TXNIP inhibition in the treatment of diabetes. Verapamil as a novel therapeutic modality in diabetic patients

Agnieszka Magdalena Borowiec, Adam Właszczuk, Edyta Olakowska, Joanna Lewin-Kowalik

Department of Physiology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland

Abstract

Loss of pancreatic β–cell is a critical factor in the pathogenesis of type 1 diabetes and it also occurs in type 2. TXNIP (*thioredoxin – interacting protein*), also known as vitamin D3-upregulated protein 1, or thioredoxin-binding-protein-2, regulates this process and modulates cellular redox balance. TXNIP is localized primarily in the nucleus, but under oxidative stress it moves to mitochondria, where it interacts with mitochondrial thioredoxin 2. Overexpression of TXNIP induced by hyperglycaemia is typical for diabetes and insulin resistance and leads to apoptosis of pancreatic β–cell, cardiomyopathy, metabolic disorders and multiple harmful effects. It activates NLRP3 inflamasomme and IL–1β, a cytokine involved in type 2 diabetes and insulin resistance. TXNIP influences peroxisome proliferator-activated receptor alpha transcriptional activity, expression of glucose transporter–1, nitric oxide production in endothelium and insulin production in β–cells. TXNIP overexpression leads to diabetic retinopathy, nephropathy, atherosclerosis, it occurs in cancers and autoimmune diseases, while its deficiency protects β cells. Reduction of TXNIP is an important target in diabetes treatment. In this mechanism insulin, metformin and inhibitors of dipeptydylopeptydase IV are involved. It has been observed that calcium channel blockers (CCB) used in hypertension also inhibit TXNIP expression in cardiomyocytes. L–type channels identification in pancreatic β-cells revealed that CCB inhibit TXNIP expression also in β–cells. For the first time, verapamil was distinguished as an agent that not only inhibits TXNIP expression in pancreatic β-cells, but also enhances β cell survival and function, and possibly prevents diabetes.

Keywords: diabetes, TXNIP, verapamil, calcium channel blockers

Introduction

β–cell apoptosis is a key factor in the pathogenesis of type 1 diabetes. Currently, the main strategy of treatment is to preserve glycaemia as close to the reference standard as possible. However, none of the therapeutic models target directly the maintenance of endogenous β-cell mass. Therefore, finding an agent that blocks β-cell apoptosis is a new therapeutic goal.

β–cell mass formation is regulated by proliferation, differentiation and apoptosis, and it changes depending on environmental and physiological conditions. Critical factor in this process is thioredoxin–interacting protein, TXNIP - a part of α-arrestin family. TXNIP binds and negatively modulates thioredoxin, a redox–protein, influencing the cellular redox balance [1]. Overexpression of TXNIP induced by increased glucose level is typical for diabetes, insulin resistance and triggers cytotoxic effect. TXNIP deficiency protects β cells against oxidative stress, glucose toxicity

and apoptosis [2]. Drugs that inhibit TXNIP expression became an important therapeutic target in diabetes. Lately, verapamil - a calcium channel blocker, used in treatment of hypertension has been examined as a potential therapeutic agent in diabetes.

Mechanism of TXNIP activity

TXNIP acts mainly by inhibition of thioredoxin (Trx) activity. There is thioredoxin-1 in the cytosol and thioredoxin-2 in the nucleus, which have been identified in pancreatic β-cells. Overexpression of TXNIP decreases thioredoxin reductive activity, binds 2 cysteine residues at the Trx active center and cells become more susceptible to oxidative stress and apoptosis [1]. Oxidative stress, due to the overproduction of reactive oxygen species which leads to the induction of protein modification and lipid peroxidation and subsequent cellular dysfunction, has been implicated in numerous human diseases. Thioredoxin system plays an important role in counteracting cellular oxidative stress [3]. It is crucial in maintaining the balance of intracellular reduction/oxidation. Thioredoxin-interacting protein induces oxidative stress by inhibiting thioredoxin activity by direct binding to Trx1, Trx2 and thus inhibits the reducing activity of thioredoxin through their disulfide exchange [4].

It has been revealed that under physiological conditions, TXNIP is localized primarily in the nucleus of pancreatic β-cells and acts as a transcriptional co-repressor by interacting with histone-decetylase-1. Its nuclear localization is mediated mainly by importin-α1, a part of α-arrestin family. In response to oxidative stress, TXNIP moves from nucleus to mitochondria, where interacts with mitochondrial thioredoxin-2, oxidizes it and inhibits ASK-1 kinase activity [5]. TXNIP competes with ASK-1 to bind to thioredoxin-2, which results in the release of ASK-1, its activation, phosphorylation and initiation of the apoptotic signalling cascade in β–cells [6]. Decreased level of thioredoxin-2 leads to increased cytochrome c release, a mitochondrial death marker [7]. Knockdown of thiodredoxin-2, but not thioredoxin-1 results in activation of caspase-3 [5].

TXNIP is also involved in NOD-like receptor protein-3, NLRP3 inflammasome activity in a redox dependent system [8]. NLRP3 inflammasome activation is initiated by TXNIP and it has been implicated in obesity-induced insulin resistance and β cell dysfunction [9]. Increased TXNIP-NLRP3 activation, nitrative stress and impaired vascular endothelial growth factor-receptor 2, VEGF-R2 activation are involved in high-fat diet-induced obesity that compromise vascular recovery in response to ischemic insult. Deletion of TXNIP restores blood flow, reduces nitrative stress and inhibits inflammasome-mediated inflammation, but it does not impact VEGF/VEGF-R2 [10]. NOD-like receptors are localized in the cytosol, where they act as molecules detecting signals from cells undergoing stress, damage or death [11]. Activated NLRP3 forms inflammasome, responsible for activation of caspase-1 and -5, which leads to proteolytic activation of the proinflammatory cytokines, i.e. IL-1β, a cytokine involved in type 2 diabetes, insulin resistance and acute liver failure. Increased level of IL-1β directly inhibits insulin signalling cascade by serine phosphorylation of insulin receptor substrate (IRS)-1 and indirectly induces TNF-α, which is an inducer of insulin resistance [12]. Targeting TXNIP-NLRP3 inflammasome is a potential therapeutic target also in obesity-induced vascular complication and acute liver failure, as well [13].

TXNIP function

TXNIP is expressed in peripheral tissues: skeletal muscles, adipocytes, liver, central nervous system, cardiomyocytes, endothelium**.** Thioredoxin system plays a significant role in carbohydrate metabolism, insulin production and sensitivity, blood pressure regulation, atherogenesis, inflammation, chemotactic activity of macrophages and elevated triglyceride–HDL ratio, in addition to central obesity and often accompanied by non-alcoholic steatohepatitis [14]. TXNIP is associated with glucose and lipid metabolism through pleiotropic actions including influence on the β-cells function, hepatic glucose production, adipogenesis, decrease of energy expenditure, reduction of insulin sensitivity in peripheral tissues (Table I) [15,16]. TXNIP is also a critical factor in diabetic cardiomyopathy [2]. In malignant tumors, such as acute lymphocytic leukemia, lung carcinoma, breast cancer, colorectal cancer, hepatocellular and gastric cancer, non-Hodgkin lymphoma, pancreas cancer, myeloma, an increased level of thioredoxin have been reported in association with aggressive tumor behavior. Thus, Trx system is also targeted for the prevention and treatment of cancer, autoimmune and chronic inflammatory diseases [14]. Expression of TXNIP is induced by UV radiation, γ-radiation, high temperature, hypoxia, H_2O_2 and hyperglycemia. TXNIP expression in pancreatic β-cells generated by high glucose levels is critical for glucotoxicity–induced apoptosis of β cells [17]. TXNIP does not harm cells, when it is within reference levels, but when its level increases, there are many undesired metabolic effects. TXNIP also serves as a key regulatory point for cells in deciding how to respond to acute energy stress. It has been reported that the modulation of the thioredoxin system may be considered as a new target in the management of the metabolic syndrome, insulin resistance, and type 2 diabetes, as well as in the treatment of hypertension and atherosclerosis.

TXNIP regulates peripheral glucose uptake, influencing expression and localization of GLUT-1 (glucose transporter-1). TXNIP suppresses glucose uptake directly by binding to the GLUT-1 inducing its internalization (Figure 1) and indirectly, by reducing the level of GLUT-1 mRNA.

TXNIP is phosphorylated by AMP-activated protein kinase, AMPK under energy stress. Phosphorylation of TXNIP leads to degradation of TXNIP, its dissociation from the glucose transporter GLUT1. Thus, it prevents GLUT1 from being endocytosed, substantially increased expression of GLUT-1 and consequently permitting an acute increase inglucose influx (Figure 2) [18,19]. Phosphorylation on the same TXNIP site by protein kinase B (AKT) results in an equivalent increase in glucose influx. Activation of AKT increases glucose uptake for energy storage in muscle and adipose tissues, as well as for energy consumption in fast dividing cells, such as in cancer. TXNIP is a direct substrate of protein kinase B and is responsible for mediating AKT-dependent acute glucose influx after stimulation by growth factor. TXNIP is also an adaptor for the basal endocytosis of GLUT-4 and its absence allows rapid glucose uptake in muscle and adipose tissues, which causes hypoglycemia during fasting. TXNIP is as a key node of signal regulation and response for modulating glucose influx through GLUT1 and GLUT4 [20].

It was observed that reduction of TXNIP expression by siRNA gene silencing enhanced both basal and insulin-stimulated glucose uptake [15]. TXNIP mediates glucose dependent up-regulation of islet amyloid peptide, that promotes inflammation and β-cell toxicity by aggregating into insoluble amyloid fibrils found in islets of most patients with type 2 diabetes [21]. The occurrence and development of diabetes and its chronic complications are closely related to the oxidative stress reaction and the increase of oxygen free radical production in diabetic patients. TXNIP facilitates the oxidative stress response in glomerular mesangial cells through AMPK pathway and leads to diabetic nephropathy. Thus TXNIP is a therapeutic target for diabetic nephropathy treatment [4].

It has been argued that TXNIP inhibition enhances β-cell survival and function [22]. It is still unclear whether it is caused by their increased proliferation or only by inhibition of β-cell lost. TXNIP deficiency prevents insulin resistance and diabetes type 2 [23]. In Shalev's studies, reduction of TXNIP levels was beneficial, animals improved insulin sensitivity, did not develop diabetes or metabolic disorders. TXNIP–deficient mice had increased β cell mass [2]. Overexpression of TXNIP has been evidenced in patients with diabetes and glucose intolerance [15].

Table I. TXNIP influence on metabolism.

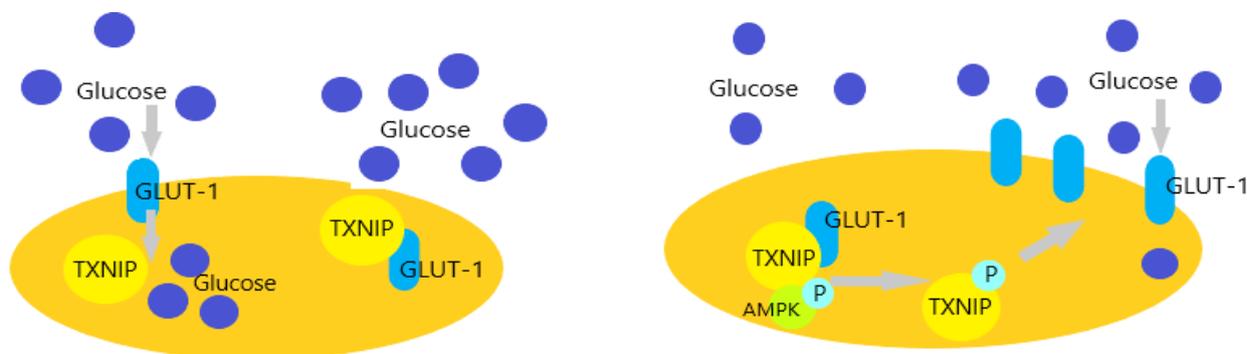| INHIBITS	ACTIVATES
GLUT–1 expression	
GLUT–4 expression	
Insulin production	
MafA	**NLRP3**
Thioredoxin activity	**IL–1β**
ASK-1 activity	**Cytochrome c release**
PPAR–α transcriptional activity	**Apoptosis**
HIF-1a	**Caspase 1, 5**
NO production	**AMPK activation**
VEGF signalling	**miR–204**
p38 MAPK	**LDL, VLDL increased level**
PI3KIAKT pathway	
Glycolisis	
Gluconeogenesis	

Figure 1 (left). Direct supression of glucose uptake by TXNIP binding to GLUT-1. It leads to its internalization.
Figure 2 (right). Phosphorylation of TXNIP by AMP-activated protein kinase, AMPK leads to its degradation and increased expression of GLUT-1.

TXNIP is expressed in the endothelium, where its raised level results in pro–inflammatory factors activation. During prolonged hypoxia TXNIP expression is increased, as a result of inhibition of the mTORC1 signaling pathway [24]. TXNIP overexpression reduces hypoxia-inducible factor 1a activity. There are multiple interactions between thioredoxin–interacting protein and nitric oxide signalling. TXNIP inhibits NO production in endothelium and the production of iNOS is significantly increased in TXNIP gene knockout mice [25]. It has been shown that not only TXNIP suppresses NO generation and its biological effects, but also NO inhibits expression of TXNIP mRNA [26,27]. Those changes are accompanied by decreased vascular endothelial growth factor, concurrent with increased expression of reactive oxygen species and vascular cell adhesion molecule–1 in the aortic endothelium. In addition, TXNIP overexpression in primary human aortic endothelial cells induced by hyperglycaemia or overexpression of carbohydrate response element binding protein (ChREBP) promotes early apoptosis and impaired NO bioactivity. The correlation between TXNIP expression levels and endothelial dysfunction suggests that TXNIP is a biomarker for vascular complications in diabetic patients [28].

Overexpression of thioredoxin-interacting protein contributes to the development of diabetic retinopathy [29]. Genetic inhibition of TXNIP preserved retinal neuronal function in retinopathy. Lina et al. discovered that in diabetic retinopathy treatment with verapamil through inhibition of TXNIP enhanced thioredoxin reductase, significantly inhibited toll-like-receptor-4 mediated NLRP3-inflammasome assembly with subsequent diminishing of inflammatory markers (TNF-α and IL-1β) release into the vitreous, suppression of pathological angiogenesis and preservation pancreatic islets diameter [30].

Perrone et al. investigated whether hyperglycemia and activation of the receptor for advanced glycation end products (RAGE) cause inflammation in rat retinal endothelial cells through TXNIP activation. They discovered that RAGE activation by its ligand S100B and hyperglycemia in retinal endothelial cells induces the expression of TXNIP and inflammatory genes: Cox2, VEGF-A, ICAM1 and leads to endothelial dysfunction in diabetic retinopathy [31]. Duan et al. investigated whether the absence of TXNIP alters diabetes-associated retinal angiogenesis. They observed that knockdown of TXNIP in mouse model suppresses moderately high glucose-induced reactive oxygen species generation, migration, tube formation and activation of Akt/mTOR pathway. Gene silencing of TXNIP blocks VEGF-induced angiogenic response by inhibiting VEGF-R2 and downstream signal pathway Akt/mTOR activation [29]. In conclusion, TXNIP deficiency inhibits vascular endothelial growth factor and moderately high glucose-induced angiogenic response in mice retinas and suggests TXNIP is a potential therapy target for treatment of proliferative diabetic retinopathy.

Pharmacological regulation of TXNIP
Calcium channels blockers

First class calcium channels blockers (CCB) inhibit L-type calcium channels which are present in the cardiomyocytes and also in pancreatic β-cells. It has been proved that CCBs reduce TXNIP expression in β-cells and enhance their survival [32]. Verapamil is first class L–type calcium channel blocker and reduces cellular calcium influx, which results in vasodilatation, lowering blood pressure and in reduction of cardiac muscle contractility and atrioventricular conduction slowing [33]. β–cell insulin release is also dependent on calcium influx. Inhibition of calcium entry is expected to inhibit β-cell insulin release. However, significantly increased insulin–serum levels were observed in verapamil–treated animals. In an experiment with severe–insulin–resistant mice, verapamil improved insulin sensitivity and contributed to overall improved glucose homeostasis [32].

Ovalle et al. assessed the efficacy and safety of oral verapamil added for 12 months to a standard insulin regimen in adult patients with recent-onset of type 1 diabetes. All patients were screened with mixed-meal tolerance tests, MMTT for the presence of a minimal stimulated C-peptide value of ≥ 0.2 nmol/L and at least one positive auto–antibody associated with type 1 diabetes. To assess endogenous beta cell function, the primary endpoint, they measured the MMTT stimulated C-peptide area under the curve at baseline, after 3 months and 12 months. The results showed that verapamil group had lower mean fasting plasma glucose - 6.4 mmol/L, while the control group achieved 6.9 mmol/L. HbA1C level was non-significantly diminished: the verapamil group 6.6 %, while in the placebo group 6.8 % HbA1C. Hypoglycemic events defined as blood glucose episodes < 2.2 mmol/L was 0.5 events/month in the verapamil group, and 2.7 events/month with placebo. Moreover, exogenous insulin requirements after 12 months increased 27.0 % in the verapamil group, but in control group 69.8%. In 3 months insulin requirements were raised by 26.0 % in placebo group and only by 5.9% in the verapamil group. The endogenous β cell function determined by meal–stimulated C-peptide AUC in verapamil group was significantly larger: 0.74 nmol/L than in control group 0.46 nmol/L at both 3 and 12 months [34]. The authors revealed that an addition of verapamil may be a safe and effective novel approach to promote endogenous beta cell function and reduce insulin requirements and hypoglycemic episodes in adult patients with recent-onset of type 1 diabetes.

The molecular mechanism of verapamil influence on pancreatic β–cells was explained in Shalev's study. It has been proved that verapamil and other calcium channel blockers decrease TXNIP expression and prevent β-cells apoptosis in mouse model and in humans, as well. Verapamil and other CCBs preserve functional β-cell mass through inhibiting TXNIP expression, prevent

from streptozocin-induced diabetes and enhance glucose homeostasis in obesity-induced diabetes. This protection was accompanied by 80% reduction of TXNIP levels in isolated islets of verapamil–treated animals [32]. Thus, verapamil is as an agent delaying β-cell apoptosis.

It has been shown that verapamil acts through reduction of intracellular calcium level, inhibition of calcineurin signaling, reduction of carbohydrate response element binding protein, ChREBP nuclear entry and its binding to the E-box repeat in the TXNIP promoter in a time–dependent manner, which results in the inhibition of TXNIP transcription (Figure 3). In response to glucose, ChREBP dephosphorylates, translocates from the cytosol into the nucleus and binds to E-box repeat [35]. Verapamil leaves ChREBP mRNA expression and total ChREBP protein levels unchanged, while nuclear ChREBP is significantly reduced. Calcium regulates gene expression by two pathways, the calcium-dependent Ser-Thr protein phosphatase 2B, PP2B/calcineurin and calcium/calmodulin-dependent protein kinase, CaMK. It has been found that the calcineurin inhibitor-CyA reduced TXNIP mRNA levels and TXNIP promoter activity. It also led to decreased carbohydrate response element binding protein nuclear levels, thereby mimicking verapamil effects [36].

To study the influence of other calcium channels blockers on β-cell TXNIP expression, Shalev et al. incubated INS-1 rat β-cells with diltiazem or verapamil and measured changes in endogenous TXNIP mRNA. Both agents decreased TXNIP mRNA, but verapamil resulted in 50% reduction. In contrast, propranolol (β-blocker) had no effect on TXNIP expression. The studies were performed at 11 mmol/l glucose, however verapamil had no effect on TXNIP expression under normal glucose level (5 mmol/l) [32]. In a cohort study REGARDS (Reasons for Geographical and Racial Differences in Stroke), Khodneva et al. examined the relationship between the use of various calcium channel blockers and fasting serum glucose levels in diabetic patients [37]. Patients treated with verapamil and other calcium channels blockers had lower fasting serum glucose by 9.7 mg/dL and 7.3 mg/dL respectively, compared to non-calcium channels blockers users. Additionally, verapamil users had 9.6 mg/dL lower blood glucose compared to diabetic without calcium channels blockers therapy. There was no statistically significant difference in serum glucose level between verapamil-users and non-users. Dissimilarity in serum glucose in verapamil-users and non-users was greater for those on insulin therapy only. The differences were greater between insulin–users and patients on insulin in combination with oral therapy. The largest difference [37 mg/dL] was in patients treated insulin and verapamil alone. Similar but less pronounced differences in fasting serum glucose levels were found for the all examined calcium channels blockers.

Influence of calcium channels blockers on the development of diabetes was also measured in INternational VErapamil SR-Trandolapril STudy [INVEST] trial. Predictors of development of type 2 diabetes among patients treated for high blood pressure were examined. Participants treated with verapamil and trandolapril were less likely to develop diabetes than those treated with atenolol [38].

Verapamil reduces TXNIP expression in cardiomyocytes [39]. Administration of verapamil in mice reduced cardiac TXNIP expression and cleaved caspase-3, even in severe diabetes and diminished TXNIP levels were associated with decreased apoptosis. The effect was dose–depended [40]. Cardiomyocyte-specific TXNIP gene knock-out reduced caspase-3 level in the heart, diminished apoptosis and reduced cardiac hypertrophy in response to hypertension [39]. TXNIP reduction is a powerful aim to enhance survival of cardiomyocytes and agents such as calcium channels blockers become an important therapeutic target. Xu et al. investigated the role of TXNIP in prediabetic neuropathy and therapeutic potential of verapamil. Verapamil, a known inhibitor of calcium channels, also improved prediabetic neuropathy in the high-fat-diet-fed mice by inhibiting the upregulation of TXNIP. This finding suggests that TXNIP might be a potential target for the treatment of neuropathy in prediabetic patients with dyslipidemia as well [41].

Carbohydrate response element binding protein (ChREBP) whose nuclear entry is significantly reduced in response to verapamil is mainly expressed in β-cells, liver and adipose tissue. Minghying et al. investigated that the TXNIP antagonist verapamil inhibits activation of the NLRP3 inflammasome, inflammatory responses and oxidative stress in acute liver failure, ALF. After confirming the optimal concentration of verapamil for ALF it was evident that, compared with the control and verapamil groups, serum levels of ALT and AST were significantly reduced by verapamil treatment [13]. Verapamil plays a protective role during the treatment of non-alcoholic fatty liver disease, NAFLD. Its administration significantly improves glucose control, body weight, and serum triglyceride levels and reduces pro-inflammatory marker levels [42].

Insulin

TXNIP and diabetes induces in β-cell expression of a specific microRNA, miR-204, which blocks insulin production by directly targeting and down-regulating *MafA*, insulin transcription factor [23]. It has been found that insulin represses TXNIP gene expression (Figure 4). It has been observed that in TXNIP gene knockout mice the serum insulin and C–peptide levels were elevated and prolonged secretion of insulin has been shown [22]. It was unclear if suppression of TXNIP by insulin was secondary to lower glucose level or due to a direct effect of insulin influence. It has been shown in mouse model that the influence of insulin on TXNIP expression is strongly related with insulin receptor signaling. In mouse skeletal muscle with insulin receptor gene knockout, treatment with insulin failed to suppress TXNIP expression [15].

Metformin

Metformin, dimethylbiguanide, is commonly used in the treatment of diabetes mellitus type 2. It decreases glucose-induced TXNIP mRNA and protein levels in beta cells through its ability to activate AMPK [35]. Metformin suppresses the recruitment of ChReBP and FOXO1 on TXNIP promoter in endothelial cells, as well as verapamil,
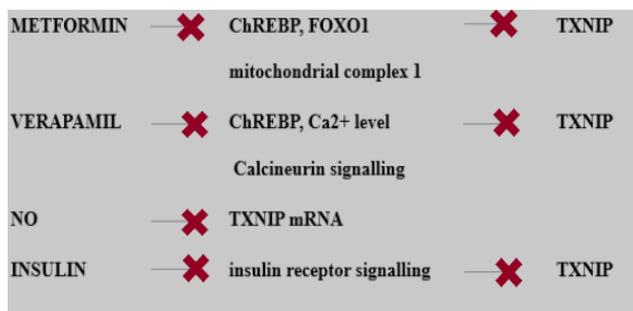
partially by reducing their nuclear entry and is mediated by the AMP activated kinase, AMPK (Figure 4) [43,44]. Metformin suppresses not only the expression of TXNIP, but also the interaction between TXNIP and NLRP3, which leads to activation of proinflammatory cytokines, involved in type 2 diabetes and insulin resistance [45].

Mechanisms of inhibition TXNIP

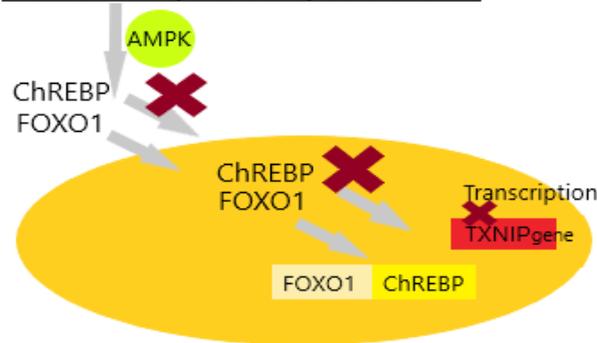**Figure 3.** Possible mechansms of inhibition of TXNIP.
[——✖ - inhibition]

Figure 4. Inhibition of TXNIP transcription through supression the nuclear entry of ChREBP and FOXO1 by verapamil, metformin and insulin.

Conclusions

TXNIP has a pleiotropic influence on metabolism (Table I). Overexpression of TXNIP is typical for patients suffering from diabetes type 1 and 2 and its chronic complications, as well as in cancers, autoimmune diseases, elevated triglyceride–HDL ratio and atherosclerosis. It has been proved that TXNIP inhibition is a critical mechanism preventing β-cell apoptosis, cardiomyopathy, diabetic retinopathy, nephropathy, neuropathy, autoimmune disorders, cancers and acute liver failure. It reduces nitrative stress, inhibits inflammasome-mediated inflammation and improves insulin sensitivity. Commonly used anti–diabetic drugs, i.e. insulin, metformin, DPP–IV inhibitors act in this mechanism. It has been observed that first class calcium channels blockers inhibit L-type calcium channels which are present not only in the cardiomyocytes, but also in pancreatic β-cells. Decreased expression of TXNIP in cardiomyocytes in diabetic patients treated with calcium channels blockers because of hypertension finally focused the attention on verapamil. It acts through TXNIP inhibition to promote β-cell survival and improve glucose-homeostasis. In patients treated with verapamil and insulin, better glucose profile was observed as well as higher C-peptide levels. Moreover, it has been proved that inhibition of TXNIP by verapamil improves retinal neuronal function and mitigates diabetic retinopathy, as well as pancreatic injury. Numerous studies proved that verapamil enhances β-cell function by inhibition of TXNIP and has

a wide positive effect on whole metabolism. It helps to prevent serious diabetic complications – cardiomyopathy, neuropathy, retinopathy, nephropathy, acute liver failure, non-alkoholic fatty liver disease, while other calcium channels blockers do not present such an influence. Verapamil added to standard insulin therapy is effective in reducing of insulin requirements, hypoglycemic episodes and lowering fasting serum glucose. The multiple effects of verapamil, because of its inhibition of TXNIP, brings new information regarding its administration. Verapamil used as an additional drug or as a preferred drug in concomitant hypertension affects positively the diabetes treatment. Through its mechanism of action verapamil became an interesting object of further studies in multiple diseases.

Abbreviations
AKT – protein kinase B
ALF – acute liver failure
ALT – alanine aminotransferase
AMP – adenosine monophosphate
AMPK – AMP-activated protein kinase
ASK-1 – apoptosis signal regulating kinase 1
AST – aspartate aminotransferase
AUC – area under curve
CaMK – calcium/calmodulin-dependent protein kinase
CCB – calcium channel blockers
ChREBP – carbohydrate response element binding protein

COX2 – cyklooxygenase 2
CyA – cyclosporin A, calcineurin inhibitor
[DPP-IVi] – inhibitors of dipeptidylopeptydase
FOXO1 – forkhead box O1
GLUT-1 – glucose transporter 1
H_2O_2 – Hydrogen peroxide
HbA1C – hemoglobin A1c
HDL – high-density lipoprotein
HIF-1a – Hypoxia-inducible factor 1-alpha
ICAM 1 – intercellular adhesion molecule 1
IL-1β – Interleukin 1 beta
iNOS – Inducible nitric oxide synthase
INS-1 rat β-cells – rat insulinoma cell line INS-1
IRS – insulin receptor substrate
LDL – low-density lipoprotein
MafA – Transcription factor
miR–204 – MicroRNA 204
MMTT – mixed-meal tolerance tests
mRNA – Messenger RNA
mTOR – mammalian target of rapamycin complex
NAFLD – non-alkoholic fatty liver disease
NF-kB – nuclear factor kB
NLR – nucleotide oligomerization domain-like receptor
NLRP 3 – nucleotide oligomerization domain-like receptor pyrin 3
NLRP3 – NOD-like receptor protein-3
NO – Nitric oxide
NOD-like receptor protein – nucleotide oligomerization domain-like receptors
p38 MAPK – p38 mitogen-activated protein kinase
PI3KIAKT – phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)
PP2B/calcineurin – protein phosphatase 2B
PPAR–α – Peroxisome proliferator-activated receptor alpha
RAGE – receptor for advanced glycation endproducts
Ser-Thr protein phosphatase 2B – Serine/threonine-protein phosphatase 2B
siRNA – small interfering RNA
TBP-2 – thioredoxin-binding-protein-2
TLR-4 – toll-like receptor 4
TNF-α – tumor necrosis factor alpha
Trx – thioredoxin
TrxR – thioredoxin reductase
TXNIP – thioredoxin–interacting protein
UV – Ultraviolet
VEGF – vascular endothelial growth factor
VEGF-A – vascular endothelial growth factor A
VEGF-R2 – vascular endothelial growth factor-receptor 2
VLDL – very low density lipoprotein

References

1. Junn E, Han SH, Im JY, Yang Y, Cho EW, Um HD, et al. Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. J Immunol. 2000;164:6287-6295.

2. Chen J, Hui ST, Couto FM, Mungrue IN, Davis DB, Attie AD, et al. Thioredoxin-interacting protein deficiency induces Akt/Bcl-xL signaling and pancreatic beta-cell mass and protects against diabetes. FASEB J. 2008;22:3581-3594.

3. Yodoi J, Matsuo Y, Tian H, Masutani H, Inamoto T. Anti-Inflammatory Thioredoxin Family Proteins for Meidcare, Healthcare and Aging Care. Nutrients. 2017;9:1081.

4. Xu LW, Jimin L, Yingying C, Yaoming X. TXNIP mediated the oxidative stress response in glomerular mesangial cells partially through the AMPK pathway. Biomed Pharmacother. 2018;107:785–792.

5. Saxena G, Chen J, Shalev A. Intracellular shuttling and mitochondrial function of thioredoxin-interacting protein. J Biol Chem. 2010;285:3997-4005.

6. Lu J, Holmgren A. Thioredoxin system in cell death progression. Antioxid Redox Signal. 2012;17:1738-1747.

7. Tanaka T, Hosoi F, Yamaguchi-Iwai Y, Nakamura H, Masutani H, Ueda S, et al. Thioredoxin-2 (TRX-2) is an essential gene regulating mitochondria-dependent apoptosis. EMBO J. 2002;21:1695–1703.

8. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol. 2010;11:136-140.

9. Mori MA, Bezy O, Kahn CR. Metabolic syndrome: is Nlrp3 inflammasome a trigger or a target of insulin resistance? Circ Res. 2011;108:1160-1162.

10. Elshaer SL, Mohamed IN, Coucha M, Altantawi S, Eldahshan W, Bartasi M, et al. Deletion of TXNIP Mitigates High-Fat Diet-Impaired Angiogenesis and Prevents Inflammation in a Mouse Model of Critical Limb Ischemia. Antioxidants (Basel). 2017;6:47.

11. Schroder K, Zhou R, Tschopp J. The NLRP3 inflammasome: a sensor for metabolic danger? Science. 2010;327:296-300.

12. Abderrazak A, Syrovets T, Couchie D, El Hadri K, Friguet B, Simmet T, et al. NLRP3 inflammasome: from a danger signal sensor to a regulatory node of oxidative stress and inflammatory diseases. Redox Biol. 2015;4:296-307.

13. Han M, Li S, Li L. Verapamil inhibits early acute liver failure through suppressing the NLRP3 inflammasome pathway. J Cell Moll Med. 2021;25:5963-5975.

14. Tinkov AA, Bjørklund G, Skalny AV, Holmgren A, Skalnaya MG, Chirumbolo S, et al. The role of the thioredoxin/thioredoxin reductase system in the metabolic syndrome: towards a possible prognostic marker? Cell Mole Life Sci. 2018;75:1567–1586.

15. Parikh H, Carlsson E, Chutkow WA, Johansson LE, Storgaard H, Poulsen P, et al. TXNIP regulates peripheral glucose metabolism in humans. PLoS Med. 2007;4:el58.

16. Alhawiti NM, Al Mahri S, Aziz MA, Malik SS, Mohammad S. TXNIP in Metabolic Regulation: Physiological Role and Therapeutic Outlook. Curr Drug Targets. 2017;18:1095-1103.

17. Chen J, Saxena G, Mungrue IN, Lusis AJ, Shalev A. Thioredoxin-interacting protein: a critical link between glucose toxicity and beta-cell apoptosis. Diabetes. 2008;57:938-944.

18. Wu N, Zheng B, Shaywitz A, Dagon Y, Tower C, Bellinger G, et al. AMPK-dependent degradation of TXNIP upon energy stress leads to enhanced glucose uptake via GLUT1. Mol Cell. 2013;49:1167-1175.

19. Lee YS, Jun HS. Anti-diabetic actions of glucagon-like peptide-1 on pancreatic beta-cells. Metabolism. 2014;63:9-19.

20. Waldhart AN, Dykstra H, Peck AS, Boguslawski EA, Madaj ZB, Wen J, et al. Phosphorylation of TXNIP by AKT Mediates Acute Influx of Glucose in Response to Insulin. Cell Rep. 2017;19:2005–2013.

21. Jing G, Westwell-Roper C, Chen J, Xu G, Verchere CB, Shalev A. Thioredoxin-interacting protein promotes islet amyloid polypeptide expression through miR-124a and FoxA2. J Biol Chem. 2014;289:11807-11815.

22. Oka S, Yoshihara E, Bizen-Abe A, Liu W, Watanabe M, Yodoi J, et al. Thioredoxin binding protein-2/thioredoxin-interacting protein is a critical regulator of insulin secretion and peroxisome proliferator-activated receptor function. Endocrinology. 2009;150:1225-1234.

23. Xu G, Chen J, Jing G, Shalev A. Thioredoxin-interacting protein regulates insulin transcription through microRNA-204. Nat Med. 2013;19:1141-1146.

24. Wong RW, Hagen T. Mechanistic target of rapamycin (mTOR) dependent regulation of thioredoxin interacting protein (TXNIP) transcription in hypoxia. Biochem Biophys Res Commun. 2013;433:40–46.

25. Park YJ, Yoon SJ, Suh HW, Kim DO, Park JR, Jung H, et al. TXNIP deficiency exacerbates endotoxic shock via the induction of excessive nitric oxide synthesis. PLoS Pathog. 2013;9:e1003646.

26. Chong CR, Chan WP, Nguyen TH, Liu S, Procter NE, Ngo DT, et al. Thioredoxin-interacting protein: pathophysiology and emerging pharmacotherapeutics in cardiovascular disease and diabetes. Cardiovasc Drugs Ther. 2014;28:347-360.

27. Schulze PC, Liu H, Choe E, Yoshioka J, Shalev A, Bloch KD, et al. Nitric oxide-dependent suppression of thioredoxin-interacting protein expression enhances thioredoxin activity. Arterioscler Thromb Vasc Biol. 2006;26:2666–2672.

28. Li X, Kover KL, Heruth DP, Watkins DJ, Guo Y, Moore WV, et al. Thioredoxin-interacting protein promotes high-glucose-induced macrovascular endothelial dysfunction. Biochem Biophys Res Commun. 2017;493:291–297.

29. Duan J, Du C, Shi Y, Liu D, Ma J,Thioredoxin-interacting protein deficiency ameliorates diabetic retinal angiogenesis; Int J Biochem Cell Biol. 2018,94:61-70.

30. Eissa LD, Ghobashy WA, El-Azab MF. Inhibition of thioredoxin-interacting protein and inflammasome assembly using verapamil mitigates diabetic retinopathy and pancreatic injury. Eur J Pharmacol. 2021,15:901:174061.

31. Perrone L, Devi TS, Hosoya K, Terasaki T, Singh LP. Thioredoxin interacting protein (TXNIP) induces inflammation through chromatin modification in retinal capilary endothelial cells under diabetic conditions, J Cell Physiol. 2009;221:262-272.

32. Xu G, Chen J, Jing G, Shalev A. Preventing β-cell loss and diabetes with calcium channel blockers. Diabetes.

2012;61:848–856.

33. Elliott WJ, Ram CV. Calcium channel blockers. J Clin Hypertens (Greenwich). 2011;13:687–689.

34. Ovalle F, Grimes T, Xu G, Patel AJ, Grayson TB, Thielen LA, et al. Verapamil and beta cell function in adults with recent-onset type 1 diabetes. Nat Med. 2018;24:1108-1112.

35. Thielen L, Shalev A. Diabetes pathogenic mechanisms and potential new therapies based upon a novel target called TXNIP. Curr Opin Endocrinol Diabetes Obes. 2018;25:75-80.

36. Davies MN, O'Callaghan BL, Towle HC. Glucose activates ChREBP by increasing its rate of nuclear entry and relieving repression of its transcriptional activity. J Biol Chem. 2008;283:24029–24038.

37. Khodneva Y, Shalev A, Frank SJ, Carson AP, Safford MM. Calcium channel blocker use is associated with lower fasting serum glucose among adults with diabetes from the REGARDS study. Diabetes Res Clin Pract. 2016;115:115–121.

38. Cooper-Dehoff R, Cohen JD, Bakris GL, Messerli FH, Erdine S, Hewkin AC, et al. Predictors of development of diabetes mellitus in patients with coronary artery disease taking antihypertensive medications (findings from the INternational VErapamil SR-Trandolapril STudy [INVEST]). Am J Cardiol. 2006;98:890–894.

39. Chen J, Cha-Molstad H, Szabo A, Shalev A. Diabetes induces and calcium channel blockers prevent cardiac expression of proapoptotic thioredoxin-interacting protein. Am J Physiol Endocrinol Metab. 2009;296:E1133–E1139.

40. Yoshioka J, Imahashi K, Gabel SA, Chutkow WA, Burds AA, Gannon J, et al. Targeted deletion of thioredoxin-interacting protein regulates cardiac dysfunction in response to pressure overload. Circ Res 2007;101:1328–1338.

41. Xu L, Lin X, Guan M, Zeng Y, Liu Y. Verapamil Attenuated Prediabetic Neuropathy in High-Fat Diet-Fed Mice through Inhibiting TXNIP-Mediated Apoptosis and Inflammation. Oxid Med Cell Longev. 2019;10:1896041.

42. Zhou F, Zhang Y, Chen J, Hu Y, Xu Y. Verapamil Ameliorates Hepatic Metaflammation by Inhibiting Thioredoxin-Interacting Protein/ NLRP3 Pathways. Front Endocrinol (Lausanne). 2018;9:640

43. Chai TF, Hong SY, He H, Zheng L, Hagen T, Luo Y, et al. A potential mechanism of metformin-mediated regulation of glucose homeostasis: inhibition of Thioredoxin-interacting protein (Txnip) gene expression. Cell Signal. 2012;24:1700-1705.

44. Li X, Kover KL, Heruth DP, Watkins D, Moore WV, Jackson K, et al. New Insight Into Metformin Action: Regulation of ChREBP and FOXO1 Activities in Endothelial Cells. Mol Endocrinol. 2015;29:1184–1194.

45. Jia Y, Cui R, Wang C, Feng Y, Li Z, Tong Y, et al. Metformin protects against intestinal ischemia-reperfusion injury and cell pyroptosis via TXNIP-NLRP3-GSDMD pathway. Redox Biol. 2020;32:101534.