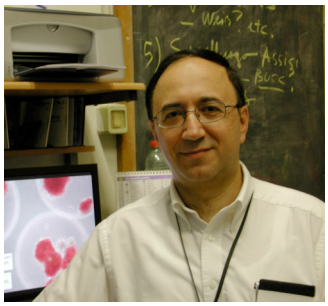
Immunoprotection of human islet grafts in alginate/polyornithine microcapsules: preliminary results



R. Calafiore, G. Basta, G. Luca, L. Racanicchi, P. Montanucci, F. Mancuso, F. Santeusanio.
Department of Internal Medicine, University of Perugia, Perugia, Italy (islet@unipg.it)

Introduction

Recent results of the Immunotolerance Network (ITN) multicenter clinical trial on human islet allografts into totally immunosuppressed patients with type 1, insulin-dependent diabetes mellitus (T1DM), have clearly shown limits of this approach for the potential cure of this metabolic disorder ((Shapiro AM, 2006). In fact less than 8% of the transplanted recipient could enjoy remission of hyperglycemia and insulin independency at 5 years post-transplant. If causes for this long-term failure associated with human islet allografts are still being under scrutiny, it is increasingly evident that total immunosuppressive regimen therapy to prevent graft rejection will result in severe damage at level of several organs, including the islet graft itself. Hence new approaches to avoid recipient's imunosuppression clearly are necessary. During the last years important advances have been made in the knowledge of the characteristics and requirements capsules have to meet in order to provide optimal biocompatibility and survival of the enveloped tissue. Novel insight shows that not only the capsules material but also the enveloped cells should be hold responsible for loss of a significant portion of the immunoisolated cells and, thus, failure of the grafts on the long term. We have developed for the last two decades alginate (AG)-based microcapsules that by enveloping each individual islet would prevent the islet graft-directed immune destruction. Ultra-purification of alginates by methods developed in our laboratory, in association with a unique poly-aminoacidic cation coating, based on poly-L-ornithine (PLO), have progressively made the finite capsule product an extremely biocompatible and selective permeable membrane which effectively turns down the islet cell graft-directed immunity with no need for the recipients' general immunosuppression.

Feedback from several years of pre-clinical experimental studies in our laboratory (Calafiore R, 2004) has permitted us to initiate a pilot closed, phase-1 clinical trial into 10 patients with T1DM receiving AG/PLO microencapsulated islet grafts and no immunosuppression, under permission and monitoring by the Italian Ministry of Health.

Rationale, research design and methods

Human islet procurement – Human islets were isolated from donor pancreases according to the Edmonton protocol (Ryan EA, 2005). The islets were cultured for 24 h in HAM F12 (Celbio, Milano, Italy), supplemented with antibiotics and 1.25% human albumin (Kedrion Spa, Milano, Italy) at 37°C in 95% air/CO2. Islet isolation final yield was quite variable depending upon organ retrieval conditions, and donor age and clinical history. Moreover, variables associated with collagenase lots also explained differences in islet cell yield between and among preparations. However, only those preparations complying with quality control standards, in terms of islet cell viability, morphologic integrity and functional competence were considered for transplant.

Microencapsulation – The vast majority of groups nowadays apply pure alginates with low content of endotoxins and lacking immunogenic effects. Islet microencapsulation was conducted according to our method (Basta G, 2004). Briefly, the islets were carefully washed to remove medium proteins and thereafter thoroughly mixed, by gentle stirring with endotoxin-and pyrogen-free, 1.8% (w/v)

Keltone LVCR sodium alginate (Stern, Milano, Italy). The raw alginate powder underwent ultra-purification process set-up in our laboratories, according to guidelines of U.S. Pharmacopeia. Upon extrusion through a microdroplet generator, with the droplets being generated by combined mechanical and air shearing forces, the microdroplets, each containing one or two human islets were collected upon a $CaCl_2$ bath which immediately turned them into gel microspheres. The gel beads, measuring an average 500μm in equatorial diameter, were sequentially coated with double 0.12% and 0.06% poly-L-ornithine (Sigma) and finally with 0.04% sodium alginate (Basta G, 2004) (Fig.1).

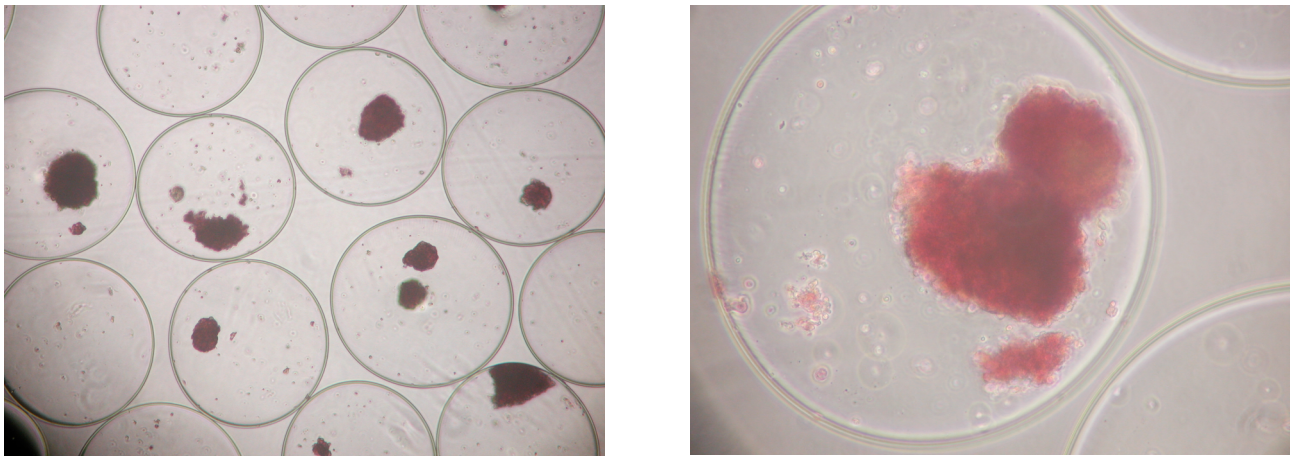


Figure 1: Encapsulated HI stained with DTZ.

Patient enrollement into study – Ten patients with long-standing T1DM, on intensive insulin therapy regimens (4 injections a day) and undetectable serum C-peptide (CPR) levels (testifying for total suppression of endogenous insulin production) underwent complete blood chemistry testing, including islet β-cell related immunity (ICA, anti-GAD65). Imaging of the abdomen (CT scan, ecography) which was selected as implant site, was performed before the study.

Pre- and post-transplant patient assessment - The patients were admitted to our hospital clinic the night before the graft, in order to monitor blood glucose overnight, and keep it on near normoglycemic values (range : 80-150 mg/dl) by exogenous insulin infusion, through the subsequent morning.

Serum CPR were taken before and twice a day after graft for the first 7 days, and once a week thereafter, both in basal and at 90 minutes upon meal consumption. To test graft function, an oral glucose tolerance tests (OGTT, 75 g) and glucagon test (1 mg i.v.) were schedules at several weeks/months of transplant. At 7 days of transplant, all patients were discharged from hospital and carefully instructed to monitor their own home blood glucose with adjustment of insulin requirement. At 30 fays of transplant, all patients were readmitted to hospital to check an abdominal ecography. Glycated haemoglobin (GHb) a parameter of medium-/long-term metabolic control was measured prior to (in at least three instances) and thereafter transplant at monthly time intervals.

Preparation of microcapsule for transplant and transplant procedure – Islet preparations ranging from 400000 to 600000 islet equivalents (islets normalized as all measured 150μ in diameter) were grafted, so far, in 4/10 patients. The final islet graft preparation, comprised of an average 50 ml of microcapsules, was suspended in 100 ml of saline. Empty capsules did not exceed 5%.

3/4 patients were grafted, under local anesthesia (2% xylocaine), at the Department of Radiology and Imaging of our School, by direct trans-cutaneous puncture, by a 14G needle, of the abdominal cavity, under echography guidance. Initially, a few ml of saline were injected between the peritoneal leaflets to create a virtual space where subsequently the capsules were deposited. The injection was performed after placing the encapsulated islets with saline in a 60 ml plastic syringe connected with the needle. Injection time lasted for an average 15 minutes. Patient#4 underwent mono-laparoscopy to exactly locate the area where the capsules would be injected within the mesentery. The procedure held the potential advantage to permit deposition of the capsules in selected graft areas, rather than blindly, as granted by direct abdominal puncture, under echography guidance.

Results

All patients, CPR-negative prior to transplant, showed post-transplant rise in serum CPR that overall lasted for 12 months (Calafiore R, 2006). All recipients also were able to substantially reduce their exogenous insulin daily dosage until 50-75% for many months post-transplant. CPR levels were higher in post-absorptive rather than fasting conditions. Pt#1 showed increase in CPR following OGTT, 60 days post-transplant (peak value 0-9 ng/ml) indicating the presence of well differentiated grafted islet β-cells. None of the grafted patients were able to suspend exogenous insulin administration, except for pt. n.4 were the encapsulated islet grafts were performed by laparoscopy. This patient that showed a serum CPR rise from 0 to over 2.5 ng/ml, was able to discontinue exogenous insulin for a few days. Thereafter insulin consumption resumed although at consistently lower levels throughout 1 year of post-transplant follow-up. GHb declined in all transplanted patients as a prove of clearly better metabolic control in the recipients, throughout 6-12 months of post-transplant clinical follow-up. One of the patients affected by serious weekly hypoglycaemic episodes showed disappearance of such acute events in the post-transplant follow-up. No side effects emerged during transplant procedure or the post-transplant time period. Patients #2, 3 and 4 were tested by i.v. glucagon (1 mg) to assess CPR secretion capacity by the encapsulate islet grafts. Of course, in control T1DM patients CPR response to glucagons test is completely flat. On the contrary, the transplanted patients showed an increase in serum CPR that assumed a double peak profile as under physiological conditions in normal subjects, with the only difference residing in lower absolute numbers, in some instance, but perfectly associated with efficient functionally competent secretory kinetics.

Discussion and Conclusions

Favourable, two-decade lasting pre-clinical study on AG/PLO microencapsulated islet allo- and xenografts into experimental animal models of diabetes allowed us to reach adequate safety and efficacy criteria so as to comply with regulations of the Italian Ministry of Health for human application. Certainly AG ultra-purification greatly facilitated acceptance of the clinical protocol by the Italian Health Authorities. Consequently, we have been granted permission to initiate a pilot phase-1 clinical trial of AG/PLO microencapsulated human islet graft into nonimmunosuppressed patients with T1DM. Current data in 4/10 cases, seem to demonstrate first that the graft procedure is simple, painless, minimally invasive, and absolutely devoid of unwanted side effects. Although none of the treated patients was able, thus far, to withdraw exogenous insulin supplementation, except for one case, multiple evidence indicated graft function, namely : significant GHb decline in all cases for months after transplant; responsiveness to OGTT and glucagons test; disappearance of acute hypoglycaemic episodes. It is likely that adjustments in grafted viable islet cell mass dosing might further improve clinical results in the remaining cases to come.

References

1. (Shapiro AM et al 2006) *International Trial of the Edmonton Protocol for islet transplantation*. NEJM (355), 13 : 1318-1830.
2. (Calafiore R at al 2004) *Grafts of microencapsulated pancreatic islet cells for the therapy of diabetes mellitus in nonimmunosuppressed animals*. Biotechnol Applied Biochem (39) : 159-164.
3. (Ryan EA et al. 2005) *Five-year follow-up after clinical islet transplantation*. Diabetes (54) : 2060-2069.
4. (Basta G et al. 2004) *Optimized parameters for microencapsulation of pancreatic islet cells : an in vitro study clueing on islet graft immunoprotection in type 1 diabetes mellitus.* Transpl Immunol (13) : 289-296.
5. (Calafiore R at al 2006) *Microencapsulated pancreatic islet allograft into nonimmunosuppressed patients with type 1 diabetes*. Diabetes Care (29), 1 : 137-138.