JQ1 Epigenetic Modulation of Pancreatic β-Cells (INS-1) Normalizes Glucose Sensitivity under Hyperglycemia: Therapeutic Preventive Implications for Type II Diabetes Mellitus

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Background

Chronic hyperinsulinemia and insulin resistance are prequels to type II diabetes mellitus (T2D), a disease that affects over 10% of Americans and is closely associated with obesity, as nearly 90% of diabetic patients are overweight or obese. Chronic excess nutrient exposure leads to glucolipotoxicity in pancreatic β-cells, which is characterized by a left shift in glucose concentration-dependent insulin secretion—a process controlled by transcription where bromodomain has been shown as metabolically relevant.

The exciting development of small molecule inhibitors of Bromodomain and Extra Terminal (BET) protein binding to chromatin has revealed that histone-protein interactions can be beneficially drugged. These inhibitors compete for the acetyl-lysine binding pocket of the bromodomain and thereby displace the BET protein from chromatin, which alters the transcriptional activity of the target gene.

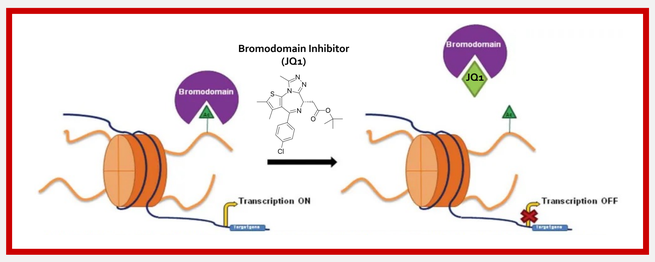

Fig. 1. Molecular approach of JQ1 inhibiting bromodomain (BET)—found in epigenetic factors that control transcription. The bromodomain is a 110 amino acid motif comprised of four anti-parallel α-helices with two connecting loops that form a binding pocket for ε-acetyl-lysines of histones present in nucleosomal chromatin.

Recently, particular attention has been centered on the activity of BET proteins as transcriptional co-activators for cell cycle and proliferation genes in oncological contexts. Rather, we further our investigation on the concept of BET proteins as co-repressors of transcription depending on the signal transduction, cellular, and gene context. Interesting therapeutic opportunities may lie with small molecule inhibition of the co-repressor functions of BET proteins. These new developments have prompted a shift in attention beyond chromatin regulator function in malignancy to a broader concern that includes metabolism.

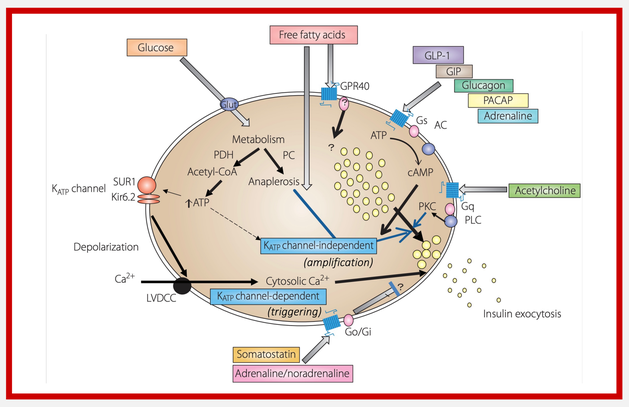

Fig. 2. Physiological signaling Mechanism of GSIS in Pancreatic β-Cells. Glucose enters the pancreatic β-cell via the membrane glucose transporter, is phosphorylated by glucokinase, and undergoes glycolysis to form pyruvate. After its metabolization, pyruvate undergoes cellular respiration in the mitochondria, amplifying ATP concentration, which closes ATP-sensitive K^+ channels, causing membrane depolarization that opens Ca^{2+} channels, increasing Ca^{2+} levels and thereby triggering insulin exocytosis.

A notably promising inhibitor, JQ1, was first synthesized in 2010 by the James Bradner Laboratory at Brigham and Women's Hospital of Harvard Medical School and named after Lead Chemist Jun Qi, Ph.D.. Since the beginning of the previous decade, exciting advancements have been made in the comprehension of the nature and action of this novel compound. Previous studies from our laboratory have demonstrated that the BET protein inhibitor JQ1 at high concentrations (400 nM) increases fatty acid oxidation in clonal pancreatic β-cells (INS-1), leading to a partial reversal of glucolipotoxicity.

The present research investigates the effect of JQ1 on the glucose sensitivity of INS-1 cells under hyperglycemic conditions. We investigate whether BET protein inhibition confers metabolic protection that may prevent the development of T2D.

Results

Insulin secretion from INS-1 cells cultured in 11 mM glucose was maximally stimulated at 4 mM glucose. Treatment with JQ1 for one day right-shifted maximal glucose-stimulated insulin secretion (GSIS) to 6 mM glucose compared to control but did not prevent stimulated insulin secretion at 4 mM glucose. Two-day pretreatment with JQ1 reduced secretion at 4 mM glucose to basal level (1 mM) while maintaining the right-shifted maximal GSIS.

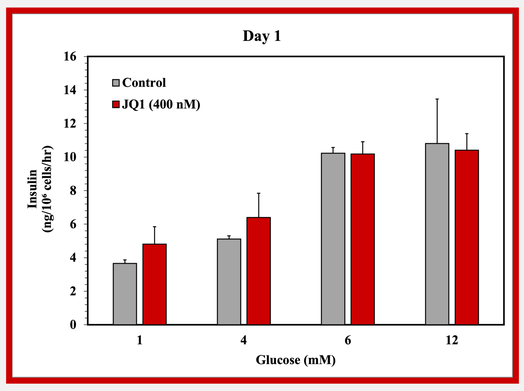

Fig. 4. GSIS 1 day after JQ1 treatment.

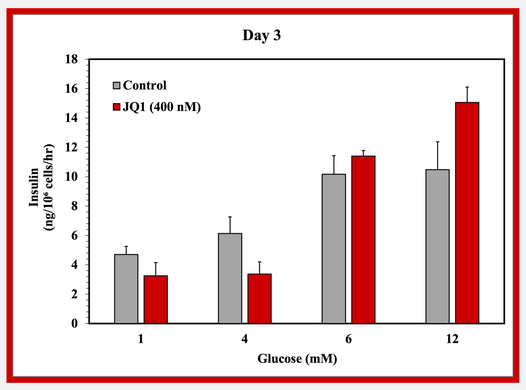

Fig. 5. GSIS three days after JQ1 treatment.

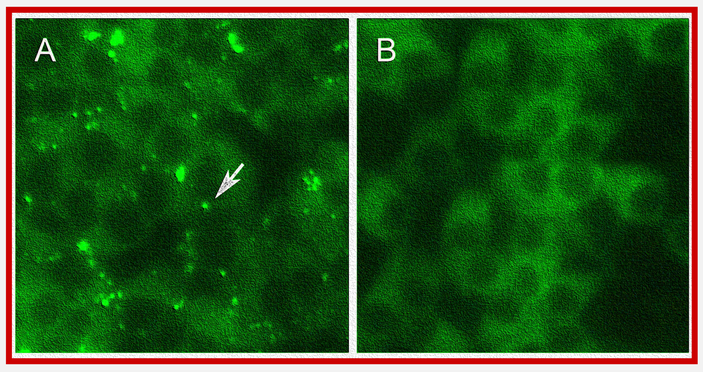

Fig. 6. JQ1 reduces lipid accumulation in INS-1 cells cultured in 11 mM glucose. *A* shows non-treated control cells; *B* shows cells with JQ1 after three days. The white arrow points to a lipid droplet in A.

Discussion

The results imply that epigenetic modulation of pancreatic β-cells with JQ1 beneficially alters signal transduction pathways that maintain insulin-glucose homeostasis by ameliorating glucolipotoxicity, indicating an increased maximal GSIS.
and inferred reduced lipid accumulation.

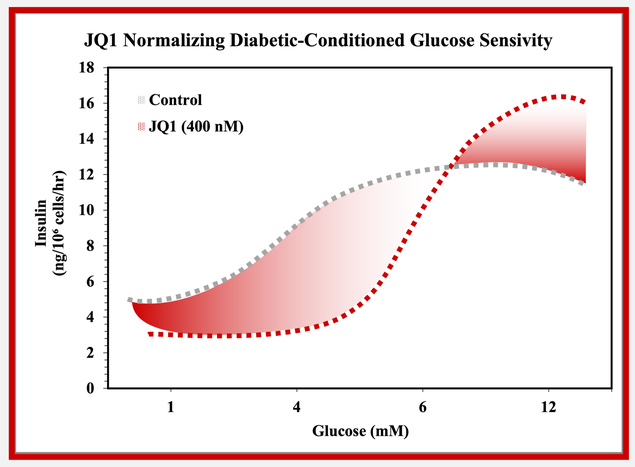

Fig. 7. The "right-shift" effect of JQ1 upon concentration-dependent GSIS (from Day 3).

Conclusions

The results imply that epigenetic modulation of pancreatic β-cells with JQ1 beneficially alters signal transduction pathways that maintain insulin-glucose homeostasis by ameliorating glucolipotoxicity, indicating an increased maximal GSIS. Suggested future directions include investigating the efficacy and accuracy of JQ1 to reverse glucolipotoxicity in isolated rat and human islets.

Methods

INS-1 cells were pre-treated with dimethyl sulfoxide (DMSO)-based active (+) and non-active (-) JQ1 enantiomer (400 nM) for 3 days prior to measurement of insulin secretion and content. INS-1 cells were pre-incubated at 37°C in RPMI 1640 media (11 mM glucose) for 5 days. Posteriorly, INS-1 cells were incubated at 37°C in 0.05% bovine serum albumin (BSA) Krebs-Henseleit (KREBS) solution buffer for 2 periods of 30 min, changing and replenishing the media in-between. Then, INS-1 cells were incubated at 37°C in different glucose concentrations of 1 mM glucose to 12 mM glucose in 0.05% BSA-KREBS solution buffer for 60 min.

Subsequently, specific antibodies were added to samples for measurement of insulin release and cellular content by a homogeneous time-resolved fluorescence (HTRF) insulin assay kit and incubated overnight. Using a TECAN M1000 Pro Plate Reader, the fluorescence-resonance energy transfer (FRET) signal between two separately labeled insulin-binding antibodies was thus measured.

Additionally, INS-1 cells were stained with Nile Red (1 μg/ml) for 20 min at 37°C in RPMI media without serum. After stain removal, cells were imaged using a Nikon TE200 fluorescence microscope (20X magnification) equipped with a cooled Olympus DP72 CCD camera and Cellsense imaging software.

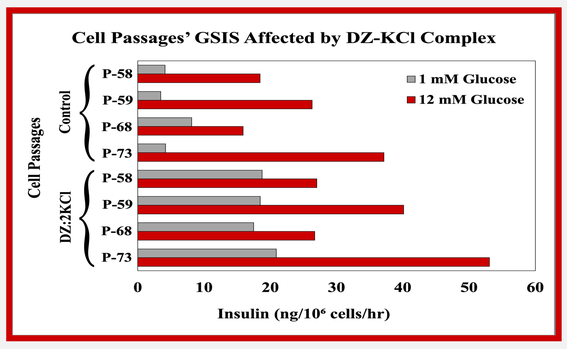

Fig. 3. Selection of Cell Passage. INS-1 cell passage for the experiments was selected by testing the efficacy of four samples of P-58 through P-73 in GSIS directly affected by diazoxide (DZ) counteracted by potassium chloride (KCl)—P-73 was ultimately selected for the following experiments.

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