Diabetes Mellitus and Glucose Metabolism

BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

Cornus Officinalis Induction of Pancreatic β-cell Autophagy as Revealed by Increased LC3 Expression and P62 Phosphorylation

Justin D. Fletcher, B.S.[1], Arielle E. Sharp-Tawfik, PhD[2], Brant Roger Burkhardt, PhD[1].
[1]University of South Florida - Tampa, Tampa, FL, USA, [2]University of South Florida, Tampa, FL, USA.

Type 1 diabetes (T1D) is an autoimmune disease that results in the destruction of pancreatic beta cells, diminishing the body's ability to produce insulin. Currently, over 1.2 million people are affected by this disease in the United States with the only treatment being exogenous insulin. The ideal preventative treatment of diabetes would be to target the interventional window during the critical pre-diabetic stage to inhibit the complete loss of pancreatic β-cells. *Cornus officinalis* (CO) is considered a source of novel therapeutics for this purpose due to prior reports and work from our laboratory (*Mol. and Cell. Endo.*2019:494:110491) demonstrating that CO may protect β-cells from autoimmune mediated cytotoxicity and enhance function. To elucidate the molecular mechanism of how CO may be biologically impacting a human pancreatic β-cell line (1.1B4), we previously performed a global and phosphorylation mass spectrometry analysis revealing an increased phosphorylation of p62, which is an important regulator of autophagy. To validate the mass spectrometry results and determine if CO is truly capable of inducing pancreatic β-cell autophagy, we examined LC3 expression which serves as a typical marker for autophagic membranes. 1.1B4 cells were treated with CO for 6 and 12 hours with at concentrations of 500 and 1000 μg/ml, respectively. Cells were fixed with formaldehyde and permeabilized with Triton X-100 followed by fluorescent detection with an LC3 antibody along with DAPI nuclear staining. The cells and nuclei were then imaged using a florescent microscope and it was discovered that upon treatment of CO, an increase in cytoplasmic LC3 puncta was markedly observed as compared to untreated controls. Western analysis also demonstrated increased expression of intracellular LC3 upon CO induction. We then went on to confirm the autophagic process by immunoblotting of p62, which is a cargo receptor for autophagy. Immunoblotting demonstrated increased expression of phosphorylated p62 in a concentration and time dependent manner after treatment with CO. Altogether, CO appears to increase expression in the critical markers of autophagy and suggests that CO may have potential as a T1D interventional therapy by promoting protective autophagy in the pancreatic β-cells.

Diabetes Mellitus and Glucose Metabolism

BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

Definition of Norma / Prediabetes Cut-off Point for Fasting Glycaemia on the Basis of Glucose Tolerance Test and HbA1c Interrelationships

Sevil Ismayilova, MD, Sadaqat Sultanova, PhD, Aygun Huseynova, MD, Valeh Mirzazade, professor.
Azerbaijan State Advanced Training Institute for Doctors, Baku, Azerbaijan.

Unification of the approach to the diagnosis of prediabetes (PD) is hardly in doubt. The borderline between PD and diabetes is recognized by all, as well as the upper and lower bounds of PD according to the results of glycemia 120 minutes after 75 g glucose loading (GL120). There are still ambiguities regarding glycohemoglobin (HbA1c) and fasting glycemia (FG). For determination of the Norma/PD cut-off point for FG, we analyzed 85 nondiabetic glucose tolerance test results (75.0 glucose; Samples of fasting blood, and 30, 60, 90, 120 minutes after glucose loading) by using correlation and regression analysis. Glycemic values were measured in mg/dl, HbA1c values were measured in %. The fact of identifying the relationship between FG and Gl120 ($r=+0.52$ [95%CI +0.346, +0.659]; $p<0.001$), as well as between FG and HbA1c ($r=+0.59$ [95%CI +0.432, +0.713]; $p<0.001$) were the basis of this study. As a result of using regression analysis, multiple regression equations were obtained. GL0 =-4.2439 + 0.1927 * GL120 + 15.462 * HbA1c If GL120 is equal to 139 mg/dl (in accordance with all recommendations), and HbA1c is equal to 5.9% (in accordance with the recommendations of NICE, Canadian Diabetes Association, Australian Diabetes Association, et al.), the maximal normal value for FG should be equal to 114 mg/dl. If GL120 is 139 mg/dl and HbA1c is 5.6% (as recommended by the American Diabetes Association), the maximum normal value of FG should be 109 mg/dl. The optimal upper limit of normal carbohydrate metabolism is levels of GL120 equal to 139 mg/dl, HbA1c - equal to 5.6%, and FG - equal to 109 mg/dl. Values above these and below diabetic levels (200 mg/dl, 6.5%, and 126 mg/dl, respectively) can be considered as prediabetes.

Diabetes Mellitus and Glucose Metabolism

BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

Functional Heterogeneity Among Pancreatic Alpha Cells

Glyn M. Noguchi, B.S.[1], Rosie Ou, B.S.[1], Vincent Castillo, N/A[1], Cynthia J. Donaldson, B.S.[2], Alexander M. Mawla, B.S.[1], Mark O. Huising, PhD[1].
[1]University of California - Davis, Davis, CA, USA, [2]Salk Institute for Biological Studies, La Jolla, CA, USA.

Historically, endocrine cells in the pancreatic islets have been assumed to function as relatively homogenous populations largely because we lacked the ability to measure individual cell activity with sufficient throughput to reliably detect heterogeneity within each population. The glucagon-secreting alpha cells play a vital role in regulating glycemia, but the mechanisms that control alpha cell activity and whether the alpha cells behave as a single unit or heterogeneously remain incompletely understood. To overcome the limitations in throughput that have to date prevented the study of alpha cells at the

population level, we used genetically-encoded fluorescent indicators selectively expressed in alpha cells. Imaging intact mouse islets with these indicators in 3D responding to treatments in real time yields hundreds of individual alpha cell recordings per experiment. Calcium imaging showed reproducible heterogeneous responses to a panel of known physiological potentiators of glucagon secretion such as arginine vasopressin, epinephrine, and amino acids. Separate dose response experiments revealed that the proportion of alpha cells responding to each signal plateaus at different proportions of alpha cells. The calcium data correlate both with direct glucagon secretion levels as well as cAMP measurement. Our findings highlight previously unappreciated levels of functional heterogeneity among alpha cells and demonstrate that alpha cells are not a single uniform unit. Our observations suggest that dose-dependent increases in glucagon secretion in response to different physiological cues may be the result of mobilizing progressively larger proportions of the total alpha cell mass. We hypothesize that this functional heterogeneity is a built-in mechanism through which different physiological cues elicit graded glucagon responses from the alpha cells.

Diabetes Mellitus and Glucose Metabolism

BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

Hyperglycemia-Induced Metabolic Reprogramming Mediates a Proatherogenic Phenotype in Healthy Human Monocytes

Anabel Lourdes Castro-Grattoni, PhD[1], Jisu Oh, MS[1], Rong Mei Zhang, MD[1], Adriana Dusso, PhD[1], Amy Elizabeth Riek, MD[1], Kevin Thomas Bauerle, MD, PhD[2], Monika Bambouskova, PhD[1], Pamela Stone, RN[1], Ana Maria Arbelaez, MD, PhD[1], Carlos Bernal-Mizrachi, MD[2].
[1]Washington University in St Louis School Medicine, St Louis, MO, USA, [2]Washington University in St Louis School Medicine, VA Medical Center, St Louis, MO, USA.

Introduction: Poor glycemic control is considered an important contributor to cardiovascular disease in patients with diabetes. Episodic hyperglycemia as a surrogate for glycemic variability promotes monocyte adhesion and increases the prevalence of proinflammatory monocytes within atherosclerotic plaques of patients with diabetes. We previously found that acute hyperglycemia-induced a pro-inflammatory phenotype and promoted the development of foamy monocytes by increasing total cholesterol deposition, cholesterol ester, and free cholesterol content by enhancing oxidized LDL uptake. However, the mechanism by which acute hyperglycemia induces monocyte cholesterol deposition and inflammation remains unknown. **Methods:** Monocytes isolated from healthy individuals (age range 20–40; n=5) were cultured in low (5mM) or high (16.7mM) glucose conditions with or without a glycolysis inhibitor (2-deoxyglucose, 2DG, 5 mM) or an endoplasmic reticulum stress inhibitor (4-phenylbutyric acid, PBA; 20mM) for 6 hrs. After treatment, cytokine release, oxidized LDL uptake, and metabolic assays using Seahorse Technology were performed. **Results:** Healthy human monocytes exposed under high glucose conditions showed a pro-atherosclerotic phenotype with higher levels of the pro-inflammatory cytokines, TNFα(median of differences 6.34 pg/ml, p=0.002) and IL1β(12.04 pg/ml, p=0.003), and increased oxidized LDL uptake (5062ug Dil-Ox LDL/mg, p=0.001). Furthermore, hyperglycemia resulted in higher levels of glycolysis (basal glycolysis 12.94 pmol/min, p=0.01; basal proton efflux rate 15.5 pmol/min, p=0.03) and mitochondrial respiration (percentage of respiratory capacity 16pmol/min p=0.04), suggesting a significant alteration in the metabolic programming of these monocytes. Treatment with 2-DG or PBA attenuated the pro-atherosclerotic phenotype induced by hyperglycemia, promoting a reduction of cytokine release, a reduction of oxidized LDL uptake, and near normalization of the glycolic rate and mitochondrial respiration, stabilizing cellular bioenergetics. **Conclusions:** Altogether, our results suggest that monocyte ER stress in response to acute hyperglycemia promotes a hypermetabolic state characterized by a proinflammatory and proatherogenic monocyte phenotype. Therefore, acute hyperglycemia is a potential mechanism promoting atherosclerosis in patients with type 2 diabetes.

Diabetes Mellitus and Glucose Metabolism

BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

LGR4 and Its Extracellular Domain as Novel Regulators of ß-Cell Survival and Proliferation

Joanna K. Filipowska, PhD, Nagesha G. Kondegowda, PhD, Rupangi C. Vasavada, PhD.
City of Hope Beckman Research Institute, Duarte, CA, USA.

Our lab has shown that RANK (Receptor activator of the NF-κB) by interacting with its ligand, RANKL, inhibits ß-cell proliferation and survival; which can be reversed by Osteoprotegerin (OPG). Recently, the G protein-coupled receptor LGR4 (leucine-rich repeat-containing G protein-coupled receptor 4), which binds R-spondin (RSPO), was identified as a novel receptor for RANKL in osteoclast precursor cells. Thus, RANKL can bind two distinct receptors, RANK and LGR4 in osteoclasts, leading to opposite effects on osteoclastogenesis. LGR4 is expressed in rodent and human ß-cells, but the role of this receptor in ß-cells remains unknown. We postulated that LGR4 through its interaction with RANKL is involved in regulating ß-cell survival and proliferation. Our data indicate expression of specific LGR4 family members, Lgr4, Rank, Rankl, is modulated by stressors, such as cytokines, ER stress, diabetes and aging, in INS1 cells, rodent and human islets. Knocking down Lgr4 in INS1 cells or rodent islets has no significant effect on ß-cell proliferation but is detrimental for ß-cell survival in basal and cytokine-stimulated conditions. We also propose that the soluble extracellular domain of LGR4 (LGR4-ECD), which binds to its ligands (RSPO/RANKL), holds therapeutic potential like OPG, by inhibiting the interaction between RANKL/RANK. At 200ng/ml LGR4-ECD significantly enhances young adult (8-12-week-old) and aged (1.y.o.) rodent ß-cell proliferation, as well as human ß-cell proliferation, in islets from not only control subjects (45±17 y.o.), but also with Type 2 diabetes (48±7 y.o.). Additionally, LGR4-ECD significantly promotes