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CSII IN TUSCANY, ITALY

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CSII is a valuable approach in the treatment of diabetes. Little information is currently available on the use of this therapy in Italy. Hereby we report on the use of CSII in Tuscany, Italy, where, according to a very recent rule from the Regional Government, the Regional Reference Centers for Diabetes screen patients who might benefit from this treatment, and local health care administrations pay for the pumps and consumables, based on a year by year budget. By sending a specific questionnaire to all the diabetes centers of the region, it has been found that the number of patients on CSII in Tuscany are 31, with age of 49 ± 11 years, males/females ratio of 10/12, duration of diabetes of 22 ± 10 years, and duration of CSII therapy of 6.3 ± 5.2 years. The models used are: 5 Minimed, 8 Microjet Quark, and 18 H-Tron Disetronic. Ten patients are on rapid insulin, and 21 on lyspro. All the patients are on a strict home glucose monitoring schedule (3-4 times/day) and refer to the diabetes center 7 ± 3 times/year. HbA1c before CSII was $9.3 \pm 1.7\%$; after starting CSII, HbA1c values decreased to $8.6 \pm 1.7\%$, $8.2 \pm 1.4\%$ and $8.0 \pm 1.2\%$ respectively at 3, 6 and 12 months, and $7.5 \pm 1.1\%$ at the time of preparation of the present report ($p < 0.05$ or less vs pre-CSII for any time point). Hypoglycemic episodes (classified as in the DCCT) decreased significantly upon CSII starting, and no episode of chetosis has been reported. The current cost of CSII treatment ranges from 2 to 3.5 millions Italian lire (approximately 1,500 to 1,800 Euro) per year, depending on the model applied. These results show that the use of CSII in Tuscany is still limited, probably due to cost-related problems; where employed, CSII is very effective in improving metabolic control and reducing hypoglycemic episodes.

XENOTRANSPLANTATION OF MICROENCAPSULATED NEO-NATAL PORCINE ISLETS (NPI) IN DIABETIC RECIPIENTS: PRE-CLINICAL TRIALS

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Pig islets, immunoprotected in alginate (AG)/poly-L-ornithine (PLO) microcapsules could circumvent problems actually posed by both the restricted availability of cadaveric human donor organs and need to treat the recipients with general immunosuppression. To develop a reliable islet source, in full compliance with the principle of large-scale availability and suitability for the human system, we have started to employ NPI, separated from the pancreas of newborn pigs. NPI released physiologic insulin patterns in response to glucose at different concentrations, under static incubation (50-300-50 mg/dl). So far, 16 NOD mice with spontaneous and 17 rabbits and 13 CD-1 mice with streptozotocin-induced diabetes underwent intraperitoneal TX of AG/PLO NPI. Following TX all the recipients achieved normoglycemia that was long-term sustained. In particular, at 180 days post-TX, 8 NOD mice (50%), 8 rabbits (48%) and 10 CD-1 mice (77%) were still euglycemic. Microcapsules explanted at 120 d post-TX from remitter NOD mice were freely floating in the peritoneal cavity and contained NPI that were well stained in red with DTZ, and upon static incubation with glucose were associated with physiological insulin secretory patterns. The microcapsules retrieved from failed animals were not surrounded by any inflammatory tissue cell reaction. In conclusion, NPI encapsulated in AG/PLO may represent a potential answer to the islet mass and TX immunoprotection problems in the treatment of type I diabetes.

CHARACTERIZATION OF ISLET AFTER KIDNEY RECIPIENTS: IDENTIFICATION OF IN VIVO PREDICTORS OF GRAFT FUNCTION

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Islet transplantation is a minimally invasive approach to cure type 1 diabetes. Despite its potential, the success rate of islet transplantation is often unpredictable. A longitudinal study of the metabolic profile was performed in fifteen islet transplanted patients, already under immunosuppressive therapy for kidney graft. Nine patients became insulin-independent and five halved their insulin requirement during the follow up; c-peptide remained >0.17 nM in all recipients. HbA1c and insulin sensitivity (HOMA analyses) improved after transplantation. In insulin independent recipients insulin responses to glucose (IVGTT) showed bi-phasic kinetics with a first transient response, followed by a sustained plateau. The areas of the two phases showed similar progression at all times and were significantly correlated with each other ($r = 0.73-0.98$, $p < 0.001$). Interestingly, both the mean values of AUC of first and of second phase at 3 and 6 months also showed correlation with corresponding proinsulin ($r = 0.64$, $p < 0.05$), a figure reported to be representative of functioning β cell mass, with a decline of graft function that is paralleled by an increase of proinsulin. A multiple regression analysis on rank-transformed data showed a significant correlation of insulin requirement and fasting glycemic values at 1 month (cumulative $r^2 = 0.94$) as well as proinsulin values at 3 months ($r^2 = 0.93$) with 6 months' insulin requirement. Our study shows that islet transplantation can be successful also in type 1 diabetic patients under conventional immunosuppression therapy and proposes some *in vivo* parameters of β cell function as predictors of clinical outcome.

CONTINUOUS INTERSTITIAL GLUCOSE DYNAMICS FOLLOWING SUBCUTANEOUS INSULIN INJECTION

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Recently a continuous glucose monitoring system (CGMS) has become available (MiniMed Inc.) that records current values in nA, proportional to subcutaneous glucose levels, every 5 minutes for several days. By downloading the data to a computer the values are retrospectively calibrated against reference blood glucose measurements, and the resulting glucose curve is displayed. The present study investigated several real time calibration algorithms. Hypoglycemic excursions were induced in rats (Sprague Dawley) by injecting insulin (Lispro or Velosulin; N=5 each) subcutaneously. For each experiment 2 sensors were inserted and tail-vein catheters used for blood sampling. Following a 1-hr sensor stabilization period, blood samples (0.1 ml) were collected at -120, -80, -40, -20, -5, and -1 min. At $t=0$ min insulin (1 U/kg) was injected and further samples collected at 5, 10, 15, 20, 30, 40, 60, 80 min and every 40 min until 400 min. Plasma glucose was measured with a YSI glucose analyzer and sensors were calibrated using a one-point real-time algorithm or a retrospective linear regression algorithm. The real-time algorithm calculated sensor glucose as $CF \cdot (I - OS)$, where CF was the calibration factor determined from plasma glucose (YSI) and sensor current (I) immediately prior to the insulin injection and OS was an arbitrary offset of 3 nA estimated empirically. For the retrospective algorithm, CF and OS were determined from the slope and intercept of the sensor current vs. YSI glucose regression line. Results showed that subcutaneous glucose sensors calibrated with a one-point real time algorithm accurately follow the hypoglycemic excursion ($MAD = 15.0 \pm 20.6\%$ with a correlation coefficient $r^2 = 0.70$) but with a slight bias in regression slope (0.91 ± 0.028 , different from 1; $p < 0.05$). Retrospective calibration removed bias in the regression slope (1.0 ± 0.26 , not different from 1; $p > 0.05$). Sensor accuracy was similar to the real-time calibration ($MAD = 14.6 \pm 18.7\%$) with a slight improvement in the correlation coefficient ($r^2 = 0.77$). These data show that the one-point (real-time) calibration is similar to the retrospective linear regression calibration. Therefore, the MiniMed Continuous Glucose Monitoring System is able to detect hypoglycemic excursions in real time.

ATG VS. BASILIXIMAB INDUCTION IN SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION

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Objectives Induction of tolerance is a crucial issue in simultaneous pancreas-kidney (SPK) transplantation (Tx). Until recently ATG or ALG have been the mainstay of tolerance induction and remain the standard with which any new treatment should be compared. This open study evaluates the efficacy and safety of Basiliximab (Bsx) compared to ATG for tolerance induction in SPK Tx when associated with Neoral combined with mycophenolate mofetil and low dose steroids.

Materials and methods Tolerance was induced in 20 consecutive SPK recipients (R) by either a 12 to 14 day course of ATG (n=10) or 2 Bsx doses (n= 10). Treatment efficacy was evaluated on the basis of the number and severity of acute rejection (AR) episodes at 1 and 3 months after Tx. Diagnosis of AR was done on a clinical basis and only steroid-resistant (SR) episodes were biopsy-proven. Safety was defined by the number of infections. The groups were well-matched for all baseline characteristics.

Results During the first month after Tx 5 R (50%) treated with ATG developed 5 AR. Equivalent figures for Bsx were 4 (40%) and 5. One AR in each group was SR. On the whole, during the first 3 post-Tx months there were 7 AR (2 SR occurring in the same R) in 5 R with ATG induction and 5 AR (1 SR) in 4 R with Bsx. No graft was lost to AR in both groups. During the first month after Tx 3 R (30%) developed 6 infections in the ATG group: 4 viral, 1 bacterial and 1 micotic. Equivalent figures for Bsx were 4 R (40%), 4 infections: 1, 3 and 0. One R in the ATG group died from sepsis. At the end of the follow-up period no further infections were recorded in both groups. Three month pancreas and kidney survival rates were 100% and 90% for ATG induction and 89% and 89% for Bsx.

Conclusions This study suggests that Bsx is equivalent to ATG for tolerance induction in SPK Tx. Final validation of these data requires a prospective randomized study including a group without induction.

SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION FROM MARGINAL DONORS

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Objectives Despite the fact that the number of simultaneous pancreas-kidney (SPK) transplantations (Tx) is continuously growing, many more grafts are needed than are actually available. As evidence accumulates that pancreas (P) Tx may be actually life-saving new strategies should be developed to expand the donor pool. We herein report our experience with SPK Tx from "marginal" donor organs (over 45 years of age, with history of cardiac arrest and/or marked hemodynamic instability requiring high dose vasopressors).

Materials and methods Between March 1998 and October 2000 24 SPK Tx were done in 24 recipients using organs procured by our own surgeons, exclusively with an original rapid en-bloc technique, from either nonmarginal (NM) (n=12) or marginal (M) (n=12) donors. P Tx outcome was evaluated as incidence of delayed endocrine graft function (DEGF), actuarial graft survival and complication rate.

Results One-year, 2-year and 31-month overall insulin independence rate was 92%. DEGF incidence was 8% and 17% for P Tx from NM and M donors, respectively ($p=0.5$). P Tx from either NM or M donors had an actuarial graft survival of 92%. Grafts procured from M donors aged more than 45 years had an actual survival of 100% at 29 months. Those procured from hemodynamically unstable donors had an actuarial graft survival rate of 90%. Cumulative relaparotomy rate was 12.5% (17% in NM vs 8% in M group). On the whole there was one partial portal thrombosis (NM P Tx) rescued by prompt total heparinization. No graft pancreatitis, pancreatic fistula, or peripancreatic fluid collection

occurred. Infection rate was 42% and 17% (cumulative 29%) for P Tx from NM and M donors, respectively.

Conclusions M organ donors allow a substantial increase of donor pool for P Tx that was doubled in this series. The use of a rapid en bloc technique of procurement allows us to postpone the final decision to accept a M graft for P Tx to ex-vivo evaluation of gross P texture and quality of flush, independently of donor age or hemodynamic stability. With this policy results of P Tx from NM and M organ donors do not differ significantly.

EXCELLENT OUTCOME OF RENAL TRANSPLANTATION IN TYPE II-DIABETICS

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Objectives Since the long-term outcome of renal transplantation in type II diabetics has been reported to be less favourable when compared to non-diabetics, we have retrospectively analyzed our respective patient population.

Materials and methods Between 1981 and 1998 a total of 27 kidney transplants were performed in 26 type II diabetics. 17 of them were insulin dependent [mean dosage 24.9 (10-48) units/day] prior to transplantation, 9 on oral medications. Immunosuppression consisted of cyclosporine A + prednisolone + azathioprine in 24 and of cyclosporine A + prednisolone in 3 patients.

Results Five year patient survival was 83%, graft survival 75%. We were able to control all 6 cases of acute rejection as well as all infectious complications. All patients were insulin dependent [mean dosage 36.0 (8-62) units/day] post-transplant. Within the observation period, 5 patients died (3 from cardiac failure and 2 from cerebrovascular accident), all of them with stable graft function.

Conclusions Renal transplantation seems to be an excellent option for type II diabetics if they are carefully selected with regard to their cardiovascular risk and carefully followed after transplantation with optimal control of diabetes and hypertension.

AUTO-ANTIBODY RESPONSE AFTER ISLET TRANSPLANTATION IN PATIENTS WITH TYPE 1 DIABETES

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The aim of this study was to determine whether islet transplantation (Tx) affects the humoral immune response to islet auto-antigens. Antibodies to GAD (GADA) and IA-2 (IA-2A) were measured before and after Tx in 33 cases of islet Tx performed in 28 patients with type 1 diabetes who received islet Tx together with (n=5) or after (n=25) kidney Tx or as islet Tx alone (n=3). Immunosuppression included azathioprine or mycophenolate and anti-lymphocyte globulins in all cases, associated with cyclosporin in kidney Tx cases. Complete independence of insulin therapy was obtained in 13 (39%) cases for a median of 0.8 year (range 0.1-3.6). Either GADA or IA-2A were found in 14 (42%) cases prior to Tx and did not influence graft outcome. In sequential follow-up samples after Tx, antibodies were persistently negative in 16 (48%) cases, stable positive in 2, declined in 1 and fluctuated at low titres in 6 (18%). The remaining 8 (24%) cases had increments of GADA after Tx: in 6 cases (including the 3 islet Tx alone, none receiving cyclosporin) increments were very remarkable, starting within post-transplant day 6 in 5 cases and within day 30 in 1 case, persisting at high levels for 4 to 6 weeks, progressively declining thereafter. In one other case GADA increment was early, but less intense, while in the last patient GADA increased 3.3 years after Tx. IA-2A levels significantly increased in 3 of these 8 cases, always after GADA had already peaked. Only one of these cases reached insulin independence for 2 weeks. These findings indicate that islet auto-immunity after islet Tx: 1) is

more frequent than previously observed after pancreas Tx; 2) could be a marker of insufficient immunosuppression.

HSP-70 OVEREXPRESSION REDUCES NECROSIS TRIGGERED BY INFLAMMATORY MEDIATORS BUT SIMULTANEOUSLY INCREASES CASPASE-3 LIKE ACTIVITIES

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Previous studies demonstrated that the heat-induced overexpression of HSP-70 in rat and pig islets reduces cell lysis mediated by nitric oxide and reactive oxygen. In contrast, prior heat shock reduced islet survival after transplantation into non-diabetic rats. The present study was performed to investigate the resistance of heat-exposed pig islets to cytokines and the expression of apoptotic markers after heat shock.

Methods Freshly isolated adult pig islets treated by heat shock (43°C/90 min) and subsequent overnight culture at 37°C were either subjected for 24 hours to 0.1-0.5 mM H₂O₂ and 0.2-1.2 mM sodium-nitroprusside (SNP, n=6) or treated for 48 hours with 1000 U/mL of IL-1β, IFNγ or TNFα used individually or in combinations (n=5). Viability and survival after cytotoxic treatment were determined by respectively measuring the portion of trypan-blue positive cells and the recovery of initially incubated insulin, related to corresponding parameters of heat-shocked and sham-heated islets under control conditions. Caspase-3-like activities were calculated by determination of DEVD-p-nitroaniline cleavage. To determine the recovery of initially grafted insulin during a 10 day period, heat-shocked and sham-heated islets were grafted contralaterally beneath both kidney capsules of non-diabetic C57/Bl6j mice.

Results Compared to sham-heated islets survival and viability were significantly increased (P<0.05) by prior heat shock for respectively 44% and 48% after treatment with 0.5 mM H₂O₂, and for respectively 26% and 31% after incubation in 0.8 mM SNP. HSP-70 overexpression was associated with a significant (P<0.05) increase in viability after cytokine treatment ranging from 17% to 28% depending on the cytokine combination used. Although viability was not changed by prior heat shock, caspase-3-like activities were found to be doubled in heat-exposed islets (242±22%, P<0.05). This observation might explain at least in part the reduced survival of heat-shocked islets in mice after 6 days of engraftment (P<0.05).

Conclusions The present study indicates that sublethal stress can simultaneously trigger apoptotic processes as well as defence mechanisms which are effective in prevention of necrotic cell death. Therefore, both viability and apoptotic markers should be considered in islet quality control.

CORTICOSTEROID-FREE IMMUNOSUPPRESSIVE REGIMEN AFTER RENAL TRANSPLANTATION: BENEFICIAL EFFECT OF A SHORT ATG COURSE AND DELAYED CSA INTRODUCTION

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Background Recent published data on recipients of kidney, liver and pancreas transplants indicate a 7 to 25% incidence of acute rejection. To further investigate the safety of a corticosteroid-free regimen after kidney transplantation, we conducted two prospective pilot studies differing in Thymoglobulin induction duration and post-transplant cyclosporin introduction.

Methods Between November 1998 and May 2000, 23 patients with end-stage renal failure underwent primary kidney transplantation in conjunction with a corticosteroid-free immunosuppressive regimen consisting of cyclosporin microemulsion (CsA), mycophenolate mofetil (MMF), and Thymoglobulin (ATG). The first trial included 11 patients (group 1: 9 women and 2 men; 9 cadaver and 2 living donors; no hyperimmunized patient) who received ATG every day for 10 days and CsA from postoperative day 11. The second trial included 12

patients (group 2: 5 women and 7 men; all cadaver donors; no hyperimmunized patient) who received ATG on day 0, 2, 4 and 6, and CsA on postoperative day 4. MMF was given to all 23 patients from day 0 at usual recommended doses.

Results All 23 patients were able to receive the entire 10- and 4-day ATG course. Three patients (27%) from group 1 experienced an acute serum sickness versus none from group 2 (ns). Delayed graft function was similar in both groups (18 and 17%). Biopsy proven acute rejection occurred in 3 patients (27%) from group 1 and in 2 (17%) from group 2 (ns). All these episodes were reversible. CMV infection was higher in group 1 than in group 2 (45 and 8%; ns). No PTLD was yet encountered. All 23 patients are currently alive and have functioning grafts (follow-up ranges from 3 to 21 months). Corticosteroids were given to 3 patients in each respective group because of rejection (n=4), recurrence of the negative of the native glomerulonephritis (n=1), and MMF withdrawal (n=1).

Conclusions Our observations with two different corticosteroid-free immunosuppressive regimens indicate that a short ATG induction course on alternate days, associated with a delay in CsA introduction on day 4, can result in a better patient tolerance profile and less CMV infection. The long-term impact of these regimens on graft and patients survival, as well as cardiovascular and bone morbidity, remains to be evaluated.

IMPROVEMENT OF HbA_{1c} AND BLOOD GLUCOSE STABILITY IN TYPE 1 DIABETIC PATIENTS TREATED WITH IMPLANTABLE INSULIN PUMPS IN COMPARISON WITH EXTERNAL PUMPS USING LISPRO

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Objective Short-acting insulin analogs were developed to circumvent delayed subcutaneous insulin absorption of regular insulin. Particularly in the setting of continuous subcutaneous insulin infusion (CSII) the short insulin analog lispro has been shown to provide a better glycemic control. Similarly to lispro, insulin profiles generated by implantable insulin pumps (IP) are characterized by a more rapid, sharp insulin peak early after the meal and lower fasting levels. We therefore compared the efficacy of CSII using lispro to IP in type 1 diabetic patients.

Study design and methods Fifteen type 1 diabetic patients (age 50.6±12.6 years, 6 men and 9 women, diabetes duration 28.2±12.9 years) were moved from CSII with lispro to IP. Blood glucose (BG) was monitored under routine condition first during 45 days with CSII, then during 45 days after implantation with IP. HbA_{1c} was measured at the end of each period.

Results A total of 6303 BG measurements (3123 with CSII, 3180 with IP) were recorded during the study and analyzed after a logarithmic type symmetrization. The use of IP resulted in a significant decrease in mean preprandial BG levels (139.3±19.4 vs. 146.5±21.4 mg/dl, p=0.03) while postprandial BG values were not different between the two pumps (153.2±25.7 vs. 157.6±15.14 mg/dl). Mean daily BG measurements were lower with IP at the very limit of statistical significance (147.1±18.8 vs. 152.9±16.8 mg/dl, p=0.08). The SD of all BG values was lower with IP (70.5±11.4 vs. 78.1±18.8 mg/dl, p=0.03). HbA_{1c} values were lower at the end of the IP period (7.4±0.9 vs. 7.9±0.9%, p=0.02). Low blood glucose index was comparable with the two pumps (3.1±1.4 vs. 2.8±1.5, p=0.29).

Conclusion IP seems to provide a better glycemic control and stability than CSII using lispro. However, the best way to mimic endogenous insulin secretion nowadays remains to be confirmed by a randomized prospective study.

METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASE IN RECIPIENTS OF SUCCESSFUL KIDNEY OR KIDNEY-PANCREAS TRANSPLANTATION

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Cardiovascular disease (CD) is the main cause of morbidity and mortality in patients with organ transplantation, due to several mechanisms, including the high prevalence of metabolic risk factors in these patients. We compared the prevalence of a number of risk factors for CD in 99 non-diabetic recipients of functioning (at least 6 months) kidney graft (KG, age 44–10 yrs, BMI 24.8–3.8 kg/m², M/F 75/24), and in 18 previously diabetic recipients of functioning (at least 6 months) kidney-pancreas graft (KPG, age 40–8 yrs, BMI 23.5–2.1 kg/m², M/F 12/6). The two groups were on stable and similar immunosuppressive therapy, based on prednisone and cyclosporin or tacrolimus. Total cholesterol (188–42 vs 247–52 mg/dl, $p < 0.001$), LDL-cholesterol (110–30 vs 152–40 mg/dl, $p < 0.001$), triglycerides (119–31 vs 181–88 mg/dl, $p < 0.001$) and fasting plasma glucose (88.5–10.2 vs 96.9–14, $p = 0.035$) were significantly lower in KPG than KG patients, whereas fasting C-peptide levels (6.4–3.6 vs 2.9–1.0 ng/ml, $p < 0.001$) were significantly higher in KPG patients; HDL-cholesterol (53–19 vs 57–17 mg/dl), fibrinogen (377–135 vs 358–81 mg/dl), and HbA1c (5.4–0.4 vs 5.6–0.5%) did not differ significantly between the two groups. These results show that recipients of kidney-pancreas graft, despite the previous presence of diabetes, have a post-transplant metabolic risk factor profile better than non-diabetic recipients of kidney graft.

DOES MICROENCAPSULATION PROTECT HUMAN ISLETS FROM GLUCO- AND/OR LIPOTOXICITY?

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The aim of the present study was to investigate whether microencapsulation of human islets (HI) is able to prevent the deleterious effects of prolonged exposure to glucose (G) or free fatty acids (FFA). HI were isolated by collagenase digestion and density gradient purification, and then suspended in 2% sodium alginate; beads of approximately 600 μ m diameter were obtained by converting this suspension into droplets by means of an air-driven droplet generator; the formation of a poly-L-lysine (PLL) membrane was successively induced by suspending the beads in a 0.1% PLL solution, and an outer alginate layer was finally applied by suspending the capsules in a 0.3% alginate solution. Within 7 days from the preparation, free or microencapsulated (mc) HI were incubated for 24h in the presence of either 400 mg/dl G, 0.5 mmol FFA or 1.0 mmol/l FFA, and insulin release (IR) in response to acute 3.3 and 16.7 mmol/l G stimulation was assessed. The results (mean-SD of 5 experiments) are summarized in the table and show that, as expected, free HI exposed to G or FFA had altered glucose-stimulated IR, compared to control (ctrl) HI; microencapsulation protected HI from the deleterious action of 0.5 mmol/l FFA, but not of G or 1.0 mmol/l FFA.

	Free HI			mc HI		
	400G	0.5 FFA	1.0 FFA	400G	0.5 FFA	1.0 FFA
IR (% of ctrl)						
3.3G	<u>+34-11</u>	+7-2	<u>-21-7</u>	+30-15	-2-0.4	-18-6
16.7G	<u>-57-13</u>	<u>-31-8</u>	<u>-65-19</u>	<u>-53-19</u>	-6-2	<u>-49-14</u>

The values underlined are statistically different ($p < 0.05$ or less) vs ctrl

This suggests that microencapsulation can partially prevent the phenomenon of lipotoxicity, whereas it does not seem to protect the islets from glucotoxicity.

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DETECTION OF HYPO- AND HYPERGLYCEMIA WITH THE GLUCOWATCH BIOGRAPHER

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Hypo- and hyperglycemia are common acute complications of diabetes that are difficult to detect with current infrequent blood glucose (BG) testing. The GlucoWatch (Cygnus, Inc., Redwood City, CA) biographer provides frequent, automatic and non-invasive glucose readings.

Detection of post-prandial hyperglycemia using the biographer was assessed in a home use trial in which 111 subjects with diabetes wore the device for 5 days. Hourly BG tests were done to generate 3060 paired (BG-biographer) measurements. Out of 947 post-meal periods, 456 (47%) had BG > 200 mg/dL, and 238 (24%) had BG > 250 mg/dL. To assess Biographer performance, results were analyzed using a definition of hyperglycemia as BG > 300 mg/dL. A receiver operating characteristic (ROC) curve (see figure) was prepared by determining both the sensitivity and specificity at various alert levels. For example, with an alert level set at 270 mg/dL the biographer had a sensitivity (true positive fraction) of 79% and 1-specificity (false positive fraction) of 6%. Similar results were obtained for hypoglycemia.

These results demonstrate

two important points. First, post-prandial hyperglycemia occurs commonly in diabetic subjects and often goes undetected with typical pre-meal testing. Second, high sensitivity for detection of hypo- and hyperglycemia can be achieved with the GlucoWatch biographer.

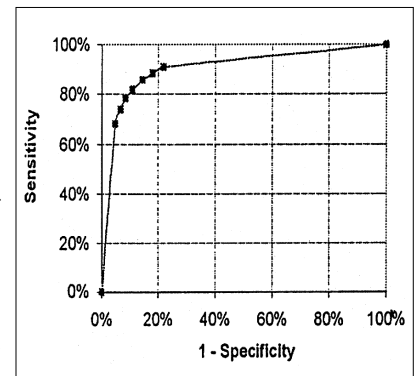


Fig. 1 ROC curve for high glucose alert.

LONG-TERM FOLLOW-UP IN 42 CONSECUTIVE KIDNEY-PANCREAS TRANSPLANTS

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Objectives Recovery from some systemic pathologies and improvement of quality of life, together with independence from dialysis and insulin therapy, are expected after simultaneous pancreas-kidney transplantation (SPK). The aim of the study is to evaluate long term results in a group of 42 patients (pts) submitted to SPK. **Material and methods** Forty-six SPK on 42 pts have been performed in our unit between October 1993 and December 1999. Mean age was 38.1 ± 7.6 years; 31 were male, 11 female; dialytic age was 21.2 ± 26 months, insulin requirement before tx was 43 ± 10 U/day; all pts required 1-4 antihypertensive drugs before tx; 13 pts were bladder drained; in 29 pts an enteric diversion was performed. All pts received quadruple immunosuppression (RATG, Azathioprine, steroids, cyclosporin) and steroid boluses or OKT3 to treat rejection episodes. Micofenolate Mofetil and FK were used as a rescue therapy during dysfunction of the graft and in all pts unresponsive to standard immunosuppressive therapy. **Results** Four pts lost their graft due to early vascular thrombosis. At 5 years pt survival is 95.2% and for free insulin pts is 83.4%. Average serum creatinine in 40 surviving pts was 1.6 ± 0.6 mg/dl with a mean follow-up of 37.9 months (range 6-79). Pancreatic function as demonstrated by HbA1c ($6.3 \pm 0.7\%$) is still good in 37 out of 40 pts. Only 5 out of 40 surviving patients required 1-2 antihypertensive drugs. So far no cases of de novo tumors were evidenced. Two pts required arterial angio-

plasty of the lower limbs 1 and 2 years after tx. Quality of life (based on questionnaire SP 36) was very satisfactory. **Conclusion** Long-term follow-up confirms that SPK is a good option in uremic patients with IDDM. Quality of life seems to be good and diabetic complications can be reduced if SPK is performed early.

HUMAN FETAL ISLET TRANSPLANTATION IN TYPE 1 DIABETICS: MULTIPLE VS. SINGLE IMPLANTATION REGIMEN

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Previous studies have suggested that a single implantation and a multiple implantation regimen might be equally efficient in human islet transplantation in type 1 diabetics. Therefore, the aim of this study was to compare metabolic effects of (a) single islet transplantation (group A) and (b) multiple islet implantation regimen (group B) in human fetal islet transplantation in type 1 diabetics (C peptide undetectable). The human fetal islets were isolated by collagenase digestion, cultured for 14 days at 37°C, 5% CO₂, and implanted under fascia of m. rectus abdominis. In group A (8 pts), 180±20x1000 islet equivalents (detected by dithionite staining) were implanted in a single injection. In group B (6 pts), the islets were implanted in 3 consecutive injections (60±10x1000 islet equivalents) at 7 day intervals. All patients were submitted to intensified insulin therapy immediately before transplantation. In each patient, we analysed: (1) insulin secretion capacity (C peptide levels determined with RIA, 0 vs 6 min after 1 mg glucagon iv), (2) metabolic control (HbA1c levels detected by chromatography) and (3) insulin daily requirements (insulin daily dose monitoring) on days -1, 30, 60, 90, 120, 150 and 180 after transplantation. We found an increase of both basal and glucagon-stimulated C peptide levels, which peaked on day 90 and then rapidly decreased by day 120 reflecting the loss of graft function, in both groups without significant differences between the groups (day 90: A: 0.31±0.12 vs 0.42±0.15 nmol/l; B: 0.28±0.15 vs 0.40±0.14 nmol/l; day 120: A: 0.14±0.10 vs 0.16±0.11 nmol/l; B: 0.13±0.10 vs 0.19±0.10 nmol/l; day 360: A: 0.05±0.05 vs 0.06±0.05 nmol/l; B: 0.05±0.05 vs 0.05±0.05 nmol/l, p=ns). Moreover, we did not detect any difference between the groups either in HbA1c levels or in insulin daily dose changes. Our results signify that both procedures of human fetal islet transplantation, the single as well as the multiple islet implantation regimen, were equally efficient in temporarily restoring a significant insulin secretion capacity in Type 1 diabetics.

REDUCTION OF ACUTE REJECTION EPISODES AFTER SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION WITH TACROLIMUS AND MYCOPHENOLATE MOFETIL

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Objectives Immunosuppression after simultaneous pancreas-kidney transplantation (SPK) has changed during the last 20 years. The aim of this retrospective study was to assess the incidence of acute rejection episodes (ARE) with two different immunosuppressive regimes. **Materials and methods** From January 1987 to July 2000 145 SPK were performed. All pancreas grafts were transplanted in pancreaticoduodenal technique by the two senior authors (U.T.H. and W.D.S.). Eighty patients (group A) received cyclosporine A (CSA), azathioprin (AZA), steroids (PRED) and a 10-14 day course of antithymocyte globulin (ATG). Immunosuppressive protocol of group B (n = 42) consisted of tacrolimus, mycophenolate mofetil (MMF), PRED and either single shot ATG or daclizumab. The remaining 23 patients were treated with CSA, MMF, PRED and ATG and were not considered in this analysis. Both groups were comparable regarding age, duration of diabetes and gender proportion. ARE were proven by histologic examina-

tion. Kidney biopsy was taken every time ARE was expected by clinical signs. **Results** One-year pancreas and kidney graft survival was 83% (group A) and 80% (group B), 86% (group A) and 90% (group B), respectively. 60% of patients of group A but only 19% of group B had one or more ARE. The difference was statistically significant (chi-square test). 22% of patients of group A but none of the patients in group B had more than one ARE during the first 12 months after transplantation. Histological signs of mild acute rejection were found in 44% of positive biopsies in group A and in 50% of group B. **Conclusions** In spite of a reduced induction therapy with only single shot ATG or daclizumab, acute rejection episodes after SPK were significantly decreased with an immunosuppressive protocol of TAC/MMF/PRED. Further studies are required to demonstrate positive impact on long-term function of pancreas and kidney transplant.

IMPROVEMENT OF THE ENCAPSULATION OF ISLETS IN SMALL-SIZED ALGINATE-MICROBEADS BY A PRECOATING PROCEDURE

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Objective Microencapsulation is one of the most powerful tools for the immunoseparation of islets in vivo. Reduction in size of microbeads offers a wide range of advantages for the encapsulated transplant, e.g. optimised oxygen- and nutrition supply, reduction in transplantation volume and the opportunity to use more favoured transplantation sites than the peritoneal cavity. Unfortunately the decrease of microbead diameter leads to a significant increase of imperfectly covered islets. Therefore, in this study we investigated a precoating procedure to improve the encapsulation of islets in small sized capsules. **Materials and methods** Isolated adult rat islets were used for microencapsulation by an air-driven droplet-generator. One fraction of the islet preparation was directly microencapsulated without a pre-treatment. The other islet-fraction was pre-coated by rotating in an alginate-solution on a spinning disc, washed several times and then gelled with Ca²⁺ afterwards. These pre-coated islets were then also microencapsulated to 300-350 µm capsules. Afterwards the microencapsulated islets were scored (**A**: central, well-covered; **B**: decentralized, well-covered; **C**: decentralized, slightly covered; **D**: decentralized, partly non-covered; **E**: non-covered islets). **Results** We showed a striking decrease of non- and partly non-covered islets by our precoating technique [Group **D** + **E** 13% vs. 51%]. Furthermore a significant increase in well encapsulated islets could be detected [Group **A+B** 62% vs. 27%].

	A	B	C	D	E	
Non-treated I.	10.7±5.5	16.5±2.5	22.0±3.4	27.0±2.0	23.8±9.4	%±SD
Pre-coated I.	29.6±0.6	32.4±0.5	24.9±4.4	8.4±6.2	4.7±1.7	%±SD

Our pre-coating technique has no negative side-effects on vitality and insulin-secretion in vitro. **Conclusion** For a long-term survival of microencapsulated islets in vivo a completely covered tissue is obligate. This study indicates that our pre-coating procedure is a safe and powerful tool to improve small-sized microencapsulation of islets.

USEFULNESS OF TC-99 MIBI GAMMAGRAPHY IN THE CLINICAL NON-INVASIVE EVALUATION OF SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTS

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Objectives Despite recent improvements achieved in immunosuppression and surgical technique of simultaneous pancreas-kidney (SPK) transplantation, rejection and surgical complications rates are still very high, most of them occurring during the first 3 months post-transplant.

The recent re-introduction of enteric drainage of exocrine secretions in SPK has reduced urological complications but prevent us measuring urinary amylase, one of the best markers of rejection. In this setting, its diagnosis depends on serum creatinine (Cr), amylase (Am) and lipase (Lp) and CT-guided pancreas biopsy. Simultaneous kidney and pancreas rejection does not always occur, serum levels of Cr, Am and Lp are non-specific, and percutaneous CT-guided biopsy is an invasive procedure associated with several complications and not always possible to perform. We present our experience obtained in the clinical non-invasive evaluation of SPK patients with the use of Tc-99 MIBI gammagraphy, Doppler Ultrasound (DU) and serum levels of Cr, Am and Lp.

Materials and methods Twenty MIBI nuclear scintigraphy scans were performed on two SPK patients. The grafts were placed intraperitoneally at the right iliac fossa, and both of them were enteric drained. The nuclear scans were indicated as part of a protocol evaluation (2nd, 5th p.o.d. and at the moment of discharge from the hospital), and whenever clinically necessary. The patients received 15 to 25 mCi of MIBI, having obtained static and dynamic images. Time to peak flow in the graft (in comparison with aorta-iliac segment) and washout were measured using time-activity curves. Static images were examined for extent of uptake, homogeneity and washout. The patients underwent DU after scintigraphic evaluation, the patency of the vascular flow and the resistive index (RI) having been measured. Serum levels of Cr, Am and Lp were obtained.

Results We found four main patterns. Normal pattern is characterized by normal levels of Cr, Am and Lp, with DU-RI less than 0.7 and with a peak activity less than 2 seconds, good definition and homogeneous graft uptake and rapid washout at scintigraphy. Pancreatitis pattern is characterized by high levels of Am and Lp but normal levels of Cr, with increased DU-RI (>0.7). Scintigraphy shows peak activity of more than 4 seconds with a slow washout and poor and non-homogeneous (patched) graft definition. Rejection pancreatitis pattern is characterized by high serum levels of Cr, Am and Lp, with DU-RI>0.7 and a decreased uptake and slow washout with poor homogeneous graft definition at scintigraphy. Finally, surgical pancreatitis pattern is characterized by normal Cr levels with high levels of Am and Lp, DU-RI between 0.5-0.7 with a peak activity between 2 and 4 seconds and a localized graft indefiniteness with near-normal washout.

Conclusions The use of TC-99 MIBI scintigraphy with other imaging modalities (DU) is useful in the clinical non-invasive evaluation of SPK patients.

THE REACTION AGAINST TRANSPLANTED MICROCAPSULES IS DEPENDENT ON THE RECIPIENT'S RAT STRAIN

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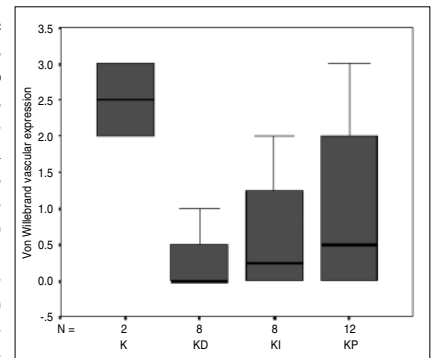
Alginate microencapsulation allows for successful transplantation of xenogenic islets but graft function is limited by fibrosis, the consequence of a non-specific body reaction against the microcapsules. The aim of the present study was to investigate whether capsular overgrowth on alginate microcapsules is dependent on the presence of islet tissue and on the recipient's strain. Microcapsules containing bovine islets (1000 islet equivalents) or empty microcapsules were implanted in the peritoneal cavity of streptozotocin-diabetic Sprague-Dawley (CD) and Munich Wistar Frömter (MWF) rats. Recovered microcapsules were analysed under light microscopy using paraffin-embedding and hematoxylin-eosin staining to estimate the percentage of capsules with fibrotic overgrowth. In CD rats the mean percentage of explanted capsules containing islets significantly decreased with implantation time (61±1.9%, 45±6.6% and 20±5.5% at 2, 4 and 10 weeks, respectively; p<0.01, 10 vs. 2 weeks) while in MWF rats the percentage of recovered capsules did not change significantly with implant time (64.7±4.5%, 52±6.6% and 44.3±8% at 2, 4 and 10 weeks, respectively). We found no statistically significant difference in percentage of recovered microcapsules between those containing pancreatic islets and empty microcapsules. In CD rats at 2 weeks the percentage of complete overgrowth around capsules averaged 67±33% in microcapsules containing islets and 37±31% in empty capsules. At 4 and 10 weeks in both

capsules with and without islets complete overgrowth reached 100%. At variance, in MWF rats at 2 weeks only 2±1% of recovered microencapsulated islets showed complete overgrowth (p<0.05 vs. CD at the same time), 5±2% showed partial overgrowth and in 92±3% of the capsules the external surfaces were free of pericapsular cellular infiltrate. At 4 and 10 weeks the percentage of capsules with complete overgrowth reached 53±26% (p<0.01 vs. CD at the same time) and 63±26% respectively. Comparable results were obtained in empty capsules. Our data suggest that fibrotic overgrowth is the consequence of a foreign body reaction that is not dependent on the presence of islet tissue. Moreover, the reaction against the capsule is rather dependent on the recipient's strain. MWF rats are a more suitable model for islet transplantation investigation with alginate microcapsules.

EFFECTS OF LONG-TERM EUGLYCEMIA ON VON WILLEBRAND AND ENDOTHELIN-1 VASCULAR EXPRESSION IN UREMIC TYPE 1 DIABETIC PATIENTS AFTER SUCCESSFUL KIDNEY-PANCREAS TRANSPLANTATION

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Recent studies have shown that successful kidney-pancreas transplantation could improve endothelial function and reduce atherosclerosis progression. Our aim was to evaluate if long term euglycemia could have positive effects on vascular expression of molecules known as markers of endothelial vitality and function. All the patients considered underwent a skin biopsy to assess vascular expression of von Willebrand factor (vWF) and endothelin-1 (ET-1). An immunohistochemistry study has been done, with evaluation of vWF and ET-1 expression. 30 patients were considered in our study, 28 of them were uremic type 1 diabetes mellitus patients who underwent surgery as follows: 12 kidney-pancreas transplantation (KP), 8 kidney-islets transplantation (KI), 8 kidney-alone (KD) transplantation. Moreover, 2 uremic non-diabetic patients (K) were considered as controls. The mean follow-up was 3.2±0.7 yrs for



K, 4.8±1.4 yrs for KD, 4.5±1.2 yrs for KI and 4.0±1.0 for KP. None of the patients presented ET-1 vascular expression, while vWF was expressed differently. vWF vascular expression was statistically higher in K (2.5±0.5) rather than in KI (0.62±0.30, p<0.05 vs K) and KD (0.50±0.30, p<0.05 vs K), but not in KP (0.91±0.31). Uremic type 1 diabetic patients presented a reduced vWF vascular expression rather than uremic non-diabetic. Kidney-pancreas transplantation seems to prevent vWF vascular depletion which is evident in the progression of diabetic vasculopathy.

ISLET TRANSPLANTATION IMPROVES NA⁺-K⁺-ATPASE ACTIVITY AND INCREASES SODIUM HANDLING IN UREMIC IDDM KIDNEY TRANSPLANTED PATIENTS

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The activity of Na-K-ATPase in the kidney is involved in the modulation of natriuresis, as more than 50% of renal sodium handling is mediated by this membrane transport. Type 1 diabetes mellitus is characterized by an impaired Na-K-ATPase activity, possibly because of the reduced secretion of C-peptide, known to activate the Na pump. Islet transplantation has been recently suggested as an alternative tool to vas-

cularized pancreas transplantation. Our aim was to assess the effects of C-peptide secretion obtained with islet transplantation on Na-K-ATPase, on sodium handling and on renal function in uremic type 1 diabetic patients. Sixteen uremic type 1 diabetic patients submitted to islets-kidney transplantation were enrolled. Patients were divided according to success of islets transplantation (C-peptide > 0.5 ng/ml) in KI (kidney-islet: 9 patients) and in KA (kidney-alone islet failure: 7 patients). The following parameters were assessed: HbA1c, C-peptide, insulin levels, insulin requirements, total cholesterol, triglycerides, emochrome, creatinine, cyclosporine, liver function, urinary albumin excretion rate, plasmatic and urinary electrolytes (Na and K), urinary fractional sodium excretion (FeNa) and urinary 24h excretion (UNaV). Finally, Na-K-ATPase activity was assessed in the red blood cell as ouabain-sensitive Na efflux from Na-loaded cells. Patients with a long term partial function of transplanted islet showed a better glycometabolic control, with a partial improvement of glycated hemoglobin. In KI group mean C-peptide secretion was higher than in the KA group. Almost all the patients in KA group lost islet function during the first 6 months after islet transplantation, while in KI group C-peptide secretion persisted higher than 1 ng/ml for the whole follow up. No differences were evident as regards creatinine or creatinine clearance at 1, 2 and 4 years. The mean dosage of furosemide was similar in the 2 groups of patients (KI=12.5±8.5 vs KA=18.6±8.3 mg/die, ns) for the whole follow up. Na-K-ATPase assessed in red blood cells was statistically higher in KI patients rather than in KA pts (KI=4.29±0.30 vs KA=3.31±0.21 mmol/Na/L cell/hour, t=1.9, p=0.03). Moreover, Na-K-ATPase activity is positively correlated with C-peptide/creatinine values in KI patients (r=0.82, p=0.006). In KI group a progressive reduction in natriuresis was evident, particularly after 4 years of follow up. In KI group, but not in KA, UNaV showed a decrease of 21.2 mmol/24h at 2nd year and of 74.6 mmol/24h at 4th year (p<0.05), with an analogue reduction in FeNa (see Table). Our study showed that islet transplantation, by restoring partial C-peptide secretion, improved glycometabolic control. Moreover an increase of red blood cells Na-K-ATPase activity, with reduction of urinary fractional sodium excretion and sodium excretion rate in uremic type 1 kidney transplanted patients could be obtained. Whether islet transplantation might restore Na-K-ATPase activity at multiple levels with potential beneficial effects on long term diabetic complications remains to be evaluated by further studies.

FeNa(%)	Basal	4 years	P value
KI	1.44%	1.12%	<0.05
KA	1.44%	1.45%	ns

LONG TERM ISLET FUNCTION COULD IMPROVE ACTUARIAL SURVIVAL AND CARDIOVASCULAR OUTCOME IN UREMIC IDDM KIDNEY TRANSPLANTED PATIENTS

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Recent studies have shown that C-peptide administration in type 1 diabetic rats could improve neural and vascular function. Our aim was to evaluate whether partial function of transplanted islet, established on the basis of a long term C-peptide secretion higher than 0.5 ng/ml, could have effects on actuarial survival, cardiovascular death rate and cardiac function. Patients were enrolled from our waiting list of uremic IDDM. Thirty-two type 1 uremic patients underwent kidney transplantation according to HLA match. Kaplan-Meier actuarial survival of the two populations and Cox regression analysis were performed. Cardiovascular death causes were considered in our analysis (sudden death, myocardial infarction, arrhythmias). All the patients underwent a myocardial scintigraphy at baseline and at each year of follow up. Patients were divided according to the presence of partial islet function, i.e. C-peptide secretion > 0.5 ng/ml (group KI, n=20), regardless of insulin independence or metabolic control, and without islet function (group KA, n=12). Only patients with at least 1 year of follow up were considered. KI patients showed a better survival than KA pts.

Moreover, a partial improvement of glycated hemoglobin was evident in KI, though not significant. Survival at 1, 4 and 7 years was in KA group 91.6%, 82% and 72%, respectively, while in KI group it was 100%, 100% and 90%. Furthermore, no differences were evident as regards creatinine levels at 1, 4 and 7 years (1 yr: KA=1.24±0.18 vs KI=1.30±0.12, ns; 4 yrs: KA=1.66±0.43 vs KI=1.46±0.17, ns; 7 yrs: KA=1.71±0.67 vs KI=1.54±0.25, ns). Cardiovascular death rate (according to ICD) was higher in KA group than in KI group [KI=1 (after 7 years) vs KA=4, p=0.05, X²=2.6]. We can observe an improvement of ejection fraction in the KI group (from 70% at baseline to 78% after 2 years) while in KA group EF declines from 73 to 69%. Moreover, indexes of diastolic function improved in KI group but not in the KA group: in KI peak filling rate improved from 3.9 to 4.1 EDV/sec after 2 years, while it declined from 4.2 to 3.5 EDV/sec in KA group. Time to peak filling rate (tPFR) worsened in KA from 153 to 183 msec after 2 years, while in KI it remained stable (from 160 to 170 msec). Finally, diastolic ratio, an index of global left ventricular function, remained stable in KI (from 1 to 0.9) while it worsened in KA (from 1.2 to 0.9) after 2 years. Our study showed that restoring of partial islet function with C-peptide secretion could improve survival, reduce cardiovascular death rate, and ameliorate cardiac function in type 1 diabetic patients.

MULTIVARIATE ANALYSIS OF MAJOR PRE-TRANSPLANT RISK FACTORS FOR PATIENTS: KIDNEY AND PANCREAS SURVIVAL IN KIDNEY-PANCREAS TRANSPLANTED PATIENTS

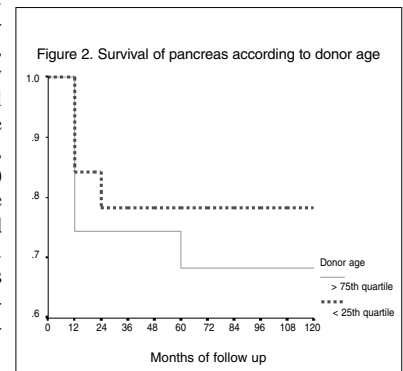
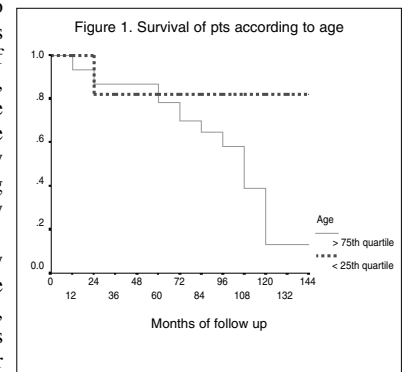
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One hundred and thirty-three uremic type 1 patients were enrolled on a waiting list for kidney-pancreas transplantation. Actuarial survival and causes of death were recorded over a period of 7 years. 7 year survival rate was 76.2% for kidney-pancreas transplants, 63.5% for kidney alone, while it was 39.6% for the dialysed group (p=0.001). A multivariate analysis has been done to assess which risk factors

(among them: duration of IDDM, duration of dialysis, donor age, age of the patients and ischemia time of the graft) are principally involved in conditioning survival of patients, kidney and pancreas respectively. Patient survival is mainly influenced by age of the patients (p=0.008, r=0.15), but this correlation was smoothed if corrected for donor age (Figure 1).

Kidney survival is influenced mainly by donor age, even if not statistically (p=0.14). Pancreas survival is mainly influenced by age of the patients (p=0.05), duration of IDDM (p=0.05) and ischemia time (p=0.03), even if corrected for all the other factors. Quintiles analysis allows us to appreciate the importance of donor age for pancreas survival (Figure 2).

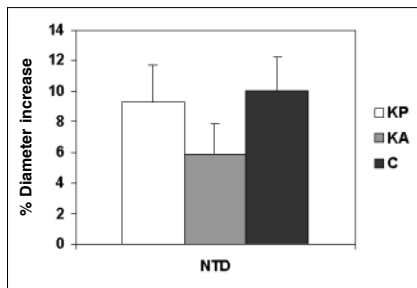
Age of the patients, donor age and ischemia time need to be carefully evaluated when choosing a candidate for transplant.



NITRIC OXIDE RESISTANCE IS EVIDENT IN KIDNEY ALONE BUT NOT IN KIDNEY-PANCREAS TRANSPLANTED PATIENTS

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A reduced dilation to nitrates challenge is likely to be associated with nitric oxide (NO) resistance. This is a well established fact associated with diabetes mellitus and related conditions. Little is known about the effects of kidney-pancreas (KP) or kidney alone transplantation (KA) regarding these conditions in uremic type 1 diabetic patients after transplantation. We assessed 30 uremic type 1 diabetic patients who underwent KA (n=14) or KP (n=30) tx. They underwent nitrates challenge with assessment of brachial artery dilation as diameter increase. A challenge with 0.4 mg nitroglycerin sublingual was given. Furthermore in basal condition and after 5 minutes a Doppler-echography evaluation was carried out. In kidney-pancreas transplanted patients a higher nitrates dilation was obtained ($8.5 \pm 1.1\%$) as compared to kidney-alone patients ($5.5 \pm 1.7\%$). This partially reduced nitrates dilation could be explained on the basis of a nitric oxide resistance present in uremia and type 1 diabetes mellitus, which could be partially overwhelmed after KP transplant, though not wholly, but not with kidney-alone transplant. This reduced dilation, could be important in establishing nitrate therapy, during angina and as protection against acute diastolic dysfunction and pulmonary oedema, which are frequent in transplanted patients due to overt cardiovascular dysfunction. This reduced dilation seems to be related to hyperglycaemia and hyperhomocysteinemia, which are better after KP transplant. Moreover, calcium overload, present in uremic patients, and not completely reversed by transplantation, could be the cause of this resistance.



ISLET AUTOTRANSPLANTATION COMBINED WITH TOTAL PANCREATECTOMY FOR TREATMENT OF PANCREATIC ADENO-CARCINOMA

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Total or near-total (>95%) pancreatectomy (TP) often results in unstable diabetes. Islet autotransplantation (auto-Tx) after major pancreatic resection for chronic pancreatitis has offered the potential to prevent this difficult metabolic disorder. The fear of infusion of occult carcinoma cells has been hampering this treatment in patients with pancreas adenocarcinoma (PA) even though TP may increase resectability to local radicality. We herein report three cases of non-diabetic patients, who underwent TP combined with islet auto-Tx in emergency operations following histologically proved R₀-resection for PA. Islets were isolated from the excised pancreata in a continuous digestion filtration device. The resultant preparation was injected into the portal vein. Metabolic evaluation included basal and stimulated C-peptide after oral glucose tolerance test (oGTT) and glucagon stimulation (GS). At 1 year follow-up the fasting C-peptide level from the first patient (63-year-old, male, pT₃pN₁G₂) was 0.66 ng/ml, the peak value in response to oGTT was 0.87 ng/ml and 0.84 ng/ml after GS. Although, the patient still remained insulin-dependent (14 U/d) he was metabolically very stable (HbA_{1c}: 5.7%). Ca19-9 was 13.1 U/ml and CEA 5.7 ng/ml. K-ras mutations were not detected. CT imaging revealed neither local tumor recurrence nor liver metastases. The second patient (52-year-old, male, pT₂pN₀G₃) had a similar stable metabolic situation at 6-month follow-up. Despite disturbed glucose tolerance he was well and gaining weight. The third patient (63-year-old, female, pT₃pN₂G₃) died at the

5th p.o. day due to septic complications not related to the islet auto-Tx. The stable metabolic situation of the first two cases indicates functioning intrahepatic β-cells or the potential effect of counterregulatory β-cell secretion. We assume the resected parts of all pancreata were free of carcinoma cells. Histology and detection of K-ras mutations might be useful techniques to prevent carcinoma cell contamination. Studies on strategies to exclude possible contamination of islet tissue with carcinoma cells are critically important. However, islet auto-Tx after TP for adenocarcinoma should currently only be regarded for rescue therapy.

ANTITUMORIGENIC ACTIVITY OF CALCITRIOL ON INSULINOMA CELLS AND SOLID β-CELL TUMORS

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Over the past decade it has become evident that calcitriol, the active form of vitamin D, exhibits antiproliferative activities and stimulates apoptosis in a variety of tumorigenic cell lines. These evidences raised the possibility that calcitriol, besides its classical use in osteoporosis and renal osteodystrophy, might be useful in the treatment of a wide spectrum of malignancies. Malignant insulinoma is a rare form of cancer with poor prognosis and a reported 5-year survival rate of 35%. Relatively little is known about the oncogenes and tumor suppressor genes that participate in its genesis and progression although strong immunoreactivity for c-myc, TGF-α, N-ras, K-ras and p53 has been reported. We examined the antitumorigenic properties of calcitriol on the murine β cell line βTC₃, and the effect of calcitriol administration into recombinant insulin/SV40 oncogene-expressing transgenic mice (RIP1Tag2), which heritably develop pancreatic β cell solid tumors. We demonstrate that calcitriol induces *in-vitro* dose-dependent inhibition of cell growth, apoptosis, and downregulation of insulin gene expression in tumorigenic pancreatic βTC₃ cells. Calcitriol-induced βTC₃ cell death is associated with modest increases in caspase-3 and nitric oxide synthetase activities. Partial (90%) prevention of calcitriol-induced cytotoxicity is obtained with protein kinase C (staurosporine) and MEK (UO126) inhibitors, but not with Verapamil, Forskolin, Wortmannin and N-methylarginine. Over-expression of Bcl-2 completely prevents calcitriol-induced cell death. A short course of calcitriol treatment of transgenic RIP1Tag2 mice causes a significant reduction in the volume of the β cell tumors consequent to an increased rate of apoptotic cell death. These results provide the rationale for testing the efficacy of calcitriol in the treatment of malignant insulinomas in the clinical setting.

SURGICAL COMPLICATIONS ARE THE MAIN CAUSE OF GRAFT LOSS IN SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANTATION

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Objectives According to the International Pancreas Registry even if the incidence of technical failure and rejection in pancreas transplantation have both decreased in these last few years, the surgical complications continue to occur at high rate compared to other organ transplants and still remain the main cause of graft loss. **Material and methods** Between October 1993 and December 1999, 46 simultaneous pancreas-kidney transplants (SPK) have been performed in our unit on 42 patients (pts). Thirteen pts were bladder drained (group A); 29 had an enteric diversion, 9 of them had a side to side duodeno-ileal anastomosis (group B) and in the remaining 20 a Roux en Y anastomosis with a head-up position was used (group C). All pts received cyclosporine

based immunosuppression and steroid boluses or OKT3 to treat rejection episodes. **Results** Out of 42 SPK, we lost 5 (11.9%) graft due to technical complications. Four were lost due to a vascular thrombosis; among them 3 pts belonged to group B and 1 to group A. One graft was lost due to a duodeno-ileal fistula followed by an acute graft pancreatitis (group C). Patients that lost their graft because of vascular thrombosis were all retransplanted but 1 patient died in first post-operative day due to a massive pulmonary embolism; 2 lost their pancreatic function within 1 year and 1 within 2 years due to chronic rejection. The overall acute rejection incidence is 57.14% but all the episodes were successfully treated and none of the graft was lost. **Conclusion** So far in our experience technical failure remains the only cause of graft loss in SPK and early venous thrombosis is the most frequent post-operative complication. Pancreas retransplantation does not seem an advisable rescue procedure as late new graft rejection seems unavoidable.

CSII IN A PATIENT WITH PARTIAL ENDOCRINE PANCREATIC GRAFT FUNCTION

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Failure of pancreatic graft function occurs in approximately 15% and 25% of recipients after respectively 1 and 5 years from implantation, and usually requires exogenous insulin administration. Little information is available on the use of CSII in such patients. We report on DG, a young female patient, who was diabetic since the age of 10, developed chronic renal failure, and started dialysis treatment at the age of 25. When she was 26, she received combined pancreas-kidney transplantation, with satisfactory kidney function (creatinin levels less than 1.8 mg/dl), but insufficient endocrine pancreas function (stimulated C-peptide levels less than 2.5 ng/ml, fasting plasma glucose levels higher than 160 mg/dl, HbA1c higher than 7% at 3 months from grafting). This required exogenous insulin administration, and at 6 months from transplantation we registered the following: daily insulin dose 36 IU, fasting plasma glucose 183 mg/dl, HbA1c 9.0%. CSII treatment was started, by which a marked improvement of diabetes control was achieved, as proved by the successive clinical evaluations (data at 3 months from CSII starting: daily insulin dose, 31 IU; fasting plasma glucose, 138 mg/dl; HbA1c, 7.2%). No sign of local infection was observed at the site of needle placing, and no episode of hypoglycemia was reported. Due to technical failure of the pump, insulin injection therapy was restarted and maintained for 2 months, at the end of which the following values were recorded: daily insulin dose, 37 IU; fasting plasma glucose, 161 mg/dl; HbA1c, 8.6%. These findings suggest that CSII therapy can be considered an effective and safe therapy for the treatment of diabetic patients with endocrine pancreatic graft failure.

DISCARDED EXOCRINE TISSUE MAY BE A POTENTIAL SOURCE OF HUMAN PANCREATIC PRECURSORS

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As initially suggested in 1996, the "proof of principle" of endocrine differentiation of human ductal cells was recently demonstrated. Optimization of the source, expansion, and differentiation are required prior to clinical relevance. Regenerating rat ductal epithelium upregulates IPF-1/PDX-1 which is a prerequisite to its differentiation into endocrine islets. The goal of this study was to characterize an abundant cytokeratin 19 positive/IPF-1 positive precursor cell that could rapidly be obtained from healthy cadaveric donor tissue ex-vivo. **Methods** Nonpathological (n=29) and pathological cases (n=5 with nesidioblastosis) were examined with immunohistochemistry. Discarded human exocrine fractions were recovered, washed, and cultured in DMEM 10% FCS, 1% ITS, 50 µg/ml G418. **Results** In nonpathological pan-

creatic specimens, ≥ 1 IPF-1 positive cell was detected in 93+2% of main, inter and intralobular ducts; ductal IPF-1 immunolabeling was fainter than in adjacent islets. Pathological specimens with nesidioblastosis undergoing islet neogenesis from ductal cells demonstrated intense IPF-1 immunolabeling in ductal structures and in some acinar cells. Human exocrine preparations composed of 61% amylase positive cells and 30-35% ductal CK19 positive cells took on a ductal phenotype after 5 days' culture. A 92 ± 3.3 loss of amylase protein (n=4, p<0.05 vs. day 1) was detected after 3 days' culture with a simultaneous increase of ductal cytokeratin 19 protein (n=4, 3.4 fold on day 3, and 7 fold on day 9, p<0.05 vs. day 1). A 3.2 fold increase in IPF-1 protein was observed at two days of culture, corresponding to the characteristic 46 kD protein in Western blots. RT-PCR confirmed a 10.5 fold increase in IPF-1 mRNA (day 3, n=5, p<0.001 vs. day 1). Double immunocytochemistry directly confirms that IPF-1 appeared during culture in these exocrine derived ductal cells (cytokeratin 7.19 positive). Exocrine pellets yield ($1.7 \pm 0.5 \times 10^9$) viable, CK19 positive ductal cells. **Conclusion** We have shown that re-expression of IPF-1 can be obtained ex vivo from exocrine preparations isolated from healthy donors. This IPF-1 expression in exocrine derived ductal cells may suggest their endocrine precursor potential, as suggested in rodents. Similar intense labeling of IPF-1 in ducts of pancreases with nesidioblastosis and overt hyperinsulinism further supports the clinical relevance of this hypothesis. This model is clinically relevant since partial pancreatectomy can be performed with little morbidity, offering a realistic perspective for autologous somatic cell therapy of IDDM.

DNase ACTIVITY IN MONOCYTES AND T LYMPHOCYTES FROM DM1 PATIENTS AS A POSSIBLE MEDIATOR OF BETA-CELL APOPTOSIS

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DNA fragmentation is a hallmark of apoptosis, executed by selectively activated endonucleases (DNases). The aim of this work was to study whether the granule-associated DNase activity is present in monocytes and T lymphocytes from diabetes mellitus type 1 (DM1) patients and the associated phenotypic characteristics in these cells. DNase activity was determined by the capacity of extracts from isolated secretion granules to degrade protein-free genomic DNA; phenotypic characteristics of mononuclear cells from 20 patients with DM1 and 20 healthy donors were determined by flow cytometry analysis. DNase activity was higher in DM1 patients than in healthy subjects (p<0.05). Data permitted us to distinguish two distinct lymphomononuclear profiles that are associated with clearly different DNase activity. Group A is characterised by a higher proportion (p<0.05) of monocytes (CD14⁺ cells), B cells (CD19⁺ cells) and activated B cells (CD19⁺CD5⁺ cells) and a lower proportion of suppressor cells (CD4⁺CD45RO⁺) than healthy donors and patients of group B. DNase activity in granule-associated monocytes of group B is higher (p<0.05) than in those of group A. This DNase activity could play a key role in the lysis or apoptosis mediated by cytolytic cells conferring cytotoxic capacity to infiltrating monocytes and T lymphocytes which could in turn display their destructive potential against pancreatic β cells.

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INDUCTION OF T CELL-MEDIATED TRANSFERABLE TRANSPLANTATION TOLERANCE TO ISLET ALLOGRAFTS

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1,25-Dihydroxyvitamin D₃ [1,25(OH)₂D₃], the active form of vitamin D₃, is a potent immunomodulatory agent able to modulate APC function and to induce dendritic cells (DCs) with a tolerogenic phenotype.

We have tested the capacity of 1,25(OH)₂D₃, administered alone or in combination with mycophenolate mofetil (MMF) - a selective inhibitor of T and B cell proliferation - to induce tolerance to fully mismatched pancreatic allografts in mice. A short treatment with these agents induces tolerance to islet allografts that is stable to challenge with donor-type spleen cells and allows acceptance of donor-type vascularized heart graft. Tolerance is associated with massive peri-transplant lymphomononuclear cell recruitment. The recruited cells fail to infiltrate the transplanted islets and are characterized by CD4⁺ cells with a memory resting phenotype and a reduced proportion of antigen-presenting cells, expressing down-regulated costimulatory molecules. CD4⁺ T cells from tolerant mice transfer long-term protection to islet grafts with an allo-antigen-specific active tolerogenic mechanism, leading to impaired development of IFN- γ -producing type 1 CD4⁺ and CD8⁺ cells in syngeneic recipients. Recipient mice show a two-fold increased percentage of CD4⁺CD25⁺ regulatory cells surrounding the tolerated, compared to rejected, islet grafts. Consistent with the increased percentage of CD4⁺CD25⁺ regulatory cells, CD4⁺ T cells from mice rendered tolerant by adoptive transfer of CD4⁺ cells fail to proliferate in response to donor-type APCs and also show a reduced response to third-party APCs. These results suggest that a short-term treatment with 1,25(OH)₂D₃ down-regulates the expression of costimulatory molecules on APCs, leading to peri-graft accumulation of CD4⁺CD25⁺ regulatory T cells. The possibility that this cell population mediates tolerance to islet allografts is currently being tested.

THE VISCOMETRIC GLUCOSE SENSOR FOR THE INTEGRATION INTO A CONTROL LOOP

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Objectives The goal was to develop a system which meets the prerequisites to be included into an automated glucose control set-up:

- Minimally invasive glucose monitoring system
- Components integrated to handy and wearable system size
- Reliable, stable sensor signal during a several days
- Avoidance of re-calibration during the monitoring
- Instant warning of critical glucose concentrations (hypo and hyper)

Such a system has not been available up to now.

Materials and methods An affinity reaction between lectin ConA and glucose modulates the viscosity of a perfused sensitive liquid. After the in-vitro calibration microdialysis sampling is performed in the subcutaneous tissue with a flux of 5 μ L/h leading to a complete equilibration. Differential pressure measurements generate the signal in situ.

Results A type 1 diabetic patient monitored his glucose level subcutaneously for 48 h. The patient is leading a normal life. Repeated SBGM determinations during the trial proved excellent coincidence ($r^2=0.83$) with the sensor signal without any correction of the data for the time delay. No re-calibration was necessary because the stability remained unchanged. An otherwise unnoticed hypo during sleep was detected by the sensor system, and the patient was alarmed to ingest carbohydrates after SBGM control.

Conclusions The viscometric sensor offers advantages compared to sensor systems based on enzymatic detection principles. It allows for glucose on-line monitoring with immediate warning. On-line evaluation of the data could be used to improve glycaemic control by automated adaptation of insulin requirement to a patient's actual need.

HYPEROXIA POST-TRANSPLANTATION IMPROVES THE SURVIVAL OF INTRAPORTALLY TRANSPLANTED ISLET GRAFTS IN DIABETIC RATS

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Hypoxia in the portal vein may compromise the survival of intraportally transplanted pancreatic islets. We therefore compared the outcome of

intraportal islet transplantation in rats housed under hyperoxic or normoxic conditions post transplantation. Diabetes was induced in male Lewis rats by i.p. injection of streptozotocin (55 mg/kg) and confirmed after 3-4 days by plasma glucose determination (33.2 \pm 1.4 vs 9.5 mmol/l in controls). After 7 days 500, 700 or 1000 islets were transplanted into the liver via the portal vein and the animals housed for 48 hours under hyperoxic (100% O₂) or normoxic (21% O₂) conditions.

In normoxic diabetic rats, the smallest graft size to consistently restore normoglycemia measured at 6 weeks after transplantation was 1000 islets (non-fasting plasma glucose, 9.8 \pm 0.8 mmol/l). In contrast in hyperoxically housed rats, a graft size of 700 islets restored normoglycemia in 8/9 animals compared to only 1/7 in normoxic animals (plasma glucose, 10.5 \pm 2.6 vs 28.7 \pm 4.1 in normoxic rats, $p<0.05$). Even a graft of 500 islets restored normoglycemia in 5/8 hyperoxically housed animals compared to 0/7 in normoxically housed rats. The glucose tolerance of the hyperoxically treated rats receiving 700 islets was similar to that of normoxic animals receiving 1000 islets; the AUCs were 1129 \pm 105 and 1263 \pm 69 mmol/120 min in hyperoxic and normoxic animals respectively, compared with 650 \pm 50 mmol/120 min in non-diabetic controls. The islet-cell mass was quantified morphometrically in liver sections collected post-mortem after 6 weeks. The total islet area in hyperoxically treated rats receiving 700 islets was not significantly different from normoxic recipients of 1000 islets and was 534, 510 \pm 62, 110 μ m² (compared to 660, 237 \pm 99, 320 μ m² in normoxic animals). The average size of the islets was the same. These results indicate that hyperoxia post-transplantation increases the number of islets that survive the engraftment process and allows normalisation of plasma glucose levels with a smaller number of transplanted islets.

XENOTRANSPLANTATION ON THE ARENA OF A UNIVERSITY HOSPITAL

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Objectives To evaluate, from cultural and ethical viewpoints, the way xenotransplantation and especially the transplantation of porcine islets is perceived among medical personnel. We used free interviews in order to catch the cultural ambivalences that are characteristic of the perception of xenotransplantation. **Material and methods** Free interviews have been done with 20 medical personnel at Huddinge Hospital in Sweden on the subject of possible clinical xenotransplantation. The interviewees were: seven nurses on a transplantation ward, six nurses with experience of working with diabetic patients but not within transplantation, five researchers/doctors within (xeno)transplantation, two individuals preoccupied only with research. **Results** The interviewees and their attitudes of xenotransplantation are viewed as being a part of a morally charged arena where different groups and individuals interact with each other on the basis of what is called "the reality of illness". Such an arena is characterized by its close contacts between different social actors as well as by a specific kind of engagement where the patients, the individuals carrying the illness, are in the centre. The arena creates, from a normative point of view, a culturally related desire for overcoming diseases, namely a desire for realizing among the patients what everybody agrees is a healthy and normal life. Under influence of this arena the medical personnel view the possibility of clinical xenotransplantation in a rather positive manner. Still, their participation in the context of the university hospital has the effect that their opinions on xenotransplantation are permeated by ambivalences rather than strong opinions. **Conclusion** The method of free interviews has shown the fine nuances of the matter of xenotransplantation. It has uncovered the cultural ambivalences that are present even among people working with or in close connection to the development of xenotransplantation. The method of free interviews is therefore an important tool for understanding the ethical charges of xenotransplantation and the reason why people are prepared to accept the transgression of the border between man and animal.

SINGLE DONOR ISLET ALLOTRANSPLANTATION IN THE STZ-DIABETIC RAT

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In both clinical and rat islet allotransplantation, islets from more than one donor pancreas are usually needed to achieve normalisation of blood glucose in the islet recipients. This study aimed to define whether optimised pre-transplant islet culture conditions may reduce the number of islets necessary for successful islet transplantation in the MHC-incompatible Lewis-to-Wistar Furth rat combination.

Lewis rat islets were isolated under low-endotoxin conditions and cultured in moistured air in carbonate-free TCM-199/5% fetal calf serum for one day at 37°C, followed by one day culture at 22°C. This temperature regimen was chosen because it combines the advantages of 37°C cultured islets (matrix regeneration, enhanced islet cell resistance to damage by cytokines, and oxygen or nitrogen radicals) with that of 22°C cultured islets (reduced capacity for Th-1 cytokine mRNA induction at the graft site). Islet quality was proven by the near complete absence of trypan blue stainable (lytic) or annexin-binding (apoptotic) cells, and an insulin secretion index >10 for incubations at 300 vs. 30 mg/dl glucose. When those islets from single donors were allotransplanted intraportally into single STZ-diabetic Wistar-Furth rats (n=7), complete normoglycemia consistently was restored within one day after transplantation, and persisted up to immunological rejection about one week later. These data demonstrate, for the first time, successful one-donor-to-one recipient transplantation of allogeneic rat pancreatic islets. The pre-transplant islet culture regimen outlined in this paper may lead to a more efficient use of donor pancreatic islet tissue in the clinical setting, too.

COMPARISON OF SEQUENTIAL CO-TRANSPLANTATION OF HEPATIC CELLS AND ISLETS VIA PORTA/PORTA AND CAVA/PORTA VEIN IN RATS WITHOUT IMMUNOSUPPRESSION

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Objective We have previously reported partial reversion of diabetes in rats, after co-transplantation (co-Tx) of islets and hepatic cells when they are mixed before implantation and also when injected separately after a period of 15 min (sequential Tx). In this study we aimed to compare the porta/porta sequential Tx (both injections in porta vein) (P/P Tx) with cava/porta sequential Tx (C/P Tx).

Material and method Diabetes was induced by i.p. streptozotocine injection (60 mg/kg) and confirmed after 3 or more blood glucose measures of 350 mg/dl over different days. Islets were isolated with collagenase solution, purified with BSA gradients and cultured in CMRL-1066 medium for 3-4 days. Fresh hepatic cells were isolated after double perfusion with saline/heparin and collagenase solutions. Non-singeneic Wistar rats were used as donors and recipients.

Four groups of Wistar rats were studied: A) P/P Tx with a ratio of 100 hepatic cells per islet (100:1) (N=10); B) P/P Tx with a ratio of 200:1 (N=9); C) C/P Tx with a ratio of 100:1 (N=8) and D) C/P Tx with a ratio of 200:1 (N=8).

In groups A and B, hepatic cells were injected via porta vein and after a period of 15 min islets were injected into the same vein. In groups C and D, hepatic cells were injected via inferior cava vein and after 15 min islets were injected via porta vein. No immunosuppressive drugs were employed. The average islet number (ENI) transplanted was 1533.2 ± 111.31 in group A, 1595 ± 125 in group B, 1632.5 ± 133 in group C and 1845 ± 195 in group D. All groups had an islet purity of 90%. The ratios of fresh hepatic cells were 100:1 in groups A and C and 200:1 in groups B and D.

Blood glucose was measured with Gluco Touch strips at 1, 2, 3, 4, 6, 7, 9, 11, 18, 25 and 30 days after Co-Tx in all groups. SPSS have been applied for statistical study.

Results Reversion of diabetes (blood glucose less than 150 mg/dl) was observed among the four groups with some differences. All groups showed a similar behaviour for the first 5-6 days after Co-Tx with blood glucose levels about 116-238 mg/dl. However, 4 rats of group A showed euglycemia (< 150 mg/dl) during 25 days, while none of the other groups was euglycemic for a period longer than 11 days.

Discussion and conclusions The results confirm some useful influence of hepatic cells on islet implantation without immunosuppressive drugs. Moreover, induction of tolerance is also present when hepatic cells are injected via inferior cava vein instead of porta vein, although sequential P/P Tx showed better results, with the method and cells employed.

ANALYSIS OF THE EFFECTS OF DIFFERENT GLUCOSE CONCENTRATIONS IN CULTURE MEDIA ON INSULIN SECRETORY CAPACITY OF ISOLATED HUMAN FETAL ISLETS

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Our previous studies have shown that isolated human fetal islets, cultured for 2 weeks, might be suitable for clinical transplantation. However, the effects of the use of different glucose concentrations in culture media on insulin secretion capacity of the islets have not yet been extensively evaluated. In this study we analysed the capacity of insulin secretion of the islets after 14 days of culture with 6 different glucose concentrations: 5 mmol/l (protocol A), 8 mmol/l (protocol B), 11 mmol/l (protocol C), 14 mmol/l (protocol D), 16 mmol/l (protocol E) and 22 mmol/l (protocol F). The islets were isolated by collagenase digestion and cultured in the media with 10% FCS at 37°C and 5% CO₂. The secretory capacity was evaluated by RIA detection of insulin levels in the culture media after 1 h incubation with 1.67 and 16.7 mmol/l glucose + 5 mmol/l theophyllin sequentially and expressed as a percentage of the increase in insulin levels after high-glucose + theophyllin stimulation. We have found that insulin secretion capacity of the islets was similar during the study period in protocols A, B, and C (day 1: A: 341.6±35.7%, B: 338.3±34.6%, C: 339.5±36.1%; day 7: A: 421.3±40.7%, B: 415.6±49.1%, C: 419.5±47.1%; day 14: A: 389.1±33.1%, B: 382.6±34.3%, C: 383.4±38.3%; A vs B, C, p=ns). In protocols D, E, and F, insulin response significantly decreased during the culture period becoming significantly lower compared to protocols A, B and C (day 1: D: 337.7±38.2%, E: 340.3±39.1%, F: 338.6±37.7%; day 7: D: 206.3±20.5%, E: 202.6±13.2%, F: 07.9±19.1% and day 14: D: 154.5±12.6%, E: 148.2±11.3, F: 149.7±17.6%; protocols D, E and F: day 1 vs day 14: p<0.01; day 14: A vs D, E, F; B vs D, E, F and C vs D, E, F p<0.05, D vs E vs F p=ns). Our data showed that insulin secretion from the islets remained stable within a wide range (5-11 mmol/l) of ambient glucose levels. However, the incubation with glucose concentrations above 11 mmol/l strongly inhibits the insulin secretion which might be of important relevance for the preparation of islets in clinical transplantation.

EFFECT OF COLD STORAGE SOLUTIONS ON PANCREATIC ISLET RECOVERY AND EVALUATION OF EXOCRINE TISSUE INJURY

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Clinical success of transplantation of pancreatic islets depends on many factors including type of preservation solution and cold storage conditions. During cold ischemia irreversible changes in the pancreas occur that cause decrease of islet yield and deterioration of their endocrine function. The aim of our study was to compare three preservation solutions (University of Wisconsin Solution - UW, Histidine-Tryptophane-Ketoglutarate - HTK, Euro-Collins - EC) used for rat pancreas cold

storage before islet isolation. 6 WAG rats were used as pancreas donors in each group. Islets were isolated using collagenase digestion and Histopaque gradient separation. Glucose static test was used to assess islet endocrine function. Three hours after cold storage at 4°C samples (n=6) from each preservation solution before isolation as well as from supernatants from tissue mass washing after pancreas digestion with collagenase, were frozen and collected to measure amylase and lipase levels. Our experiments indicate that three hours of cold ischemia cause decrease of islet yield (control [680±21 islets/pancreas] vs. UW [508±139; p<0.02]; vs. HTK [344±103; p<0.01]; vs. EC [322±113; p<0.01]). Deteriorating type of insulin release in glucose static test was observed in islets isolated from pancreases preserved in EC and HTK. The highest level of amylase in preservation solution was observed in the group with EC (9173±2597 U/L). No statistically significant differences between groups were observed. In supernatants from tissue mass washing, increased enzyme levels corresponding with prolongation of preservation time were observed. The amylase activity in supernatants in the group preserved in UW solution was lowest (36733 ± 3151 U/L; HTK 39562 ± 7541 U/L; EC 43478 ± 11199 U/L). Concentration of lipase activity ranged from 4358 ± 856 U/L (HTK); [4492 ± 493 U/L] (UW); [4956 ± 1524 U/L] (EC). We observed that UW solution has a beneficial effect for protection of pancreas integrity during 3h of preservation, which was confirmed by measurement of enzymes activity. Recovery and yield of islet cells depends on the type of solution used for pancreas cold storage.

CONTROL OF β -CELL PROLIFERATION BY CCK-8 PEPTIDE IN TYPE 1 AND TYPE 2 DIABETIC RATS

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Aims The adult pancreatic β -cell is normally virtually quiescent, but can be stimulated to replicate *in vitro* by a number of growth factors. Cholecystokinin (CCK), a gastrointestinal peptide, has been shown to induce growth of the exocrine pancreas and to stimulate insulin secretion. Thus, the aim of this study was to investigate a possible role of the CCK-octapeptide (CCK-8) to generate islet cell proliferation in normal and diabetic rats. **Materials and methods** Type 1 diabetes was induced in adult Wistar rats by an I.V. injection of 65 mg/kg streptozotocin. The type 2 diabetes model was obtained by an I.P. injection of 260 mg/kg nicotinamide given 15 min before streptozotocin. 14 days thereafter, CCK-8 (1, 2 and 4 μ g/kg) or saline (control group) was injected S.C., three times daily, for 8 successive days, in 7 animals per group. Then, the animals were sacrificed and blood was collected. Light microscopic immunocytochemical and morphometric analysis was carried out on the whole pancreas using proliferating cell nuclear antigen (PCNA) as a marker for cell proliferation and insulin as a marker for β cells. **Results** 1) In the control group, glycemia was 99.17 ± 4.04 mg/dl, a value that was not significantly affected by CCK-8. However, from 1 μ g/kg, CCK-8 increased the surface of the pancreas by 18.97% (p<0.05); but neither the surface and number of islets expressed per μ m² of total pancreas nor the number of PCNA labelled β -cells per μ m² of islet surface were altered. 2) In streptozotocin-induced diabetic rats, glycemia was 471 ± 28 mg/dl, a value that was not significantly altered by CCK-8. The surface of the pancreas increased by 48.24% (p<0.05) from 2 mg/kg. The PCNA β -cell labelling increased in parallel with CCK-8 concentrations and was maximum at 2 μ g/kg. 3) In nicotinamide treated diabetic rats, glycemia was 154 ± 8 mg/dl. CCK-8 did not modify significantly this parameter. The surface of the pancreas increased by 56.76% (p<0.05) from 2 μ g/kg CCK-8. The number of PCNA labelled β -cells increased by 31% (p<0.05) from 2 μ g/kg CCK-8 and the surface of the islets increased by 42% (NS). **Conclusion** CCK-8 exerts a trophic effect on the islets of Langerhans in both type 1 and type 2 diabetic rats but not in normal rats. Thus CCK-8 acts in concert with glucose to stimulate β -cell proliferation in adult rats.

DOSE DEPENDENCY OF THE BENEFICIAL EFFECT OF LONG-TERM INCUBATION WITH GROWTH HORMONE ON INSULIN SECRETION FROM ISOLATED HUMAN FETAL ISLETS

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We have previously shown that long-term incubation (15 days) with growth hormone (GH) exerts an important beneficial effect on insulin secretion capacity from isolated human fetal islets by preventing its decline during cultivation *in vitro*. Therefore, the aim of this study was to analyse dose dependency of the potentiating effect of the long-term incubation with GH (Genotropin, Kabi Pharmacia) on insulin secretion capacity from isolated human fetal islets, evaluated by glucose+theophylline stimulation. The islets were isolated from pancreata of the fetuses by collagenase digestion, and cultured in the media containing 10% fetal calf serum at 37°C, 5% CO₂. The effect on insulin secretion was evaluated by using a 15 day incubation with 250, 500, 1000 and 2000 μ g/l GH. The insulin secretion capacity was evaluated by determining insulin levels in culture media after 1 hour incubation sequentially with 1.67 mmol/l glucose and 16.7 mmol/l glucose+5 mmol/l theophylline, and expressed as a percentage of the increase in insulin levels after stimulations. We found that insulin response increased and remained stable at the dose of 500 μ g/l GH (day 1: 532.6±52.2%, day 15: 529.4±62.3%; p=ns), with 1000 μ g/l GH the response was higher and remained stable (day 1: 738.8±59.2%, day 15: 733.7±63.5%; p=ns), which was similar with the use of 2000 μ g/l GH (day 1: 734.7±55.4%, day 15: 731.6±60.3%; p=ns). In contrast, the insulin response initially increased but then declined at the dose of 250 μ g/l GH (day 1: 726.2±42.3% vs day 15: 473.7±41.3%, p<0.05). Our results have demonstrated that beneficial effect of long-term incubation with GH on preventing the decline of insulin secretion capacity of isolated islets is dose dependent. The results imply that the effect was not achievable with the very low doses of GH.

ACTIVATION OF THE COAGULATION CASCADE AFTER INTRAPORTAL ISLET ALLOGRAFT

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An activation of the coagulation cascade by allogenic islets in contact with human blood has recently been reported *in vitro*. We studied the systemic coagulation activation after injection of allogenic islet preparation into the portal vein.

Method Systemic markers of haemostasis were measured in pig (n=29) during 72 hours after the injection of inert microbeads (160-300 μ m) or islet allogenic preparations (Ricordi's method), with or without anticoagulant treatment (heparin 100 IU/kg or lepirudine 0.8 mg/kg). We also measured the same parameters following sequential (n=4) intraportal islet transplantations, preceded by heparin administration, in one diabetic patient with a functioning kidney graft. **Results** The mean results (\pm sem) observed 30 minutes after injection in pigs are summarised below [basal value of thrombin-antithrombin (TAT) complexes: 13±1 mg/l].

Material	Volume injected (ml/kg)	Treatment	Portal pressure increase (mmHg)	TAT (μ g/l)	Decrease of platelets (%)
Microbeads	0.3	none	1.3 ± 0.9	58 ± 10	0 ± 3
Islet prep.	0.3	none	11.2 ± 3.0	665 ± 122	41 ± 6
Islet prep.	0.15	none	1.7 ± 0.8	163 ± 14	26 ± 3
Islet prep.	0.3	heparin	4.7 ± 0.9	119 ± 19	12 ± 1
Islet prep.	0.3	lepirudine	3.1 ± 1.0	38 ± 9	8 ± 5

In the patient, 30 minutes after the injection of islets, the middle rate of the TAT increased from 2.4 ± 0.3 to 21.2 ± 11 mg/l. Platelet count decreased from 3 to 19% between 30 min and 6h.

Conclusion Intraportal infusion of allogenic islet preparations activates the coagulation cascade in an immediate, specific, and volume dependent mode, that is only partially reduced by heparin. This reaction could play a crucial role in primary non function after intraportal islet allotransplantation.

SCREENING OF NEW BIOMATERIALS FOR MACROENCAPSULATION OF PANCREATIC ISLETS

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Objectives Transplantation of immuno-isolated islets has the potential to restore endogenous insulin secretion in type I diabetics with no need for lifetime immunosuppression. A material screening is presented which aims to identify a new biomaterial combining immunoprotection with optimal diffusion properties for glucose and insulin. **Materials and methods** The impact of chemical modifications of polysulphone (PSU) capillary polymers with a cut-off of 50 kD on glucose induced insulin secretion of macroencapsulated rat islets was studied in perfusion experiments. **Results** Insulin release of free floating islets showed the typical rapid response to glucose stimulation with a lagtime of insulin release of 5 min and a maximal release after 30 min of stimulation. Total insulin release (AUC) reached 117 ± 22 ng/ml x 90 min. Macroencapsulation in capillaries made of PSU/polyvinylpyrrolidone or PSU/sodium-dodecyl-sulphate prevented glucose induced insulin release. Hydroxy-methylation (CH_2OH) of PSU improved the secretory behaviour of macroencapsulated islets. At 0.8 degrees of PSU-substitution macroencapsulated islets responded with a total insulin release of 62 ± 15 ng/ml x 90 min. At maximal degrees of PSU-substitution (1.7) the kinetics and efficiency of glucose induced insulin release were very similar to that observed with free floating islets (AUC: 111 ± 24 ng/ml x 90 min). Repeated glucose stimulations of encapsulated islets are paralleled by concomitant insulin release even after 2 days of macroencapsulation. **Conclusions** Highly substituted-hydroxy methylated PSU capillary material for islet macroencapsulation combines immunoprotection with optimal diffusion properties for glucose and insulin and can be considered as optimal prerequisite for the development of a bioartificial pancreas.

CONTINUED PRODUCTION OF XENOIMMUNE ANTIBODIES 6-8 YEARS AFTER CLINICAL TRANSPLANTATION OF FETAL PIG ISLET-LIKE CELL-CLUSTERS

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Objectives The humoral immune response in ten type I diabetic patients, transplanted with fetal pig islet-like cell clusters was followed up to 8 years after transplantation. Before transplantation all patients had xenoreactive IgM and IgG2 antibodies. 30-50 days after transplantation all patients had elevated levels of xenoreactive IgM, IgG2 antibodies and also IgG1 antibodies, mainly directed against Gal α 1, 3Gal-containing epitopes.

Materials and methods Hemagglutinating antibodies were measured using pig erythrocytes. Titers of cytotoxic antibodies and antibody dependent cellular cytotoxicity (ADCC) were examined using pig lymphocytes and adult pig islet cells as targets. Titers of antibodies reactive against Gal α 1, 3Gal were investigated using a human B-cell line, transfected with pig α 1, 3-galactosyltransferase, giving expression of Gal α 1, 3Gal epitopes (RajiGT cells). Absorption of Gal α 1, 3Gal-specific antibodies was made using RajiGT cells. Subclass distribution and

specificity differences were investigated in Western blot experiments.

Results Nine patients had higher than pre-transplant levels of xenoreactive IgM antibodies in hemagglutination and microcytotoxicity tests, 6-8 years after transplantation. Levels of Gal α 1, 3Gal-reactive antibodies were also higher, using RajiGT cells as targets in cytotoxicity tests. ADCC activity against RajiGT cells was detected in four patients while ADCC activity against pig islet cells was only detected in two patients 6-8 years after transplantation. Western blot experiment showed that all patients have xenoreactive IgM and IgG2 while only four patients have xenoreactive IgG1 antibodies 6-8 years after transplantation. Specific absorption of Gal α 1, 3Gal-reactive antibodies removed IgM and IgG2 antibody reactivity while leaving IgG1 antibody reactivity essentially unaffected.

Conclusion 6-8 years after xenotransplantation patients still produce higher than pre-transplant level of xenoreactive antibodies. Xenoreactive IgM and IgG2 antibodies are still mainly directed against Gal α 1, 3Gal while IgG1 antibodies are directed against other, as yet unidentified, epitopes.

FUNCTIONAL EVALUATION OF ISOLATED HUMAN ISLETS OF LANGERHANS WITH ALPHA-CELL DEFICIENCY

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During the isolation procedure for islet transplantation, the architecture of the isolated islets could be disturbed by enzyme digestion, with loss of peripheral glucagon-secreting cells. We investigated whether changes in islet cell composition could affect the function of islets in vitro and in vivo. Human islets with alpha cell deficiency were prepared by prolonged enzyme digestion as confirmed by immunocytochemistry and islet insulin/glucagon content analysis. In vitro, human islets with alpha cell deficiency had a lower insulin response to glucose stimulation compared with intact islets. In islets deficient in alpha cells, exogenous glucagon did not directly stimulate insulin release in the absence of glucose, but increased the glucose-induced insulin release in a dose-dependent manner. In intact islets, glucagon did not significantly change glucose-stimulated insulin release. In vivo, transplantation of 1000 human islets with alpha cell deficiency did not effectively correct hyperglycemia in diabetic mice. In diabetic nude mice transplanted with islets deficient in alpha cells, i.p. administration of low dose glucagon significantly decreased glycemia, as compared with relevant controls. In addition, the survival of diabetic nude mice grafted with islets deficient in alpha cell was significantly shorter than the survival of mice grafted with intact islets. These results indicate that glucagon secreting alpha cells have an important role in insulin release from beta cells, and that alpha cell loss during isolation has a deleterious effect on islet function.

INTRAPORTAL TRANSPLANTATION OF ADULT PORCINE ISLETS INTO CYNOMOLOGUS MONKEYS. EFFECTS OF COMPLEMENT INHIBITION AND A HIGH DOSE OF ISLETS

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Objectives Porcine islets exposed to fresh human blood, in vitro, elicit an instant blood mediated inflammatory reaction (IBMIR) resulting in islet damage. Complement inhibition with sCR1 can reduce this damage. Here, we investigated whether this could also be achieved in a clinically relevant in vivo model. We also wanted to see how a high dose of islets and the assumed complement activation would affect the liver cir-

ulation. **Method** Approximately 20000 IEQ/kg BW of adult porcine islets were transplanted intraportally into four pairs of cynomolgus monkeys. One monkey in each pair was pre-treated i.v. with sCR1 (40 mg/kg BW) 1 hour prior to islet injection; the paired control monkey received an equal volume of saline. Flow and pressure measurements in the hepatic ligament and blood sampling from the v cava were carried out during the 1 hour experiment. **Results** Complement activation in the control was extensive. In the sCR1 treated monkeys it was significantly reduced. With regards to insulin release, which was taken as a measure of islet damage, the AUC for the control group was 48823 ± 14672 pmol/L/65 min compared to 6779 ± 1863 pmol/L/65 min in the sCR1 treated group ($p=0.026$). Histology of islets recovered in the liver of control monkeys revealed damaged islets whereas the islets in the sCR1 treated group had significantly better preserved morphology. Portal pressure and the blood flow in the major hepatic vessels were stable throughout the experiment in both groups. **Conclusion** When adult porcine islets were transplanted intraportally into cynomolgus monkeys IBMIR occurred and severe islet damage ensued. Complement inhibition with sCR1 prevented this chain of events. Although a high dose of islets was given and there was a substantial complement activation the hepatic circulation was not affected, indicating that high xeno islet doses can be given without achieving portal hypertension or major effects on the systemic circulation.

CRYOPRESERVATION OF DISPERSED HUMAN AND BOVINE PANCREATIC ISLET CELLS

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Objectives Cryopreservation of human islets has given inconsistent results in terms of yield and in-vitro as well as in-vivo function. We assessed whether using dispersed islet cells could represent an alternative approach to bank insulin-secreting tissue by cryopreservation. **Materials and methods** Human (HI) and bovine (BI) islets were prepared by collagenase digestion and density gradient purification, and within 3-5 days from isolation they were dispersed by trypsin and DNase (final viability >90%). The next day aliquots of approximately 1×10^6 /ml cells were placed into sterile cryopreservation bags, and DMSO was added to achieve a final concentration of 10% in M199 medium. Then the preparation was placed at 4°C for 5 min, cooled to -7°C (0.3°C/min), kept at this temperature for 3 min, cooled to -40°C at 0.3°C/min, and then frozen to -70°C at 5°C/min. Thawing was quickly performed at 37°C, with progressive dilution of DMSO. **Results** Recovery was $68 \pm 15\%$ and $68 \pm 8\%$ for HI and BI dispersed cells, respectively, and post-thawing viability $56 \pm 4\%$ and $58 \pm 5\%$ for HI and BI, respectively. The frozen-thawed cells were placed in M199 medium at 37°C for 7 days, and during this period HI-derived cells remained dispersed, whereas BI-derived cells re-aggregated into discrete pseudoislets, ranging approximately 50-100 µm in diameter. Insulin secretion studies showed no glucose sensitivity from dispersed human cells, whereas re-aggregated BI cells were able to sense glucose stimulation (insulin release at 3.3 mmol/l glucose: 28.4 ± 6.6 µU/ml/10 aggregates; insulin release at 16.7 mmol/l glucose: 62.8 ± 4.4 µU/ml/10 aggregates, $p < 0.01$ vs 3.3 mmol/l glucose). **Conclusion** At the described experimental conditions, cryopreservation of dispersed human islet cells remains elusive, whereas frozen-thawed bovine islet cells might represent an additional tool for xenotransplantation studies.

FREE FATTY ACID-INDUCED APOPTOSIS OF ISOLATED HUMAN ISLET CELLS

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Objectives Experiments in rodents have shown that prolonged exposure of pancreatic islets to high concentrations of free fatty acids (FFA) causes reduced islet cell survival (lipotoxicity). We evaluated the phe-

nomenon of lipotoxicity in isolated human islets (HI), and investigated some of the possible mechanisms. For the purpose of this study, HI were prepared by collagenase digestion and density gradient purification and then incubated for 24h with 1.0 mmol/l FFA (oleate-to-palmitate, 2-to-1). Compared to control islets, FFA-exposed cells exhibited a significant increase in the amount of dead cells (see table), and electron microscopy showed the involvement of beta-cells, with morphological appearance compatible with the presence of apoptotic phenomena.

	Amount of dead cells	
	Controls	FFA-exposed
TUNEL (%)	12.3±3.0	44.0±5.0*
ELISA (OD)	0.6±0.2	3.3±0.5*

* $p < 0.01$ vs Controls

FFA-induced islet cell death was completely prevented by inhibition of upstream caspases. RT-PCR studies revealed no major change of iNOS and Bax mRNA expression, and a marked decrease of Bcl-2 mRNA expression in the islets cultured with FFA. Thus, prolonged exposure to FFA has caspase-mediated, pro-apoptotic effects on human pancreatic beta-cells; these alterations are accompanied by no major change in iNOS and Bax mRNA expression, and decreased Bcl-2 expression.

CONSTRUCTION OR RECONSTRUCTION OF THE ISLET VASCULAR BORDERS IN THE SPLEEN ENVIRONMENT WITH PRODUCTION OF MATRICES AND ENDOTHELIALIZATION: THE PLAYERS

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Objectives It is proposed that adult islet revascularization is fast but not a totally achieved process in the reported mean time of 7-14 days after inoculation in several locations. As with reinnervation the process may be greatly influenced by the peculiarities of the receptor territories and the type of islet preparation. In this complex bimodal process we have extrinsic and intrinsic players, some significantly influenced by previous manipulation, the age and growing potential of the inoculated islet tissue. In the spleen with near-ideal conditions of nutrition, hormonal regulation and oxygen delivery we may have islet units from the discrete islet cell to the mega-islet with very different critical levels (nutrients/oxygen) for survival. The complex morphogenesis of the vascularization process implies ECM production, its assembly and endothelialization, with reconstruction of basement membranes, interstitial matrix and capillary neogenesis. **Methods and results** In an organ with a high blood flow and a great diversity of mobile and fixed blood elements enmeshed in a reticular frame with an abundant mesenchymatous cellular population we intended to clarify their prospective roles in the revascularization process by light and electron microscopy at 4, 8, 24 and 38 weeks after autotransplantation into spleen of a polymorph preparation of adult pancreatic fragments whose global fate, previously reported, was denoting an exuberant exocrine and endocrine regenerative process sustained at all times during the study. In an environment without immunological reactivity we found evidence that in the construction or reconstruction of the islet vascular borders, inside and at the periphery, with production of matrices and endothelialization, were fundamental players: islet cells, Schwann cells, endothelial cells, blood cells (lymphocytes, granulocytes, plasmocytes, mastocytes), macrophages, fibroblasts, spleen reticular cells, pericytes, and myofibroblasts. In contrast to exocrine structures, the islet cells appeared to be strong inducers (not explained by a mass effect) of neocapillaries with circulating red blood cells in the core and at the periphery of islet structures. Permeabilization of the islet core was made through the intercellular spaces (naked channels), establishing close contact between islet cells, Schwann cells, circulating blood cells, migrating fibroblasts and remodeling endothelial cells, recreating identical conditions for the islet

core and periphery. Production of neuro-endocrine and endothelial ECMs on the periphery and on intra-islet channels defined the islet compartment. **Conclusions** All epithelial phenotypes and cells with a mesodermal ancestry may have a significant primordial role in the production and ECM assembly and islets and islet cells are strong inducers of Schwann and endothelial cell tropism and capillary neogenesis, despite some presumed criticism for the compact and more voluminous islet organoids. The complex potential interactions of the players deserve further studies at ultrastructural and molecular levels.

UPTAKE OF D-[³H]MANNOHEPTULOSE BY TRANSPLANTED ISLETS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS AND MICE

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D-mannoheptulose is apparently transported into cells, mainly if not exclusively, by GLUT2. It was recently proposed, therefore, as a tool to label preferentially insulin-producing B-cells in the pancreatic gland, in the perspective of the non-invasive imaging and quantification of the endocrine pancreas. The aim of the present study was to investigate whether such an approach could be extended to transplanted islets. For such a purpose, D-[³H]mannoheptulose was injected intraperitoneally in streptozotocin-induced diabetic rats, that had been transplanted with groups of 1,500 islets each placed in an implantation module. One day after injection of the tritiated heptose, the radioactive content of the transplanted islets was one order of magnitude higher than that of the pancreatic gland. Likewise, when D-[³H]mannoheptulose was injected intravenously in streptozotocin-induced diabetic mice transplanted under the kidney capsule with groups of 300-550 islets each, the radioactive content of the liver and transplanted islets was, one hour after injection of the tritiated heptose, 5-8 times higher than that of the pancreatic gland. It is proposed, therefore, that a suitably radiolabelled D-mannoheptulose analog, e.g. 1-deoxy-1-[¹²³I]iodo-D-mannoheptulose, could be used to label transplanted islets *in vivo* and assess by a non-invasive method their functional integrity.

MULTICENTRIC EVALUATION OF CONTINUOUS SUBCUTANEOUS GLUCOSE MONITORING IN DIABETIC PATIENTS

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Aims To assess accuracy and precision of a new subcutaneous glucose sensor in comparison to the reference glucose-oxidase method in type 1 and type 2 diabetic patients. **Methods** 70 diabetic patients were recruited. We reported data from 53 diabetic inpatients (27 M and 26 F; 60% type 1 diabetes), age 47 ± 15 (SD) years, BMI = 24.9 ± 3 (SD) kg/m². In each patient a microdialysis probe was inserted in the subcutaneous abdominal tissue and connected to a portable microperfusion pump running at 10 μ l/min (Glucoday) for at least 24 hours. Subcutaneous glucose concentration was measured continuously and recorded every 3 minutes. Throughout the day 9 venous samples were collected (1 hour after probe insertion, before lunch, 1, 2 and 3 hours after lunch, before dinner, 2 hours after dinner, 3 a.m., and 7 a.m.) for later glucose measurements using the reference method (Beckman, Ohio, USA). **Results** Probe insertion was well tolerated by all patients. Over a range 45-450 mg/dl of plasma glucose levels the correlation coefficient between the sensor and the reference method was $r = 0.778$ ($p < 0.001$). Error grid analysis showed >95% of glucose values in the A and B regions. The glucose sensor detected 5.5% of values in the low range (<65 mg/dl). **Conclusion** Subcutaneous glucose monitoring with microdialysis technique accurately reproduces values obtained from

circulating glucose. Early detection of daily and nocturnal glucose fluctuations could improve metabolic control both in type 1 and type 2 diabetic patients.

CYTOSTATIC AND CYTOTOXIC EFFECT ON HUMAN PANCREATIC BETA CELLS OF PERIPHERAL BENZODIAZEPINE RECEPTORS ACTIVATION

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Objectives The peripheral benzodiazepine receptors (PBRs) are proteins located on the mitochondrial membrane where they take place in the formation of the mitochondrial permeability transition (PT) pore. PT pore regulates the function and integrity of mitochondria, that, in turn, have a crucial role on cell function and survival. We have previously demonstrated that PBRs are present on human pancreatic islets (HI). In the present study we evaluated the effects of a prolonged (12h) exposure of purified HI to a specific PBRs agonist (PK-11195), used at varying concentrations. **Materials and methods** HI were prepared by collagenase digestion and density gradient purification, and functional, survival and PCR studies were performed with control and PK-11195 exposed islets. **Results** Insulin release (IR, μ U/ml, mean \pm SD) in response to 16.7 mmol glucose was significantly ($p < 0.05$) lower after exposure to PK-11195 0.5 μ M (222 ± 46 , n=12), 1.0 μ M (160 ± 71 , n=10) and 50 μ M (132 ± 34 , n=8), compared to control HI (348 ± 188 , n=14). By electron microscopy we observed typical apoptotic changes (cellular shrinkage, chromatin condensation and apoptotic bodies) in human beta-cells exposed to PK-11195. By the TUNEL technique (n = 5) we evaluated the percentage of dead cells, which was significantly higher ($p < 0.02$) in HI exposed to PK-11195 ($42.3 \pm 7.9\%$) compared to control HI ($20.1 \pm 7.6\%$). This finding was confirmed by the ELISA cell death detection method. Messenger RNA expression (evaluated by RT-PCR) of inducible nitric oxide synthase (iNOS), Bax and Bcl-2 was similar in HI cultured in the presence of PK-11195 and control HI. The use of specific caspase-3, but not caspase-6, inhibitor significantly reduced the amount of dead cells. **Conclusions** These results demonstrate, for the first time, that PBRs activation causes human beta-cells functional damage and apoptosis, a phenomenon that occurs without iNOS, Bax and Bcl-2 involvement, and requires specific caspases activation.

CLINICAL EVALUATION OF A MODIFIED SIDE PORT CATHETER FOR INSULIN IMPLANTABLE PUMP: THE EVADIAC EXPERIENCE

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Increase in refill error rate has been observed and not resolved by the flush procedures through side port. This underdelivery was explained by side port compliance which was too low to pass the stroke volume in 2 ms. The Minimed company modified the catheter side port with the adjunction in the side port of three small titanium "pillows" that act as an accumulator to store the initial impact of the hydraulic force pression. EVADIAC designed a protocol study to examine the effect of this new side port.

Forty type 1 diabetic patients were enrolled for a new implantation with the Minimed implantable pump MMT 2001 and the modified side port catheter MMT 4027. They were monitored every 45 days (11 refill procedures).

HbA1c levels remained stable at $7.7 \pm 0.9\%$. Refill accuracy remained stable between $8.31\% \pm SD 7.3$ at the 6th refill and $11.3\% \pm 9.3$ at the 11th refill. During the same period the number of insulin units really infused varied from 46 ± 20 at the 6th refill to 62 ± 28 at the 10th refill. Eight adverse events occurred between the 9th and the 14th month (1 encapsulation, 1 obstruction of the catheter and 6 catheter/pump obstructions all solved by pump rinse or catheter flush procedures), a

number of adverse events similar to that observed before 1994.

On the basis of 450 days follow up in 40 patients, we may conclude that the modified side port catheter with the titanium "pillow" restores the performance of the implantable pump system without increasing device complications.

STUDIES OF EFFECT OF XANTURENIC ACID ON PANCREATIC ISLETS

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Xanturenic acid (XA) or 4,8-dihydroxyquinolin-2-carboxylic acid, a derivative of 8-oxyquinolin (D8OX), an abnormal product of Tryptophan metabolism, is formed in humans deficient in vit. B6. More than 10 mg/kg of D8OX in doses of 40-60 mg/kg induced diabetes due to ability to form toxic chelat complexes with Zn-ions in cytoplasm of B-cells which results in selective destruction of B-cells 15-30 min post-injection. We studied the action of XA on models in vivo and in vitro. Diabetes induced by endogene synthesized XA in rats contained on special diet for 2 months, accompanied by normoglycemia (35% animals) and hyperglycemia (6.3-13.6 mmol/l) in others, and partial or total decreasing amount of insulin deposited in B-cells by degenerative changes in B-cells. In vitro on a model of isolated islets we used synthetic XA in doses of 500-750 mcg/ml which is equivalent to injection of diabetogenic doses of other D8OX. 1-3 h later we observed a complete degranulation and destruction of B-cells analogically to the action of other D8OX. XA formed in B-cells complex 1:1 with Zn-ions, the most toxic for cells, with fixing of the Zn-atom between the active group in position 8 and position 1 or 2 of the quinolin ring. XA formed the same complex. Extraction of the active group from position 8 was accompanied by complete disappearance of diabeto-genic properties of D8OX as of XA. We suppose that diabeto-genic action of XA is determined by the same mechanisms as other D8OX added to inactivation of insulin by XA and overload of B-cells showed by Kotake Y. Our special interest in XA is determined by the following facts: 1. XA contrary to all other D8OX is easily formed in humans. 2. Xanturenicuria as a deficiency of Vit. B6 is discovered often in patients with diabetes aged 45-50 and over both confirmed and undiagnosed and may have significance in the pathogenesis of human diabetes.

EFFECT OF CSII IN NEWLY DISCOVERED IDDM PATIENTS ON GLUCOSE EFFECTIVENESS: MINIMAL MODEL ANALYSIS

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Intensive insulin regime from the beginning of newly diagnosed IDDM may have beneficial effect on the outcome of the illness. The aim of our study was to evaluate the effect of initial 2-week CSII treatment (Novo Nordisk MKII or MiniMed 506 Infuser using Velosulin) on glucose effectiveness (Sg) in a group of newly diagnosed IDDM patients. Forty-nine patients (24 males, 25 females) were included in the study: age: 22.39 ± 0.69 yr; BMI: 21.28 ± 0.36 kg/m². Modified minimal model (MINMOD) (copyright R.N. Bergman) was used for determination of Sg (10^{-2} min^{-1}) at: 0; 2; 12; 24 and 52 weeks. Patients were divided into those who entered clinical remission (Group A; n=12) and those who did not enter remission (Group B; n=37). Mann-Whitney and Wilcoxon's tests were used for statistical analysis. The following results were obtained for Sg in Group A vs. Group B: 0 week: 1.33 ± 0.26 vs. 2.08 ± 0.25 , $p > 0.05$; 2 weeks: 1.95 ± 0.26 vs. 1.82 ± 0.14 , $p > 0.05$; 12 weeks: 1.82 ± 0.37 vs. 2.21 ± 0.21 , $p > 0.05$; 24 weeks: 1.32 ± 0.20 vs. 1.12 ± 0.15 , $p > 0.05$; 52 weeks: 1.57 ± 0.36 vs. 1.43 ± 0.45 , $p > 0.05$. In conclusion, we did not obtain significant change in Sg between patients who entered clinical remission and those who did not enter clinical remission during the entire period of follow-up.

INTERIM RESULTS FROM MULTI-CENTER STUDY INTO THE USE OF NEAR-INFRARED SPECTROSCOPY FOR THE NON-INVASIVE MEASUREMENT OF BLOOD GLUCOSE

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The non-invasive proper measurement of blood glucose levels in people with diabetes using near-infrared (NIR) diffuse reflectance spectroscopy is a challenging and complex task that has attracted investigators from a broad range of scientific and engineering backgrounds. The measurement requires a solid understanding of the living sample and an application of methods from a broad field from such diverse perspectives as disciplines including tissue optics, spectroscopy, chemometrics multivariate statistics, signal processing, scattering theory and analytical chemistry. The non-linear effect of variable tissue scattering on the near-infrared measurement light near-infrared measurement complicates the detection extraction of the blood glucose signal as the near-infrared light propagates through the from the dynamically varying tissue background. This The dynamic nature and heterogeneous composition of change in skin tissue can leads to a variations in the effective tissue sample through changes in the relative photon flux delivered to individual skin layers. the sampled tissue volume elements. that is sampled. It is likely that the sampling protocol governing the preparation of the measurement site and the coupling of the instrument to the skin are of critical importance. Furthermore, advanced signal processing and multivariate analysis is are necessary for the extraction of the spectral contribution due to glucose amid the complex and varying background signals.

This research presented here represents an investigation will examined light into into the the complexity of the NIR-based, non-invasive this problem measurement, and a discussion of potential solutions for and solutions for reducing the complexity simplifying the measurement will be proposed of the multivariate model required for prediction reasonable solutions to achieving the non-invasive measurement of blood glucose levels accurately and precisely. The effect of Experience with the variations in inter- and intra-patient tissue variation samples will be presented and how these variations affecton the calibration and blood glucose and prediction of blood glucose will be examined discussed.

Interim results from a multi-center study involving incorporating over 100 subjects with diabetes are examined. presented and discussed. Of particular focus are quantitative results from the individual calibration models based on individual of the subjects and the quantitative results obtained during the non-invasive prediction of blood glucose levels. Factors influencing the reliability of the non-invasive glucose measurement system are discussed and solutions for improving long-term performance are proposed. The causes of failed calibrations are also investigated and actions designed to reduce the likelihood of failures in calibration are proposed. For those subjects in which calibration models could not be developed, the investigation into the reasons for the failed calibrations (ancillary correlations, skin thickness, skin hydration) will be presented. Interim results on the robustness of those individuals whom a calibration model could be developed will be presented.

TACROLIMUS VERSUS CYCLOSPORINE IN PRIMARY SIMULTANEOUS PANCREAS-KIDNEY (SPK) TRANSPLANTATION: PRELIMINARY RESULTS AT 3 MONTHS

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We present the 3-months interim analysis of an open, randomised, prospective, parallel-group study, that has been designed to include 200 SPK transplant recipients from 10 centres in Europe and 1 in Israel. Patients received either tacrolimus or cyclosporine-microemulsion concurrently with antithymocyte globulin, mycophenolate mofetyl and steroids. We studied 177 patients who achieved a 3 months' follow up:

89 in the Tacro group and 88 in the Ciclo group. Despite randomisation, significant differences in baseline demographics appear: recipients in the Tacro group were older, less likely to have received Tx before dialysis and more likely to have received portal venous drainage. Rejection-free kidney survival was 58.1 % in the Tacro group and 44.8 % in the Ciclo group. One patient died in the Tacro group. Kidney graft survival rate at 3 months was 97.7 % in the Tacro group and 95.2 % in the Ciclo group. Pancreas graft survival rate at 3 months was 92 % in the Tacro group and 81.3 % in the Ciclo group ($p = 0.027$). The most frequently reported adverse events were urinary tract infection (34.5 %), CMV infection (29.5 %), abdominal infection (11 %) and abdominal drain contamination (17%). There are no significant differences between the 2 groups. Whatever the treatment group was, the incidence of urinary tract infection was greater in patients with bladder drainage of the pancreatic duct than enteric drainage (62 % vs. 30 %, $p=0.0344$). The incidence of abdominal infection was increased in patients previously treated by peritoneal dialysis versus hemodialysis or no dialysis (24 % vs. 8 %, $p=0.0092$). At 3 months, serum creatinine level was 1.3 ± 0.4 mg/dl in the Tacro group and 1.6 ± 0.9 mg/dl in the Ciclo group, fasting glucose was 95 mg/ml in the Tacro group and 110 mg/dl in the Ciclo group, C-peptide was 4.4 ± 1.8 ng/ml in the Tacro group and 5.6 ± 3.3 ng/ml in the Ciclo group, HbA1C was 5.1 % in both groups. The first hospital stay was 34 days in the Tacro group and 41 days in the Ciclo group ($p=0.014$). **CONCLUSION** This 3-month analysis of 177 patients needs to be interpreted with caution. Nevertheless, peritoneal dialysis and bladder drainage increased the infection risk in primary simultaneous pancreas-kidney transplantation.

OPTIMIZATION OF HUMAN ISLET ISOLATION PROCEDURES FOR CLINICAL TRIALS OF TRANSPLANTATION IN TYPE 1 DIABETIC PATIENTS

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Successful isolation procedures represent a prerequisite for successful clinical trials of human islet transplantation in type 1 diabetic patients. To allow broad application of the procedures, success should be based not only on isolations of many and viable islets, but also on continuous and reproducible results. We proposed an isolation method that with a broad selection of donors (age 18-65; serum amylase < 3 times the normal range, cold ischemia time <12 hours) was able to guarantee a high islet yield in 17 consecutive isolations sufficient to transplant 7 recipients. These procedures are based on i) the introduction of a transparent digestion chamber to follow the digestion of the gland, ii) the addition of proteases to Liberase batches characterized by low proteases content, iii) the reduction of mechanical digestion. This method, compared to our previous procedures, obtained higher islet yield, both in terms of equivalent ($354,750 \pm 38,920$ versus $249,650 \pm 25,670$), absolute number of islets ($388,780 \pm 41,270$ versus $204,350 \pm 20,000$) and number of islet equivalents per gram of pancreas ($3,409 \pm 1,370$ versus $2,381 \pm 1,744$). The range of digestion times is also different between the two procedures, ranging from 9 to 43 (old method) and from 20 to 30 minutes (new one). All recipients showed a functioning graft: in particular two of them have already reached insulin independence within three months from the transplant. The reproducibility of the successful results represents a base for further large clinical trials of human islets transplantation in diabetic patients and for the application of novel immunosuppression strategies.

ADULT ISLET ALLOTRANSPLANTATION IN TYPE 1 DIABETIC PATIENTS: RESULTS FROM THE GRAGIL MULTICENTRIC NETWORK

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Improvement of islet transplantation requires clinical series large enough to implement controlled strategies. The goal of this study was to demonstrate the feasibility of GRAGIL, a multicentric network for islet transplantation in type 1 diabetic patients. Gathering five centers (Besançon, Geneva, Grenoble, Lyon, Strasbourg) this network allows pancreas procurement, recipient recruitment, transplantation procedure and follow-up while islet isolation is performed in one single laboratory (Geneva). Pancreata were procured in each of the 5 centers and transported with an ischemia time inferior to 8 hours to Geneva. Islets were isolated by a standard automated method and cultured during 2 days for microbiological and insulin secretion tests. If the islet number was too low for a graft (< 6000 islet-equivalent/kg) culture period was extended up to 12 days until another isolation became available. The islets were transplanted by percutaneous transhepatic intraportal injection. Immunosuppression was performed with cyclosporine, mycophenolate mofetil, steroids and an anti-interleukin 2 receptor antibody (Basiliximab, Simulect, Novartis). Intensive intravenous insulin therapy was associated with an antioxidant adjuvant treatment. From November 1998 to June 2000, 56 pancreas procurements were performed. The average yield was 234,500 IEQ, with 32 preparations > 200,000 IEQ. Ten C-peptide negative type 1 diabetic patients with a kidney graft could be transplanted with 9030 ± 1090 islet equivalent/kg with a mean purity of 70%. In 5 cases, two pancreata were required for the graft; the other 5 cases were 1:1 grafts. With a median follow-up of 12 months, we observed 50% graft survival and 20% insulin-independence. In conclusion, with the applied immunosuppression, graft loss could not be constantly prevented and insulin-independence was rarely achieved. However, within this network primary graft function could be achieved in all patients. This network with a large donor and recipient pool will allow us to study new immunosuppression protocols for clinical islet transplantation in type 1 diabetic patients.

MORTALITY AFTER SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANTATION

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We conducted a retrospective analysis of the timing and causes of death after cadaveric simultaneous kidney-pancreas transplantations (SKPTs). From July 1985 through November 2000, 135 SKPTs were performed at our centre: 33 segmental with duct occlusion by Neoprene (DO), 77 whole organ transplantation with bladder diversion (BD) and 25 with enteric diversion (ED). Recipient characteristics were 70 male and 65 female, mean age 38 ± 8 years (range 25-59), mean time of diabetes 25 ± 6 years and mean time of dialysis 29.2 ± 10 months. Immunosuppressive protocol consisted of a quadruple sequential regimen of Azathioprine, Prednisone, Antilymphocyte globuline (ATG or ALG) and Cyclosporine A. From January 1998, MMF replaced Azathioprine and from January 1999, FK506 (Tacrolimus) replaced Cyclosporine A. The recipients were divided into three groups: 33 patients with duct occlusion (DO), 77 bladder drained (BD) and 25 enteric drained (ED). In DO group, perioperative mortality was 12% (4/33: 2 myocardial infarction, 1 post-anoxic coma, 1 bleeding by mycotic aneurysm); in the follow-up of 3-180 months early deaths were 25% (8/33: 2 stroke, 4 neoplasia, 1 pulmonary infection, 1 suicide). In BD group, perioperative mortality was 4% (3/77: 1 tubercular sepsis following a lymphoma, 1 cardiac arrhythmia, 1 acute pulmonary edema), while in the follow-up of 19-112 months late deaths were 12.5% (9/77: 3 strokes, 2 acute pulmonary edema, 3 myocardial infarction and 1 neoplasia). In the ED group there were no perioperative and late deaths (follow-up of 3-22 months). Patient, kidney, pancreas survival rates in DO were, respectively 100%, 90%, 61% at one year and 71%, 71%, 6% at ten years; patient, kidney, pancreas survival rates in BD were respectively 97%, 94%, 84% at one year and 82%, 69%, 68% at ten years; in the ED patient and kidney survival rate was 100% at one year and pancreas survival rate was 85%. Unfortunately, patient mortality is substantially higher in SKPTs when compared with other types

of transplant with an increased incidence of technical failure, acute rejection, sepsis and death. The developments in organ retrieval technology, in clinical immunosuppression and in surgical techniques have allowed us to improve our success rates.

RESULTS OF SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION ACCORDING TO THREE DIFFERENT SURGICAL TECHNIQUES: THREE YEARS OF FOLLOW UP

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Simultaneous pancreas-kidney transplantation (SKPT) is a well established surgical technique which allows us to obtain insulin independence and restores the renal function. Recently, in the International Pancreas Transplant Registry it has been reported that the graft survival rate is >80% at one year. Unfortunately, the Achilles' heel remains the high rate of surgical complications, frequently correlated to the treatment of exocrine pancreas secretions. To further define patient, pancreas and kidney allograft 3-years survival in regard to the method of exocrine drainage, we retrospectively analysed our experience from July 1985 to November 2000. 135 patients were enrolled. They were 70 male with a mean age of 40 years and 65 female with a mean age of 37 years, mean duration of diabetes of 25 ± 6 years and a mean duration of dialysis of 29 ± 10 months. The patients were divided into three groups according to the surgical technique applied: 33 were submitted to duct occlusion (DO), 77 to bladder diversion (BD) and 25 to enteric diversion (ED). Patient survival rate was: 60%, 78%, 96%, in DO, BD and ED respectively; pancreas survival rate was: 60%, 73%, 95% in DO, BD, ED; and kidney graft survival rate was: 85%, 93%, 85% in DO, BD and ED at three years. A positive effect on patient survival is evident in ED versus OD ($p=0.01$), but not versus BD and on pancreas graft survival in ED versus OD ($p=0.01$) and versus BD ($p=0.04$). Because of the high rate of urological complications (72%) in BD, the fibrosis induced by neoprene in DO and the results in terms of patient and pancreas survival, ED seems to be the better technical choice in the treatment of exocrine secretions drainage.

Figures 1 and 2 Patient actuarial survival following SKPT (DO vs BD, $p=0.008$; ED vs BD, $p=0.08$; ED vs DO, $p=0.01$) and pancreas actuarial survival (ED vs OD, $p=0.01$; ED vs BD, $p=0.04$)

UROLOGICAL COMPLICATIONS AFTER SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION

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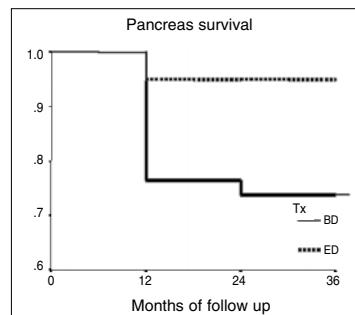
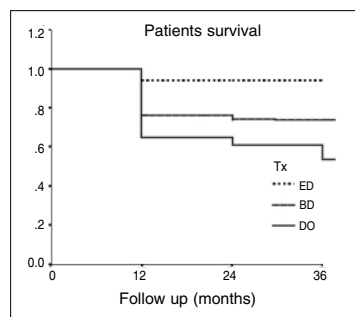
The urological complications of 135 consecutive simultaneous kidney-pancreas transplantations (SKPT) were reviewed. The mean age of the patients (70 men and 65 women) was 38.8 years (range 25.7-59.7

years). The mean duration of type I IDDM was 25.08 years. The patients were divided into three groups according to the duct management surgical technique: group I (33 segmental pancreas-kidney transplantations with duct occlusion), group II (77 whole pancreas-kidney transplantations with bladder diversion, BD) and group III (25 whole pancreas-kidney transplantations with enteric diversion, ED). Urological complications were divided into three types: related to the pancreas transplant (A), to the kidney transplant (B) and unrelated to the transplant procedure (C). In group I and III, obviously, no type A complications were observed. On the contrary in group II, type A complications were common, overall 56/77 (72.7% with at least one complication): hematuria 18/77 (23.4%) with at least one episode, bladder-duodenal segment leak 1/77 (1.3%), recurrent UTI 53/77 (68.8% at least one episode, range of complications 1-11) or cystitis 16/77 (20.7%), dysuria 12/77 (15.6%), duodenal-bladder anastomosis leakage 3/77 (3.9%), urinary retention 11/77 (13%), reflux pancreatitis 8/77 (10.4%). The overall type B complications were observed in 6/33 (18.1%), 27/77 (35%), 9/25 (36%) in group I, II, III respectively. Type C complications occurred in 6/77 patients (7.8%) in group II. Out of group II, 5/77 (6.49%) underwent cystoenteric conversion. SKPT with BD is the most commonly employed technique. The major disadvantage of BD is the high rate of urological complications. In our experience the technical failure rates of pancreas graft for BD and ED are similar. For this reason, we conclude that ED seems to be the more physiological technique for duct management.

THE "DIAS" CAN IMPROVE INSULIN DOSE ADJUSTMENT IN TYPE I DIABETICS

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The DIAS is a decision support system, based on a model of glucose metabolism, that can generate adjustments of insulin doses in type 1 diabetics. 19 Italian type I diabetics were studied to evaluate the safety and efficacy of adjustments by the DIAS. Insulin dose adjustment was performed during 4 visits at weekly intervals (V0-V4), based on data from 3 consecutive days of home BG measurements (HBGM). Dose adjustment was done in the DIAS group by a diabetologist according to the advice from DIAS, and in the controls by another diabetologist according to his/her clinical experience. In the following 8 weeks the insulin dose remained unchanged; results were assessed 4 weeks after V4 (V5) except for HbA1c evaluated at the final visit (V6). In the DIAS group total insulin dose was reduced from 33.8 ± 10.6 U at V1 to 28.8 ± 9.3 U at V5 ($p=0.03$) mainly due to a reduction of morning (6.5 ± 3 U at V1 vs 4.1 ± 1.7 at V5; $p=0.01$) and noon (9.2 ± 2.4 U at V1 vs 6.6 ± 2.5 at V5; $p=0.01$) insulin doses. In the controls the total dose was increased from 37.9 ± 10 U at V1 to 39.3 ± 10.9 U at V5, primarily due to an increase in dinner insulin dose (7.4 ± 3.6 U at V1 vs 8.6 ± 2.8 U at V5; $p=0.01$). The variability of BG measurements in the previous 3 days before each visit was assessed through the number of low (<3.5 mmol/l) and high (>15 mmol/l) BG measurements and the standard deviation of mean of the BG measurements. In the DIAS group the hypoglycaemias were reduced from 9 at V1 to 0 at V5 ($p=0.00001$) and the hyperglycaemias (21 at V1 vs 2 at V5; $p=0.0001$) and standard deviation of BG measurements (3.2 mmol/L at V1 vs 2.2 at V5; $p=0.03$). In the controls no significant changes were observed in these three parameters. At V0 HbA1c was $7.8 \pm 1.7\%$ in the DIAS group and $8.0 \pm 1.9\%$ in the controls ($p=ns$). At V6 HbA1c was reduced in respect to V0 by 0.7% ($p=0.07$) in the DIAS group and increased by 0.5% ($p=0.48$) in the controls thus resulting in a significant difference between the two groups ($p=0.05$). The DIAS can generate a safe and effective insulin adjustment. Advice based on DIAS affected the variability of BG by reducing both hypo and hyperglycaemias, thus ameliorating the overall metabolic control.



INSULIN SECRETORY RESERVE OF PORCINE ISLETS AFTER INTRAPORTAL TRANSPLANTATION

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Objective Adult porcine islets may represent an abundant source of insulin secreting cells for diabetes cell therapy. Using autotransplantation to avoid confounding immunological factors, we studied the metabolic function of isolated porcine islets transplanted intraportally. **Methods** Islets were isolated after total pancreatectomy in adult minipigs (37±3 kg, n=9) and autotransplanted in the portal vein through a percutaneous catheter. Islet metabolic function was evaluated during an IVGTT (0.5g of glucose/kg) at 3 months after transplantation (T). Functional insulin secretory reserve was estimated by the first phase acute insulin response (AIR) and glucose tolerance was assessed by the glucose decay constant between 5 and 30 mn (K value). Results were compared to those of non diabetic (ND, n=14), pancreatectomized (P, n=8), and hemi-pancreatectomized (HP, n=5) minipigs. **Results** (Mean±SEM, *: P<0.05 vs P). All pancreatectomized controls and three animals that received 1582±437 150µm-islet equivalents (IE)/kg became hyperglycemic (fasting blood glucose >250 mg/dl at 1 week) with progressive acidocetosis and cachexia within respectively 14±3 days and 38±12 days. Six animals which received 4557±1185 IE/kg became euglycemic within one month after T (fasting blood glucose: 106±11 mg/dl at 3 months for T*, vs 259±12 mg/dl for P, 83±3 mg/dl for HP*, and 84±3 mg/dl for ND*). K value was 1.01±0.12 %/min for T*, vs 0.39±0.07 %/min for P, 1.19±0.18 %/min for HP* and 1.35±0.17 %/min for ND*. Acute insulin response was 11±5 mU/L for T*, vs -1±1 mU/L for P, 18±3 mU/L for HP* and 27±4 mU/L for ND*. Both AIR and K values were significantly correlated (P<0.001) with the islet mass transplanted after pancreatectomy. The presence of islets in the liver was confirmed by immunohistochemistry in 4 euglycemic animals at sacrifice, 4 to 6 months after T, and by intraoperative simultaneous sampling in portal and suprahepatic veins after arginine injection in two chronic experiments (> 1 year). **Conclusion** The intraportal Tx of 5000 IE/kg porcine islets can achieve long term control of fasting glycemia but only 10 000 IEQ/kg restores normal functional insulin secretory reserve and glucose tolerance. These figures should be considered when clinical islet xenoT is envisaged.

FEASIBILITY, EFFICACY, ACCEPTABILITY, AND IMPACT ON QUALITY OF LIFE OF CSII TREATMENT

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Background and aims Continuous subcutaneous insulin infusion (CSII) with pumps is often used to improve metabolic control in type 1 diabetic patients. We studied CSII treated diabetic patients of the Veneto region to assess feasibility, efficacy, acceptability and impact on quality of life of this therapy. **Material and methods** Retrospective data were obtained from 11 diabetes clinics using a questionnaire designed to get clinical information before and during CSII as well as the degree of patients' acceptance. **Results** We collected a total of 138 patients (M/F: 49/89; age: 40.1±1.0; diabetes duration: 13.1±0.7; CSII duration: 7.4±0.4 yrs, range: 0.3-19.9 yrs). 90% of patients used a multiple basal rate profile of insulin infusion. HBA_{1c} was significantly reduced after the first year of therapy (9.0±0.2% vs 7.9±0.1%, p<0.0001) and this improvement was maintained afterwards (year 10 = 8.0±0.2%). A reduction of both severe hypoglycemic (0.26±0.07/yr vs 0.07±0.02, p<0.006) and ketoacidotic events (0.4±0.1/yr vs 0.10±0.04 p<0.034) was observed as well. Daily insulin requirement decreased after the first year of CSII (49.2±2.0 vs 42±1 U/day, p<0.0001) without further changes. Nonetheless, a progressive increase of body weight

(+0.5 kg/yr) occurred. The number of outpatient consultations also decreased (7.5±0.4 vs. 6.5±0.4 consultation/yr, p<0.05). According to patients' judgement, CSII provided better glycemic control (72.6%), greater wellbeing (42.7%), together with an increased sense of freedom (41.9%). The risk of subcutaneous abscesses at the site of infusion was 0.20±0.05 event/patients yr (mild: 72%, moderate: 18%, severe: 10%). Evaluation of the quality of life resulted in a score of 2.13±0.05 (max =1, min =5). **Conclusions** In type 1 diabetic patients CSII treatment: 1) improves metabolic control in the short- and long-term; 2) reduces serious hypoglycemic and ketoacidotic events; 3) is well accepted; 4) provides good quality of life with a reduction in the number of outpatient consultations and admissions to hospital.

HUMAN PANCREATIC ISLETS PRODUCE AND SECRETE MCP-1: APPLICATION IN HUMAN ISLET TRANSPLANTATION

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A significant portion of the implanted islet mass is lost, immediately as a result of hypoxic and an inflammatory response to the graft mainly mediated by macrophages. We have found that human islets release large amounts of active Monocyte Chemoattractant Protein-1 (MCP-1), the most powerful chemokine for macrophage. Our aim was to determine whether the amount of MCP-1 secretion by the islet preparations before the transplantation was predictive of the clinical outcome of the graft and whether recipient's MCP-1 serum levels were a marker of islet engraftment. 16 islet preparations were transplanted in 11 type 1 diabetic patients already immunosuppressed for a previous kidney graft. MCP-1 was measured in the supernatants of the islet preparations before transplantation and in the serum of the recipients (time 0, +1, +3, +5 and +7 after transplantation). Islet preparations were divided into two groups below (group 1, mean MCP-1 value of 2.6±0.7 pg/islet/24hours) or above (group 2, mean MCP-1 value of 21.6±4.3 pg/islet/24hours) a threshold MCP-1 level of 4.73 pg/islet/24hours (50 percentile). Group 2 showed a decrease in their insulin requirement that was significantly lower than that of group 1 both at 3 (44±20% n=6 versus 83±6% n=5, p=0.07) and 6 months (16±16% n=3 versus 92±8% n=5, p=0.003). Furthermore fasting glycemic values after overnight insulin withdrawal at 1 month (a parameter we found to be predictive of the fate of the graft) were different between the two groups (216±50 versus 124±9 mg/dl in group 2 and 1, respectively). Assessment of IL8 on islet supernatants showed a strict correlation with MCP-1 values. Serum MCP-1 levels did not correlate with in vitro MCP-1 levels of the islet preparations nor with the function of the graft. We want to confirm these results in a larger number of transplanted patients. To this aim a multicentric trial called "Laura", started two months ago, has involved the main islet centers (Edmonton, Giessen, Miami, Minneapolis) to collect data from a big number of islet recipients and further study the role of MCP-1 in the transplantation outcome.

HUMAN PANCREATIC ISLETS PRODUCE AND SECRETE MCP-1: BIOLOGICAL RELEVANCE

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Monocyte chemotactic protein 1 (MCP-1) is a CC chemokine active on monocytes, activated T lymphocytes and NK cells and is involved in the regulation of monocyte recruitment in a variety of pathological conditions. Since monocyte/macrophages are involved in autoimmune diabetes and in islet transplant rejection we have investigated the capacity of human islets to produce MCP-1. Primary cultures of pancreatic islets produced and secreted MCP-1 as determined by Northern blot, immunohistochemistry and ELISA. By immunohistochemistry and electron microscopy, the endocrine cells appeared positive for MCP-1. In chemotaxis assay MCP-1 secreted by pancreatic islets was able to

attract human monocytes; mAb anti-MCP-1 completely blocked their migration, suggesting that only MCP-1 was present as monocyte chemotactic factor. Human islets do not possess CCR2 (Northern blot, Ca⁺⁺ influx) and MCP-1 does not modulate insulin secretion. MCP-1 release is upregulated by cytokines (IL-1, TNF alpha and INF gamma), by LPS, by adhesion to laminin, fibronectin, vitronectin, collagen and pancreatic fibroblasts, but is not induced by metabolic stimuli (arginine, glucose). Preliminary data showed that human islets secrete other chemokine-like IL-8 and MIF. Finally, MCP-1 positivity confined to islets (immunohistochemistry) was found in various clinical conditions (pancreatic adeno K, chronic pancreatitis, pancreas from multiorgan donor) suggesting a constitutive secretion of MCP-1 by the endocrine cell population of the pancreas. This finding opens new perspectives in all the endocrine pancreatic diseases.

FEEDBACK-REGULATION OF SUBCUTANEOUS INSULIN INFUSION BY CONTINUOUS SUBCUTANEOUS GLUCOSE MEASUREMENT

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A closed-loop system for diabetic patients suitable for feedback-regulation under daily life conditions should include continuous subcutaneous (sc.) glucose monitoring and sc. insulin infusion. We tested a feedback algorithm specifically designed for subcutaneous insulin lispro infusion using tissue glucose (TG) concentrations measured by microdialysis technique. This sc. feedback regulation should achieve near physiological control without hypoglycaemic events.

The algorithm is based on the calculation of a prospective glucose value leading to an insulin dose calculation. The current glucose concentration and its gradient, carbohydrate intake and patient specific factors were used to determine the prospective glucose concentration and the insulin dose. The target glucose concentration was 120 mg/dl. We performed a study in 8 type 1 diabetic patients (2W:6M) with an age of 39 ± 9 (mean ± SD) years, a BMI of 24.4 ± 2.0 kg/m² and HbA1c of 7.1 ± 1.2%. Experiments lasted 22.0 ± 6.1 hours including 3 meals. The glucose concentration was measured subcutaneously every minute by microdialysis technique. Venous glucose concentration was measured every 12 or 36 min (during the day or night) and compared with the actual TG value. TG concentrations were used for the algorithm regulation if the deviation from the blood glucose (BG) value was less than 40%, otherwise the BG value was used.

During the feedback periods 91.4% of TG values were used for the feedback regulation. The mean BG concentration (MBG) of all experiments was 126.7 ± 51.8 mg/dl with a MBG range of individual experiments between 104.9 and 196.8 mg/dl. The standard deviation of all BG values of one experiment ranges between 31.6 and 60.8 mg/dl. We observed a postprandial BG increase of 68.9 ± 42.3 mg/dl. Throughout the experiments there were three hypoglycaemic events (BG < 50 mg/dl).

In the present study using a feedback regulation we observed mean glucose concentrations near the target value, low glucose fluctuations, postprandial glucose increases in almost physiological ranges and very few hypoglycaemic events. With a sc. closed-loop system, i.e. a combination of continuous sc. glucose monitoring and sc. insulin infusion based on a specific algorithm, improved metabolic control in diabetic patients should be possible.

ISLET-AUTOTRANSPLANTATION IN PATIENTS UNDERGOING TOTAL PANCREATECTOMY FOR CHRONIC PANCREATITIS

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There are only few indications for total pancreatectomy because this surgical procedure is followed by severe (brittle) diabetes mellitus.

Therefore it is only performed in exceptional cases. By combining total pancreatectomy with islet autotransplantation a brittle diabetes could probably be avoided. We performed total pancreatectomy in 4 patients with chronic pancreatitis over a period from 1998 until 2000. All patients had pancreatic resection of the head part due to chronic pain or duodenal obstruction previously. Indication for total pancreatectomy now was persisting pain in 2 cases, an anastomotic breakdown postoperatively following a Whipple-procedure and life-threatening complications due to pancreatitis (mediastinal pseudocysts) in one case. All patients showed severe fibrotic changes of the pancreatic tissue (50-90%) histologically. Three patients already suffered from IDDM before islet autotransplantation. In two cases (diabetes mellitus preoperatively, fibrotic changes > 80%) only few islets could be isolated (<10.000 IEQ) and no fasting C-peptide was measured postoperatively. These patients required more insulin postoperatively and showed no acute insulin response due to L-arginin stimulation. In two patients (pathological oral glucose tolerance test preoperatively in one case and Type-II-diabetes mellitus in the other patient, fibrotic changes 50-80%) we were able to isolate more than 200,000 IEQ. These patients showed a fasting C-peptide of 0.66 ng/ml and 0.81 ng/ml half-a-year postoperatively, an acute insulin response due to stimulation with L-arginin, and a stable metabolic situation without any episodes of hypoglycaemia. They require 16 i.U. (one-year-result) and 30 i.U. insulin per day (half-year-result) postoperatively. Our results demonstrate that the amount of islets which can be isolated from fibrotic pancreatic tissue is essential for the metabolic outcome. Thus, insulin independency cannot be achieved by islet autotransplantation, in spite of the counted amount of islets, but metabolic instability of patients with total pancreatectomy can be prevented.

ENGINEERED PITUITARY CELLS FOR ENCAPSULATION-BASED DIABETES CELL THERAPY

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Successful β cell replacement therapy in insulin-dependent (type 1) diabetes is hindered by the scarcity of human donor tissue and by the recurrence of autoimmune destruction of transplanted β cells. Availability of non- β cells, capable of releasing insulin and escaping autoimmune recognition, would therefore be important for diabetes cell therapy. We developed rat pituitary GH3 cells stably transfected with a furin-cleavable human proinsulin cDNA linked to the rat PRL promoter. Mature insulin secretion was obtained in both clones accounting for about 40% of total released (pro)insulin-like products. Secretagogue-stimulated insulin secretion was observed in both InsGH3 clones indicating that insulin was targeted also to the regulated secretory pathway. Proinsulin mRNA levels were elevated in InsGH3 cells, being significantly higher than in pancreatic β TC3 cells; moreover, proinsulin gene expression increased in response to various stimuli thereby showing the regulation of the transfected gene at the transcriptional level. Compared to β TC3 cells, InsGH3 cells showed in vitro a higher rate of replication, an elevated resistance to apoptosis induced by serum deprivation and proinflammatory cytokines, and significantly higher anti-apoptotic Bcl-2 protein levels. Moreover, InsGH3 cells were resistant to the streptozotocin toxicity that, in contrast, reduced β TC3 cell viability to 50-60% of controls. Subcutaneous implantation of 2 x 10⁶ InsGH3 cells resulted in the progressive reversal of hyperglycemia and diabetic symptoms. Proinsulin transgene expression was maintained in harvested InsGH3 grafts that, conversely, lose the expression of the prolactin (PRL) gene. Elevated concentrations of circulating mature human insulin were detected in graft recipients, demonstrating that proinsulin processing by InsGH3 cells did occur in vivo. InsGH3 cells might represent a potential β cell surrogate because, being more resistant than pancreatic β cells to different apoptotic insults, they might be particularly suitable for encapsulation.

USE OF CONTINUOUS SUBCUTANEOUS GLUCOSE MONITORING DURING PROLONGED EXERCISE IN TYPE 1 DIABETES

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Little scientific information is available regarding the changes in insulin or carbohydrate intake which are necessary to maintain euglycaemia during prolonged exercise. We took advantage of the availability of continuous subcutaneous glucose sensors (MiniMed, Sylmar, USA) to study 8 runners with Type 1 diabetes and 4 non-diabetic controls during a marathon race, run in ambient temperatures of 6-12°C. Sensors were fitted the day before the race and recording was continued for up to 24h afterwards. Runners were asked not to include finger pricks done during or immediately after the race in the sensor calibration in case there was any change in the relationship between blood and subcutaneous glucose levels. There were no cases of sensor failure and 9 runners (6 diabetic) finished the race with sensors in place. In controls, tissue glucose levels varied by less than 2 mmol/l. In four diabetic runners who had not reduced their insulin doses and started the race with glucose levels of >11 mmol/l, glucose levels began to fall after 30 mins and fell gradually to a plateau of 5-7 mmol/l. One runner who reduced her insulin had a tissue glucose of >22 mmol/l for >2h during the race, and one runner who gave himself extra insulin at the start had to stop because of hypoglycaemia 1h later. There were no other cases of hypoglycaemia during the race, and none during the following night, but two runners had prolonged episodes of asymptomatic hypoglycaemia during the night before the race. During the race some runners who did fingerpricks had very low glucose values yet had no symptoms of hypoglycaemia. A systematic comparison between fingerprick results and simultaneous subcutaneous readings showed a consistent disparity, finger pricks often reading substantially lower (5.5±0.8 mmol/l v 10.0±1.3, p<0.002). Conclusions: 1. Patients who are taking subcutaneous insulin cannot mimic the physiological fall in insulin levels which is normally seen at the start of exercise and only avoid hypoglycaemia by starting with elevated glucose levels. 2. Finger prick glucose levels give spuriously low values in cool ambient temperatures.

EXPRESSION OF BCL-2 FAMILY PROTEINS AND CASPASE 3 IN INTERLEUKIN 1 β -INDUCED APOPTOSIS

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Cytokine-induced β -cell apoptosis is one of the main events in the development of IDDM. In particular, Interleukin 1 β (IL1 β) initiates a signal transduction process resulting in the expression of inducible Nitric Oxide Synthase (iNOS), N-Mono-Methyl-L-Arginine (NMMA), an iNOS inhibitor, and Nicotinamide (NA), a PARP inhibitor, were used to prevent oxidative damages. The Bcl-2 family of proteins and the ICE family of cysteine protease are important in the control of apoptosis. The aim of this work was to investigate whether NMMA and NA can regulate expression of proteins related to apoptosis in an insulin-producing β -cell line. β -TC cells were exposed for 3 days to 5 mM NMMA, 5 mM and 25 mM NA alone or in combination with 50 U/ml IL1 β . Expression of Bcl-2, Bad, Bax, Caspase 3 and PARP was analysed by western-blot. Caspase 3 activation and DNA fragmentation were evaluated in all tested conditions. Results showed that: 1) exposure to IL1 β increased expression of Bax, Bad and caspase 3 activation; 2) β -TC cells cultured with 5 mM NMMA or NA showed pro-caspase 3 and PARP cleavage and DNA fragmentation; 3) 5 mM NA increased Bcl-2 expression without preventing IL1 β -induced apoptosis; 4) 5 mM NA and NMMA increased expression of Bad and Bax; 5) 25 mM NA decreased expression of Bad and Bax, both constitutive and IL1 β -induced, preventing IL1 β -induced apoptosis. These results indicate that Bad and Bax downregulation prevents IL1 β -induced apoptosis, while increased expression of Bcl-2 is unable to do that. NA prevents apoptosis in a dose-dependent manner. Since apoptosis occurs even in presence of NMMA these data suggest that strategies involving inhibition of NO production are not so beneficial as previously thought.

ASSESSMENT OF SUBCUTANEOUS GLUCOSE SENSING FOR USE IN A CLOSED-LOOP INSULIN DELIVERY SYSTEM

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We evaluated a subcutaneous glucose sensor (MiniMed Inc) in 5 non-diabetic men (age 53.3±11.6 years, mean±SD) with varying adiposity (BMI: 23.5 to 35.4 kg/m²) under dynamic hyper- and hypoglycemic conditions. Subjects underwent hyperglycemic clamps with 2 sensors inserted in the abdominal area. Sensors were allowed 2 h to stabilize after which blood samples were collected at -30, -20, -10, -1, 2, 4, 6, 8, 10, 15 min and every 5 min thereafter. Plasma glucose was clamped at ~10 mM with an exogenous glucose infusion. Sensors were calibrated retrospectively using a two-point algorithm. Plasma glucose increased from 5.5±0.07 mM (basal) to 17.2±0.99 mM within 2 min of initiating the clamp and stabilized at 10.2±0.22. Subsequently glucose was allowed to fall to 4.4±0.6 mM (range 2.8-5.4 mM). The subcutaneous sensor signal was well correlated with the plasma dynamics within ~6 min of initiating the clamp (r²=0.91) with a regression slope of 0.99±0.014 (not different from 1, p>0.05; 505 paired samples) and a mean absolute difference (Beckman vs. Sensor) of 8.1%. A sensor delay of 2.9±0.6 min was estimated (T_{1/2}) using a first-order differential equation. The sensor signal was then used as an input to a closed-loop insulin delivery algorithm designed to emulate the β -cells' natural first and second phase insulin responses. The algorithm is based on a proportional/integral/derivative (PID) control equation. The algorithm's ability to emulate endogenous insulin secretion, using the subcutaneous glucose signal as the input, was then evaluated by comparing the theoretical plasma insulin response to the measured plasma insulin response during the clamp (a first order model of insulin clearance was used to simulate plasma insulin concentration from the insulin appearance profile). For all subjects, the plasma insulin profile was well fit by the PID model using the sensor signal as input (0.82 < r² < 0.96). We conclude that the subcutaneous glucose-sensing site can serve as a surrogate for blood glucose measurements for closed-loop insulin delivery.

A NOVEL IMMUNO-ISOLATION DEVICE TO TRANSPLANT PANCREATIC ISLETS

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Isolation of pancreatic islets from the host's immune system by a semi-permeable membrane is a particularly attractive method of xenogenic islet transplantation. The aim of our study was to develop an immunoprotection device for the transplantation of pancreatic islets using synthetic hollow fibers. The device must have an internal volume that allows introduction of an adequate number of islets to induce a glycemic control in diabetic rats. Moreover the system should occupy a small volume and have a planar geometry to be transplanted under the skin, reducing the need for traumatic surgery and facilitating the removal of the device in case of malfunction. We have constructed a planar and parallel array of hollow fibers made of polyethersulfone (Amicon). Hollow fibers had an internal diameter of 500 μ m. To introduce the islets in the fibers we have designed a perfusion system that allows insertion inside the fibers of a sodium alginate solution containing pancreatic islets. Histological examination of the pancreatic islets before and after insertion into the device showed that perfusion procedure does not alter morphological appearance. A total number of 8,000 islet equivalents can be inserted in a planar device (5 x 2 cm). To evaluate survival and function of immuno-isolated islets, we placed 14,000 bovine islet equivalents in two devices and transplanted them under the dorsal skin of a streptozotocin-treated diabetic rat. After implantation of the device blood glucose decreased to normal levels in two days (from 587 mg/dl to 140 mg/dl). Normoglycemia was maintained for eight days post implantation, then progressively increased toward pre-implantation levels. These results indicate that this device allows an adequate exchange of glucose and insulin in order to obtain metabolic control. The morphologic study on hollow fibers recovered after failure

of transplanted material showed that the fibers were covered with fibrotic tissue while enclosed islets showed a good preservation of their structure. Our data show that islets contained within this planar device and transplanted under the skin survive and regulate blood glucose levels at least for a short period of time. We are testing different membranes with the aim of extending the time of glucose metabolic control.

FIRST HUMAN EXPERIENCE WITH COMBINED IMPLANTATION OF A LONG-TERM IV GLUCOSE SENSOR AND AN IP INSULIN PUMP

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Prevention of chronic complications, lowered risk of hypoglycaemia and improved patient convenience are the ultimate goals of type 1 diabetes treatment, that might be achieved by an artificial implantable pancreas elaborated from using improvements in available IP insulin pumps. Following successful animal trials and short-term use in humans, the present study was designed to assess the accuracy of an implanted long-term IV glucose sensor and the feasibility of its combined implantation with a pump for IP insulin delivery in diabetic patients. We performed a first implantation of this long-term sensor system (LTSS) in a type 1 diabetic man, aged 57, with a disease history of 38 years including stabilized retinopathy, previously treated by IP insulin from MIP2001 devices since 1990. Under general anesthesia, the glucose sensor was inserted under visual control into the right jugular vein and connected via a SC tunneled lead to a MIP2007B pump, located in an abdominal pump-pocket at the right flank. Surgical implantation of LTSS lasted 45 min. The single adverse event device malfunction was a disconnection between sensor and SC lead that was fixed under local anesthesia at day 2. During the first month, while insulin delivery was still controlled by using an external pump communicator from the results of at least 6 daily capillary blood glucose (CBG) measurements, CBG was 140 ± 57 mg/dl with 48-53 IU of insulin/day. Correlations between sensor data and HemoCue-measured CBG were excellent ($r=0.95$, $n=170$, range= 35 to 325 mg/dl), with 98% of paired points within A and B regions of Clarke Grid. Although limited to a short follow-up, our initial clinical and sensor data support LTSS implantation feasibility and sensor accuracy and open favourable perspectives for allowing insulin delivery based upon sensor data.

SHORT-TERM EXPERIENCE WITH NEW IMPLANTABLE PUMP MIP2007A SHOWS NEAR-OPTIMUM ACCURACY OF INSULIN DELIVERY

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Recurrent incidents of underdelivery related to insulin-pump incompatibility have been among the main adverse events of insulin infusion from implantable pumps during recent years. Acquired experience with previous devices allowed the design of the new implantable system MIP2007A, which may be expected to become a key element of the future artificial implantable pancreas. We assessed the accuracy of insulin peritoneal delivery from MIP2007A over a 3-month period in 10 type 1 diabetic patients. Among these patients (4 males, 6 females, aged 51 ± 8 , with a diabetes duration of 31 ± 12 years), 6 previously used the implantable system MIP2001. Both pump models infused U400 HOE21PH-variant 3 insulin and underwent refills of reservoir every 45 days. Accuracy of infusion was assessed by the percentage of programmed insulin delivery that was really infused, calculated from remaining insulin in the reservoir at pump refill. Optimum accuracy corresponds to 100% delivery. Refills of MIP2007A indicated near-optimum accuracy of insulin delivery after 45 days (102 ± 6 %) and 90 days (98 ± 4 %), whereas previous data obtained with MIP2001 showed significant impairment of accuracy between refills at 45 days (93 ± 17

%) and 90 days (85 ± 15 %, $p=0.006$). HbA1c levels were similar with MIP2001 (7.3 ± 0.3 %) and MIP2007A (7.6 ± 0.7 %), but precocious impairment of infusion accuracy with MIP2001 resulted in hazardous compensatory overprogramming of insulin doses. Although limited to a still short period of follow-up, our data suggest that significant technical improvements have been made with MIP2007A. Confirmation after a longer duration of use might generate favourable perspectives for the artificial implantable pancreas project.

THE INABILITY OF PANCREATIC β -CELLS TO EXPRESS ANTI-APOPTOTIC MOLECULES CAUSES THEIR SELECTIVE DESTRUCTION IN TYPE 1 DIABETES (INSULIN-DEPENDENT)

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Apoptosis has been identified as the principal mechanism of pancreatic islet β -cell death in auto-immune diabetes through pro-inflammatory cytokines. To further investigate the role of Fas-mediated apoptosis in determining β -cell destruction, we aimed to establish the different sensitivity of α - and β -cell lines to different apoptotic stimuli and the differential expression of *pro*- and *anti*-apoptotic molecules.

For this purpose, α -TC1-6 and β -TC3 cell lines after treatment with different cytokines (IL-1 α , IL-1 β , IFN- γ) were examined for cytotoxicity (MTT), apoptosis and expression of *pro*- (Fas, FasL, BAX) and *anti*-apoptotic (bcl-2, bcl-2 x_L , FLIPs) molecules by flow-cytometry, RT-PCR and WB.

The most potent inducers of apoptosis in β -TC3 cells were IFN- γ +IL-1 α or IL-1 β after a 72 h culture. In contrast, α -TC1-6 cells were strongly resistant to apoptosis induced by the cytokines used. Both α -TC1-6 and β -TC3 cell lines were Fas-negative in basal conditions, but they expressed Fas protein and mRNA after cytokine stimulation. β -cells expressed FasL on their surface but the expression was dramatically downregulated after cytokine treatment. Bcl-2 and bcl-2 x_L were detected in α -cells, but not in β -cells studying mRNA, protein content, and surface expression. FLIPs mRNA and protein content were found in both cell lines.

In conclusion, we suggest that β -cell destruction involves mechanisms subjected to regulation by Fas and FasL apoptotic molecules at the pancreatic level. These findings confirm the hypothesis that the selective destruction of pancreatic β -cells in type 1 diabetes is mediated by IL-1 β -induced apoptosis. In this light, β -cells are selectively sensitive to apoptosis because of their inability to express *anti*-apoptotic molecules. This study provides new insights into potential new therapeutic fields for the study of preventive tools in islet transplantation.

PANCREAS RESECTION AND ISLET CELL AUTOTRANSPLANTATION AFTER ACUTE NECROTISING PANCREATITIS

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Islet cell autotransplantation (ICA) after pancreas resection can prevent diabetes. However, there have been reports of significant complications after intraportal islet cell infusion. More recently with the introduction of safer endotoxin free isolation reagents these complications are now rarely reported. We report of a case of ICA after pancreas resection for acute necrotizing pancreatitis.

A 53-year-old female presented as an emergency with acute abdominal pain and vomiting. APACHE II scoring classified her as having severe acute pancreatitis. Abdominal CT identified necrosis with liquefaction in the central portion of the pancreatic body. The tail and head remained viable though disconnected from each other. She was initially managed conservatively; however, the necrotic region within the body of the gland showed no resolution. During a formal necrosectomy and distal pancreatectomy, islet cells were harvested from the viable tail and infused into the left branch of the portal vein. Approximately 350

IEQ/kg patient was infused in a total volume of 1.5 mL. There was no elevation in portal venous pressure or alteration in liver function tests. The patient made an uneventful postoperative recovery, and remains insulin independent.

Patients requiring necrosectomy after acute pancreatitis have a high risk of developing diabetes. When areas of the pancreas remain viable, islet harvesting using endotoxin free reagents and intraportal infusion is a safe procedure without any additional risk to the patient.

OVERALL SURGICAL COMPLICATIONS IN SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION

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Objectives To investigate the incidence of early surgical complications after pancreas transplantation (PT) and their incidence according to different surgical procedures utilized. **Materials and Methods** We have retrospectively analysed a group of 50 patients (pts) who received 54 PT between March 1993 and June 2000: 46 simultaneous pancreas-kidney transplantation (PKT), 4 pancreas transplantation alone (PTA), 4 pancreas retransplantation (RePT). The patients were divided into three different groups according to exocrine secretions treatment: Group A, 15 pts with bladder drainage (BD); Group B, 10 pts with side-to-side duodenal enteric drainage (SSED); Group C, 21 pts with duodenal enteric drainage on a Roux-en-Y loop (RED). All surgical complications requiring either relaparotomy or conservative treatment were recorded.

Results 16 pts (32%) developed at least one major surgical complication: 4 pts (8%) had venous thrombosis (1 BD and 3 SSED) and were retransplanted (1 patient died of pulmonary embolism, 3 pts are still alive but lost their graft function after 3, 9 and 27 months due to chronic rejection); 3 pts (BD) developed anastomotic leak of the bladder treated conservatively by a Foley catheter (all 3 pts maintained good graft function); 3 pts (6%; RED) with enteric leak required relaparotomy (1 pt developed associated pancreatitis and was submitted to pancreatectomy while the other 2 pts still have graft function); 3 pts (6%; RED) developed bowel occlusion requiring laparotomy (all 3 pts still have a good graft function). Surgical complications concerning kidney graft affected 3 pts: in one case (RED) ureteric stenosis required a Casati-Boari procedure, in the other pt (BD) with ureteric leak a re-anastomosis (Gregoir procedure) has been performed. With the last one who developed a lymphocele, surgical drainage was necessary. All pts have a good graft function. The overall incidence of major surgical complications in BD, SSED and RED groups was respectively 10%, 6% and 16%. **Conclusions** Despite a significant decline in the incidence during the last years, early surgical complications after PT still remain a major concern. In our experience retransplantation following venous thrombosis was associated with the worst results. Patients in the RED group developed a higher incidence of surgical complications (16%) but no mortality and a lower incidence of graft loss (1 pt) affected this group.

THE EFFECT OF BONE MARROW CO-TRANSPLANTATION ON ISLET SURVIVAL UNDER TEMPORARY IMMUNOSUPPRESSION WITH TACROLIMUS

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Objectives The aim of the present study was to assess the effect of pre-conditioning with donor specific bone marrow on the survival of allo-transplanted pancreatic islets in rats that received only temporary immunosuppressive therapy. We assumed that specific immunological tolerance could be achieved by the induction of peripheral mixed lymphocyte chimerism.

Methods As donors male Lewis x Brown Norway (BN) F1 hybrids were used; female BN rats served as recipients. In all animals diabetes was induced by streptozotocine. In Group I, 10 animals were only treated with transplantation (Tx) of 1000 freshly isolated pancreatic islets into the portal vein. In Group II, 15 rats underwent islet Tx after previous 45-day therapy with tacrolimus 1 mg/kg i.m. and hydrocortisone 2 mg/kg i.m. which was stopped 6 days after transplantation. Group III consisted of 15 rats that were treated as Group II and, in addition, underwent Tx of 1×10^8 bone marrow cells 10 days after initiation of immunosuppressive therapy. Peripheral microchimerism was detected using nested PCR technique specific for SRY locus on Y chromosome 1, 7 and 28 days following marrow administration. **Results** In Group I, the islets were rejected in all animals 8-10 days after Tx (blood glucose >9 mmol/L). In Groups II and III, 12/15 and 13/15 animals were still normoglycemic 34 days after cessation of immunosuppressive therapy. In Group III, chimerism was detected in most animals 1, 7 and 28 days following bone marrow injection. **Conclusion:** Temporary pre- and post-transplant therapy with tacrolimus and hydrocortisone was able to prevent rejection of allogeneic islets and development of graft versus host disease after bone marrow Tx for at least 5 weeks after therapy withdrawal. Only long term follow up will show whether peripheral microchimerism induced by bone marrow transplantation will prolong the survival of islet cells in non-immunosuppressed animals.

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INTRAHEPATIC TRANSPLANTATION OF PANCREATIC ISLETS ENCAPSULATED IN ALGINATE MICROCAPSULES IN DIABETIC RATS

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Objectives The survival of encapsulated islets transplanted into the peritoneal cavity is limited, even if capsular overgrowth is restricted to a minimum. Therefore, research should focus on finding a transplantation site that permits closer contact between the encapsulated islets and the blood. The most successful transplantation site for non-encapsulated islets is the liver. In this study, the effect of intraportal injection of encapsulated islets in diabetic rats was evaluated. **Materials and methods** Lewis rats served as islet donors and diabetic Sprague Dawley rats as recipients. A bolus of 1200 islets containing alginate microcapsules (350 μ m diameter) was injected intraportally (Group A; N=5). Animals transplanted with 1200 non-encapsulated islets served as controls (Group B; N=5). In defined time-intervals blood samples were taken for the evaluation of liver-enzymes and blood sugar. After graft failure the liver was explanted for histological evaluation. **Results** In diabetic rats the intraportal transplantation of non-encapsulated islets (Group B) leads within 24 hours to normoglycemia. This good metabolic control could be maintained for at least 8 days. In contrast to this, the transplantation of encapsulated islets (Group A) leads within 4 hours to blood sugar concentrations <35 mg/dl. This hypoglycemic status lasts up to 24 hours. Afterwards, an acute graft failure occurred in these animals. Histological studies revealed only a slight concentration of neutrophils surrounding islets which were fully covered by the alginate capsule. In contrast, islets not fully covered by the capsule were infiltrated by neutrophils leading to an acute cell lysis. In both groups (A and B) no substantial changes were observed concerning the estimated liver parameters, confirming the absence of relevant cholestase or cellular necrosis. **Conclusions** Histological studies revealed that the intraportal transplantation of 350 μ m islets containing barium alginate capsules is feasible but failed to induce long-term normoglycemia due to an acute cell lysis. Therefore, in ongoing studies we are developing a pre-coating technique to increase the number of well encapsulated islets in small sized capsules.

LONG-TERM EFFECTS OF PANCREAS TRANSPLANTATION ON PREVENTION OF DIABETIC NEPHROPATHY ON RENAL ALLOGRAFT

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Recurrence of diabetic nephropathy has been described on type 1 diabetic patients receiving a kidney allograft and pancreas transplantation is believed to be able to prevent it. The aim of the present study was to evaluate long-term protective effect of pancreas graft against the recurrence of the diabetic nephropathy in kidney transplant.

The study included 19 patients with diabetes type I and end-stage renal disease who underwent simultaneous pancreas-kidney (SPK) transplantation between 1984 and 1992: 11 recipients had functioning pancreas and kidney allografts (PK group) while 8 recipients lost their pancreas allografts in the post-operative period and they had a functioning kidney graft alone (K group). In both groups we measured glomerular filtration rate (GFR) and renal blood flow (RBF) by inuline and para-aminohippuric acid clearances and albumin excretion ratio (AER) by albumin/creatinine ratios. In 12 patients (6 of each group) kidney biopsies for detection of developing diabetic nephropathy were performed. Light and electron microscopy studies were performed to evaluate glomerular and tubular basement membrane thickness and the relative volume of mesangial tissue. Nonparametric test U of Mann-Whitney was employed for statistical analysis. Mean GFR and RBF were not statistically different between the two groups while AER was significantly higher in K group. In one biopsy from PK group a lesion score of >2 was demonstrated, while it was present in 5 of the 6 biopsies from K group. The morphometry studies showed a mean glomerular and mesangial volume significantly higher in the K group; a significant difference was also demonstrated for the glomerular and tubular basement membrane thickness. Ten years after transplantation the SPK recipients who have lost the pancreas allografts showed higher protein urinary excretion and substantial morphological alteration in the biopsies. These findings support the hypothesis that SPK transplantation could prevent the development of diabetic nephropathy in kidney allografts.

PROGRESS ON A FULLY IMPLANTED ARTIFICIAL β -CELL

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The efficacy of an implantable device capable of regulating glucose levels in insulin-dependent diabetic canines is presented. Device components include an intravascular glucose sensor, a sensor-enabled implantable insulin pump (MiniMed MMT 2007-B), and a "closed-loop" algorithm which allows the pump to automatically deliver insulin based on glucose sensor output. The biomechanical pancreas has been implanted in four diabetic canines for a 6-month period. Over the 6-month period, control was initiated or terminated on demand through an RF telemetry link.

Feedback control implementation produced an immediate lasting reduction in blood glucose values. Meals containing more than 100 g of carbohydrates were provided three times a day in order to challenge the algorithm and assess system response time. Prior to automatic control, average blood glucose values ranged from 155 to 393 mg/dl. For periods of automatic control, average blood glucose ranged from 69 to 123 mg/dl. Target glucose values for all experiments ranged from 80 to 100 mg/dl. Use of the artificial β cell reduced the number of reference blood glucose samples above 200 mg/dl from more than 40% to less than 5%.

Pharmokinetic and pharmacodynamic studies of plasma insulin levels were also performed during the control studies to assay for hyperinsulinemia. Under euglycemic conditions, insulin levels were consistently below 10 μ U/ml. Maximum levels never exceeded 50 μ U/ml. Plasma insulin profiles lagged programmed insulin delivery patterns by approximately 30 minutes. No clinical signs of hypoglycemia were observed during any of the control periods.

INTEREST OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN TRANSPLANTATION OF ENCAPSULATED PANCREATIC ISLETS: INDUCTION OF ANGIOGENESIS IN EPIPLOIC TISSUE

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Transplantation of encapsulated pancreatic islets has been proposed as a treatment for type 1 diabetes to limit rejection. Insufficient blood supply caused the loss of viability of grafted islets. The aim of our study was to evaluate the effect of vascular endothelial growth factor (VEGF) delivery on angiogenesis of tissues surrounding encapsulation device, containing or not islets. The encapsulation device (AN69 membrane, HOSPAL) containing 50 rat islets immobilized into collagen in the presence or not of VEGF was implanted in the peritoneal cavity of Wistar rats (n=6). Devices containing collagen supplemented or not with VEGF served as controls. After 7, 14 and 28 days of implantation, encapsulation devices and epiploic tissue surrounding the capsules were removed. Scanning electron microscopy analysis of the membrane surface and epiploic tissue histological examinations were performed. To analyse the course of the angiogenic process, 3 parameters were determined: the number of granulation tissue buds, the diameter of such buds and the distance between devices and buds. At each step of the study, an increase by 49.5 % of the number of buds was observed in the epiploic tissue surrounding the islets containing device supplemented with VEGF. After 7 and 14 days: (i) VEGF increased significantly the buds' diameter: 15.4 \pm 4.9 vs 5.3 \pm 2.4 μ m (p<0.001) and 25.4 \pm 2.5 vs 10.9 \pm 0.9 μ m (p<0.001), (ii) the distance between devices containing VEGF and buds was significantly lower than that observed with encapsulated islets alone: 16.2 \pm 5.6 vs 51.6 \pm 10.1 μ m (p<0.001) and 10.3 \pm 2.6 vs 30.7 \pm 2.8 μ m (p<0.001). From 14 to 28 days, no change was observed in either of parameters. Furthermore, the study of the controls revealed an increase in bud formation under the influence of VEGF alone emphasized by the presence of islets. The structure of the membrane remained unmodified by the addition of VEGF and there was no cellular adhesion at its surface. In conclusion, VEGF increased vascularization of the tissue embedding the devices. Local delivery of VEGF proved to be a relevant approach to ameliorate the outcome of islets transplantation.

NON-VIRAL GENE DELIVERY TO HUMAN PANCREATIC ISLETS

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Cellular transplants, such as pancreatic islets, may be manipulated *in vitro* prior to transplantation, improving graft survival without exposing the recipient to systemic therapy. One such manipulation is non-viral gene therapy. Ultrasound, liposomes and non-lipid polyamine transfection reagents may enhance non-viral gene delivery. Addition of microbubble echocontrast reagents (Optison[™]) enhances the effects of ultrasound, by facilitating cavitation. Here we describe preliminary results investigating delivery of the luciferase expressing pGL3 vector to human islets using a range of transfection conditions.

Human islets were purified from a pancreas from a cadaveric donor at the University of Giessen and transported to Bristol. Transfection was performed either with naked plasmid or after addition of a commercial non-lipid polyamine transfection reagent (*TransIT-LT1* [Mirus Corp, USA]), with or without 10% v/v Optison[™] microbubbles. Three different ultrasound conditions (60s exposure 956kHz: Mechanical index 1.1 6% duty cycle, MI 2 6% duty cycle or MI 3 1% duty cycle) were then compared with no ultrasound for each of these conditions. Islets

were aliquoted to wells of a 12 well tissue culture plate and luciferase expressing pGL3 plasmid added with or without adjuvant reagents. Ultrasound was applied to the cells, using a probe adapted to fit the wells. Following transfection cells were cultured for 48h and then lysed and luciferase expression assayed. An aliquot of islets was stained with FDA/EtBr to assess viability. Luciferase expression was enhanced by ultrasound and adjuvant reagents. Ongoing studies are optimising these transfection conditions.

PROLONGATION OF PORCINE ISLET XENOGRAFT SURVIVAL BY NON-CYTOTOXIC ANTI-MHC CLASS II ANTIBODIES

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Secondarily vascularised cellular xenografts are not a target for antibody mediated hyperacute rejection. In keeping with this, conventional T cell targeted immunosuppressive therapies are effective in preventing islet xenograft rejection in murine and primate models.

We have studied the efficacy of a non-cytotoxic anti-MHC class II antibody (OX6) in delaying porcine islet xenograft rejection in NOD mice. Rejection of this tissue is dependent on indirect T cell mediated responses. Furthermore, donor MHC molecules are not recognised by the antibody used and indirect recognition of graft antigens alone is therefore targeted by the immunotherapeutic regimen.

Cultured fetal porcine grafts were placed under the kidney capsule of NOD mice. One mg of OX6 was given ip the day before, day of and day after grafting. Grafts were scored according to an accepted scheme at days 7, 14 and 21 after grafting. Data are representative of two similar experiments.

Treatment	Number of grafts surviving		
	Day 7	Day 14	Day 21
OX6	5/5	6/8	1/7
PBS	3/5	0/8	1/6

At day 7 control grafts showed an eosinophil infiltrate with graft necrosis. OX6 treated grafts showed eosinophilic infiltrates but no necrosis. At day 14 both control and OX6 treated grafts showed heavy infiltrates but only OX6 treated grafts showed intact endocrine tissue. By day 21 OX6 treated and control grafts showed heavy infiltrates with loss of endocrine tissue.

This work demonstrates the potential for the use of immunomodulatory agents targeting indirect antigen recognition for prevention of rejection of xenogeneic islets.

EFFECTS OF SULFORAPHANE ON LYMPHOCYTE T PROLIFERATION INCUBATED WITH ALLOGENEIC PANCREATIC ISLETS

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Sulforaphane (SF) [1-isothiocyanato-4-(methylsulfinyl)butane] is known as a protective agent to prevent proliferation of tumor cells. The aim of our study was to evaluate the inhibitory effects of SF on lymphocyte T proliferation stimulated by allo-immune response of isolated pancreatic islets. Lymphocytes were isolated from spleen of Lewis rats (male, 250-300g). Pancreatic islets were isolated from WAG rats (male, 250-300g) using standard procedures and then separated by spinning on discontinuous Histopaque gradients. Islets and lymphocytes were conjugated with [methyl-3H] thymidine after 24h,

48h and 72h incubation at 37°C, 5% CO₂. The cells were collected and counted using a liquid scintillation counter. Group I: control group (n=4); Group II: SF was administered i.p. (25mg/kg/b.w.) a day before harvesting the pancreata (n=6); Group III: SF was administered i.p. (50 mg/kg/b.w.) a day before harvesting the pancreata (n=6). Allo-immune response of islets isolated from each group to lymphocyte proliferation was assessed by standard proliferation test. Statistical analyses were carried out using Students' T test. Differences were considered significant at $p < 0.05$. Our data indicate that the control group (mean lymphocyte number 3077.41 ± 1125.59) compared with lymphocyte proliferation in group II (mean lymphocyte number 331.41 ± 226.14) and group III (mean lymphocyte number 164.65 ± 77.34) shows statistically significant differences (control group vs group II $p < 0.0019$ and control group vs group III $p < 0.0013$). **Conclusions** 1. Islets isolated from rats treated with sulforaphane reduce lymphocyte proliferation. 2. Immunocompetency of allogeneic pancreatic islets is decreased by sulforaphane. The inhibitory effect of sulforaphane on T cells response needs in vivo confirmation by treatment of allograft islets recipients including drug mechanism study.

INDUCTION OF TOLERANCE TO PANCREATIC ISLET TRANSPLANTATION WITH DIFFERENT HEPATIC CELL SUBPOPULATIONS, IN WISTAR RATS, WITHOUT IMMUNOSUPPRESSIVE DRUGS

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Objective Looking for tolerance to pancreatic islets without using immunosuppressive drugs, we have tried to inject different hepatic cell subpopulations to determine which is the best form to generate tolerance for these islets.

Material and method Diabetes was induced by i.p. streptozotocine injection (60 mg/kg) and confirmed after 3 or more blood glucose measures of 350 mg/dl over different days. Islets were isolated with collagenase solution, purified with BSA gradients and cultured in CMRL-1066 medium for 3-4 days. Fresh hepatic cells and hepatocytes were isolated after double perfusion with saline/heparin and collagenase solutions. Fibroblasts were obtained after 1 month of culture in CMRL-1066 medium. Non-singeneic Wistar rats were used as donors and recipients.

Three groups of Wistar rats were studied: A) Co-Tx with a pool of hepatic cells (N=8); B) Co-Tx with only (pure) hepatocytes (N=8) and C) Co-Tx with liver fibroblasts (N=6).

In all groups, different hepatic cell subpopulations, with a ratio of 200 cells per islet (200:1), were injected via inferior cava vein and after a period of 15 min islets were injected via porta vein. No immunosuppressive drugs were employed. The average islet number (ENI) transplanted was 1845 ± 195 in group A, 1552 ± 109 in group B and 1472 ± 125 in group C; with purity of 90%. The ratio hepatic cells/islet was 200:1 in all groups.

Blood glucose was measured with Gluco Touch strips at 1, 2, 3, 4, 6, 7, 9, 11, 18, 25 and 30 days after Co-Tx in all groups. SPSS have been applied for statistical study.

Results Reversion of diabetes (blood glucose less than 150 mg/dl) was observed among the three groups with some differences. All groups showed a decrease of blood glucose levels for the first 5-6 days after Co-Tx, increasing at day 6 and finally reaching high levels. However, 2 rats of group A showed euglycemia (< 150 mg/dl) during 18 days, 3 rats of group B showed euglycemia during all the study and none of the group C was euglycemic for a period longer than 7 days.

Discussion and conclusions The results confirm some useful influence of hepatic cells on islet implantation without immunosuppressive drugs. Moreover, induction of tolerance is better with pure hepatocytes compared with the other types of hepatic cell subpopulations. More studies are needed in order to clarify the possible mechanism implicated.

A MULTICENTER TRIAL OF TWO DACLIZUMAB DOSING STRATEGIES VERSUS NO ANTIBODY INDUCTION IN SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANTATION: INTERIM ANALYSIS

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Introduction The safety and efficacy of daclizumab (DAC) (1 mg/kg/dose every 14 days for 5 doses) have been established in kidney and heart transplant recipients. Alternative dosing regimens based on pharmacokinetic simulation and limited clinical trials are being investigated. The purpose of this ongoing multicenter study is to determine the safety and efficacy of two dosing regimens of DAC as an adjunctive immunosuppressive agent compared to no antibody induction in simultaneous kidney-pancreas transplant (SKPT) recipients receiving tacrolimus (TAC), mycophenolate mofetil (MMF) and steroids as primary therapy. **Methods** This is an interim report of a multicenter, prospective, open-label, randomized study with a target enrollment of 290 patients. Eligible SKPT patients were randomized to one of three groups: DAC 1 mg/kg/dose every 14 days for 5 doses (Group I), DAC 2 mg/kg/dose every 14 days for 2 doses (Group II) and no antibody induction (Group III). The primary endpoint of the study is a composite of the incidence of presumed or biopsy-proven kidney or pancreas rejection, graft loss or death within the first 6 months post-transplant. **Results** A total of 239 patients were randomized into the 3 groups [Group I (n=91), Group II (n=96), Group III (n=52)]. Demographic and transplant characteristics were similar among the groups. At a minimum follow-up of 3 months (n=166), patient, kidney and pancreas graft survival rates were similar among the three groups. However, the rates of acute renal allograft rejection were 18% (Group I), 8% (Group II), and 36% (Group III) (p=0.006). The probability of either kidney or pancreas allograft rejection was 22% (Group I), 8% (Group II), and 38% (Group III). At 3 months, the actuarial event-free survival (no acute rejection, allograft loss or death) was 67%, 81%, and 50% in Groups I, II, and III, respectively (p=0.02). **Conclusion** The 2-dose regimen (Group II) appears to be as effective as the 5-dose regimen (Group I) in preventing acute rejection after SKPT and is associated with the lowest acute rejection rate and the highest rate of event-free survival. However, the benefits of DAC compared to no antibody induction await larger sample size accrual.

A PROSPECTIVE COMPARISON OF SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANTATION WITH SYSTEMIC-ENTERIC VERSUS PORTAL-ENTERIC DRAINAGE

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Although renewed interest has occurred in enteric exocrine drainage, most pancreas transplants (PTXs) are performed with systemic venous delivery of insulin (systemic-enteric [S-E]). To improve the physiology of PTX, we developed a technique of portal venous delivery of insulin and enteric drainage of the exocrine secretions (portal-enteric [P-E]). The purpose of this study was to compare PTX with S-E versus P-E drainage in a prospective fashion. Over a 26-month period, we alternated 54 consecutive simultaneous kidney-PTXs (SKPTs) to either S-E (N=27) or P-E (N=27) drainage. The two groups were well matched. Maintenance immunosuppression in both groups consisted of Tacrolimus, Mycophenolate Mofetil, and steroids. **Results** Patient and kidney graft survival rates are 96% and 93%, respectively, in both groups. One early death occurred in each group (both with functioning grafts). PTX survival (complete insulin independence) is 74% after S-E versus 85% after P-E drainage with a mean follow-up of 14 months. The mean length of initial hospital stay was 12.4 days in the S-E and 12.8 days in the P-E groups, respectively. The S-E group had a slight increase in the number of readmissions (mean 2.8 S-E versus 2.2 P-E, P=NS). The incidence of acute rejection (33%) was similar in both groups, with immunologic pancreas graft loss occurring in 3 S-E

patients versus 1 P-E patient. The incidence of major infection was 52% in both groups, with 1 CMV infection (4%) in each group. The incidence of intra-abdominal infection was slightly higher in the S-E group (30% S-E versus 11% P-E, P=NS). However, the early relaparotomy rate was similar between groups (30% S-E versus 26% P-E). Mean hospital charges were also comparable between groups (\$102,255 S-E versus \$105,789 P-E). The composite endpoint of no rejection, graft loss, or death was attained in 56% of S-E versus 59% of P-E patients. **Conclusions** These results suggest that SKPT with S-E or P-E drainage can be performed with comparable short-term outcomes.

SPECIFIC BINDING OF PORCINE C-PEPTIDE TO THE HUMAN C-PEPTIDE RECEPTOR

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Background The use of porcine islets of Langerhans would offer an unlimited source of tissue for transplantation of patients with type 1 diabetes. Porcine insulin is structurally very similar to human insulin and it is well confirmed that porcine insulin functions in humans. In contrast, porcine and human C-peptide have major structural differences. However, in the active carboxy terminal pentapeptide, 3 of 5 residues are homologous. C-peptide has until recently been considered to be biologically inert. During the last 5 years, a series of reports have presented data regarding beneficial effects of C-peptide on the microcirculation of kidney, nerve, retina and skeletal muscle in patients with type 1 diabetes. These effects are mediated via C-peptide's ability to stimulate endothelial nitric oxide synthase and Na⁺, K⁺-ATPase activities. It may thus be of value to evaluate whether porcine C-peptide can exert similar biological effects in future xenoislet recipients. As a first step, the binding of porcine C-peptide to human cell membranes has been examined using fluorescence correlation spectroscopy (FCS).

Material and methods By using the FCS technique - measuring the ligand-membrane interactions at single-molecule detection sensitivity in 0.2- fL confocal volume elements - specific binding of fluorescently labelled (tetramethylrhodamine = Rh) human C-peptide to human renal tubular cells could be demonstrated. Full saturation of the C-peptide binding to the cell surface was obtained at low nanomolar concentrations (1 nM). When an excess of unlabelled pig C-peptide (5 µM) was added the Rh- labelled human C-peptide was completely displaced. **Conclusion** Our results indicate binding of the porcine C-peptide to human C-peptide binding sites. However, further experiments are needed to evaluate whether biological effects are obtained.

FACTORS AFFECTING HUMAN ISLET ISOLATION YIELD

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Objectives Yields of human islet isolation are highly changing from one isolation to another. We identify criteria for the selection of donors in order to increase isolation outcomes.

Materials and methods From 11.91 to 10.00, 234 islet isolations have been performed in our laboratory according to a semi-automated technique. The first 50 pancreata used to establish the technique (from 11.91 to 10.93) and 16 procured for autotransplantation (isolation without purification) have been discarded from the study. Yields (EIN/g) from 168 isolations are compared to donor and procurement characteristics.

Results

		EIN/g		EIN/g	p
Age (years)	>=20 (n:155)	1763	<20 (n:13)	1011	<0.01
BMI (kg/m ²)	>=25 (n:52)	2135	<25 (n:117)	1508	<0.01
Hypotension	No (n:99)	1873	Yes (n:65)	1512	0.06
Cold ischemia (h)	<8 (n:135)	1906	>=8 (n:25)	1037	<0.005
Secondary warm ischemia (min)	<30 (n:13)	2434	>=30 (n:93)	1625	0.05

In this study, the sex, the cause of death, the blood glucose and the LDH levels had no significant effect on yield.

Conclusion A selection of donors according to age, the BMI and the haemodynamic stability increase isolation yields. The duration of harvesting (secondary warm ischemia) and of transport (cold ischemia) also has significant effects on the outcome. These last parameters are important, as it is possible to shorten them.

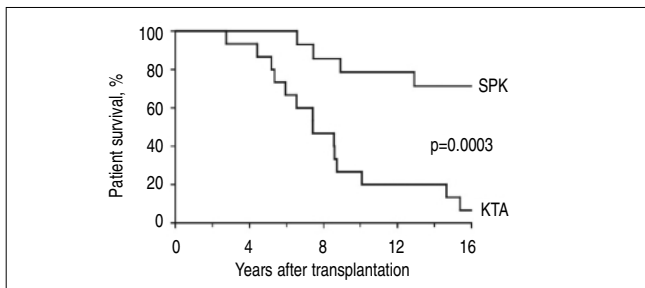
MAINTAINED HIGH SURVIVAL RATE IN DIABETIC RECIPIENTS OF COMBINED RENAL AND PANCREATIC GRAFTS 16 YEARS AFTER TRANSPLANTATION

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Background We have followed two groups of patients with insulin-dependent diabetes mellitus (IDDM) and end-stage diabetic nephropathy. As previously reported, patients undergoing simultaneous pancreas and kidney (SPK) transplantation had significantly better survival at 10 years than those receiving a kidney transplant alone (KTA). These patients have now been followed up to 16 years.

Material and method This prospective study involved all diabetic patients accepted for combined renal and pancreatic transplantation between 1982 and 1986 and who were evaluable two years after transplantation. The SPK group consists of 14 patients given segmental pancreatic grafts with enteric exocrine drainage. The KTA group consists of 15 patients who lost their pancreatic grafts within the first year because of technical complications (n = 9) or rejection (n = 1) or opted for a kidney alone in spite of being accepted for the combined procedure (n=5).

Results At present, 10 of 14 patients in the SPK group are alive compared to 1 of 15 in the KTA group.



Conclusion As previously reported, patients undergoing SPK have an improved long-term survival, compared to diabetic patients given renal grafts alone. A high survival rate is maintained in the SPK group for at least 16 years after transplantation.

PANCREAS TRANSPLANTATION WITH ENTERIC EXOCRINE DRAINAGE: FROM AN EXPERIMENTAL TECHNIQUE TO A ROUTINE PROCEDURE

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Background Our group has performed pancreatic transplantation with enteric drainage technique since 1974. Using segmental pancreatic grafts, this technique was initially associated with a high incidence of pancreatic fistulas and infections. With refinements in technique and by the use of pancreatic duct catheter for temporary drainage, the results gradually improved. In 1984, pancreatico-duodenal grafts were reintroduced. Initially a protecting pancreatic duct catheter was still used. However, the incidence of early severe graft pancreatitis increased and

the catheter was considered to be a possible contributing factor. In our most recent series we have performed pancreatico-duodenal grafts without catheter and without Roux-en-Y loop. The technical complication rate for a pancreatic transplantation with exocrine enteric drainage using different surgical techniques is shown in the table below:

	Pancreatitis	Infection	Thrombosis	Leakage	Total
Segmental grafts with Roux-loop (n=20)	5%	25%	15%	10%	55%
PD grafts without Roux-loop, with catheter (n=20)	20%	10%		5%	35%
PD grafts without Roux-loop, without catheter (n=41)	5%	2%	7%		15%

Conclusion Pancreatico-duodenal transplantation with enteric exocrine drainage by direct side-to-side bowel anastomosis has developed into a reliable technique that can be applied routinely with a low incidence of technical complications.

A DIFFERENT MODEL PREDICTS HbA1c IN TYPE 1 AND TYPE 2 DIABETES

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Background Elevated HbA1c is associated with long-term complications in both type 1 (DCCT, 1993) and type 2 (UKPDS, 1998) diabetes mellitus (DM). The physiology that underlies variations in glucose levels is different for these two DM types. Continuous glucose monitoring can be used to identify expected differences in glycemic patterns for the two types of DM.

Purpose The purpose of this study was to model the multivariate relationship between continuous glucose profiles and HbA1c and to identify differences in the model as a function of DM type. We can also predict the optimal timing of finger-stick samples for both types.

Method Glucose profiles from a continuous glucose monitoring system (MiniMed, Northridge, CA) were reviewed for 174 patients with type 1 DM and 26 patients with type 2 DM. Subjects wore the sensors for between 1 and 7 days and all subjects had an HbA1c measurement obtained no more than 90 days (mean \pm SD = 23 ± 24) prior to the sensor's use. Data from each subject's sensor use were reduced to four quantitative variables: average glucose (AVG), glucose standard deviation (SD), area under the curve for values ≥ 140 mg/dL (AUC HI) and AUC for values ≤ 60 . A Stepwise Multiple Least Squares Regression was then used to predict HbA1c from these four variables.

Results AUC, HI and SD were the only significant predictors of HbA1c for patients with type 1 diabetes ($R^2=0.30$) conversely, AVG was the only significant predictor of HbA1c for patients with type 2 diabetes ($R^2=0.55$).

Conclusion These results suggest that the relationship between patients' pattern of glycemic control and their HbA1c values depends on the type of diabetes they possess. The use of continuous glucose sensing confirms that degree and frequency of glucose excursions are more important than overall level of glucose for patients with type 1 DM, whereas the opposite is true for patients with type 2 diabetes. These results suggest that only glucose testing that captures postprandial and nocturnal glucose excursions will adequately reflect a patient's risk of long-term complications in the case of type 1 DM. The importance of average glycemia for type 2 DM and the variability of glycemia for type 1 DM also suggest that the optimal strategies for insulin delivery may be different for the two types of diabetes.

A MICRODIALYSIS TECHNIQUE FOR CONTINUOUS SUBCUTANEOUS GLUCOSE MONITORING IN DIABETIC PATIENTS

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Aim To assess accuracy and precision of a new subcutaneous glucose measuring system in comparison to the reference glucose-oxidase method in type 1 and type 2 diabetic patients. **Methods** In each patient a microdialysis probe was inserted in the subcutaneous abdominal tissue and connected to a portable microperfusion pump coupled with a biosensor and a microdialysis system (GlucoDay, A. Menarini I.F.R. S.r.l., Florence Italy) for 24, 48 and 72 hours. Subcutaneous glucose concentration was measured continuously and recorded every 3 minutes. Throughout the day venous samples were collected for later glucose measurements with the reference method (Beckman, Ohio, USA). The reproducibility study was performed using two instruments on the same patient. **Results** Probe insertion was well tolerated by all patients. The linearity is extended to 27 mM for glucose concentration in-vivo, and the sensitivity concerning blood glucose is better than 0.1 mM. The excellent biosensor stability at room temperatures with minimal maintenance procedures simplifies the management of the system. The intra-series reproducibility study showed a good agreement with bias below 10%. **Conclusion** Subcutaneous glucose monitoring with microdialysis technique accurately reproduces venous glucose values.

48-72 hours of continuous monitoring of glucose could improve metabolic control both in type 1 and type 2 diabetic patients.

AUTOIMMUNE REACTIVITY AFTER HUMAN ISLET CELL AUTOTRANSPLANTATION

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Human islet cell autotransplantation (ICA) combined with pancreas resection can prevent the onset of diabetes. However, not all patients remain insulin independent. The aim of this study was to assess autoimmune reactivity, after human ICA, as a cause of beta cell destruction and insulin dependence.

A series of different patient cohorts were assessed for autoreactivity. These included control subjects n=14, chronic pancreatitis (no diabetes) n=20, chronic pancreatitis (with diabetes) n=4, autoimmune disease n=20, cancer surgery n=11, total pancreatectomy (TP) alone n=7, pancreas resection with ICA n=20 and finally diabetic subjects n=6. All patients were assessed for GAD-65 antibodies and islet associated-2 (IA-2) antibodies. HLA-DR and DQ type 1 diabetes-associated alleles

were also evaluated in ICA recipients (data not shown). The results are summarised in the table below.

	Mean (SEM)	
	GAD-65	IA-2
CP (no diabetes)	5761 (193)	288 (12)
CP (diabetes)	5566 (396)	357 (15)
Controls	5818 (150)	285 (14)
Autoimmune	5761 (104)	216 (6)
Cancer surgery	5315 (106)	189 (3)
TP (no ICA)	5417 (178)	330 (37)
TP with ICA	5761 (104)	278 (13)
IDDM	8123 (1748)	1386 (349)

These results suggest that autoreactivity is not a significant cause of insulin dependence after pancreas resection combined with human islet cell autotransplantation.

IMPACT OF WARM-ISCHEMIA TIME ON ISLET ISOLATION AND PURIFICATION OUTCOME IN RATS

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Objectives Many factors influence the outcome of islet isolation and purification. Impact of warm-ischemia time has always been regarded as one of the most important factors. But up to now no clear relationship between warm-ischemia time and outcome of islet isolation and purification has been reported. The aim of the present study was to clarify this. **Materials and methods** 60 adult Lewis rats (weight 350-500 g) were randomly divided into 3 groups. After liproctomy, a catheter was cannulated into the pancreatic duct. 25 mg collagenase (Sigma, Type V) was dissolved into 15 ml 4°C RPMI1640 solution (Sigma). 10 ml solution was injected into the pancreas via the catheter to distend the pancreas while the remaining 5 ml was used during incubation. In the first group, after opening the thoracic cavity and bloodletting, collagenase solution was immediately infused. In the second group, collagenase was infused 8 minutes after bloodletting was finished. In the third group, collagenase was infused 15 minutes after bloodletting. Islet isolation and purification outcome were compared. **Results** In the first group, 800±120 islets were isolated and 680±80 islets were recovered after purification. In the second group, only 400±80 islets were procured and 260±100 islets remained after purification. In the last group, few islets were observed. There was significant difference between the first and the second group (P<0.05). **Conclusion** Warm-ischemia time has significant influence on the outcome of islet isolation and purification. With time prolonged, islets recovery after isolation and purification also decreased and few islets could be found after 15 minutes.