Poster abstracts

Basic and clinical science posters: basic science of diabetes complications

P1

Effects of xenin and neurotensin on pancreatic islet function and beta cell survival

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Aims: In the present study we examined effects of the neurotensin (NT) receptor modulators, NT and xenin, on beta cell function, proliferation and apoptosis, as well as islet distribution in insulin deficient and resistant diabetic mice.

Methods: Insulin secretion studies were performed in rodent BRIN-BD11, human 1.1B4 beta cells and isolated mouse islets. Beta cell proliferation and apoptosis was assessed in vitro by Ki-67 staining and the Comet assay, respectively. Insulin-deficiency was induced by multiple low dose streptozotocin (50mg/kg bw, for 5 days), and insulin resistance by hydrocortisone (70mg/kg bw, for 10 days), injection in C57BL/6 mice.

Results: Xenin and NT stimulated (p<0.001) insulin secretion from BRIN-BD11 cells at 5.6mM glucose, with xenin having similar significant (p<0.001) insulinotropic actions at 16.7mM glucose. In contrast, NT inhibited (p<0.001) glucose-induced insulin secretion. Essentially similar observations were made in human 1.1B4 beta cells and isolated mouse islets. Further investigations revealed that xenin and NT protected BRIN-BD11 and 1.1B4 beta cells against streptozotocin-induced cytotoxicity. In addition, xenin augmented (p<0.05) rodent and human beta cell proliferation, whereas NT displayed proliferative actions (p<0.05) only in human beta cells. As expected, streptozotocin decreased (p<0.01) and hydrocortisone increased (p<0.001) cell mass in mice. Interestingly, xenin co-localisation with glucagon was increased (p<0.05) by streptozotocin, but unaltered by hydrocortisone. This corresponded to elevated (p<0.05) plasma xenin levels in streptozotocin treated mice. Both streptozotocin and hydrocortisone had increased immunoreactivity of NT in exocrine and endocrine pancreatic tissue.

Conclusion: These data highlight the important involvement of NT receptor signalling for the modulation of beta cell function.

P2

Soluble Nogo-B ameliorates angiogenesis and albuminuria in experimental models of diabetic glomerulopathy

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Aims or Objectives: Diabetic glomerulopathy (DG) is characterised by defects in the glomerular filtration barrier resulting in albuminuria. Neurite Outgrowth Inhibitor (Nogo)-B, a molecule involved in vessel remodelling, is expressed in glomeruli and is downregulated in diabetes. We postulate that soluble Nogo-B (sNogo-B), a circulating isoform, may be protective in DG. Our aims were, in experimental models of DG, to determine: (a) in vivo, whether overexpression of sNogo-B ameliorates albuminuria; (b) the role of Nogo-B in angiotensin-II (Ang-II)-mediated changes in podocyte actin cytoskeleton; (c) the role of sNogo-B on impaired angiogenesis observed in human umbilical vein endothelial cells (HUVEC) incubated with serum of patients with Type 1 diabetes and history of albuminuria (DN).

Method: sNogo-B was overexpressed (AAV-vector) in diabetic mice. Plasma sNogo-B levels were analysed by ELISA, full-length Nogo-B levels with immunoblotting, and albuminuria by fluorescence. Podocytes Nogo-B expression was downregulated with siRNA technique. Cells were incubated with 100nM Ang-II for 24h and actin cytoskeleton visualised with phalloidin. Angiogenesis was studied in HUVEC incubated with Type 1 diabetes serum (4%vol/vol) with or without DN. sNogo-B was overexpressed with viral vector.

Results: (a) sNogo-B overexpression was paralleled by inhibition of diabetes-induced albuminuria (p=0.38) and Nogo-B downregulation in kidney cortex (p=0.021); (b) downregulation of Nogo-B was paralleled by a worsening of Ang-II-mediated disruption of podocytes’ actin cytoskeleton; (c) overexpression of sNogo-B rescued the altered angiogenesis in HUVEC incubated with T1DM/DN+ serum (p<0.02).

Conclusion: Nogo-B is implicated in DG. Overexpression of sNogo-B, by preventing diabetes-mediated full-length Nogo-B downregulation, appears to confer a degree of glomerular protection in diabetes.

P3

Effects of intravitreal Anti-VEGF therapy on the vasculature in Ins2Akita diabetic and wild-type (WT) non-diabetic mice

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Aim: Anti-VEGF (vascular endothelial growth factor) therapy has become a standard therapy for the management of diabetic macular oedema. In a previous study we have shown that sustained VEGF neutralisation induced retinal neurodegeneration in the Ins2Akita diabetic mouse. The aim of this study was to understand how sustained VEGF neutralisation may affect retinal blood vessels under diabetic conditions.

Methods: Two months old Ins2Akita mice were injected intravitreally with either 1 μl of anti-VEGF antibody (0.2μg/μl) or rat IgG once every 2.5–3 weeks for a total of five injections alongside age matched un-injected Ins2Akita mice as controls. Non-diabetic mice were received the same treatment. Two week following the last injection, eyes were enucleated and the different vascular plexuses
were analysed in retinal flatmounts. The percentage of vasculature coverage in each vascular plexus and albumine leakage was determined.

**Results:** A slight reduction was detected in vessel density in diabetic eyes in the superficial vascular plexus. Whereas there were no differences in the vessel density in the inner and deep vascular plexuses. Sustained VEGF neutralisation had no effect on vessel density in all layers in diabetic or non-diabetic mice. Albumin leakage was increased in diabetic eyes following anti-VEGF injections, suggesting that the blood retinal barrier (BRB) is disrupted in anti-VEGF treated diabetic eyes.

**Conclusion:** Our results suggest that sustained VEGF neutralisation may disrupt the integrity of the BRB, but do not cause significant retinal vascular structural changes in diabetic and non-diabetic mice. The effect of sustained VEGF neutralisation in the BRB function warrants further investigation.

**P4**

Extracellular nicotinamide phosphoribosyltransferase (eNAMPT) plays a dose- and time-dependent role in pancreatic islet function

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**Background and Aims:** Serum levels of extracellular nicotinamide phosphoribosyltransferase (eNAMPT; visfatin/PBEF) are elevated in Type 2 diabetes. However, the role of eNAMPT in pathophysiology remains unclear. Here we examined acute and chronic dose-dependent effects of eNAMPT on mouse and human pancreatic islet function.

**Methods:** Mouse and human islets were treated with dimeric eNAMPT for 1–72h, at concentrations of 0.5–10ng/ml (mouse) and 1–50mg/ml (human). Glucose-stimulated insulin secretion (GSIS) was measured by radioimmunoassay. Intracellular Ca2+ was measured in MIN6 cells using Fura-2 and wide-field microscopy. Apoptotic activity was measured using Caspase-Glo 3/7 assay and NAD/NADH expression by colorimetric assay. Gene expression analysis was assessed by qPCR.

**Results:** GSIS was largely unchanged after 1h of eNAMPT exposure at all concentrations. However, GSIS was increased after 24–72h treatment with 1ng/ml eNAMPT (p < 0.05) versus NAD-dependent effects. In contrast, islets treated with higher concentrations of eNAMPT (<5ng/ml) showed NAD-independent impairment of GSIS and reduced intracellular [Ca2+]o, (p < 0.001). Gene expression analysis revealed that exposure to eNAMPT (<5ng/ml) leads to an enhanced inflammatory response (MCP1: p < 0.0001, IL1β: p < 0.05). Correspondingly, apoptosis is enhanced in islets treated with high concentrations of eNAMPT in the presence of the TNFα and IFNγ (p < 0.05).

**Conclusions:** Chronic dimeric eNAMPT-exposure is detrimental to normal beta cell function. However, moderate exposure enhances insulin secretion. This data begins to clarify previous conflicting findings regarding eNAMPT in Type 2 diabetes. Additional mechanisms of eNAMPT action are being investigated, including the role of monomeric eNAMPT in beta cell function.

**P5**

A proteomics analysis to determine the role of copper in diabetic nephropathy

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**Introduction:** Approximately 40% of patients with diabetes develop diabetic nephropathy (DN). Elevated copper is seen in patients and the renal cortices of rats with DN. We hypothesise that diabetes alters metabolism of redox active copper increasing oxidative stress and renal remodelling. This study aims to quantify protein expression changes and copper metabolism in DN using Triethyltetramine (TETA), a copper chelator.

**Methods:** Four controls, six diabetic and six TETA treated diabetic rats were studied using the STZ model. Tissue copper was measured using inductively coupled plasma mass spectrometry (MS). Renal cortex protein was iTRAQ labelled and analysed by 2D-LC-MS/MS. Data was analysed using ProteinPilot with dysregulated proteins further analysed using Ingenuity Pathway Analysis.

**Results:** Renal cortical copper increases in diabetes and was partially corrected by TETA treatment. Mass Spectrometry analysis of protein expression quantified 4,143 proteins. 189 upregulated and 58 downregulated proteins were associated with diabetes. This identified diabetes-related pathways including retinoate signalling, glutathione mediated detoxification, fatty acid oxidation and dermatan sulfate degradation. TETA treatment upregulated 26 and downregulated 25 proteins compared to levels in diabetes, including adipogenesis, retinoate pathways and glutathione-mediated detoxification. Proteins with antioxidant functions Gstm1 and Gstm2 were increased in diabetes and further increased after TETA suggesting improved response to oxidative stress.

**Conclusions:** DN modifies protein expression in pathways involved in altered lipid metabolism, glomerular basement membrane degradation, oxidative stress and inflammation all of which except GBM degradation are improved by TETA. We surmise that ameliorating copper build-up improves antioxidant response, decreasing inflammation and lipid pathway alterations in DN.

**P6**

A role for ATP in tubular fibrosis in the diabetic kidney

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**Aims:** Recent studies link elevated hemi-channel mediated ATP release to increased tissue fibrosis in disease. This study examines a role for ATP in extracellular matrix (ECM) remodelling and increased secretion of fibrotic mediators in kidney tubular epithelial cells.

**Methods:** Human kidney (HK)2 tubular epithelial cells were treated with ATPγS (1–100μM) for 48h. Cell morphology and cytoskeletal reorganisation were assessed by phase contrast microscopy and immunocytochemistry respectively. Cell viability was determined by MTT and crystal violet assays. Collagen I, IV, Fibronectin and Laminin expression were determined by immunoblot analysis, whilst Interleukin-6 and Beta-Nerve Growth Factor (beta NGF) secretion were assessed by cytokine arrays.
Results: ATP$_{7S}$ (1–100μM) failed to evoke any significant change in HK2 morphology, cytoskeletal reorganisation or cell viability at 48h. Immunoblotting confirmed that ATP$_{7S}$ up-regulated Collagen I to 177 ± 12%, 182 ± 21%, and 187 ± 21% (n = 3, p < 0.01) and Collagen IV to 233 ± 18%, 344 ± 18.5%, and 390 ± 10% compared to control (n = 3 p < 0.001) at 1, 10 and 100 μM. In addition, ATP$_{7S}$ significantly increased expression of Fibronectin to 274 ± 47%, 350 ± 23% and 433 ± 81% of control (n = 3, p < 0.01) at 1, 10 and 100μM, yet failed to alter expression of ECM protein Laminin. Array analysis of supernatant from ATP$_{7S}$ treated cells confirmed a significant increase in secretion of both IL-6 and beta-NGF to 261/C6 47%, 350/C6 12%, 182/C6 21%, and Collagen IV to 233/C6 3p, 47%, 350/C6 12%, 182/C6 21% (n = 3, p < 0.01) at 1, 10 and 100μM. In contrast, high levels of glucose had no significant effect on collagen-induced aggregation with ADP or collagen in whole blood. Future studies will focus on chronic hyperglycaemia and endothelial dysfunction, using platelets from Type 2 diabetic patients and matched controls.

Conclusions: This work is supported by Diabetes UK (BDA:11/0004215, BDA:16/0005427).

Acknowledgement: This study was supported by Diabetes UK (BDA:11/0004215, BDA:16/0005427).

Methods: C57Bl6/J (WT), Type 2 diabetic mice Lepr$^{db/db}$, CSE deficient (CSEKO) and diabetic CSEKO Lepr$^{db/db}$/CSEKO mice were used in this study.

Results: Vascular density was reduced in Lepr$^{db/db}$ mice and further impaired upon CSE deficiency in Lepr$^{db/db}$/CSEKO (p < 0.0001; n = 5). PE marker soluble fms-like tyrosine kinase-1 (sFLT-1) was upregulated in Lepr$^{db/db}$ tissues (p < 0.01; n = 5). ER stress marker eukaryotic Initiation Factor 2 (eIF2) was upregulated in kidney and liver from Lepr$^{db/db}$ leading to increased oxidative stress through decreased expressions of activating transcription factor 4 (ATF4) and thio-redoxin reductase (Txnrd) (vs WT p < 0.01; n = 5). Similar observations were made in CSEKO mice kidney and placenta. Inhibition of Txnrd (siRNA) in HUVECs has increased PE marker sFLT1 (p < 0.0001; n = 5). H2S therapy rebalanced redox signaling through nuclear factor erythroid 2-related factor 2 (Nrf2) upregulation. Anti-angiogenic microRNAs (miRNAs) – 152 and 195 were increased in Lepr$^{db/db}$ (p < 0.001; n = 5) and human DM fat tissues (p < 0.001; n = 23).

Conclusions: Severe impairment of CSE/H2S in DM leads to deregulated redox angiogenesis signaling causing increased onset of PE. H2S and Txnrd are potential therapeutic targets to restore functions in DM/PE.
Methods: Immortalised human RPTEC (HK-2 cells) were exposed to different concentrations of glucose (5, 25, 30mM) glucose and the osmotic control 5mM t-glucose + 25mM l-glucose) and glucose consumption, proliferation/migration of HK-2 cells and the secretion of the pro-fibrotic marker TGFβ1 were measured.

Results: HK-2 cells appear to utilise the same amount of glucose, regardless of the concentration of glucose in the cell culture medium. Cell proliferation/migration was highest at 25mM glucose and was significantly inhibited at 30mM glucose. In contrast, TGFβ1 secretion was lowest at 25mM and highest at 30mM glucose.

Conclusion: The data suggest that pro-fibrotic processes are triggered between 25 and 30mM glucose and further investigation is required into these mechanisms.

P11
Targeting outgrowth endothelial progenitor cell defective function using glycomimetics for improved diabetic wound healing
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Objective: Diabetic Foot Ulcers (DFU) are a major health and economic burden. Outgrowth endothelial progenitor cells (OECs) promote endothelial repair and tissue regeneration. However, diabetic patients have impaired OEC function. We have developed state-of-the-art small molecule glycomimetic drugs that have protective effects against endothelial dysfunction. The aim of our study was to investigate the effect of a novel glycomimetic (C3) on the angiogenic potential of diabetic OECs and whether (C3) could improve their function.

Methods: Biochemical assays and functional analysis (migration, angiogenesis, mitochondrial activity) was performed on OECs harvested from blood taken from patients with Neuropathic or Neuroischaemic ulcers for in vitro analysis in the presence or absence of C3.

Results: 13 neuroischaemic, (NI) 11 neuropathic patients (NP) and four healthy controls (HC) were analysed. Neuroischaemic compared to neuropathic OECs showed: (1) enhanced migration – scratch assay: 95% closure vs 42% (p<0.05). (2) Improved angiogenesis-tube formation assay: increased closed loops (p<0.0001) and branch points (p<0.05). (3) Less Nitric Oxide (Griess assay). The basal rate of glycolysis (extracellular acidification rate: ECAR) and mitochondrial activity (oxygen consumption rate (OCR) and total mitochondrial capacity is significantly reduced in NP compared to NI or HC. The angiogenic capacity of NI OECs was significantly enhanced when treated with the glycomimetic C3.

Conclusion: OECs from neuroischaemic patients differ from neuropathic patients. The enhanced migration and angiogenic responses in vitro suggests this may be more successful in neuroischaemic patients and may be enhanced by the treatment of C3, which offers novel therapeutic potential for diabetic wound healing.

Basic and clinical science posters: beta cells, islets and stem cells

P12
The effect of glucokinase activator on insulin secretion in a model of age-related mitochondrial DNA depletion
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Introduction: Type 2 diabetes is characterised by an age-related decline in insulin secretion. Mitochondrial DNA (mtDNA) content declines with age in human pancreatic islets, and we showed that a comparable degree of mtDNA depletion in cultured beta cells directly decreased insulin secretion. Glucokinase activators (GKAs) have been developed as a potential treatment for Type 2 diabetes. However, because glucokinase lies proximal to the mitochondrial energy generation step, it is questionable whether GKAs will work under conditions of mtDNA depletion.

Aim: To investigate the effect of glucokinase activator on insulin secretion in a model of aged pancreatic beta cells.

Methods: MIN6 cells were transfected with siRNA probes against TFAM, a regulator of mtDNA biogenesis, or a scrambled control using the Neon system. Knockdown efficiency was assessed using real-time PCR. 72h after TFAM knockdown, the cells were exposed to 10mM glucose for 1h, with GKA or without GKA (DMSO control). Insulin was determined by ELISA.

Results: TFAM expression and mtDNA content were decreased by 83% (p<0.001) and 57% (p<0.001), respectively, compared with scrambled controls. At 10mM glucose, in scrambled controls, 10μM GKA increased glucose stimulated insulin secretion (GSIS) compared with DMSO (4.31 ± 0.65 vs 2.03 ± 0.94ng insulin/μg protein; Mean ± SEM; p<0.05). In the absence of GKA, mtDNA depletion impaired GSIS (0.67 ± 0.19 vs 2.03 ± 0.94ng insulin/μg protein; p<0.05) compared with scrambled controls. GKA restored the impaired GSIS in mtDNA depleted cells (2.06 ± 0.37 vs 2.03 ± 0.94ng insulin/μg protein) compared with DMSO scrambled controls.

Conclusions: Glucokinase activator can still effectively increase insulin secretion under impaired pancreatic beta cell function due to partial mtDNA depletion.