ISLET BIOLOGY—APOPTOSIS

2965-PO

Vascular and Histological Changes in Pancreatic Islets in Diabetes Caused by Xanturenic Acid

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Diabetogenic derivatives of 8-oxyquinolin (D8OX) including Xanturenic Acid (XA) result selective destruction and death of B-cells within 20-40 min. Among D8OX XA only is formed in animals and Human in deficiency of Vit.B6. Aim of work: to study vascular and histological changes in pancreas of animals contained on diet (DXA) stimulated endogene synthesis of XA approached to human. 34 rats contained 116 days on DXA. Blood glucose (BG) control weekly, XA in the urine (XAU)-monthly; histology: haematoxylin-eosin, aldehydefucshine; immunohistochemical insulin staining (IG) and Diethylpseudoisocyanine (PS) methods with measuring of absorbance. Results. *Islets:* thickening of basal membrane in 37±5.6% capillares in 36± 4.5% of islets, edema of endothelium in 29.6±4.2% capillares in 48±7.6% of islets; BG: before-4.4± 0.5mM; 112th-114th day-10.8±2.2mM; XAU: before-0,044±0.008mcg/ml; 112nd-115th day-0.376 ±39 mcg/ml. Insulin content in B-cells: IG-1.33±0.04(control: 1.91±0.08); PS-1.39±0.05 (2.04±0.07); histology: hydropic degeneration, vacuolization, necrosis and death of 35.9±6.3% isl- ets, lysis of B-granules, hydropic changes of nuclei. *Exocrine tissue.* Blood vessels: moderate arterial hyperemia in 22.5±5.8% islets, marked vein stagnant hyperemia (21.6±3.2%), fibrinoid changes of exterior part of arte- ries, interglobular arteries sclerosis (16.2±4.1%) homogenisation collagen fibers of arterial adventicium; chaotic order of collagen fibers in veins, homo- genisation and fragmentation of adventicium, thrombosis of veins; alteration of arterial endothelium (12.8±3.3%); destruction and distrophya endothelium of veins (9.2±2.1%), filtration of liquid in intercellular space. Conclusions: 1) significant vascular changes developed in islets and exocrine tissue in diabetes caused by DXA contrary to diabetes induced by injection of XA and D8OX; 2) developed vascular changes are able to aggravate diabetes caused by XA as additional cause alteration of B-cells.

Supported by: Family of Professor G. Meyramov

ISLET BIOLOGY—BETA CELL—DEVELOPMENT AND POSTNATAL GROWTH

2966-PO

Establishment of Feeder-Free Culture System of Mouse Embryonic Stem Cells

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Type 1 diabetes result from the loss of insulin-secreting beta cells. Regeneration of functional pancreatic beta-cells is one of the most attractive approaches to cure Type1 diabetes. Embryonic stem cells (ESCs) are derived from the blastocyst inner cell mass. They can differentiate into various cell types, and can be competent cell source for regenerating beta-cells. ESCs require embryonic fibroblasts as a feeder layer during the culture to keep their pluripotency. However, feeder cells include a risk of xenogenic-contamination such as transferring animal viruses and pathogens to ESCs, which is not suitable for clinical application. Therefore, the development of new feeder free culture system of ESCs is required. In this study, we validated whether ESCs can be maintained in pluripotential stage on new feeder free dish, coated with poly (N.N'-dimetylacrylamide) and hectorite to promote the self-renewal of ESCs developed by RIKEN Kawamura. Mouse ES cells (mESCs) cultured on this new dish showed undifferentiated morphology, possitive for alkaline phosphatase staining, expressed undifferentiated marker genes and protein (Figure), and formed teratoma after 2 months of culture (30 passages). Therefore, we conclude that mESCs can be maintained undifferentiated state without feeder cells on our new dishes. This data suggest that microenvironment of the new feeder free dish contribute to maintain undifferentiated state. (MH and YN contributed equally to the work)

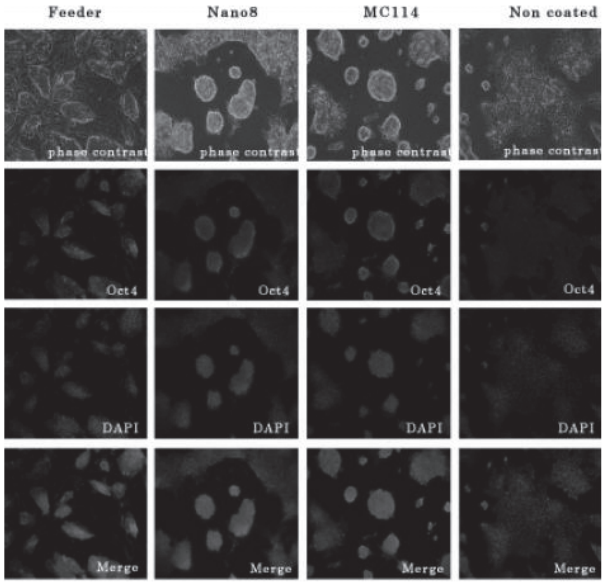

Figure Expression of undifferentiated marker gene Oct4

2967-PO

Regulation Effect of Anxa1 on Proliferation of MIN6 Cells

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Background: Anxa1 (Annexin 1, also known as lipocortin-1), is a member of calcium-dependent phospholipid-binding proteins with diverse functions. Previous study proved that Anxa1 protein had been involved in change of cell proliferation in different tissues. However, it is unclear that how Anxa1 affects proliferation of pancreatic beta cells.

Methods: In this study, after upregulation of Anxa1 expression by plasmid transfection and downregulation of Anxa1 expression by small interfering RNA (siAnxa1) in MIN6 beta cell line, CCK8 assay was used to determine the degree of cell proliferation and cell cycle analysis was performed by using flow cytometry (FCM). Subsequently, the expression of cell cycle-related proteins, such as Cyclin D1, Cyclin E and CDK2 proteins were also detected by western blot. The relationship between the PI3K/Akt/mTOR signaling pathway and Anxa1 was also evaluated.

Results: Overexpression of Anxa1 in MIN6 cells increased the extent of cell proliferation. Knockdown of Anxa1 expression using siRNA decreased the degree of cell proliferation. Overexpression of Anxa1 in MIN6 cells induced a significant increase in the percentage of cells in the S and G(2)/M phase, as demonstrated by flow cytometry. In contrast, the percentage of cells in the S and G(2)/M phase was decreased in cells when Anxa1 was inhibited, compared with control cells. Expression of Anxa1 protein in MIN6 cells was positively realted with Cyclin D1, Cyclin E and CDK2 proteins and positively regulated the PI3K/Akt/mTOR signaling pathway.

Conclusion: This study has found a positive correlation between Anxa1protein and proliferation of prancreatic beta cells line, MIN6 cells. Anxa1 protein might affect proliferation of MIN6 cells by regulation of Cyclin D1, Cyclin E and CDK2 protein and the PI3K/Akt/mTOR signaling pathway.

Supported by: NSFC (81173625), (81270424)

2968-PO

Function Turnover Observation of B-Cell on 240 T2DM Cases With 5 Years' Treatment

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Objectives: 240 T2DM cases had been given standardized treatment and 5 years' consecutive follow up to dynamically observe whether B cell function had had reversible change. Function turnover of B cell mainly affects the course change of T2DM, and as indicated by UKPDS, T2DM is a serious progressive disease, which as time goes by, the B cell function shows progressive decrease, and 50% of T2DM cases require insulin treatment after 6 years. Then how is the B cell function change situation of T2DM cases in China?

Method: 240 T2DM cases admitted in our hospital in 2007 were selected and divided, with 122 male cases and 118 female cases, 5 to 15 years' diabetes history, age range, 60±20. 96 cases received OAD treatment while 134 cases received insulin treatment, the same experiments including OGTT, INS and C-peptide release were given to all the cases, then rechecks were given

For author disclosure information, see page 829.

for each 3 to 6 months, 12 to 18 rechecks in all.Till March 2012, insulin and C-peptide change was observed, and by comparing the change of insulin use dose, the treatment change of using insulin instead of OAD was observed,the normal value range of insulin.

Results: Among the 240 cases, 110 cases showed average value increase of insulin and C-peptide, accounting for 45.8%, 51 cases had no change, accounting for 21.2%, and 89 cases had decrease of the average value, accounting for 33%. 32 cases received insulin treatment instead of OAD, and 96 cases required decreased insulin use dose.

Conclusion: B cell function of some T2DM cases in China can be reversed. The main cause of T2DM is function decrease of IR and B cell, and for the same case, it shows different insulin function in different stage, and the regular pattern is: high insulin and high C-peptide symptom in early stage, and gradually decompensation appears with low insulin and low C-peptide symptom, which after reasonable and standardized treatment to get rid of toxicity of saccharide and lipide, B cell function of some T2DM cases showed various range of recovery.

2969-PO

Effect of Handle Region Peptide (HRP) on Islet Morphology and Functions With Rats Neonatally Treated With Monosodium L-glutamate (MSG)

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Objectives: To investigate effect of blockade of the (Pro)renin receptor ((P)RR) on glucose status and islet morphology in rats neonatally treated with monosodium L-glutamate (MSG).

Methods: Newborn male rats were subcutaneously injected with MSG while the control rats with NaCl (Con group).The MSG rats were randomly divided into MSG group, handle region peptide (HRP) treated group (HRP group, mini-pump, 1mg/d/kg), losartan treated group (L group, 20 mg/kg/d) and co-treated group (HRP-L group) and fed with high-fat diet at 3rd week. Glucose status of animals was evaluated at 12th week. Islets β and α cell were marked respectively by insulin and glucogan antibody according to immunohistochemisty. Proliferation of islets cells was detected by method of PCNA.

Results: 1. Results of OGTT and insulin release test were showed in Figure 1; MSG group had lower insulin sensitivity, and both losartan and HRP improved insulin sensitivity. 2. MSG group had lower level of β-mass when compared to the Con group and HRP group. L group and HRP-L group improved β-mass. L group had higher levels of β-mass than HRP group. 3. PCNA positive cells were located in the periphery of islets and accordance with α cell. The numbers of PCNA positive cells was highest in HRP group.

Conclusions: HRP ameliorated insulin sensitivity but not improved the level of insulin releasing in the MSG rats. HRP promoted the proliferation of α cell in islets.

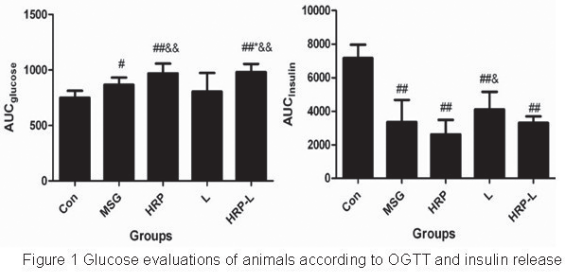

Figure 1 Glucose evaluations of animals according to OGTT and insulin release test

#: Compared with Con group, P<0.05; ##: Compared with Con group, P<0.01;
*: Compared with MSG group, P<0.05;
&: Compared with Con group, P<0.05; &&: Compared with Con group, P<0.01

Supported by: NSFC (81170711)

2970-PO

WITHDRAWN

2971-PO

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2972-PO

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2973-PO

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2974-PO

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2975-PO

Diet Control and Weight Loss Are Critical for the "Legacy" Effect of Insulin Treatment on Glycemic Control in High Fat Diet (HFD)-Induced Type 2 Diabetic Mice

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Clinical studies suggested that early insulin therapy in type 2 diabetes (T2DM) can promote long-term glycemic control by preserving β-cell function. Our pilot study (2132-P,ADA2012) in a T2DM mouse model indicated a prolonged diabetes control after short-term insulin therapy. The role of diet control in such a "legacy" effect of insulin therapy was further examined in this study. C57BL6/J male mice were fed a HFD (60% fat) for 20 weeks to induce diabetes, and then received daily sham (s.c. PBS) or Insulin Glargine (targeting glucose levels 100-150mg/dL) for 4 weeks. Mice were then kept on a HFD or switched to a LFD (chow diet, 10% fat) for 4 more weeks before sacrifice. Another group of mice fed on a LFD throughout the study served as controls. As expected, glucose tolerance (GTT) and A1c levels were greatly improved in HFD mice following insulin treatment compared to sham controls. After switching to a LFD at the end of treatment, insulin-treated mice sustained weight loss to the similar extent as sham controls, along with improved GTT and reduced blood levels of Leptin; moreover, the former demonstrated a significantly better GTT performance than the latter, indicating a "legacy" benefit distinct from diet control and weight loss. This may reflect a better β-cell function after insulin therapy, although direct evidence is lacking at present, via plausible mechanisms like inhibition of glucolipotoxicity, proinflammation, and apoptosis. Indeed, fewer TUNEL-positive cells were seen in their islets than that of sham controls. Conversely, this "legacy" benefit was totally lost in mice kept on a HFD that exhibited the worst GTT performance, regardless of therapy. Our finding of prolonged glycemic control only attained in mice switched to a LFD after insulin therapy may explain why clinically such a similar "legacy" effect was usually achieved only in a portion of cases studied, emphasizing a vital role of diet adhesion in diabetes control.

Supported by: Ohio University-Heritage College of Osteopathic Medicine (to A.G.)

2976-PO

Receptor and Second Messenger Interactions in Pancreatic Beta-Cell: A Computational System Analysis

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There are complex interdependencies between the different messengers and signaling pathways that contribute to the insulin secretory response to nutrient stimuli and hormonal modulators in pancreatic beta cell. On the base of our previous models we present an updated computational model that incorporate modern data on glucose, plasma membrane potential, G-protein-coupled-receptors (GPCR), cytoplasmic and endoplasmic reticulum calcium, cAMP, phospholipase C and the insulin receptor pathways that regulate secretion of insulin granules. The values of most of the model parameters were inferred from available experimental data. Our analysis of the dynamic data provides evidence for a pivotal role for calcium-dependent adenylyl cyclase activation in the effect of glucagon-like peptide 1 (GLP-1) on pancreatic beta-cells. The regulatory properties of various adenylyl cyclase isoforms determine fluctuations in cytoplasmic cAMP concentration and reveal a synergistic action of glucose and GLP-1 on insulin secretion. On other hand, the regulatory properties of phospholipase C isoforms determine interaction of glucose, acetylcholine and fatty acids (that act through the receptor GPR40). We evaluated also the role of the insulin receptor pathways in beta cell regulation. We test the hypothesis that activation of specific key beta-cell GPCRs in concert with the insulin receptor can be in some cases stimulate but in other combinations inhibit glucose-stimulated insulin secretion. The regulation of messenger's pathway interactions may be important pharmacological targets for improving insulin secretion in type 2 diabetes.

Supported by: NIH; Keck Foundation

2977-PO

Possible Antioxidant Activity of Epiafizelechin, a Flavonoid from Leaves of Bauhinia Forficata, in Pancreatic Beta-Cells

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Diabetes mellitus type 2 (DM2) is characterized by insulin resistance and relative insulin deficiency. The supply of synthetic drugs for the treatment of DM2 is very wide today. Despite this fact, the possibility of using a natural compound would be useful and perhaps could increase adherence to treatment

For author disclosure information, see page 829.

of the disease. Bauhinia forficata is often used as teas and other herbal preparations because of its hypoglycemic activity. However, the exact mechanism of action is still unclear. Therefore, this study objectives to investigate the antioxidant activity of the compound epiafzelechin (EEE), isolated from the leaves of B forficata, and its possible mechanisms.

EEE toxicity was evaluated by flow cytometry with 150 nM, 15 uM and 150 uM, after 1 or 24 h of treatment. Western Blotting was used to evaluate AKT and JNK phosphorylation level. INS1-E cells were used for all experiments. Using hydrogen peroxide (5 uM) or D-ribose (15 mM) to cause oxidative stress, we observed that EEE treatment (150 nM) decrease the percentage of DNA fragmentation caused by D-ribose. However, EEE is not able to reduce DNA fragmentation caused by hydrogen peroxide. Although not yet statistically significant, there is a tendency to increase the phosphorylation level of AKT and reduce phosphorylation level of JNK after incubation with D-ribose (15 mM) and EEE (150 nM).

Our preliminary results obtained so far allow us to conclude that: 1) EEE does not affect cell viability and increase DNA fragmentation at a concentration of 150 uM, and 2) EEE is able to reverse the cell death caused by incubation with D-ribose, has a tendency to increase the phosphorylation level of AKT, and decrease the phosphorylation level of JNK, showing therefore possible antioxidant activity.

Supported by: FAPESP; CNPq

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