UNIVERSITY of MINNESOTA

Barcelona - fg

Mr Chairman, Co-chairman
Ladies and Gentlemen

Jnl.

- Our aim is to optimize canine islet isolation for a comparative in vitro and in vivo study of islet function
- Major problems in islet isolation ~~are~~ from the large mammalian pancreas, are :
  - obtaining sufficient islets from 1 pancreas "the yield"
  - and purity, that is reducing the amount of tissue to be transplanted.
- Two methods for islet isolation were compared
- The next slides illustrate the procedure

Groupe - B

(1)

- First, part of the pancreas is removed,

the other part remains in situ for other experiments.

- this which demonstrates removal of the duodenal or right lobe

∴ in fact left pancreatic segments were used in this study

(2) In the first series of 6 experiments segments were intraductally distended by manual injection of a small volume, pre warmed collagenase solution,

- this is the segment prior to distension

(3) Here, the segment is distended:

- collagenase, a buffered solution, is flowing at the distal end
- note, that it is difficult to attain complete distension using this method

(4) In the second series of experiments a roller pump was used to infuse a large amount of cold collagenase,

- this slide shows the set up
  - a roller pump, over here
  - an organ perfusion box, with the pancreatic segment laying on a screen

- collagenase at the bottom of the box,
is forced into the gland using the pump
- Fluid leaking out is recycled.

(5) The next block shows the lobe prior to distension

(6) Next, the distended segment; it's volume has increased 3 to 4 times.

- Next in both series the distended gland is incubated at 37°C for 20 minutes

↓

(7) After digestion ~~of the gland~~ by collagenase of the connective tissue,

the gland is teased apart using forceps

(8) The ~~duct~~ pancreatic duct and large blood vessels are discarded

(9) The tissue is further dispersed by aspiration in syringes of various

- and screened by expulsion over a 400 μm sieve

(10) Trapped tissue is discarded

(11) This slide shows non-stained cells, in the sieved suspension.

- Cells are easily recognized from their uniform light blue appearance.

- At this stage, in the so called "digest" the amount of cells freed from senior tissue is determined, usually using a stain ... I will come to that later on.

(12) Finally, the dispersed tissue is spun in density gradients
- either Ficoll in the first series
- or dextran in the 2nd series of experiments

- Purified islets are collected from the two uppermost interfaces.

(13) This slide shows the final preparation of freshly purified islets.
- Islet are stained red by dithizone
- There is remaining acinar tissue
- "Islet Yield" is determined from the number, and volume of islets calculated from the mean diameter of islets.
- And expressed per gram processed tissue

(14) With the glucose-injection method

- islets ranged from 25 to two hundred fifty 250 μm in diameter prior to purification
  ∴ white bars.

- on Ficoll purification only islets upto a 150 μm were found.
  ∴ hatched bars

- That, looking at the isolated islet volume per diameter class

- expressed as a percentage of the total volume of isolated islets

- the islet size distribution was shifted towards smaller diameters ∴ suggesting fragmentation of islets to have occured.

(15) In the second series of experiments, no effect was seen with dextran purification on the islet size distribution (in fact this is an old slide with data obtained by isolation from the prox. duodenal tube) only islets similar data are obtained from left pancreatic lobe

(16) Prior to purification & using the roller pump

- islet yield had increased several fold as compared to manual injection of collagenase

- here, isolated islet yield is expressed as a percentage of the mean pancreatic islet content, which has been stereologically determined in a previous study.

- we now routinely obtain approx. 70% of the pancreatic islet content.

(12) However, both using Ficoll or electron for purification reduces the islet yield by ~ 50%

- This slide shows the results with the glucose infusion technique prior to - and after electron purification

- Using this new technique we now obtain purified islets amounting to 40% of the pancreatic islet content.

- Our first conclusions are:

- With glucose infusion, as compared to manual injection: Yield increased 5 fold
- Key elements appeared to be
  - A more uniform distension of the gland
  - And the higher volume ratio of collagenase for digest

(18) This slide shows one of the purest preparations obtained, with an estimated purity of 65% islets

- On the average however a purity of only 50% was obtained
- ~~purity is estimated from angiographic scoring in stained test samples~~

- Recently we adopted an entirely new approach to islet isolation

- Since UW, ~~solution~~ the new organ cold storage solution has been shown to allow long-term cold storage of the canine pancreas

- And, since islet isolation GOO is largely performed in the cold, we tested this preservation solution as the isolation medium throughout the isolation procedure (solution) in another 6 dogs.

* as compared to the common used RRPL tissue culture medium

(19) This slide shows the final distribution stained preparation after detection purification of UM-isolated islets.

- UM did not affect islet yield
- however UM markedly improved purity:
  - acinar tissue is virtually absent.

(20) Using UM:

- purity has increased from ~30% with RPMI

to over 90% with UM

(21) Immunostaining for insulin of UM isolated islets confirmed these results.

- This slide shows staining of an islet in the ~~normal~~ intact canine pancreas

(22) - And this slide UM-isolated islets

- 90% of the tissue was identified as islets
- some acinar tissue is visible now

Thank you

- These islets are tested now
(23) in vivo after auto Tx in 6 dogs
by ~~retrograde~~ retrograde venous
infusion into the spleen
- 3 dogs using RPMI
and 3 dogs using UW for isolation
- Out of each group 2 remained
normoglycemic for over 3 mo now
- One dog in the RPMI - group received
an adequate islet mass, though the
graft failed after 2 mo, probably due
to acinar tissue contaminating the graft
- The other dog received an inadequate
amount of pure islets and became
(24) hyperglycemic immediately. Two wks
later still functioning islets were
identified by insulin-staining of the splenic tissue.

Thank you

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Slides

(1) demo RL
(2) pré manual distension injection
(3) post manual injection
(4) set up roller pump
(5) pré roller pump infusion
(6) post roller infusion
(7) teasing using forceps.
(8) discard p. duct large blood vessel
(9) aspiration / expulsion screen
(10) harpoon tissue in digestion : Trial
(11) (16): non-stained islets "sieved suspension"
(12) collect islets density gradient with pure
(13) dithizone stained islets final preparation
(14) pick / sieved size distribution
(15) dextran / sieved " "
(16) yield manual vs infusion
(17) yield digest / dextran
(18) dithizone stained islets final prep
to purity

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(19) [illegible] [illegible] [illegible] : CW

(20) [illegible] RPM1 w CW [illegible]

(21) [illegible] LL

(22) [illegible] CW-[illegible].

(23) √[illegible] δx [illegible]

(24) [illegible] functionally active [illegible].
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