

Involvement of Oxidative Stress and the JNK Pathway in Glucose Toxicity

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■ Abstract

The hallmark of type 2 diabetes is pancreatic β -cell dysfunction and insulin resistance. Normal β -cells can compensate for insulin resistance by increasing insulin secretion, but insufficient compensation leads to the onset of glucose intolerance. Once hyperglycemia becomes apparent, β -cell function gradually deteriorates and insulin resistance becomes aggravated. Such phenomena are collectively called “glucose toxicity”. Under diabetic conditions, oxidative stress is induced and the JNK pathway is activated, which is

involved in “glucose toxicity”. Activation of the JNK pathway suppresses insulin biosynthesis and interferes with insulin action. Indeed, suppression of the JNK pathway in diabetic mice improves insulin resistance and ameliorates glucose tolerance. Consequently, the JNK pathway plays a crucial role in the progression of pancreatic β -cell dysfunction and insulin resistance and thus could be a potential therapeutic target for the “glucose toxicity” found in diabetes.

Keywords: diabetes · insulin resistance · insulin biosynthesis · oxidative stress · JNK pathway

Oxidative stress is induced under hyperglycemic conditions

The development of type 2 diabetes is usually associated with a combination of pancreatic β -cell dysfunction and insulin resistance. Normal β -cells can compensate for insulin resistance by increasing insulin secretion or β -cell mass, but insufficient compensation leads to the onset of glucose intolerance. Chronic hyperglycemia is a cause of impaired insulin biosynthesis and secretion; once hyperglycemia becomes apparent, β -cell function gradually deteriorates and insulin resistance becomes aggravated [1-5]. This process is called “glucose toxicity”.

Under diabetic conditions, reactive oxygen species (ROS) increase in various tissues and are involved in the development of diabetic complications [6-9]. Pancreatic β -cells have recently emerged as a target of oxi-

dative stress-mediated tissue damage [10-25]. β -cells express abundant levels of the high-Km glucose transporter GLUT2 and thereby display highly efficient glucose uptake when exposed to high glucose concentration. Accordingly, extracellular hyperglycemia causes intracellular hyperglycemia in β -cells, leading to the induction of ROS in pancreatic islets of diabetic animals. Indeed, it was shown that the presence of oxidative stress markers 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 4-hydroxy-2,3-nonenal (4-HNE) is increased in islets under diabetic conditions [13, 21]. In addition, due to the relatively low expression of antioxidant enzymes such as catalase, and glutathione peroxidase [12], β -cells are rather vulnerable to oxidative stress. Thus, it is likely that oxidative stress is involved in β -cell deterioration in type 2 diabetes. There are several sources of ROS production in cells: 1. The non-enzymatic glycosylation reaction [10, 11], 2. the elec-

tron transport chain in mitochondria [8, 22], and 3. the hexosamine pathway [19] (Figure 1). In diabetic animals, glycation can be observed in various tissues and organs, and various kinds of glycated proteins are produced in a non-enzymatical manner through the Maillard reaction. During the reaction which in turn produces Schiff base, Amadori product and advanced glycosylation end products (AGE), ROS are also produced [10]. Also, the electron transport chain in mitochondria is likely to be an important pathway triggering the production of ROS. Indeed, it has been suggested that mitochondrial overwork, which causes the emergence of ROS, is a potential mechanism causing impaired first-phase glucose-stimulated insulin secretion found in the early stage of diabetes [22] as well as diabetic complications [8, 9].

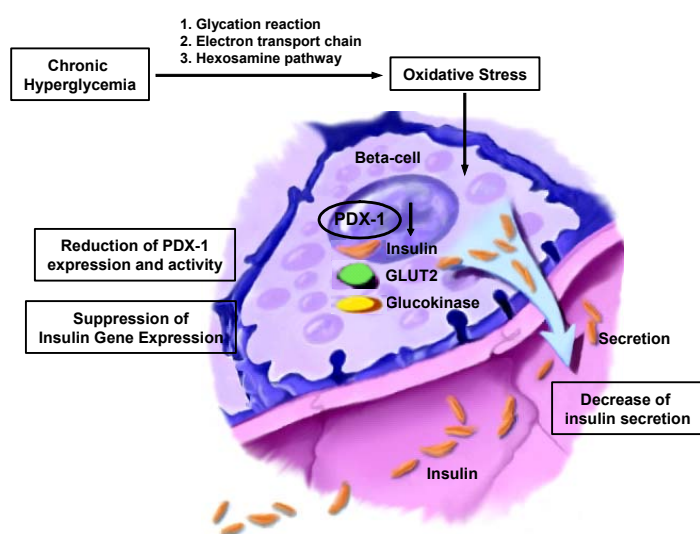


Figure 1. Oxidative stress and pancreatic β -cell dysfunction. Extra-cellular hyperglycemia contributes to intra-cellular hyperglycemia via the following ways of ROS production: 1. The non-enzymatical glycosylation reaction, 2. the electron transport chain in mitochondria, and 3. the hexosamine pathway. DNA-binding activity of PDX-1 is rather sensitive to oxidative stress. ROS production in pancreatic β -cells, therefore, leads to a decreased expression of PDX-1, which in turn causes the down-regulation of β -cell genes, such as insulin, GLUT2, and glucokinase. Thus, chronic hyperglycemia suppresses insulin biosynthesis and secretion.

Oxidative stress is involved in pancreatic β -cell dysfunction

The glycation reaction suppresses the insulin gene transcription in β -cells by provoking oxidative stress. When β -cell-derived HIT cells were exposed to D-ribose, a strong reducing sugar, insulin gene promoter

activity and mRNA expression were suppressed, whereas no such changes were observed with the β -actin gene [11]. These were neutralized with aminoguanidine, an inhibitor of the glycation reaction, or N-acetyl-L-cysteine, an antioxidant, indicating that D-ribose suppresses insulin gene transcription by provoking oxidative stress through the glycation reaction.

Pancreatic and duodenal homeobox factor-1 (PDX-1), also known as IDX-1/STF-1/IPF1 [26-28], is a member of the homeodomain-containing transcription factor family. PDX-1 is expressed in the pancreas and duodenum and plays a crucial role in pancreas development [29-38], β -cell differentiation/regeneration [39-51], and in maintaining normal β -cell function by regulating many important β -cell genes, including insulin, GLUT2, and glucokinase [52-62]. During the development of the pancreas, PDX-1 expression is maintained in multipotential precursors that co-express several hormones and later it becomes restricted to β -cells. There is no pancreas in mice homozygous for a targeted mutation in the PDX-1 gene and the mice develop fatal perinatal hyperglycemia [29]. Heterozygous PDX-1-deficient mice reveal impaired glucose tolerance [63], which also provides evidence for the crucial role of PDX-1 in pancreas development. Clinically, mutations in PDX-1 are known to cause some cases of maturity-onset diabetes of the young (MODY) [64]. As a possible cause of the reduction in the insulin gene promoter activity by oxidative stress, we found that the DNA-binding activity of PDX-1 is rather sensitive to oxidative stress; when HIT cells were exposed to D-ribose, PDX-1 binding to the insulin gene was markedly reduced, which was prevented by aminoguanidine or NAC [11]. In summary, chronic hyperglycemia suppresses insulin biosynthesis and secretion by provoking oxidative stress through various pathways, accompanied by the reduction of PDX-1 DNA-binding activity (Figure 1).

Because oxidative stress is produced under diabetic conditions and is likely to be involved in pancreatic β -cell dysfunction found in diabetes, we evaluated the potential usefulness of antioxidants in treatment for type 2 diabetes. We treated obese diabetic C57BL/KsJ-db/db mice with antioxidants (N-acetyl-L-cysteine plus vitamin C and E) and evaluated their effects [14]. The antioxidant treatment retained glucose-stimulated insulin secretion and moderately decreased blood glucose levels. The β -cell mass was

significantly larger in the mice treated with the antioxidants. The antioxidant treatment suppressed apoptosis in β -cells without changing the rate of β -cell proliferation. The amounts of insulin content and insulin mRNA were also retained by the antioxidant treatment. Furthermore, PDX-1 expression was more clearly visible in the nuclei of islet cells after the antioxidant treatment [14]. Similar effects were observed with the Zucker diabetic fatty (ZDF) rat, another animal model for type 2 diabetes [15]. In summary, these data indicate that antioxidant treatment can generate beneficial effects for diabetes with preservation of *in vivo* β -cell function.

In addition, as a step toward the clinical trial of antioxidants for type 2 diabetes, we investigated the possible anti-diabetic effects of probucol, an antioxidant widely used as an anti-hyperlipidemic agent, on the preservation of β -cell function in diabetic C57BL/KsJ-db/db mice [21]. Immunostaining for oxidative stress markers such as 4-hydroxy-2-nonenal (HNE)-modified proteins and heme oxygenase-1 revealed that probucol treatment decreased reactive oxygen species (ROS) in pancreatic islets of diabetic animals. Probucool treatment preserved β -cell mass, the insulin content, and glucose-stimulated insulin secretion, leading to the improvement of glucose tolerance [21]. These data suggest the potential usefulness of antioxidants for treating diabetes and provide further evidence for the implication of oxidative stress in β -cell glucose toxicity found in diabetes (Figure 1).

The JNK pathway is involved in pancreatic β -cell dysfunction

Several signal transduction pathways including c-Jun N-terminal kinase (JNK) (also known as stress-activated protein kinase (SAPK)) [65-68], p38 mitogen-activated protein kinase (p38 MAPK), and protein kinase C (PKC) are activated by oxidative stress in several cell types including pancreatic β -cells. It has been shown recently that activation of JNK is involved in the reduction of insulin gene expression by oxidative stress and that suppression of the JNK pathway can protect β -cells from oxidative stress [69]. When isolated rat islets were exposed to oxidative stress, JNK, p38 MAPK, and PKC pathways were activated, preceding the decrease of insulin gene expression. Adenovirus-mediated over-expression of dominant-negative type (DN) JNK, but not the p38 MAPK inhibitor SB203580 nor the PKC inhibitor GF109203X, protected insulin gene expression and secretion from oxi-

dative stress. In contrast, wild type (WT) JNK over-expression suppressed both insulin gene expression and secretion.

These results are correlated with changes in the binding of the major transcription factor PDX-1 to the insulin promoter; adenoviral over-expression of DN-JNK preserved PDX-1 DNA-binding activity in the presence of oxidative stress, while WT-JNK over-expression decreased PDX-1 DNA-binding activity. PDX-1 DNA-binding activity is decreased in association with a reduction of insulin gene transcription after chronic exposure to high glucose concentration. The impairment of PDX-1 activity and subsequent suppression of insulin gene transcription is mediated by the activation of the JNK pathway in the diabetic state. Thus, it is likely that JNK-mediated suppression of PDX-1 DNA-binding activity accounts to some extent for the deterioration of the β -cell function (Figure 2).

As a potential mechanism for JNK-mediated PDX-1 inactivation, we recently reported that PDX-1 is translocated from the nuclei to the cytoplasm in response to oxidative stress; when oxidative stress was charged upon β -cell-derived HIT cells, both intrinsically expressed PDX-1 and exogenously introduced green fluorescent protein (GFP)-tagged PDX-1 moved from the nuclei to the cytoplasm [70] (Figure 2). Addition of DN-JNK inhibited the oxidative stress-induced PDX-1 translocation, suggesting an essential role of JNK in mediating the phenomenon. Whereas the nuclear localization signal (NLS) in PDX-1 was not affected by oxidative stress, leptomycin B, a specific inhibitor of the classical, leucine-rich nuclear export signal (NES), inhibited nucleo-cytoplasmic translocation of PDX-1 induced by oxidative stress. Indeed, we identified an NES at position 82-94 of the mouse PDX-1 protein (Figure 2) [70].

To examine whether DN-JNK can protect β -cells from the toxic effects of hyperglycemia and to explore the potential therapeutic application for islet transplantation, we performed islet transplantation into diabetic mice [69]. Isolated rat islets were infected with dominant-negative JNK expressing adenovirus (Ad-DN-JNK) or Ad-GFP and cultured for 2 days; then 500 islets were transplanted under kidney capsules of STZ-induced diabetic Swiss nude mice. Blood glucose levels were not sufficiently decreased by the transplantation of islets infected with Ad-GFP, which was probably due to toxic effects of hyperglycemia upon a marginal islet number, but were markedly decreased by Ad-DN-JNK. Four weeks after the transplantation of islets infected with Ad-GFP, insulin mRNA levels in islet

grafts were clearly decreased compared with those before transplantation, but relatively preserved by DN-JNK overexpression [69]. These results suggest that DN-JNK can protect β -cells from some of the toxic effects of hyperglycemia during this transplant period, providing new insights into the mechanism through which oxidative stress suppresses insulin gene transcription in β -cells. Also, the finding that this adverse outcome can be prevented by DN-JNK overexpression suggests that the JNK pathway in β -cells could become a new therapeutic target for diabetes.

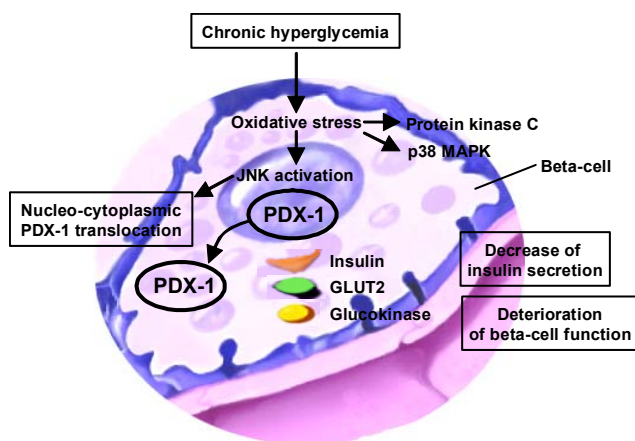


Figure 2. The JNK pathway and pancreatic β -cell dysfunction. Signal transduction pathways including JNK, p38 mitogen-activated protein kinase (p38 MAPK), and protein kinase C (PKC) are activated by oxidative stress in pancreatic β -cells. Wild-type-JNK over-expression decreases PDX-1 DNA-binding activity in association with a reduction of insulin gene transcription after chronic exposure to high glucose concentration leading to a decrease of insulin secretion. In addition, PDX-1 is translocated pathogenically from the nucleus to the cytoplasm by JNK activation.

It is known that β -cell destruction by cytokines such as interleukin-1 β (IL-1 β) [71-74] can be prevented by inhibition of the JNK pathway [75-78], implying that JNK plays a role in autoimmune β -cell destruction found in early stages of type 1 diabetes. Also, it has been reported that levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker for oxidative stress, are increased in the blood of type 2 diabetic patients as well as in islets of type 2 diabetic animal models [6, 13, 21] and that JNK activation by oxidative stress in islets actually reduces the PDX-1 DNA binding activity and insulin gene transcription [69]. In addition, the significance of JNK in the development of diabetes comes

from the result of a genetic analysis in humans; while islet-brain-1 (IB1), the human and rat homologue of mouse JNK-interacting protein-1 (JIP-1) [79, 80], was known to selectively inhibit the JNK signaling [78], it was reported that a missense mutation within the IB1-encoding MAPKIP1 gene (S59N) is associated with a late onset type 2 diabetes [81]. Thus, we assume that JNK is involved in the deterioration of β -cell function in both type 2 diabetes and the early stage of type 1 diabetes.

The JNK pathway is involved in insulin resistance

The JNK pathway is known to be activated under diabetic conditions and possibly to be involved in the progression of insulin resistance. We have recently examined the effects of a modulation of the JNK pathway in the liver on insulin resistance and glucose tolerance [82]. Overexpression of dominant-negative type JNK in the liver of obese diabetic mice dramatically improved insulin resistance and markedly decreased blood glucose levels. When C57BL/KsJ-db/db mice were treated with Ad-DN-JNK, non-fasting blood glucose levels were markedly reduced, whereas no such effect was observed in Ad-GFP-treated mice. In the intraperitoneal insulin tolerance test (IPITT), the hypoglycemic response to insulin was larger in Ad-DN-JNK-treated C57BL/KsJ-db/db mice compared to Ad-GFP-treated mice. To investigate this point further, we performed the euglycemic hyperinsulinemic clamp test. Glucose infusion rate (GIR) in Ad-DN-JNK-treated mice was higher than that in Ad-GFP-treated mice, indicating that suppression of the JNK pathway in the liver reduces insulin resistance and thus ameliorates glucose tolerance in C57BL/KsJ-db/db mice. Furthermore, hepatic glucose production (HGP) was significantly lower in Ad-DN-JNK-treated mice. In contrast, there was no difference in the glucose disappearance rate (Rd) between these two groups. These results indicate that the reduction of insulin resistance and the amelioration of glucose tolerance by DN-JNK overexpression are mainly effectuated by a suppression of hepatic glucose production (Figure 3).

It has been reported that serine phosphorylation of insulin receptor substrate-1 (IRS-1) inhibits insulin-stimulated tyrosine phosphorylation of IRS-1, leading to an increase in insulin resistance [83, 84]. IRS-1 serine 307 phosphorylation was markedly decreased in Ad-DN-JNK-treated mice. We also found an increase in IRS-1 tyrosine phosphorylation in Ad-DN-JNK-

treated mice compared to control mice. Reduction of Akt serine 473 phosphorylation was observed in Ad-DN-JNK-treated C57BL/KsJ-db/db mice. Therefore, an increase in IRS-1 serine phosphorylation may be closely associated with the development of insulin resistance induced by JNK overexpression. Next, we examined the expression levels of the key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), both of which are known to be regulated by insulin signaling. Expression levels of both enzymes were markedly decreased by Ad-DN-JNK treatment in C57BL/KsJ-db/db mice. These results indicate that suppression of the JNK pathway enhances insulin signaling which leads to a decrease in gluconeogenesis and amelioration of glucose tolerance. Similar effects were observed in high-fat/high-sucrose diet-induced diabetic mice. Conversely, expression of wild type JNK in the liver of normal mice decreased insulin sensitivity. In summary, these findings suggest that suppression of the JNK pathway in liver exerts greatly beneficial effects on insulin resistance status and glucose tolerance in both genetic and dietary models of diabetes (Figure 3).

(FFAs), tumor necrosis factor- α (TNF- α), all of which are known to be increased under diabetic conditions. Under diabetic conditions, reactive oxygen species (ROS) are produced in various tissues and are involved in the development of insulin resistance as well as the progression of β -cell deterioration. FFAs and TNF- α are also likely to be involved in the development of insulin resistance; levels of FFAs and TNF- α are increased under obese diabetic conditions with insulin resistance, which leads to a further increase in insulin resistance. Thus, we assume that the improvement of insulin resistance by suppression of the JNK pathway is, at least in part, counterbalancing the deleterious effects of several factors such as oxidative stress, FFAs and TNF- α (Figure 3).

It has been also reported recently that JNK activity is abnormally elevated in liver, muscle and adipose tissues in obese type 2 diabetic mice and that insulin resistance is substantially reduced in mice homozygous for a targeted mutation in the JNK1 gene (JNK-KO mice) [83]. When JNK-KO mice are placed on a high-fat/high-caloric nutrition, obese wild type mice develop mild hyperglycemia compared to lean wild-type

control mice. In contrast, blood glucose levels in obese JNK-KO mice were proven to be significantly lower than those in obese wild type mice. In addition, serum insulin levels in obese JNK-KO mice were significantly lower than those in obese wild type mice. Intraperitoneal insulin tolerance tests showed that hypoglycemic response to insulin in obese wild type mice is lower than that in obese JNK-KO mice. Also, the intraperitoneal glucose tolerance test revealed a higher degree of hyperglycemia in obese wild type mice than in obese JNK-KO mice. These results indicate that the JNK-KO mice are protected from the development of dietary obesity-induced insulin resistance. Furthermore, targeted mutations in JNK were introduced in genetically obese mice (ob/ob) [65]. Blood glucose levels in ob/ob-

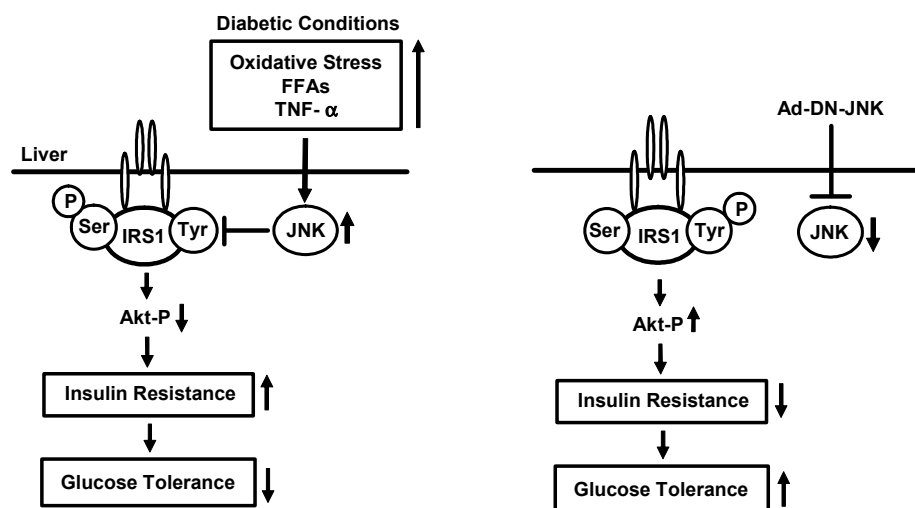


Figure 3. The effects of the JNK pathway on insulin resistance and glucose tolerance. The JNK pathway in the liver is activated by factors such as oxidative stress, free fatty acids (FFAs), and TNF- α . The levels of these factors are elevated under obese diabetic conditions. Serine phosphorylation of insulin receptor substrate-1 (IRS-1) inhibits insulin-stimulated tyrosine phosphorylation of IRS-1 and leads to an increase in insulin resistance. Expression of wild type JNK in the liver of normal mice decreased insulin sensitivity. Suppression of the JNK pathway by the treatment with Ad-DN-JNK enhances insulin signaling which leads to a decrease in gluconeogenesis and amelioration of glucose tolerance.

The JNK pathway is known to be activated by several factors such as oxidative stress, free fatty acids

mice (ob/ob) [65]. Blood glucose levels in ob/ob-

JNK-KO mice were lower than those in ob/ob wild type mice, and the ob/ob wild type mice displayed a severe and progressive hyperinsulinemia. Thus, JNK deficiency can provide partial resistance against obesity, hyperglycemia and hyperinsulinemia in both genetic and dietary models of diabetes.

Consequently, obese type 2 diabetes is associated with activation of the JNK pathway, and the absence of JNK results in substantial protection from obesity-induced insulin resistance. These results strongly suggest that JNK plays a crucial role in the progression of insulin resistance found in type 2 diabetes. Therefore, a selective interference with JNK activity is a potential therapeutic target for obesity, insulin resistance and type 2 diabetes [85, 86].

The JNK pathway as a potential therapeutic target for diabetes

Protein transduction domains (PTDs) such as the small PTD from the TAT protein of human immunodeficiency virus (HIV-1), the VP22 protein of Herpes simplex virus, and the third α -helix of the homeodomain of Antennapedia, a *Drosophila* transcription factor, are known to allow various proteins and peptides to be efficiently delivered into cells through the plasma membrane, and thus there has been increasing interest in their potential usefulness for the delivery of bioactive proteins and peptides into cells [87-93].

We have recently evaluated the potential usefulness of a JNK inhibitory peptide in the treatment of type 2 diabetes and found that the cell-permeable JNK inhibitory peptide (amino acid sequence: GRKKRRQRRPPRPKRPTTLNLFQVPRSQDT) is very effective. This peptide is derived from the JNK binding domain of JNK-interacting protein-1 (JIP-1), also known as islet-brain-1 (IB-1), and has been reported to function as a dominant inhibitor of the JNK pathway [76]. To convert the minimal JNK-binding domain into a bioactive cell-permeable compound, a 20-amino acid sequence derived from the JNK-binding domain of JIP-1 (RPK RPT TLN LFP QVP RSQ DT) was covalently linked to a 10-amino acid carrier peptide derived from the HIV-TAT sequence (GRK KRR QRR R); then to monitor peptide delivery, this JIP-1-HIV-TAT peptide was further conjugated with fluorescein isothiocyanate (FITC). First, to examine the effectiveness of the JNK inhibitory peptide *in vivo*, C57BL/KsJ-db/db obese diabetic mice were injected intraperitoneally with the JIP-1-HIV-TAT-FITC peptide. The FITC-conjugated peptide showed fluorescence signals in insulin target

organs (liver, fat, muscle) and in insulin secreting tissue (pancreatic islets). Next, we examined whether the JNK pathway is inhibited after treatment with JIP-1-HIV-TAT-FITC. In various tissues (liver, fat, and muscle), JNK activity was actually suppressed by JIP-1-HIV-TAT-FITC in a dose-dependent manner.

To investigate whether suppression of the JNK pathway exerts beneficial effects on diabetes, we treated C57BL/KsJ-db/db mice with an intraperitoneal injection of the JNK inhibitory peptide, JIP-1-HIV-TAT-FITC or a scramble peptide as a control. There was no difference in body weight and food intake between the JIP-1-HIV-TAT-FITC-treated and untreated mice. The non-fasting blood glucose levels in mice treated with JIP-1-HIV-TAT-FITC were significantly decreased compared to untreated mice or those treated with the scramble peptide. Also, the glucose tolerance test showed that glucose tolerance in JIP-1-HIV-TAT-FITC-treated mice was significantly ameliorated in comparison to untreated or scramble peptide-treated mice [94]. These data indicate that the JNK pathway is involved in the exacerbation of diabetes and that suppression of the JNK pathway could be a therapeutic target for diabetes (Figure 4).

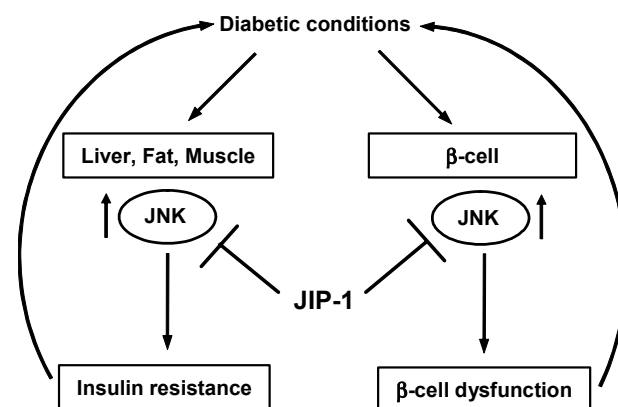


Figure 4. The JNK pathway as a therapeutic target for diabetes. Oxidative stress mediated by chronic diabetic conditions activates JNK in several cells, such as liver-, fat-, muscle-, as well as β -cells, leads to insulin resistance and β -cell dysfunction. The JNK pathway can be suppressed by JNK inhibitory peptide, JIP-1-HIV-TAT-FITC. Treatment with JIP-1 decreases non-fasting blood glucose levels and improves glucose tolerance in mice significantly.

To investigate the possible effects of the JNK inhibitory peptide on insulin action, we performed an insulin tolerance test. Reduction of blood glucose levels in response to injected insulin was much larger in

JIP-HIV-TAT-FITC-treated mice than in untreated mice, indicating that the peptide treatment improves insulin sensitivity. In contrast, no such effect was observed when non-diabetic C57BL/6 mice were treated with JIP-1-HIV-TAT-FITC. These results imply that the JNK pathway is activated under diabetic conditions and thus the JNK inhibitory peptide exerts beneficial effects on insulin action and glucose tolerance.

To further investigate the effect of the peptide on insulin resistance, we performed the euglycemic hyperinsulinemic clamp test. The steady-state glucose infusion rate (GIR) in JIP-1-HIV-TAT-FITC-treated mice was significantly higher than in untreated mice, indicating that JIP-1-HIV-TAT-FITC reduces insulin resistance in C57BL/KsJ-db/db mice. Furthermore, we evaluated endogenous hepatic glucose production (HGP) and glucose disappearance rate (Rd) in the JNK inhibitory peptide-treated mice. It is noted that Rd reflects glucose utilization in the peripheral tissues. HGP in JIP-1-HIV-TAT-FITC-treated mice was significantly lower than in untreated mice. In addition, Rd in JIP-1-HIV-TAT-FITC-treated mice was significantly higher than in untreated mice. These results indicate that JIP-1-HIV-TAT-FITC treatment reduces insulin resistance by decreasing HGP and increasing Rd. The data provide strong evidence that JNK is indeed a crucial component of the biochemical pathway responsible for insulin resistance *in vivo*. In addition, to examine the effect of JIP-1-HIV-TAT-FITC treatment on insulin biosynthesis, we measured the insulin mRNA level and content in pancreata of C57BL/KsJ-db/db mice which had been treated with the peptide. The insulin mRNA level and insulin content were significantly higher in the peptide-treated mice. Thus, we assume that the JNK inhibitory peptide exerted some beneficial effects on the pancreatic islets.

To explore the molecular mechanism of how JIP-1-HIV-TAT-FITC treatment improves insulin sensitivity and ameliorates glucose tolerance, we evaluated IRS-1 serine 307 phosphorylation in various insulin target tissues (liver, fat, and muscle) of JIP-1-HIV-TAT-FITC-treated mice. IRS-1 serine 307 phosphorylation was decreased in JIP-1-HIV-TAT-FITC-treated mice compared to control mice. We also found an increase of IRS-1 tyrosine phosphorylation in the peptide-treated mice compared to control mice. Concomitantly, an increase of Akt serine 473 and threonine 308 phosphorylation, both of which are known to be important for activation of the Akt pathway, was observed in JIP-1-HIV-TAT-FITC-treated mice.

In conclusion, the cell-permeable JNK inhibitory peptide, JIP-1-HIV-TAT-FITC, improves insulin resistance and ameliorates glucose intolerance, indicating the critical involvement of the JNK pathway in diabetes and the usefulness of the cell-permeable JNK inhibitory peptide as a novel therapeutic agent for diabetes (Figure 4).

Conclusions

Activation of the JNK pathway is involved in the progression of insulin resistance as well as deterioration of pancreatic β -cell function. Indeed, suppression of the JNK pathway in obese diabetic mice markedly improves insulin resistance and β -cell function, leading to amelioration of glucose tolerance. Taken together, the JNK pathway plays a crucial role in the progression of insulin resistance as well as β -cell dysfunction and thus could be a potential therapeutic target for the "glucose toxicity" found in diabetes.

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