

# A Systematic Review on Prevention of Beta Cell Apoptosis in Type 2 Diabetes Miletus Using Verapamil: A Calcium Channel Blocker

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**Abstract:** Type 2 Diabetes Miletus is a growing public health issue characterized by peripheral insulin resistance and decompensation of the pancreatic cells that can no longer keep up with the increased insulin requirements, resulting in hyperglycemia. These elevated glucose levels have detrimental effects on various tissues including the pancreatic cell. Cell glucose toxicity leads to progressive cell dysfunction, impaired insulin gene transcription and irreversible cell loss by apoptosis, resulting in a vicious cycle with worsening hyperglycemia. Novel approaches that could promote pancreatic beta cell reserve, protect against apoptotic beta-cell loss, and help prevent diabetes are therefore urgently needed. The calcium-channel blocker verapamil effectively lowers beta cell TXNIP expression in rodent beta cells and islets as well as in human islets. This effect is based on the established mode of action of verapamil, i.e., blockade of L-type calcium channels and the resulting decrease in intracellular free calcium leading to inhibition of TXNIP transcription. Tissues with high expression of L-type calcium channels, such as beta cells and the heart, are therefore most likely to benefit from the resulting TXNIP inhibition.

**Keywords:** Type 2 Diabetes Miletus, Oxidative stress, Glucotoxicity, Thiredoxin Interacting Protein, Verapamil, Beta cell apoptosis

## 1. Introduction

Type 2 diabetes is a growing public health issue characterized by peripheral insulin resistance and decompensation of the pancreatic cells that can no longer keep up with the increased insulin requirements, resulting in hyperglycemia. These elevated glucose levels have detrimental effects on various tissues including the pancreatic cell. Cell glucose toxicity leads to progressive cell dysfunction, impaired insulin gene transcription and irreversible cell loss by apoptosis, resulting in a vicious cycle with worsening hyperglycemia<sup>1,29</sup>. Novel approaches that could promote pancreatic beta cell reserve, protect against apoptotic beta-cell loss, and help prevent diabetes are therefore urgently needed<sup>1,29</sup>.

The loss of pancreatic beta cell is a major drawback both in the development and progression of Diabetes Miletus. Type 1 Diabetes Miletus (T1DM) occurs as a result of destruction of insulin producing beta cells due to autoimmune responses. It develops mostly in younger population and accounts for 5-10% of the diabetic subjects<sup>1,2,27,29</sup>. It may develop over a period of several years after an asymptomatic phase<sup>3,23</sup>.

In type 2 diabetes, reasons for decreased insulin secretion and loss of beta cell mass are multiple. One of the initial defects is a loss of the early phase of meal-stimulated insulin secretion. This is followed by an inability of the  $\beta$ -cell to increase insulin secretion sufficient to overcome hepatic and peripheral insulin resistance. The relationship between beta cell dysfunction and insulin resistance remains highly complex. Hyperglycemia can trigger both beta cell dysfunction and insulin resistance which in other words called glucotoxicity. With persistent hyperglycemia, increased saturated fatty acids induce a glucolipotoxic state that is detrimental to beta cells by increasing oxidative stress, subsequently reducing insulin synthesis and secretion

thereby compromising both beta cell structure and function. Beta cell dysfunction is more complex than insulin resistance. With beta cell dysfunction, insulin secretion is impaired whereas with insulin resistance, insulin may still be secreted but insulin insensitivity manifests in target tissues. As beta cell dysfunction and insulin resistance exacerbate, hyperglycemia amplifies leading to the progression to type 2 diabetes<sup>4,20,27,29</sup>.

There are two major pathways involved in the death of beta cell in case of type 2 Diabetes Miletus. A. Oxidative stress  
B. Glucotoxicity.

### Oxidative stress:

The  $\beta$  cell is particularly prone to develop oxidative stress, due to low activities of catalase, selenium-dependent glutathione peroxidase 1 and Cu/Zn-superoxide dismutase. By contrast, the NADPH-dependent oxidoreductase thioredoxin is abundant, which suggests that it has an important role in  $\beta$ -cell defense against cellular stress<sup>2,5,27,33</sup>.

Aerobic cells produce reactive oxygen species (ROS) such as superoxide anion ( $O_2^{\cdot-}$ ) and  $H_2O_2$  during oxidative phosphorylation in the mitochondria as by-products. Like in other aerobic cell types, mitochondrial electron transport is the main source of superoxide anions of pancreatic  $\beta$  cells. Superoxide anion is a reactive molecule, but it can be converted to  $H_2O_2$  by superoxide dismutase (SOD) isoenzymes and then to oxygen and water by enzymes including catalase (CAT), glutathione peroxidase (GPx), and peroxiredoxin (Prx). Beta cells have lower antioxidative enzymes to combat the continuously generated superoxide anions. This makes  $\beta$  cells highly sensitive to ROS-related signaling and distinctively susceptible to oxidative and cytotoxicity stress<sup>10,30,32</sup>.

In addition to hyperglycemia, exposure to excessive lipid (hyperlipidemia) has also been shown to activate cell stress responses including oxidative stress, which contributes to lipotoxicity in  $\beta$  cells in T2DM. An *in vitro* study using prolonged exposure to free fatty acid (FFA) exhibited increased islet ROS production in mitochondria, which was prevented by over expression of the enzyme, GPx4. Another mechanism that contributes to lipid-induced oxidative stress in  $\beta$  cells is the modulation of respiratory chain by FFA.  $\beta$  cells exposed to FFA exhibited increased ROS production, and respiratory complex I in mitochondria seemed to be the major radical source<sup>10,30,32</sup>.

### Glucotoxicity:

Glucotoxicity is a well-known perpetrator of widespread tissue damage that effects the islet both by compromising vascular and neural supply and by directly blunting  $\beta$  cell response to glucose; it does so *in vitro* where exposure to high glucose for a few days is sufficient to alter the subsequent secretory response to glucose stimulation. Obesity is a one another key risk factor for type 2 diabetes as it desensitizes glucose recipient organs to the action of insulin. Saturated fats are strongly associated with insulin resistance and beta cell dysfunction. This implies that beta cell function supersedes insulin resistance as the critical determinant of type 2 diabetes<sup>1,21,28</sup>.

Previous animal studies conducted in rodent  $\beta$ -cells highly exposed to glucose toxicity displayed several changes in glucose stimulus-secretion coupling, gene expression, cell survival, and cell growth. These alterations could be the results of cytokine production, oxidative stress, or ER stress-induced changes in gene expression and cell survival<sup>1,21,28</sup>.

## 2. A New Target for diabetes

Thioredoxin interacting protein (TXNIP) is a protein that helps in cellular oxidation reduction reactions. It's a major protein that regulates the oxidation and reduction reactions inside the cell. The TXNIP inhibits thioredoxin a thiol-oxidoreductase enzyme which plays a major role in reducing systems protecting the cells against oxidative stress<sup>6,12,17</sup>.

Evidence that TXNIP is strongly up regulated on human islet oligonucleotide gene in response to glucose by a microarray study in 2002 and raised the suggestions that it might play an important role in diabetes<sup>16</sup>. Adding to this evidence, many studies suggested that txnip expression is markedly increased in diabetes in a variety of rodent models and human insulin/glucose clamp physiological studies with genome-wide expression profiling to identify thioredoxin interacting protein.<sup>4</sup> Higher beta cell mass of TXNIP deficient mice was found which in turn promotes beta cell survival and reduction in the loss of beta cell mass. They further reveal that TXNIP mediates its effects through induction of Akt/Bcl-XL signaling, inhibition of mitochondrial cell death, and prevention of beta-cell apoptosis. This shows the critical link of glucose toxicity and beta cell apoptosis concerning the expression of TXNIP on the beta cells<sup>14,18,19</sup>.

Studies conducted in animal mouse models on TXNIP expression in human pancreatic islets, primary mouse islets and INS-1  $\beta$ -cells demonstrated that increase in TXNIP is associated with increased  $\beta$ -cell apoptosis. In addition, TXNIP expression was elevated in islets of different mouse models of diabetes. Moreover, the results of the study demonstrated that TXNIP is required for  $\beta$ -cell death caused by glucotoxicity and therefore suggest that TXNIP may represent a potential target to interfere with the toxic effects of glucose<sup>34</sup>.

A recent study identified TXNIP as a critical factor mediating glucose toxicity induced cell death and have described the pathways by which TXNIP induces cell apoptosis. We found that TXNIP over expression leads to activation of the intrinsic mitochondrial pathway of apoptosis.<sup>11</sup>

TXNIP has been discovered to control beta cell microRNA expression, beta cell function and insulin production. This interaction with thioredoxin as a cellular redox regulator, TXNIP has been thought to be localized in the cytoplasm. However, more recent findings have revealed that TXNIP can also translocate into the mitochondria where it binds to mitochondrial thioredoxin 2, releasing apoptosis signal-regulating kinase 1 (ASK1) from its inhibition by thioredoxin 2 and allowing for phosphorylation and activation of ASK1<sup>31</sup>.

These findings were consistent about TXNIP over expression causing beta cell death and lack of TXNIP preventing beta cell apoptosis and its extrapancreatic benefits. Based on the above evidence regarding the role of TXNIP in diabetes and protection of beta cell from apoptosis has given its way in developing a novel approach in the management of the disease.

The calcium-channel blocker verapamil effectively lowers beta cell TXNIP expression in rodent beta cells and islets as well as in human islets. This effect is based on the established mode of action of verapamil, i.e., blockade of L-type calcium channels and the resulting decrease in intracellular free calcium leading to inhibition of TXNIP transcription. Tissues with high expression of L-type calcium channels, such as beta cells and the heart, are therefore most likely to benefit from the resulting TXNIP inhibition<sup>3,5,15</sup>.

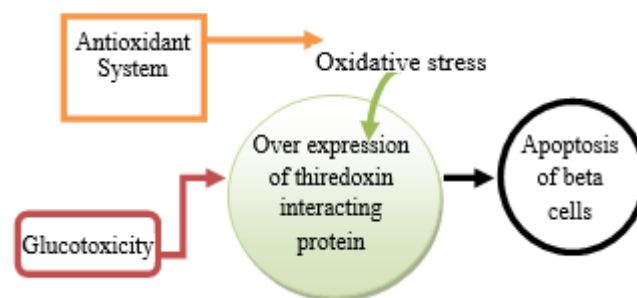


Figure 1: Pathway of pancreatic cell apoptosis through thioredoxin interacting protein

## 3. Verapamil – A Calcium Channel Blocker

In vitro studies discovered that the approved antihypertensive drug and calcium-channel blocker verapamil effectively lowers beta cell TXNIP expression in rodent beta cells and islets as well as in human islets. This effect is based on the established mode of action of verapamil, i.e., blockade of L-type calcium channels and the resulting decrease in intracellular free calcium leading to inhibition of TXNIP transcription<sup>3</sup>. Previous studies attest that calcium antagonists and combined verapamil-trandolapril as a potentially valuable therapy for hypertension accompanying diabetes mellitus. Studies comparing the efficacy of verapamil with different calcium channel blockers and antihypertensives demonstrated that verapamil may have advantages over nifedipine and diltiazem in increasing fasting serum immunoreactive insulin<sup>5,15</sup>.

A study conducted on the influence of short term verapamil in non insulin dependent type 1 diabetes mellitus has shown that verapamil decreases fasting blood glucose, glucose turnover and a tendency to improve glucose tolerance during the treatment<sup>9</sup>. Also verapamil improves the glucose tolerance in patients with NIDDM without affecting the insulin secretion<sup>26</sup>.

Use of verapamil sustained release based strategy effectively achieves BP goal in adults with diabetes, HT, and CAD<sup>7</sup>.

A randomized double-blind placebo-controlled phase 2 clinical trial (NCT02372253) have assessed the efficacy and safety of oral verapamil added for 12 months to a standard insulin regimen in adults with recent onset of type 1 diabetes mellitus. They recommended that once-daily dose of oral verapamil in a standard drug regimen may provide a safe and effective approach to promote and preserve an individual's own beta cell function, delay beta cell loss and disease progression for at least 1 year and reduce insulin requirements and hypoglycemic episodes in adults with recent-onset T1D<sup>8</sup>.

Studies found that downregulating TXNIP also improves beta cell function including insulin production and secretion, effects that may help increase the amount of insulin synthesized and secreted per beta cell, especially in the context of the dramatically decreased beta cell mass occurring in T1D<sup>13,35</sup>.

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