Study of the mechanism of action of verapamil in prevention of β-cell apoptosis in STZ-induced Diabetes in rats.

Thesis

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Abstract

Introduction: Discovered as the top glucose-induced gene, thioredoxin-interacting protein (TXNIP) has now emerged as such a key player in pancreatic β-cell biology. Pancreatic β-cell expression of TXNIP gene has been found to be tightly regulated by multiple factors and to be dramatically increased in diabetic islets. Hyperglycemia and diabetes mellitus upregulate β-cell TXNIP gene expression, and TXNIP overexpression plays a critical role in the subsequent beta cell glucotoxicity and induction of pancreatic β-cell apoptosis. This study aims to evaluate the effect of verapamil, in different doses, on blood glucose and insulin levels as well as TXNIP gene expression in the β-cells of the pancreatic islets in STZ-induced diabetes mellitus in rats, shedding light on the mechanism involved.

Methods: Diabetes was induced by a single intraperitoneal injection of freshly dissolved streptozotocin (STZ) 60 mg/kg body weight in 0.1 M sodium citrate buffer, pH=4.5, to 18 h fasted 60 male adult albino Sprague–Dawley rats. Diabetic rats received oral verapamil at variable doses (7 and 14mg/kg/day) and mixed insulin (16 unit/kg/day) subcutaneous injection; both once daily for four weeks. Fresh blood samples was obtained for estimating fasting blood-glucose at the start of the experiment, 48 h, one week, two weeks and four weeks after STZ injection. Sera were used for determination of fasting serum insulin at the start, 48 h and four weeks after STZ injection. Estimation of TXNIP gene expression levels in isolated islets by real time PCR was done after animals' sacrifaction.

Results: In the present study, oral verapamil administration for 4 weeks; in both doses used in the study (7 and 14mg/kg/day), decreased significantly mean serum glucose levels and increased significantly mean serum insulin levels in a dose

dependent manner in STZ induced diabetic rats. Both low and high doses of verapamil also managed to reduce significantly overexpressed TXNIP gene in the pancreatic β-cells of the diabetic rats' islets. The postulated mechanism that verapamil repressed pancreatic beta cell overexpressed TXNIP gene may relates directly to calcium entry, as calcium plays a very important role in linking hyperglycemia with elevated TXNIP expression in the diabetic islets and the subsequent apoptotic events in the beta cells.

Conclusion: The present study shows that oral verapamil administration may be able to significantly improve glucose homeostasis and pancreatic β-cell function in STZ diabetic rats.

Key words: Diabetes Mellitus- Beta cells apoptosis- TXNIP- Verapamil.

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List of Abbreviations

ADA: American Diabetes Association

ASK1: Apoptosis signal regulating kinase1

BAK: Bcl-2 homologous antagonist/killer

BAX: Bcl-2-associated X protein

Bcl-2: B-cell lymphoma-2

CAS-3: Caspase-3 enzyme

CD: Cluster of differentiation

ChoRE: Carbohydrate response element

ChREBP: Carbohydrate response element binding protein

DHP: Dihydropyridine

dNTP: Deoxynucleotide triphosphate

DPP-4: Dipeptidyl peptidase-4

EGFR: Epidermal growth factor receptor

ER: Endoplasmic reticulum

FFA: Free fatty acids

FPG: Fasting plasma glucose

GAD: Glutamic acid decarboxylase

GLP-1: Glucagon-like peptide-1

GLUT1: Glucose transporter-1

GTC: Guanidine thiocyanate

HDAC: Histone deacetylase

HNF: Hepatocyte nuclear factor

IFG: Impaired fasting glucose

IGT: Impaired glucose tolerance

IL-1β: Interleukin-1β

I-R injury: Ischemia reperfusion injury

JNK: c-Jun N terminal kinase

KATP channel: ATP-sensitive K+ channel

Kip: Kinase inhibitor

MAP: Mitogen-activated protein

MAPK: Mitogen-activated protein kinase

MLX: Max-like protein X

MODY: Maturity-onset diabetes of the young

NLRP3: NOD-like receptor family, pyrin domain containing 3

PPAR: Peroxisome proliferator-activated receptor-gamma

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

TBP-2: Thioredoxin binding protein 2

TNF: Tumor necrosis factor

TRAFs: TNF-receptor associated factors

TRX: Thioredoxin

TUNEL: Terminal deoxynucleotidyl transferase

TXNIP: Thioredoxin interacting protein

VDUP-1: Vitamin D-3 upregulated protein-1

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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Genuth et al., 2003).Several pathogenic processes are involved in the development of diabetes mellitus. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency in type 1 to abnormalities that result in resistance to insulin action in type 2 (Wajchenberg, 2013).

Although loss of functional β-cell mass is a hallmark of diabetes mellitus, no treatment approaches that halt this process are currently available. Thioredoxin-interacting protein (TXNIP) is recently identified as an attractive target in this regard.Discovered as the top glucose-induced gene, thioredoxin-interacting protein (TXNIP); a ubiquitously expressed cellular redox regulator, has now emerged as such a key player in pancreatic β-cell biology (Shalev et al., 2002). Pancreatic β-cell expression of TXNIP gene has been found to be tightly regulated by multiple factors and to be dramatically increased in diabetic islets. Hyperglycemia and diabetes mellitus upregulate β-cell TXNIP gene expression, and TXNIP overexpression plays a critical role in the subsequent beta cell glucotoxicity and induction of β-cell apoptosis (Chen et al., 2008).

Modern molecular interventions in medicine have now made a closer understanding to the genetic factors controlling the development and progression of diabetes mellitus, increasing the possibilities to suggest solutions that may prevent diabetes mellitus.

TXNIP gene down regulators are now claimed to be effective in preventing diabetes mellitus in animal models. Data suggested that calcium channel blockers,

verapamil in specific, are capable of reducing cardiac TXNIP overexpression and preventing apoptosis in the heart that accompanies cardiovascular complications in diabetes mellitus (Chen et al., 2009).Finding an oral medication that could inhibit TXNIP overexpression would therefore represent an important step in the context of pancreatic β-cell survival and diabetes mellitus development.

Aim of work

The present study aims to evaluate the effect of verapamil on blood glucose and insulin levels as well as to determine whether verapamil may affect the pancreatic β-cells and the mechanism by which verapamil may affect β-cell apoptosis and protect against diabetes mellitus in streptozotocin- induced diabetes mellitus in rats (an experimental model of diabetes mellitus).

Diabetes Mellitus

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia (Seino et al., 2010).

This chronic hyperglycemic condition is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Long-term complications of diabetes mellitus include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputation, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular symptoms and sexual dysfunction. Acute, life-threatening consequences of diabetes are hyperglycemia with ketoacidosis or the non ketotic hyperosmolar syndrome (Ramesh et al., 2010).

Several pathogenic processes are involved in the development of diabetes mellitus. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action (Wajchenberg, 2013). The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type 2diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. The degree of

hyperglycemia (if any) may change over time, depending on the extent of the underlying disease process (Fig. 1). A disease process may be present but may not have progressed far enough to cause hyperglycemia. The same disease process can cause impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) without fulfilling the criteria for the diagnosis of diabetes.

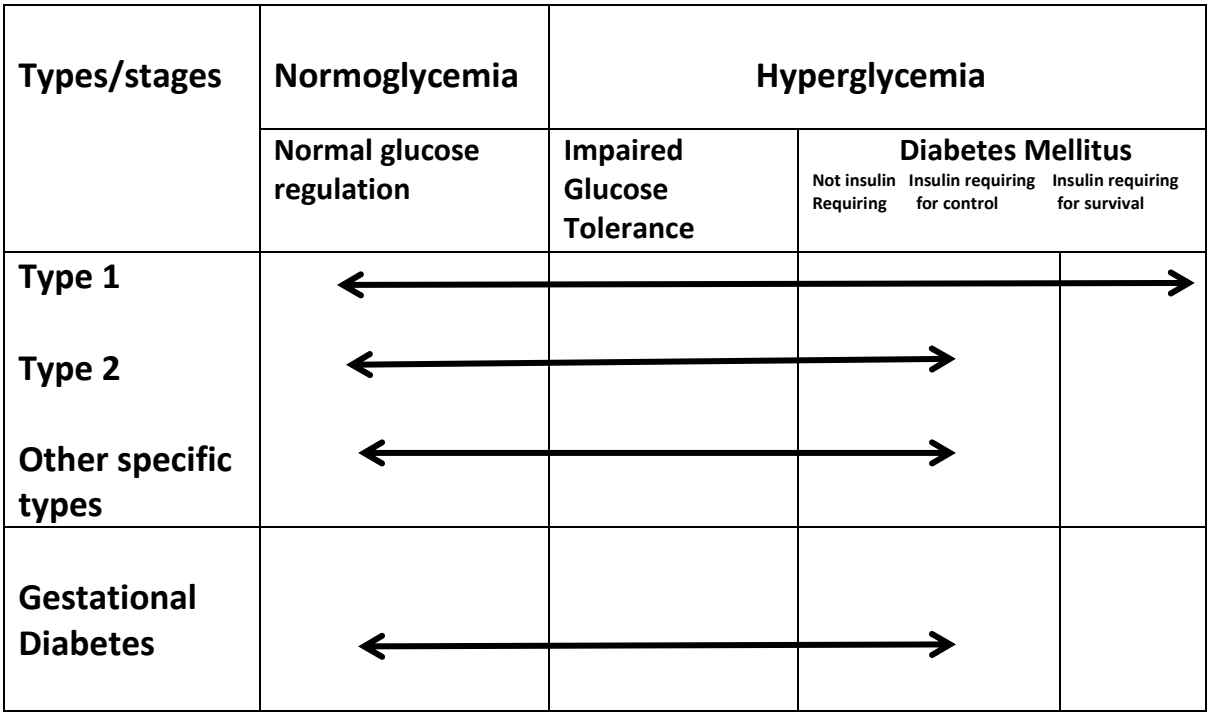

Figure (1): Disorders of glycemia: etiologic types and stages (American Diabetes Association, 2010).

In some individuals with diabetes, adequate glycemic control can be achieved with weight reduction, exercise, and/or oral glucose-lowering agents. These individuals therefore do not require insulin. Other individuals who have some residual insulin secretion but require exogenous insulin for adequate glycemic control can survive without it. Individuals with extensive β-cell destruction and therefore no residual insulin secretion require insulin for survival. The severity of the metabolic abnormality can progress, regress, or stay the same. Thus, the degree of hyperglycemia reflects the severity of the underlying metabolic