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Thioredoxin-mimetic peptides (TXM) inhibit inflammatory pathways associated with high-glucose and oxidative stress

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ABSTRACT

Impaired insulin signaling and the associated insulin-resistance in liver, adipose tissue, and skeletal muscle, represents a hallmark of the pathogenesis of type 2-diabetes-mellitus. Here we show that in the liver of db/db mice, a murine model of obesity, type 2 diabetes, and dyslipidemia, the elevated activities of *mitogen-activated protein kinases* (*MAPK*; ERK1/2 and $p38^{MAPK}$), and Akt/PKB are abolished by rosiglitazone-treatment, which normalizes blood glucose in db/db mice. This is unequivocal evidence of a functional link between the activation of the MAPK specific inflammatory-pathway and high-blood sugar. A similar reduction in ERK1/2, $p38^{MAPK}$, and Akt activities but without affecting blood-glucose was observed in the liver of db/db mice treated with a molecule that mimics the action of thioredoxin, called thioredoxin-mimetic peptide (TXM). N-Acetyl-Cys-Pro-Cys-amide (TXM-CB3) is a free radical scavenger, a reducing and denitrosylating reagent that protects the cells from early death induced by inflammatory pathways. TXM-CB3 also lowered MAPK signaling activated by the disruption of the thioredoxin-reductase-thioredoxin (Trx-TrxR) redox-system and restored Akt activity in rat hepatoma FAO cells. Similarly, two other TXM-peptides, N-Acetyl-Cys-Met-Lys-Cys-amide (TXM-CB13; DY70), and N-Acetyl-Cys-γGlu-Cys-Cys-amide (TXM-CB16; DY71), lowered insulin- and oxidative stress-induced ERK1/2 activation, and rescued HepG2 cells from cell death. The potential impact of TXM-peptides on inhibiting inflammatory pathways associated with high-glucose could be effective in reversing low-grade inflammation. TXM-peptides might also have the potential to improve insulin resistance by protecting from posttranslational modifications like nitrosylation.

1. Introduction

In the last decade a growing body of evidence indicates that obesity promotes a chronic low-grade inflammation accompanied with a greater abundance of proinflammatory cytokines [1,2], leading to insulin resistance and diabetes [3–6].

In diabetes, the long-term exposure to high glucose is associated with chronic inflammation leading to the progression of the disease state, which in patients with diabetes is in a good correlation with increased expression of oxidative stress markers [7]. Chronic inflammatory conditions result in severe alterations in insulin signaling and in extensive protein post-translational modification, which is one of the hallmarks of chronic metabolic and neurodegenerative diseases [8]. The posttranslational modifications, including carbonylation, S-nitrosylation, glycoxidation, and glycation, constitute early events that compromise the activity of the covalently modified proteins and their cellular performance.

Among the most widely used animal models used to study the role of inflammation in the obesity-induced diabetes research is the leptin receptor function-deficient db/db-mice [9,10]. The leptin receptor deficient db/db mouse displays diabetic features such as age-dependent progression, and early insulin resistance followed by an insulin secretory defect resulting in profound hyperglycemia.

Insulin-induced activation of MAPK via the RAS-MEK-ERK and of Akt/PKB via the phosphatidylinositol 3-kinase (PI3-kinase) pathways are elevated in diabetes and obesity. For example, the activities of PI3-kinase and Akt in anti-phospho-tyrosine immunoprecipitates from livers of diabetic mice (db/db) were increased 3 fold and 2 fold, respectively, compared to normal control mice [11]. In addition, in diabetic mice renal cortical activities of PI3-kinase, Akt, and ERK1/2-type mitogen-activated protein (MAP) kinase were significantly elevated 6 fold, 2 fold, and 7 fold,

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respectively. Similarly to diabetic mice ERK1/2 activity is elevated in adipose tissue, liver, and muscles of obese/diabetic patients or mice (review [Tanti, 2009 #13]). Consistent with these results, studies in primary muscle cells from type 2 diabetic patients showed more than a twofold increase in the basal phosphorylation of IRS-1 on serine 636 in myotubes, concomitant with an abnormally high basal ERK activity in these cells [12].

Similar elevation in $p38^{MAPK}$ phosphorylation was observed, which has been shown to play a distinct pathogenic role in the progression of diabetic nephropathy in db/db mice [13].

Another close correlation between high glucose and activated inflammatory pathways has been observed in the Zucker rat brain [14]. In these studies lowering of blood-glucose by rosiglitazone normalized MAPK activity in the brain of the treated Zucker rats [14]. Furthermore, Zucker rats treated with the anti-inflammatory denitrosylating, thiol-peptide, TXM-CB3 significantly reduced MAPK activation in the rat brain [14]. TXM-CB3 is a family member of thioredoxin mimetic peptides (TXM peptides) that mimics thioredoxin activity in vivo [15] and in vitro [16,17] and acts to denitrosylate proteins [18,19].

Oxidative-mediated posttranslational modifications such as the nitosylation of selective proteins link multiple stressors, such as amyloid β and hyperglycemia, to mechanisms that may ultimately lead to organ damage. This notion is consistent with our previous observations that TXM-CB3 and TXM-CB4 are able to lower MAPK activities induced by amyloid β1–42 in neuronal culture [17], in human neuronal SH-SY5Y cells [20], and high glucose in the brain of Zucker rats [14].

It appears that impaired glucose-control and insulin signaling are strongly associated with intracellular inflammatory-triggered pathways. Given our previous findings that TXM-peptides such as TXM-CB3, TXM-CB13 (DY70) and TXM-CB16 (DY71) display anti-inflammatory properties, we performed the present study in which we evaluate the effects of TXM peptides on inflammatory pathways and on glycaemic control and metabolically active organs in diabetic mice. We expanded our studies to rodent and human liver cell lines to allow for more detailed studies of the mechanisms by which the TXM-peptides could impact glucose control or organ protection.

We show that the constitutive Akt, GSK3β, TSC2 and MAPK activities in the liver of db/db mice were lowered by i.p injection of TXM-CB3 for 21 days. We also found that insulin- induced Akt phosphorylation inhibited by oxidative stress following the disruption of the thioredoxin reductase-thioredoxin (TrxR-Trx) system, was restored by TXM-CB3 and two other TXM-peptides, TXM-CB13 and TXM-CB16 in liver derived-rat hepatoma (FAO cells). We propose that TXM-peptides could become beneficial for reversing obesity-induced Akt and MAPK activation.

2. Materials and methods

2.1. Reagents

All materials were purchased from Sigma, Jerusalem, if not otherwise stated; Auranofin (Enzo life sciences, Shoham, Israel), triethylphosphine (2,3,4,6-tetra-O-acetyl-β-1-D-thiopyranosato-S) gold(I).

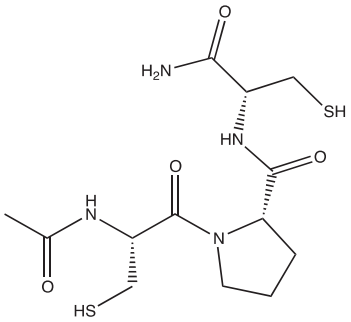
The thioredoxin mimetic peptide TXM-CB3 (Acetyl-Cys-Pro-Cys-amide) was prepared by solid-phase peptide synthesis methods. After cleavage from the resin and reversed-phase chromatography purification of the crude peptide, desired fractions were pooled and lyophilized to obtain the desired peptide powder (> 98% purity by analytical HPLC). Before initiating the in vivo study and due to the presence of two free cysteine residues in the peptide sequence, solution peptide stability studies were carried out in 20 mM citrate buffer solutions at 3 mg/mL concentration at room temperature under pH ranging from 4 to 7. Stability was reduced with increased pH due to disulfide bridge formation (presence of dimers and cyclized monomer side-products increase with time and pH). Final formulation for the in vivo study was 3 mg/mL in 20 mM citrate buffer pH 4. Solutions were prepared daily from fresh powder prior to subcutaneous injection.

TXM-CB13 (DY70) and TXM-CB16 (DY71) (Scheme 1) were obtained from OneDay Biotech and Pharma Ltd, Tel Aviv, Israel; Tissue culture serum and medium were from Biological Industries, Kibbutz Beit-Haemek, Haifa, Israel..

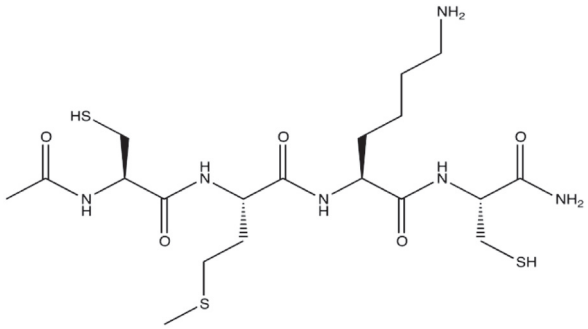
2.2. Animal model

Five-week-old C57BLKS/J *db/db* mice were purchased from Harlan Laboratories. All mice were fed LabDiet 5008, maintained under approved Animal Care and Use protocols for Lilly Research Laboratories (IACUC protocol #12-243) and housed on a 12-h light cycle from 0600 to 1800. At 6 weeks of age, TXM-CB3 was administered i.p (5 mL/kg) daily in a 20 mM citrate buffer (pH 5) for 21 days. Additionally, Rosiglitazone was administered p.o. (5 mL/kg) in HEC 1%/Tween 80 0.25%/Antifoam 0.05%. Body weight, non-fasted glucose and insulin were assessed throughout the study. On Day 19, after an overnight fast, glucose and insulin samples were

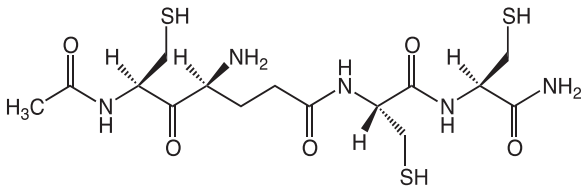
TXM-CB3, Acetyl-Cys-Pro-Cys-Amide



TXM-CB13 (DY-70), Acetyl-Cys-Met-Lys-Cys-Amide



TXM-CB16 (DY-71), Acetyl-Cys-γGlu-Cys-Cys-Amide



Scheme 1. The structure of TXM-CB3, TXM-CB13 (DY70) and TXM-CB16 (DY71).