolated by a manual sequential collagenase digestion technique. The number of islets 50–100 µm, 100–200 µm and >200 µm diameter were determined after staining with dithizone.
The majority of islets isolated were in the size range 50–100 µm – 95.83 ± 3.19 % (Landrace) and 99.09 ± 0.71 % (Welsh) (mean ± SD).
The median number of islets/gram pancreas isolated from 6 Landrace crossbred pigs was 10,499 (range 4,234–16,125) compared with 31,380 (range 14,622–82,400) from 6 Welsh crossbred pigs. Assuming a standard islet diameter of 150 µm, this is equivalent to 1,561 (range 500–2355) IE/g for Landrace and 3,414 (range 1568–8554) IE/g for Welsh pigs – these values are significantly different ($p < 0.05$; Mann-Whitney U). The ratio of I/IE was 1:7 for Landrace and 1:9 for Welsh.
The total islet volume was 2.76 mm^3/g (range 0.88–4.16) pancreas Landrace and 6.03 mm^3/g (range 2.77–15.11) pancreas Welsh.
In conclusion, significantly greater numbers of islets were obtained from Large White/Welsh pigs compared to Large White/Landrace pigs using this isolation technique.

A29

The efficacy of barium-alginate encapsulation of porcine islets of Langerhans

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Porcine islets, protected by an immunoisolation device, may provide a suitable alternative to human islets for clinical transplantation. Calcium-linked alginates form an effective immunoisolation barrier which can prevent allo and xenograft rejection. Barium linked alginates have the advantage of being stable under physiological conditions, unlike calcium-linked alginates which require a polyaminoacid to stabilise the membrane.
We have investigated the ability of two forms of barium alginate, one high in mannuronic acid (MALG) and one high in guluronic acid (GALG), to prevent complement-mediated lysis of porcine islets.
The metabolism of free and encapsulated islets was determined after exposure to autologous porcine or non-diabetic human serum by the MTT assay. Metabolism of free islets was significantly reduced following exposure to human serum compared to pig serum in all experiments ($p < 0.05$; Student's t-test; $n = 8$), the mean percentage reduction was 37.5 ± 19.3 %. In seven out of eight experiments there was no significant difference in the metabolism of MALG-encapsulated or GALG-encapsulated islets in autologous pig or human serum ($p < 0.05$).
In three experiments, the mean insulin secretion (basal to peak levels) during perifusion increased by 25 % (free islets), 66 % (GALG-encapsulated islets) and 75 % (MALG-encapsulated islets).
In conclusion, barium-alginate encapsulation prevented complement-mediated lysis induced by human serum, but did not impair insulin secretion of porcine islets.

A30

Enzyme activities in commercial collagenase preparations and their kinetics during islet isolation procedure

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One of the major problems in islet isolation, in particular in porcine islet isolation, is the quality of the collagenase preparations. Most commercial collagenases are more or less crude preparations contaminated with other enzymes, e.g., trypsin-like, neutral protease and clostripain. The importance of these enzymes for islet isolation is broadly unknown.
The aim of this study was to examine various collagenase preparations and the kinetics of the enzyme activities during the porcine islet isolation procedure. Collagenase activity was measured by three different biochemical tests with either collagen or the artificial FALGPA and PZ-Pro-Leu-Gly-Pro-Arg as substrates. Both, the trypsin-like activity and the clostripain activity were evaluated using two different assays and BAEE as substrate. Neutral protease activity was detected by the Azocoll-test. Isolations were performed using a modification of the half-automated method by C. Ricordi (Surgery 107: 688–694, 1990).
Results: (1) The examined collagenase preparations showed broadly varying patterns of enzyme activities, particularly trypsin-like activity. (2) During the isolation procedure, trypsin-like and neutral protease activities increased strongly, presumably due to the release of enzymes from the exocrine portion of the disintegrating pancreas, and collagenase activity decreased. (3) Experiments about inhibiton of trypsin-like activity showed that only one of four tested inhibitors was able to inhibit trypsin-like activity completely. (4) The yield and viability of isolated islets was insufficiently dependent on the enzyme activities investigated so far.
Discussion and Conclusion: Because of the different enzymes involved, e.g., collagenase and trypsin-like, and the lack of defined enzyme activity patterns, reproducible islet yield and viability still remain a constant problem. Aiming at selected trypsin inhibition in addition to standard collagenase digestion, it should be possible to overcome this obstacle.