OP 24

Effect of Immunorejection after Transplantation of Bone Marrow Donor's Islets in Streptozotocin-Induced Diabetes Mellitus Hematopoietic Mixed Chimerism Mouse Model

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Background and Aims. *The current success with islet allograft transplantation is reviewed however the shortage of human islet tissue and immune rejection mean that only a small fraction of people with diabetes would be able to benefit. The immune tolerance induction conducted by hematopoietic mixed chimerism with minimal invasive methods may be the ideal alternative to overcome not only immune rejection also the toxicities of immunosuppressive drugs. The purpose of this study is to investigate the effect of mixed chimerism conducted by newly developed minimally invasive methods on islet allografts rejection in streptozotocin induced diabetic mice.*

Materials and Methods. *Recipient. Balb/c(H-2Kd) mouse was injected intraperitoneally with anti-asialoGM1 antibody on day -1 before bone marrow transplantation. It received total body irradiation at a dose of 500 cGy and followed by tail vein injection of the 2x107 T-cell depleted bone marrow cells from C57BL/6(H-2Kb). Mixed chimerism mouse determined by gDNA PCR of lymphocyte MHC class I gene (H-2K) on day 21. Streptozotocin induced diabetic mixed chimera mouse was received islet transplantation from bone marrow donors. Grafts and spleen, peripheral blood were obtained from the mixed chimera mouse and there were by use of Immunohistochimerstry stain, flow cytometric analysis and gDNA PCR on day 21.*

Results. *The blood glucose level was normalized by transplantation of bone marrow donor's islets and maintained during 21 days. After removal of first islet allo-grafts, diabetes mellitus was re-established. We could confirmed donor specific tolerance of transplanted islets by second transplantation of bone marrow donor's islets. Normoglycemia was maintained for 21 days after second islet transplantation. Furthermore islet graft from mismatched third party mouse were immediately rejected. Flow cytometric analysis results suggest that T-cell depleted bone marrow induced mixed chimerism mice was maintain during the whole study period.*

Conclusion. *The mixed chimerism conducted by newly developed minimally invasive methods effectively prevent the islet allografts rejection in streptozotocin induced diabetic mice.*

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OP 25

A Robust Method for the Assessment of Beta-Cell Mass: The Influence of Altered Peripheral Insulin Sensitivity on Commonly Used Tests of Insulin Secretion

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Background and Aims. *Insulin secretion is the product of beta-cell mass and function, and decreases with the progression of diabetes, possibly due to decreasing beta-cell mass. Several methods are used to investigate insulin secretion after a period of medical therapy, putatively reflecting improvement in insulin secretory capacity. These tests are often conducted on the background of changing insulin sensitivity, and may in fact be reflecting compensatory increases in beta-cell function, therefore failing to distinguish between improved function and increased mass. Thus, an accurate test for beta-cell mass should be insensitive to changes in function, and it may be possible to render such a test if a method to maximize beta-cell insulin output were developed.*

Materials and Methods. *This was a 3 period cross-over study, with 9 healthy subjects receiving, in random order; control period, 7 days of Metformin 500 mg bid or 7 days of Prednisolone 15 mg bid, with 14 day washout between periods. The assumption is that peripheral insulin sensitivity would be altered by the prednisolone and metformin therapy whilst beta-cell mass would remain stable. The beta-cell assessment procedure consisted of the following manoeuvres performed in sequence; baseline glucose, IVGTT, hyperglycaemic clamp at 25mM glucose, arginine bolus and infusion, and glucagon bolus (1mg). Insulin secretion was assessed at each step.*

Results. *After prednisolone therapy, peripheral insulin sensitivity was decreased compared to control, and there were significant increases in insulin secretion at baseline(pmol/L) (75.7 vs 44.7, ratio= 1.69, $p<0.001$), first phase insulin secretion after IVGTT(pmol*min/L) (7112 vs 4089, ratio= 1.74, $p=0.008$), and during the hyperglycaemic clamp(pmol*min/L) (63105 vs 31923, ratio= 1.98, $p=0.001$). However, as the assessment progressed, the differences in insulin secretion diminished, such that following the glucagon bolus, the difference between prednisolone treatment and control in mean maximal insulin secretion(pmol/L) (79814 vs 68770), ratio= 1.16, p=NS) was minimal and not significant.*

Conclusion. *Hence, short-term changes in insulin sensitivity are compensated for by alterations in basal insulin secretion, insulin secretion during IVGTT and hyperglycaemic clamp. However, after the glucagon bolus, the difference in insulin secretion between the control and prednisolone arms is reduced to approximately 16%, possibly indicating that maximal insulin secretion is being approached. This suggests that the insulin secretion at this point may be truly indicative of beta-cell mass and not merely of function. A modification of this investigational technique may be useful for determination of changes in beta-cell mass following medical therapy.*

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OP 26

The Effect of Endothelin-1 on the Expression of Cd36 in Vascular Smooth Muscle Cells

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Background and Aims. *Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor. ET-1 also stimulates the DNA synthesis of vascular smooth muscle cells (VSMC) and thus is suspected to play an important role in developing various circulatory disorders including hypertension and atherosclerosis. CD36 is a highly glycosylated, single chain 88-kDa membrane protein that is widely expressed in human tissues including the heart and blood vessels. CD36 binds oxidized low-density lipoproteins(ox-LDL), fatty acids, anionic phospholipids, collagen and thrombospondin. As a result of the broad ligand specificity, multiple roles for this protein have been proposed such as transporter for long-chain fatty acids and scavenger receptor for ox-LDL. Moreover, deficiency of CD36 has been reported to associate with insulin resistance. This study is aimed to investigate the effects of ET-1 on the expression of CD36.*

Materials and Methods. *Aortic smooth muscle cells were cultured from Sprague-Dawley rats. When they were 80% confluent, ET-1 was added at different time points (0~24 h) and concentrations (0~10^{-7} M). The expression of CD36 was measured using Western blot analysis.*

Results. *The results showed that incubation with 10^{-9} M of ET-1 resulted in 20% inhibition on CD36 expression compared to the control group. This response was dose-dependent. The maximal inhibition (10^{-7} M of ET-1) was approximately 50% lower than that in controls. The time course of ET-1 effect was also examined and a significant inhibition (about 20%) on CD36 expression was found in the ET-1-treated group after 12h of incubation, with maximal inhibition (about 40%) seen at 24h of incubation. Furthermore, BQ-610(an ET_AR antagonist) completely prevented the inhibitive effect of ET-1, while BQ-788(an ET_BR antagonist) had no effect, suggesting that the effect of ET-1 on CD36 expression was mediated through the ET_AR. Finally, either Wortmannin(10^{-7} M), a PI3-kinase inhibitor, or PD98059($6x10^{-6}$ M), a selective ERK inhibitor partially reversed the effect of ET-1.*

Conclusion. *In conclusion, we found that ET-1 suppressed the expression of CD36 in VSMC, acting through ET_AR coupled with PI3- kinase and ERK dependent signaling pathways. Further studies to investigate its mechanisms in more detail and its potential physiologic and pathologic roles in the vessel wall are warranted.*

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OP 27

Glycated and Carboxy-Methylated Proteins Do Not Directly Activate Human Vascular Smooth Muscle Cells

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Background and Aims. *Advanced glycation end products (AGEs) accumulate in patients with diabetes, particularly at sites of vascular damage and within atherosclerotic lesions, but whether they have direct actions on vascular smooth muscle cells (vSMCs) is controversial. The aim of the study was to determine if AGEs have direct actions on vSMCs to elicit full cellular responses associated with cell activation and atherosclerosis*

Materials and Methods. *AGEs were constructed and characterized by protein content, level of modification, fluorescence and molecular size. Human VSMCs were derived from different vascular beds. Glucose consumption, de novo protein synthesis and proteoglycan biosynthesis were measured using a colorimetric assay and metabolic radiolabeling. Receptor for AGEs (RAGE) expression was assessed by Real Time RT-PCR and Western blot*

Results. *Treatment with AGEs under low or high glucose conditions showed no change in cellular glucose consumption or in cellular protein synthesis under low glucose conditions. Treatment of vSMCs with Ne (carboxymethyl)lysine in the presence of low glucose, increased [35S] sulfate incorporation into secreted proteoglycans by 72% ($P<0.001$) and 67% ($P<0.001$) however, the control proteins also increased [35S] sulfate incorporation into proteoglycans by 56% ($P<0.01$), with similar effects observed under high glucose conditions. Human vSMCs showed no difference in response to glycated and non glycated protein. Protein and gene expression of RAGE in vSMC was very low, consistent with the immunohistochemical staining of RAGE in a human artery. Monocytes and endothelial cells had higher levels of RAGE expression and monocytes gave a cellular response to AGEs.*

Conclusion. *vSMCs show very low levels of RAGE expression and activation of human vSMCs by AGEs does not occur. In diabetes, RAGE expression in vSM may be increased to the extent that vSMCs are drawn in to the process of atherosclerosis and the factors that induce RAGE expression in vSMC need to be identified.*

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OP 28

Humalink Web-Based System for Prediction and Management of Blood Glucose – First Steps in Europe

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Background and Aims. *Promise of the DCCT study has yet to be realized in clinical practice. Notwithstanding intensive education and therapy, there is a distinct lack of suitable alternative to the intensive decision support also provided in the DCCT. Novel glucose predicting engine HumaLink (HL) predicts expected blood glucose (BG) level at next meal as well as pending risks of hypoglycemia. Use of its predictions in decision support in respect to medication dosing, diet, exercise and stress promises to empower insulin treated patients to achieve better diabetes control while reducing hypoglycemia. Our aim was to adapt HL for access using internet and apply in first patients in Europe; evaluate validity, safety and efficacy of its predictions in dosing decision support for routine patient care.*

Materials and Methods. *The kernel of HL is database located on server accessible to both registered patients and providers. Patients can access HL and enter self-monitored BG levels anytime. In response, means to modify medication dosages (dosing decision support) and modify planned diet and physical activity (life-style decision support) are afforded the user. Up-down buttons allow adjusting dosages, change carbohydrates, exercise, and level of stress. For each adjustment, impact on medications and predicted BG outcomes is animated. Providers can access patients' data and make therapeutic changes. 1.10.2004 first 2 intensive insulin treated patients were registered to HL in Europe. Validation was done at 6-month follow-up by comparison of predicted values to subsequently observed data using Clarke Error Grid (CEG), safety focused on body weight and frequency of hypoglycemia. Efficacy was judged according HbA1c and daily insulin dosages.*

Results. *From 750 reported BG levels 510 BG predictions were made. 88,6% were in clinically acceptable zones of CEG, 9,4% in C+D and 2% in E. In the first patient HbA1c decreased from 9,9% at baseline to 7,25% at 6-months follow-up and from 8,03% to 7,69% in the second one. Total daily insulin doses increased from 26IU to 28IU in the first patient and declined from 40,3IU to 33,3IU in the second one, while body weight remained constant. Hypoglycemia dropped 3 fold.*

Conclusion. *Use of validated HL predictions in decision support for managing intensified insulin therapy in first patients in Europe proved safe and efficacious. It may help patients and their providers to realize better glycemic control and thereby achieve the promise of the DCCT.*

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OP 29

Application of Continuous Glucose Monitoring System in the Assessment of Within-Day and Day-To-Day Blood Glucose Excursions in Type 2 Diabetes

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Background and Aims. *The profile of 24h glucose is very importent to the treatment and prognosis of diabetic patients.To study the features of within-day and day-to-day blood glucose excursions in newly-diagnosed type 2 diabetic patients,we observed the change of glucose using CGMS.*

Materials and Methods. *Thirty-eight individuals with normal weight and normal glucose regulation (NGR) and thirty-nine newly diagnosed type 2 diabetic patients (N-T2DM) without previous management were measured by continuous glucose monitoring system (CGMS) for three days. The mean blood glucose (MBG), mean amplitude of glycemic excursions (MAGE), and absolute means of daily differences (MODD) for 48h were calculated in each individual.*

Results. *The MBG, MAGE, and MODD in the N-T2DM group were 11.69±2.74 mmol/L, 5.98±1.40 mmol/L, and 1.95±0.58 mmol/L respectively, all significantly higher than those of the NGR group (5.35±0.51 mmol/L, 1.96±0.71 mmol/L, and 0.81±0.29 mmol/L respectively, P<0.01). u$ There was a significantly correlation between MBG and glycosylated hemoglobin A1c (HbA1c, 9.12±0.32%) in the N-T2DM group (r =0.82 P <0.01). However, the correlation of MAGE and MODD with MBG and HbA1c was not significant. The correlation of MAGE with MODD was also not significant.*

Conclusion. *Increase in amplitude of glycemic excursions as well as increase in glycemic levels is the features of abnormal glycemic metabolism in type 2 diabetes. Diabetic patients with similar glycemic levels may differ in term of glycemic variability. In designingstrategies to achieve excellent glycemic control, both a quantitative effect of hyperglycaemia (fasting, postprandial hyperglycaemia, and HbA1c) as well as a qualitative component should be taken into account. u$The CGMS profile can help characterize intraday blood glucose variability and day-to-day blood glucose reproducibility in type 2 diabetes.*

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OP 30

Clinical Utility of the Continuous Glucose Monitoring System in Intensified Insulin Treated Patients

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Background and Aims. *Continuous glucose monitoring (CGM) system may reveal unrecognized hyper- and hypoglycemia previously unseen using self-monitoring of blood glucose (SMBG) and thus provides significantly more information on glucose patterns during daytime and sleeping hours as well as the effect of different meals. Our aim was to uncover correctable factors hidden from detection with SMBG in intensified insulin treated patients.*

Materials and Methods. *47 type 1 poorly controlled diabetics (Ø duration 7,83±7,34 years, Ø age 23,47±11,33 years, ØHbA1c=9,56±1,48%, ØBMI=21,98±3,67; 5 CSII, 42 MDI) were monitored using CGM system for 3 or more days during normal activity with SMBG tests conducted at least 4 times per day. Duration, frequency and cause of glycemic excursions were analyzed. Results were presented as means ± SD and percentage of time period spent hypo- or hyperglycemic. We compared HbAlc at baseline and 3 months after CGMS-based therapy changes. Statistical analyses included correlation, mean absolute difference (MAD) and paired Student's t-test.*

Results. *51390 CGM values (ÿ duration 97,67±28,44h) compared with 949 capillary blood glucose (BG) values (Ø r=0,86±0,09; MAD=16,37±5,31%) showed 645 hypoglycemic episodes (<3,5 mmol/l at least 10min). 63% of nocturnal hypoglycemia was during 1am-4am period, 35% of daytime hypoglycemia during 10am-1pm period. From 166 nights only 2,2% were hypoglycemic with 5 asymptomatic prolonged (>280min) episodes. 92% of nocturnal and 65% of daytime hypoglycemia were undetected by SMBG (p<0,001). CGM uncovered lowered BG post-exercise, continued fall for 1,5h post-exercise and lower BG following day, in 3 cases (ice hockey, ice skating) with nocturnal hypoglycemia. Subjects showed 38,8% prevalence of hyperglycemia (Ø 9,3h/patient/day), total of 9761 hyperglycemic episodes (>7,8 mmol/l at least 10min), mostly attributed to diet fault or insufficient lag time. Dawn phenomenon was detected in 4 subjects. 75% of post hypoglycemic hyperglycemia > 10mmol/l was caused by overeating, 20% was attributed to rebound hyperglycemia. Stress hyperglycemia (work-stress, car-driving, pit bull terriers' attack) tended to come down without additional insulin. HbA1c decreased significantly from 9,56±1,48% at baseline to 8,86±1,38% at 3 months follow-up (p=0,021).*

Conclusion. *CGM completed picture of patients' glycemic responses to sleep, work, exercise, food intake and insulin dose, leading to improved glycemic control. The study provided additional support for clinical usefulness of CGM system in intensified insulin management.*

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OP 31

Possible Cause of Glucose Oxidase-Based Sensor Failure during Clinical Use of Continuous Glucose Monitoring System

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Background and Aims. *During clinical use of continuous glucose monitoring (CGM) system in some cases glucose sensor failure occurs. To date there is no applicable explanation for this problem. Studying cases with CGM disorders, we assume that glucose oxidase (GOD) mixed with bovine serum albumin (BSA) as vital component of the sensor can be affected by various endogenous processes, which may lead to its rejection. Our aim was to evaluate discordant tracings and their relation to selected patients' characteristics in order to reveal cause of the sensor failure.*

Materials and Methods. *We investigated 70 Caucasian patients with diabetes mellitus/DM (Ø duration 6,2±6,6 years; Ø age 34,6±18,9 years; ØBMI=24,6±5,2; ØHbA1c=8,6±1,9%; 64% female; 47 type 1 DM; 37% glutamic acid decarboxylase antibody/GADA positive) with CGM system for e"3 days during normal activity, in conjunction with self-monitored blood glucose (BG) tests conducted e"4x/day. CGM data were evaluated and classified as satisfactory (A) or impossible to evaluate (B), attributed to: (B1) insufficient calibration, (B2) insufficient starting sensor electronic signal (ISIG) with no response, and (B3) sensor error of unknown origin we further investigated. Statistical analyses included correlation, mean absolute difference (MAD) and paired Student's t-test.*

Results. *320 CGM profiles (74 sensors; Ø duration 111,6±42,24h) evaluated by comparing sensor data to 1511 BG values (r=0,84; MAD=14,8±4,7%) showed total 469,5h of gaps (5,7%), 1,7% attributed to insufficient calibration (B1). 2 sensors were discarded for technical reasons (B2). During the first 48h of CGM in 8 subjects (B3) unexpected ISIG weakening occurred leading to error after calibration with steady hyperglycemia, whereas euglycemia didn't lead to error despite boundary ISIG (2,2% of gaps). This type of sensor failure (B3) strongly correlated with GADA positivity (p=0,00005), other auto-immune disease (p=0,0003), repeated CGM (p=0,0006) and type 1 DM (p=0,036). No significant correlation was observed with total daily insulin dose (p=0, 12), age (p=0,215), HbA1c (p=0,389), BMI (p=0,798), duration of DM (p=0,983). There were no significant adverse events (e.g. skin irritation).*

Conclusion. *We identified new glucose pattern specific to sensor weakening (B3), which differs from that one of failed for technical reasons. Possible immune response to BSA is a suspicious factor that could lead to eventual sensor failure. The effect of possible agents responsible for GOD degradation on CGM performance awaits further study.*

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