

**SAFETY AND VIABILITY OF MICROENCAPSULATED HUMAN ISLETS
TRANSPLANTED INTO DIABETIC HUMANS**

Short running title: Seaweed Diabetes Trial

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Additional information for this article can be found in an online appendix at

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Background: Transplantation of insulin-producing cells placed inside microcapsules is being trialled to overcome the need for immunosuppressive therapy.

Research design: 4 type 1 diabetic people with no detectable C-peptide received an intraperitoneal infusion of islets inside microcapsules of barium alginate (mean 178,200 islet equivalents on each of 8 occasions).

Results: C-peptide was detected on day 1 post transplantation, and blood glucose levels and insulin requirements decreased. By 1-4 weeks, C-peptide was undetectable. In a multi-islet recipient, C-peptide was detected at 6 weeks after the 3rd infusion, and remains detectable at 2.5 years. Neither insulin requirements nor glycaemic control was affected. Capsules recovered at 16 months were surrounded by fibrous tissue and contained necrotic islets. No major side effects or infection occurred.

Conclusions: Whilst allografting of encapsulated human islets is safe, efficacy of the cells needs to improve for the therapy to make an impact on the clinical scene.

Transplantation of insulin-producing cells placed inside capsules is a strategy that is being trialled to overcome the need for immunosuppressive therapy in insulin-dependent diabetic people (1). We have made microcapsules of barium alginate and shown that insulin-producing pig cells can function as efficiently inside such capsules, as when non-encapsulated (2). Moreover, human islets placed in these capsules normalize blood glucose levels when engrafted in diabetic mice (3,4). In the current study, we transplanted 4 type-1 diabetic humans with encapsulated human islets.

RESEARCH DESIGN AND METHODS

Cadaveric pancreases were obtained with consent, and islets isolated by digestion with collagenase NB1 premium grade and neutral protease NB (Serva, Germany). Islets were placed in barium alginate microcapsules (2), their median average diameter from 8 islet preparations being 340 μm (range 255 – 750 μm).

Median viability of the encapsulated islets, assessed with fluorescent dyes carboxyfluorescein diacetate and propidium iodide (2), was 73% (range 60-80%). Purity was 68% (range 50 – 88%), and insulin content (2) 1.1 mU/islet equivalent (IEQ) (range 0.1 – 35 mU/IEQ). The median stimulation index of the islets exposed to 20 mM glucose, compared to 2.8 mM glucose, for 1 hour was 1.22 (range 1.0 – 2.4). The median number of IEQ's transplanted on each occasion was 178,200 (range 98,200 – 227,900). Conditioned culture medium was free of microbial contamination.

Of the 14 people with diabetes screened for the phase 1 clinical trial, 7 were selected, and 4 transplanted over a period of 19 months. The 7 with long standing type-1 diabetes had no endogenous insulin production (no C-peptide in serum during an arginine tolerance test, and nil in 24 hour

urine), body mass index < 25 and weight < 70 kg. Those transplanted had antibodies to neither glutamic acid decarboxylase (GAD) nor islet cell surface antigens (ICA512). One person received 4 islet infusions over 7 months, 3 in the first month; a second received 2 infusions 10 months apart; the other 2 recipients received one infusion each (see Tables A1 and A2 in the online Appendix available at <http://care.diabetesjournals.org>). Infusions were carried out as an outpatient in Dept Medical Imaging.

No immunosuppression was used but recipients did take both a mild anti-inflammatory agent (Atorvastatin 20mg), and anti-oxidants (vitamin A 50,000 units, vitamin B6 100mg and vitamin E 750 units) after each transplant. For the last two islet infusions, exenatide 5 μg bd was administered, in an attempt to enhance beta cell survival and function.

Approval for all procedures performed was obtained from the Institutional Human Research Ethics Committee.

RESULTS

C-peptide was detected in urine on the first day after the islet infusion (median 0.59 nmol/L [range 0.11 – 1.79]; 0.15 nmol/mmol creatinine [range 0.06 – 0.25], with levels declining thereafter, becoming undetectable at 1 - 4 weeks (median 10 days) later. Both blood glucose levels and insulin requirements also were lower on the first day after transplantation, by an average (\pm SEM) of 36 \pm 8% and 22 \pm 3% respectively, but not thereafter. At day 7, when an arginine tolerance test was carried out, plasma C-peptide was undetectable.

In the recipient of four islet infusions, urinary C-peptide was detected at 6 weeks after the 3rd infusion, and continues to remain detectable at 2.5 years. C-peptide levels are 0.06 – 0.34 nmol/L or 0.02 – 0.06 nmol/mmol creatinine. The small amount of insulin being

produced did not alter insulin requirements or glycaemic control.

To understand better what was occurring in the capsules transplanted, a laparoscopy was performed in this patient at 16 months after the 1st infusion. Large numbers of capsules were found scattered throughout the peritoneal cavity, in clusters attached to the parietal peritoneum (Figure 1A), spleen, omentum and kidney. Biopsy showed the capsules to be intact and surrounded by fibrous tissue containing thin walled capillaries, with a mild histiocytic response. Islets were necrotic (Figure 1B).

Antibodies were detected to GAD but not ICA512 in three recipients. In two, the titre became elevated 4 weeks after the first infusion; and in the third, 14 wk after the 4th infusion. In all these recipients, antibodies continue to remain detectable, at 1.1 -2.5 years after the initial infusion.

Cytotoxic antibodies were detected at 4 and 8 weeks after the 1st infusion in two patients, one who received four infusions and the other a single infusion. The titre, 56% and 32% respectively, declined with time, but was still detectable when last measured at 1.9 and 0.6 years respectively (titres of 19 % for both).

There were no serious adverse events. Nausea did occur in the two recipients of exenatide, with the medication being ceased earlier than anticipated because of this symptom in one person. There was a trend for blood glucose levels to be lower when exenatide was administered, probably because of delayed gastric emptying, since urinary C-peptide was undetectable during some of this time. Wound infections did not occur, and all recipients were discharged within an hour of the completion of the infusion.

CONCLUSIONS

This phase 1 trial shows that the infusion of encapsulated human islets is safe,

although cytotoxic antibodies can develop. That all recipients had normal renal function after several decades of type-1 diabetes makes them extremely unlikely to ever develop renal dysfunction and potentially require a renal transplant. In this situation, the occurrence of cytotoxic antibodies is mostly of passing interest.

The laparoscopy performed on a recipient of encapsulated human islets was novel, with capsules being intact, and islets necrotic. This outcome is different from the one other report of a laparoscopy performed in a human receiving encapsulated insulin-producing cells; viable endocrine cells were observed in the encapsulated neonatal pig cells 9 years after transplantation (5).

Loss of graft function within days of transplantation was likely due to either ischaemic necrosis or an inflammatory process, possibly initiated by fibrinogen adhering to the capsule surface (6). Cytokines could enter the capsule through its pores (250 kDa), and destroy the β cells. That there was some late graft function lasting for years in the recipient of the 4 infusions might be explained by a small number of ductal cells in the graft differentiating into β cells (7).

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Figure

