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Therapeutic potential of benfotiamine and its molecular targets

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Abstract. – OBJECTIVE: The water-soluble vitamin, thiamine forms an important part of the diet because of its role in the energy metabolism. The protective effects of thiamine against diabetic vascular complications have been well documented. However, slower absorption and reduced bioavailability is a major limiting factor for its clinical use. To overcome this issue, lipid-soluble derivatives of thiamine (allithiamines) was developed. Among the many synthetic lipophilic derivatives of thiamine, benfotiamine (BFT) is regarded as the first choice based on its safety and clinical efficacy data. BFT facilitates the action of thiamine diphosphate, a cofactor for the enzyme transketolase. The activation of transketolase enzyme accelerates the precursors of advanced glycation end products (AGEs) towards the pentose phosphate pathway thereby reducing the production of AGEs. The reduction in AGEs subsequently decreases metabolic stress which benefits vascular complications seen in diabetes. The effects of BFT on the AGE-dependent pathway is well established. However, several studies have shown that BFT also modulates pathways other than AGE such as arachidonic acid (AA), nuclear transcription Factor κB (NF-κβ), protein kinase B, mitogen-activated protein kinases (MAPK) and vascular endothelial growth factor receptor 2 (VEGFR2) signaling pathways. In the present review, we have comprehensively reviewed all the molecular targets modulated by BFT to provide mechanistic perspective to highlight its pleiotropic effects.

Key Words:
Benfotiamine, Allithiamine, Thiamin and advanced glycation end products.

Introduction

Nutritional deficiency is one of the most common global problems, especially in the developing world. Vitamins play a vital role in maintaining the nutritional status of the body. Thiamine, also known as vitamin B1, is an important dietary nutrient due to its role in the energy metabolism[1]. Reduced dietary intake of thiamine results in defective energy metabolism and raised levels of cellular oxidative stress. Thiamine also plays an important role in neuronal health; its deficiency is associated with severe neurological side effects. Because of its role in the central nervous system (CNS), thiamine is also known as *aneurin*. In general, the conditions associated with thiamine deficiency are known as Beriberi which was first reported in the areas where white or polished rice constituted a major portion of the diet. Thiamine was first isolated as a vitamin from rice husks. Beriberi was considered the first disorder for which the term "deficiency disease" was used. The symptoms of this disease are mainly related to the autonomic nervous system and cardiovascular system, which include sinus tachycardia, vasovagal syncope, mitral valve prolapse, dysautonomia, and hypotension.

Thiamine cannot be synthesized in humans, only bacteria, fungi and plants can synthesize it. Plant eating animals obtain thiamine from the food source; therefore, this essential nutrient is also fortified in many human food products including cereals. Clinically, thiamine deficiency leads to various abnormalities that include neurological and circulatory disorders[2,3]. Many conditions are associated with deficiency of thiamine including chronic alcoholism[4,5], celiac and renal diseases as well as diabetes mellitus (DM)[6-9]. Numerous studies[10-12] have shown that low levels of intracellular thiamine triggers apoptotic machinery to induce cell death pathways. In humans, general thiamine deficiency is

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manifested as malaise, weight loss, irritability, and confusion. Clinically, when thiamine is prescribed for certain medical conditions, high levels of thiamine are required to ensure appropriate bioavailability in the tissues. In clinical settings, oral thiamine supplement has been used for the treatment of diabetic neuropathy for decades. The overt thiamine deficiency is not so common in recent times. However, the prevalence of subclinical thiamine deficiency is on the rise[13,14]. It has been shown that, in an elderly group of subjects with subclinical thiamine deficiency, the quality of their life significantly improved in general after thiamine replete in these subjects[14].

Thiamine is absorbed from both small and large intestines by utilizing thiamine transporters-1 and -2 (THTR-1 and THTR-2). These transporters belong to the family of solute carriers (SLCs) encoded by SLC19A2 and SLC19A3 genes. Both these transporters facilitate absorption of thiamine in micromolar to nanomolar range from the intestinal lumen and follow saturation kinetics principles. These transporters are also expressed in other tissues such as pancreas, kidney, and brain[15] which facilitates free thiamine transport into the tissues[16-18]. The thiamine transporters limit the rate of absorption, to overcome this problem lipid-soluble thiamine derivatives (allithiamines) have been developed with an advanced pharmacokinetic profile. Benfotiamine (BFT) is a lipid-soluble derivative of thiamine which shows enhanced bioavailability after oral administration when compared to an equivalent dose of water-soluble thiamine. The role of BFT in inhibiting the production of advanced glycation end products (AGEs) is well established. This reduces glucose-induced metabolic stress and confers beneficial effects against diabetic vascular complications. However, several studies have also shown that BFT can modulate non-AGE dependent pathways that include nuclear transcription Factor κB (NF-κB), vascular endothelial growth factor receptor 2 (VEGFR-2), glycogen synthase kinase-3β (GSK-3β) as well as pathways which play roles in cell survival, repair and cell death[19-21].

Due to its enhanced bioavailability and improved efficacy, the clinical use of BFT to treat overt thiamine deficiency or subclinical thiamine deficiency is likely to be more effective compared to water-soluble thiamine. Therefore, it is imperative to understand the various physiological pathways and the molecular targets that are modulated by BFT. In the present review, we have comprehensively reviewed all the molecular targets of BFT using up to date literature search to provide a mechanistic perspective to highlight its pleiotropic effects.

Clinical Advantages of Benfotiamine Compared to Thiamine

Chemically, BFT is known as S-[(Z)-2-[(4-amino-2-methylpyrimidin-5-yl) methylformyl-amino] -5-phosphonooxypent-2-en-3-yl] benzenecarbothioate. An open thiazole ring in the chemical structure distinguishes BFT from thiamine (Figure 1). After ingestion, BFT is dephosphorylated by the ecto-alkaline phosphatases present on the intestine brush border membrane to S-benzoylthiamine, which is highly lipophilic and readily diffuses through biological membranes. In the blood, a major portion of S-benzoylthiamine is trapped by the erythrocytes and converted to active thiamine. Pharmacokinetic studies[22-24] have demonstrated that a single dose of oral BFT results in five times higher concentration of thiamine in the plasma compared to an equivalent dose of water-soluble thiamine.

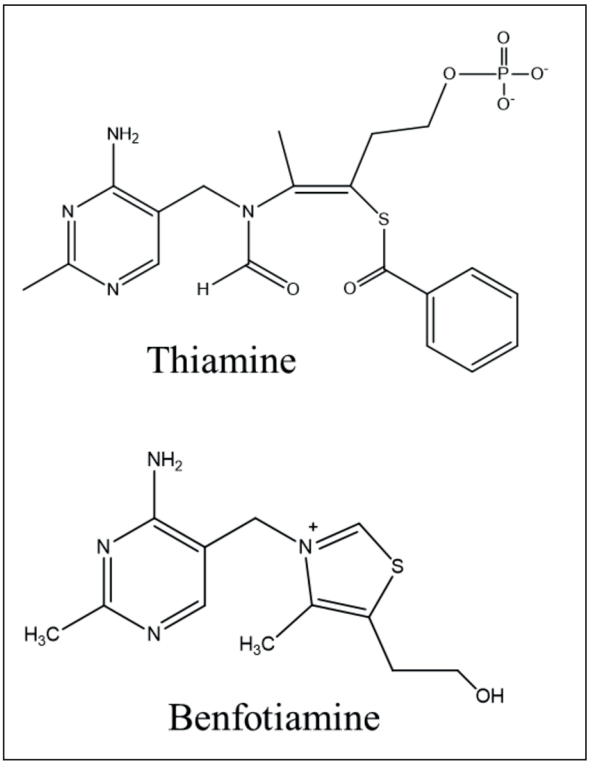

Figure 1. Chemical structure of thiamine and benfotiamine (BFT).

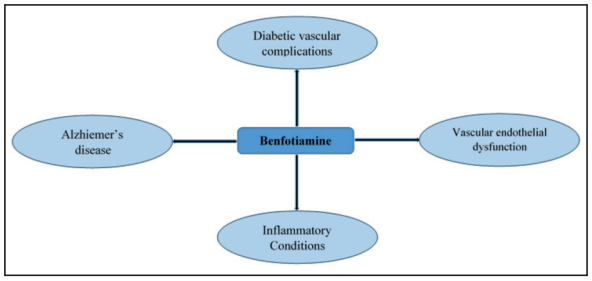

Figure 2. Therapeutic targets of benfotiamine.

Enhanced Transketolase Activity and Modulation of Advanced Glycation End Products Formation Pathways

Hyperglycemia and oxidative stress are associated with the generation of signaling proteins termed Advanced Glycation End products (AGEs). AGEs are synthesized from Amadori products by the conversion of reversible Schiff-base adducts. These Amadori products, via various rearrangement processes, results in the formation of irreversibly bound AGEs. Earlier reports have shown the role of glucose-6-phosphate, glyceraldehydes-3-phosphate, glyoxal (GO), methylglyoxal (MGO) and 3-deoxyglucosone (3DG) in the synthesis of these proteins[34]. AGEs play an important role in the progression and pathophysiology of diabetes mellitus, atherosclerosis, chronic renal failure and also enhance ageing and neurodegeneration. AGEs exert deleterious effects on cells and tissues *via* many mechanisms including (a) intracellular glycation of proteins causing defective cellular functions; (b) changes in various gene expression and activation of signal transduction pathways responsible for cells degeneration; (c) vascular complications via the accumulation of AGEs in the extracellular matrix.

AGEs are synthesized *in vivo* by a non-enzymatic reaction between reducing sugars, amino acids, lipids and nucleic acids following two major pathways[34,35]. In the Maillard pathway, ketone or aldehyde groups of glucose molecules react with the amino groups of proteins. In diabetes or oxidative stress-induced hyperglycemic conditions, reversible Schiff base adducts are formed from glucose, which are converted to covalently bound stable amadori products. Following the diverse rearrangement reactions, which include dehydration and condensation, these amadori products form irreversible AGEs[35]. Furthermore, reduction of glucose into sorbitol is facilitated by the enzyme aldose reductase. Sorbitol dehydrogenase catalyzes the conversion of sorbitol to fructose undergoes further metabolic modifications to generate fructose-3-phosphate and 3-deoxyglucosone that ultimately result in the formation of AGEs (Polyol pathway). Increased glucose levels inside the cell during hyperglycemic states disturb glucose metabolism which results due to the accumulation of super reactive glucose-metabolic intermediaries such as triose-phosphate inside the cell. The excess of triose-phosphate perturb the surrounding proteins, lipids and DNA, and results in the formation of oxoaldehydes that causes AGE damage in the cell[36,37].

Previous works[25,38] have reported that agents which inhibit AGE formation can modulate a variety of physiological and pathological manifestations of diabetes. *In vitro* and *in vivo* experiments have shown that the active form of thiamine-thiamine pyrophosphate (TPP) inhibits the synthesis of AGEs from amadori products (Figure 3). Various preclinical and clinical studies have also elucidated the beneficial effects of

Table I. Molecular targets and signaling pathways involved in the protective effect of benfotiamine.

Molecular target/ signaling pathway	Actions observed	Clinical significance	References
Transketolase	Activation	Diabetic microvascular complications	(25-27)
Arachidonic acid pathway	COX and LOX inhibition	Inflammatory conditions	(28,21,29)
NF-κB signaling pathway	Inhibits activation	Inflammatory conditions	(28,21,29)
PKB/Akt	Activation	Prevents hyperglycemia induced apoptosis	(19,30,31)
MAPK signaling pathway	Inhibits activation of MAPK, ERK1/2	Inflammatory conditions and Paraptosis (JNK1/2 activation)	(32,28)
VEGF Signaling pathway	Activation of VEGFR 2	Diabetic microvascular complications	(19,30,31)
GSK-3	Decreases activity	Alzheimer's disease	(19,33)

free, unphosphorylated thiamine administration. BFT, being more lipophilic, can diffuse through plasma membranes easily and evade the thiamine rate-limiting transport system, therefore have higher bioavailability to achieve desired therapeutic effect[39]. BFT stimulates transketolase activation forces excess triosephosphates into pentose phosphate metabolic pathway, resulting in suppression of AGE synthesis and associated sugar-induced metabolic stress[25,27,40]. The transketolase activation plays a vital role in oxidative and non-oxidative pentose phosphate pathways that suppress vascular complications in diabetes[41]. Enhanced transketolase activity also mediates the conversion of glyceraldehyde-3-phosphate and fructose-6-phosphate into xylulose-5-phosphate and erythrose-4-phosphate, respectively. Thus, BFT has been reported to limit three major molecular pathways which result in hyperglycemic damage. First, the hexosamine pathway averts the increase of UDP-N-acetylglucosamine (UDP-GlcNAc) and reduces the accumulation of glucose metabolites that can lead to AGE formation. Second, BFT decreases aldose reductase activity, which in turn normalizes the polyol pathway, sorbitol concentrations, and intracellular glucose. Thus, BFT prevents the damage induced by glucose metabolites on the endothelial cells. Third, BFT also blocks the diacylglycerol (DAG)-protein kinase C (PKC) pathway[42,26,43,39].

BFT has been acknowledged as a key molecule, when compared with water-soluble thiamine in preventing AGEs formation during diabetic-associated complications such as neuropathy, retinopathy, and nephropathy. Based on safety and efficacy data profile, BFT is as an important nutritional supplement for the prevention and progression of diabetic neuropathy[44,45]. The enhanced transketolase activity induced by BFT is found to be beneficial in experimental diabetic neuropathy, where high levels of hexose and triose phosphates are diverted into the pentose phosphate pathway, resulting in decreased tissue AGEs[46,47]. In a clinical study, high dose of BFT significantly reduce neuropathic pain[48]. High dose of BFT also results in increased transketolase expression in

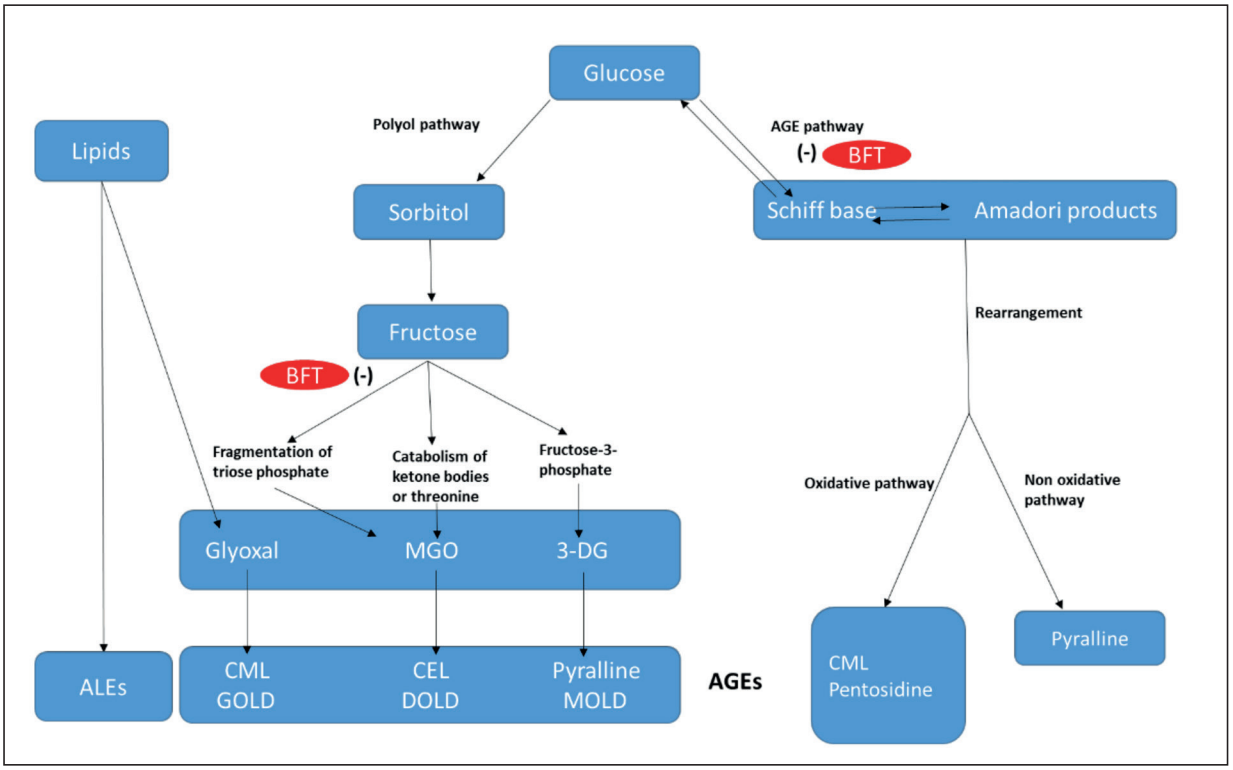

Figure 3. Role of benfotiamine to inhibit the formation of Advanced Glycation End products (AGEs). AGE Products – Pentosidine glyoxal-derived lysine dimmer (GOLD), methylglyoxalderived lysine dimmer (MOLD), 3-deoxyglucosone-derived lysine dimmer (DOLD), methylglyoxal hydroimidazolone (MGH), 3-deoxyglucosone hydroimidazolone (3DG-H), and monolysyl adducts such as N-carboxymethyl-lysine (CML), N-carboxyethyllysine (CEL) and pyrraline.

renal glomeruli that further activates the conversion of triosephosphate into ribose-5-phosphate. The scientific rationale of BFT supplementation in diabetic nephropathy patients is attributed to its ability to reduce the occurrence of albuminuria/proteinuria, increased oxidative stress and, in renal tissues, AGEs accumulation[49-51]. BFT treatment increases the transketolase expression in the retina of streptozotocin (STZ)-diabetic rats and inhibition of AGEs formation in retinal pericytes was reported to suppress the progression of diabetic retinopathy[26]. All these studies suggest that BFT enhanced transketolase activity prevents diabetic microvascular complications.

Arachidonic Acid-dependent Pathway

Arachidonic acid (AA) is a fatty acid released from membrane phospholipids where it is cleaved by the enzyme phospholipase A2. In the AA metabolic pathway, cyclooxygenases (COX) catalyze the conversion of AA into prostaglandin G2 (PGG2) and prostaglandin H2 (PGH2). PGH2 is the precursor of other prostaglandins and thromboxanes. In another pathway, 5-lipoxygenases (LOX) mediate the conversion of AA into biologically active leukotrienes (LTs). Prostaglandins and and leukotrienes are pro-inflammatory mediators. Various studies have convincingly demonstrated that the anti-inflammatory effect of BFT is achieved by the inhibition of prostaglandins and leukotrienes synthesis[28]. BFT has a significant role in AA metabolism and its anti-inflammatory action[21,28,29,52].

COX-1 and COX-2 are two isoforms of cyclooxygenases. A third variant COX-3 has been recently reported[53]. COX-1 is a constitutive physiological enzyme and COX-2 is induced by cytokines, growth-related factors, and other stimuli. Eicosanoids produced by COX-1 participate in housekeeping functions such as mucus secretion that is involved in the protection of the gastric mucosa, hemostasis and maintenance of renal function, whereas those produced by COX-2 lead to inflammatory and pathological changes. The non-steroidal anti-inflammatory drugs (NSAIDs) were reported to inhibit COX synthesis. Most of the NSAIDs are non-selective and can inhibit both cyclooxygenases (COX-1 and COX-2). A few of the most recent derivatives like celecoxib, rofecoxib are very selective for COX-2. The lipoxygenases (LOXs) are members of the non-heme iron-containing enzyme family and catalyze the deoxygenation of polyunsaturated fatty acids in membranes. There are five subclasses of LOXs—5*(S)*-LOX, 12(*S*)-LOX, 12(*R*)-LOX, 15*(S)*-LOX-1, and 15*(S)*-LOX-2. Many natural flavonoids with anti-inflammatory properties act through 5-LOX inhibition. For example, Curcumin is a widely studied natural bioactive compound, and human recombinant 5-LOX appears to be one of its prime molecular targets. Reports suggest that the dual inhibition of LOX/COX might be a unique pharmacological strategy in the development of anti-inflammatory drugs. It is reported that BFT down-regulated the expression of COX-2 and LOX-5 enzymes (Figure 4)[28]. BFT found to decrease the biosynthesis of pro-inflammatory PGs and LTB4. In addition, BFT also attenuated prostaglandin I2 (PGI2)-induced vasculopathy in bacterial infections because of its ability to decrease PGI2 expression thereby, inhibiting 6-keto PGF1 formation. These mounting evidence suggest that BFT can be a potent anti-inflammatory agent due to its dual inhibitory action on COX-2 and LOX-5 over individual specific LOX and COX inhibitors.

Nuclear Transcription Factor-κB (NF-κB) Signaling Pathway

The protein complex of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is an important pro-inflammatory signaling pathway for cytokine release and cell survival. NF-κB is becoming increasingly recognized due to its central role in mediating immune reactions against various infections. It moderates cellular responses to various stimuli like stress, cytokines, free radicals, UV rays, oxidized low-density lipoproteins (LDL) and from bacterial and viral antigens. NF-κB signaling has been implicated in the progression of cancer, inflammatory and autoimmune responses, septic shock, viral infection and improper immune enhancement. The agents which have potential to overwhelm the activated state of NF-κB could be important for the management of inflammatory conditions and many other pathological states, because failure in proper synchronization of inflammatory conditions is linked to a wide variety of diseases[52]. The anti-inflammatory role of BFT has been demonstrated through its ability to inhibit the binding of the nuclear transcription factor NF-κB[40]. Inhibition of NF-κB pathway by BFT has been shown both in experimental model of diabetes and also in lipopolysaccharides (LPS)-induced inflammation in murine macrophages[21)]. BFT also found to prevent endotoxin-induced uveitis in rats by inhibiting NF-κB signaling pathway[29]. These reports suggested that the serine/threonine kinase

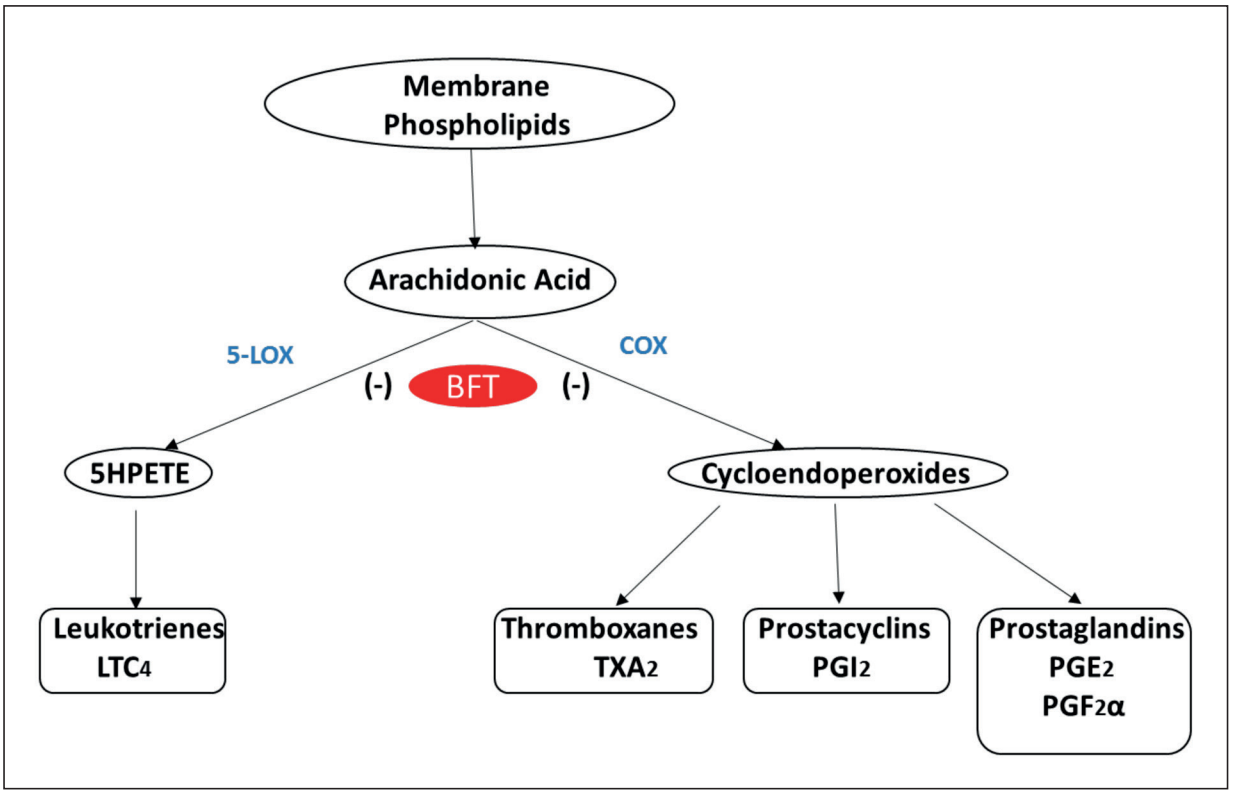

Figure 4. Dual inhibition of COX and LOX enzymes by benfotiamine.

PKC phosphorylates and activates NF-κB in the cytosol, this activation was inhibited by BFT. Inhibition of nuclear translocation of NF-*κ*B by preventing the phosphorylation helps in subsequent degradation of I*κ*B (Figure 5), which in turn decreases inflammatory cell migration and protects ocular tissues during uveitis. In addition, other studies also have shown that the BFT suppresses PKC and NF-*κ*B activation clearly demonstrating its anti-inflammatory properties[28,32].

Protein Kinase B Signaling Pathway

Protein kinase B (PKB) or in full (Akt) is a serine/threonine-specific protein kinase and plays a critical role in glucose metabolism, apoptosis, cell proliferation and migration. In mammals, Akt exists in three isoforms. Akt1 is a key signaling protein that mediates cell survival by enhancing protein synthesis thereby attenuating apoptosis. Reports have shown that overt activity of Akt2 and Akt3 isoforms are implicated in human cancers[54]. According to studies published during the past two decades, it is evident that Akt/PKB has a pivotal role in the signal transduction cascade that is activated in response to growth factors or insulin[55].

Thiamine is a cofactor of transketolase enzyme and plays a critical role in directing glucose metabolites towards the pentose phosphate pathway. Thiamine deficiency exacerbates the consequences of chronic hyperglycemia further, due to reduced transketolase activity. As a thiamine derivative, BFT has been shown to act by blocking three major pathways of hyperglycemia-induced damage. It was reported that BFT induces activation of Akt, mediates post-ischaemic healing and attenuates apoptosis in diabetic mice[19]. The reduced phosphorylation of Akt residues Ser-473 in hyperglycemic states is well documented. BFT is found to attenuate serine phosphorylation of Akt and enhance its nuclear localization, events that are critical for cell survival and also prevented angiogenesis[30,31]. BFT prevents the downregulation of PKB/Akt during diabetic complications and enhances cell survival. Thus, BFT protected human endothelial cells from hyperglycemic damage via the enhancement of nuclear translocation of phosphorylated-PKB/Akt.

Mitogen-activated Protein Kinases Signaling Pathway

Mitogen-activated protein kinases (MAPK) are eukaryotic serine-threonine kinases that me-

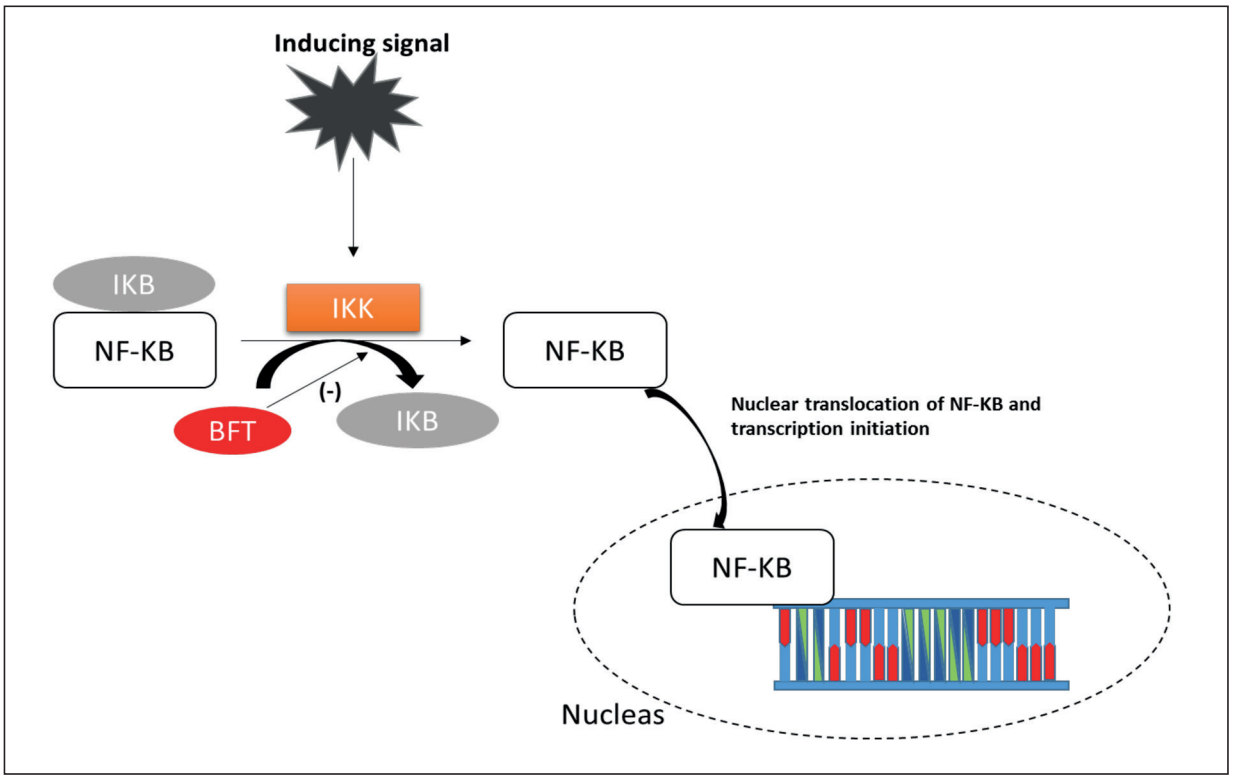

Figure 5. Inhibition of NF-κB activation by benfotiamine.

diate vital intracellular programs like proliferation, differentiation, survival, and apoptosis. There are three major families of MAP kinases which include the extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs) and p38/MAPKs. ERK[1/2] mediates cell growth, JNK mediates apoptosis and cytokine production and the p38 family also contributes to apoptosis, inflammation and cell cycle regulation[56]. The regulation of cellular processes and anti-inflammatory actions of BFT are partly mediated through MAPK signaling mechanisms. BFT plays a protective role against inflammatory responses induced by LPS activated microglia. BFT acts by down regulating pro-inflammatory mediators and cytokines *via* multiple signaling mechanisms. The amplified phosphorylation of ERK[1/2] and JNK are significantly attenuated by BFT. This, in addition to the inhibition of Akt, plays a major role in reducing tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) release in LPS stimulated BV-2 microglia[32,28]. BFT is also found to inhibit LPS-induced activation of p-38 MAPK[21] and play a significant role in attenuating apoptosis (caspase-dependent) and paraptosis (caspase independent). Furthermore, BFT induces paraptosis in leukemia cells[57] through the activation of JNK[1/2].

Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) Signaling Pathway

Vascular endothelial growth factor (VEGF) belongs to the homodimeric glycoprotein family and plays a critical role in vasculogenesis, lymphangiogenesis, and angiogenesis. The VEGFs bind to three different types of VEGF-receptor tyrosine kinases; VEGFRs 1, 2 and 3 that dimerize and activate upon ligand-binding. The active receptors initiate signal transduction pathways that direct cellular functions. VEGFR-2 is expressed in vascular and lymphatic endothelial cells and plays an important role in physiologic and pathogenic angiogenesis[58]. The relationship between glucose-6-phosphate dehydrogenase (G6PD) and VEGF mediated cell proliferation has been demonstrated earlier in the *in vivo* and *in vitro* studies. The diminished G6PD expression and activity are correlated with impaired response to VEGF-induced cell proliferation and migration by influencing tyrosine phosphorylation of VEGFR2. *In vitro* studies have demonstrated that knockdown of G6PD decreases an-

giogenesis, which was restored by forced G6PD expression[50]. In myocardial ischemia, increased G6PD activity was reported to induce VEGFR2 activation; a compensatory mechanism to counteract oxidative stress and aid cardiomyocyte survival. This response is blunted in DM due to the inhibition of the enzymes transketolase and G6PD. The restoration of G6PD levels was recognized as a specific pharmacological intervention to restore contractile dysfunction and prevent cardiomyocyte damage in DM[19]. BFT enhanced cardiomyocyte survival through increased transketolase and G6PD activities in diabetic hearts. The raised activity of G6PD leads to the phosphorylation and activation of VEGFR2. Hence, BFT appears to have a cardioprotective role in potentiating angiogenesis and inhibiting apoptosis in diabetic complications; a response mediated by VEGFR2[19,30].

Glycogen Synthase Kinase-3 (GSK-3)

Glycogen synthase kinase 3 (GSK-3) is a serine-threonine protein kinase that has been extensively studied because of its role in chronic inflammatory, immune, metabolic and neurodegenerative diseases. Among the many isoforms of GSK-3 such as GSK-3α, GSK-3β, and GSK-3 β2, the isoenzyme GSK-3β is primarily localized in neurons present in the hippocampus[59]. GSK-3 phosphorylates microtubule associated proteins and tau proteins present in the neurons. Hyperphosphorylation of tau proteins by GSK-3β results in detachment of microtubules from the neurons that are involved in the pathogenesis of Alzheimer's disease (AD). These hyperphosphorylated tau proteins accumulate and weaken the synaptic junctions between neurons resulting in the progression of neuronal death[60-63]. It was recently demonstrated that GSK-3α also contributes to the pathogenesis of AD by enhancing Amyloid β formation by interfering with amyloid precursor protein (APP) cleavage[64]. GSK-3β activity can be modulated by phosphorylation of Ser9 and Tyr216 residues which inhibit and activate GSK-3β, respectively. Whereas, the GSK-3α activity can be modulated by phosphorylation of Ser21 and Tyr279 residues, which inhibit and activate GSK-3α, respectively[65].

The role of BFT in counteracting glucose toxicity has been extensively studied[46-51]. Akt phosphorylation is one of the important steps involved in counteracting glucose toxicity by BFT[55]. The raised glucose levels in diabetes inhibit Akt^{Ser473} phosphorylation and BFT blocks this action and induce nuclear localization of active Akt that is an important upstream kinase of GSK-3β. This revelation suggests that BFT may also regulate GSK-3 activity through Akt. GSK-3 activity is implicated in β-amyloid deposition in the brain and leads to AD. BFT dose-dependently increased $GSK\text{-}3\beta^{Ser9}$ and $GSK\text{-}3\alpha^{Ser21}$ phosphorylation in the brain of APP/PS1 double-transgenic mice. BFT also increased the Akt phosphorylation and the phosphorylation of serine residues, reduced the enzymatic activity of GSK-3α and GSK-3β diminished the β-amyloid deposition in the brain and improved the symptoms associated with the pathogenesis of AD[66,67]. In a recent clinical study[68], BFT has been reported to inhibit the progression of the cognitive function impairment in AD patients, which was correlated with improved brain glucose metabolism.

Role of Benfotiamine in Regulating Programmed Cell Death

Apoptosis

Apoptosis is a set of biochemical events that occur in multicellular organisms and leads to characteristic morphological changes and programmed cell death. Unlike necrosis which is traumatic cell death, apoptosis is extremely synchronized in a controlled progressive manner which confers advantage during the lifecycle of every organism. Apoptosis can be initiated through intrinsic or extrinsic pathways. Stimulation of death receptors triggers the extrinsic pathway whereas DNA damage, UV radiation, oxidative stress and other factors trigger the intrinsic pathway[33]. The mitochondria are central to the intrinsic pathway[69]. The integrity of the outer mitochondrial membrane (OMM) is regulated by pro- and anti-apoptotic Bcl-2 proteins. Bax/Bcl-2 ratio is a major factor that regulates apoptosis because the variations in this ratio are critical in determining whether apoptosis can be initiated or not. Thiamine and BFT are effective in correcting the altered ratio to prevent apoptosis *in vitro*[19,70]. The anti-oxidant properties of BFT are responsible for its protective role against DNA damage in cisplatin-induced nephrotoxicity in rats[71]. BFT is found to inhibit excess nitric oxide (NO) production, lipid peroxidation, protein oxidation and DNA damage induced by cisplatin. BFT also modulated the expression of enzymes including superoxide

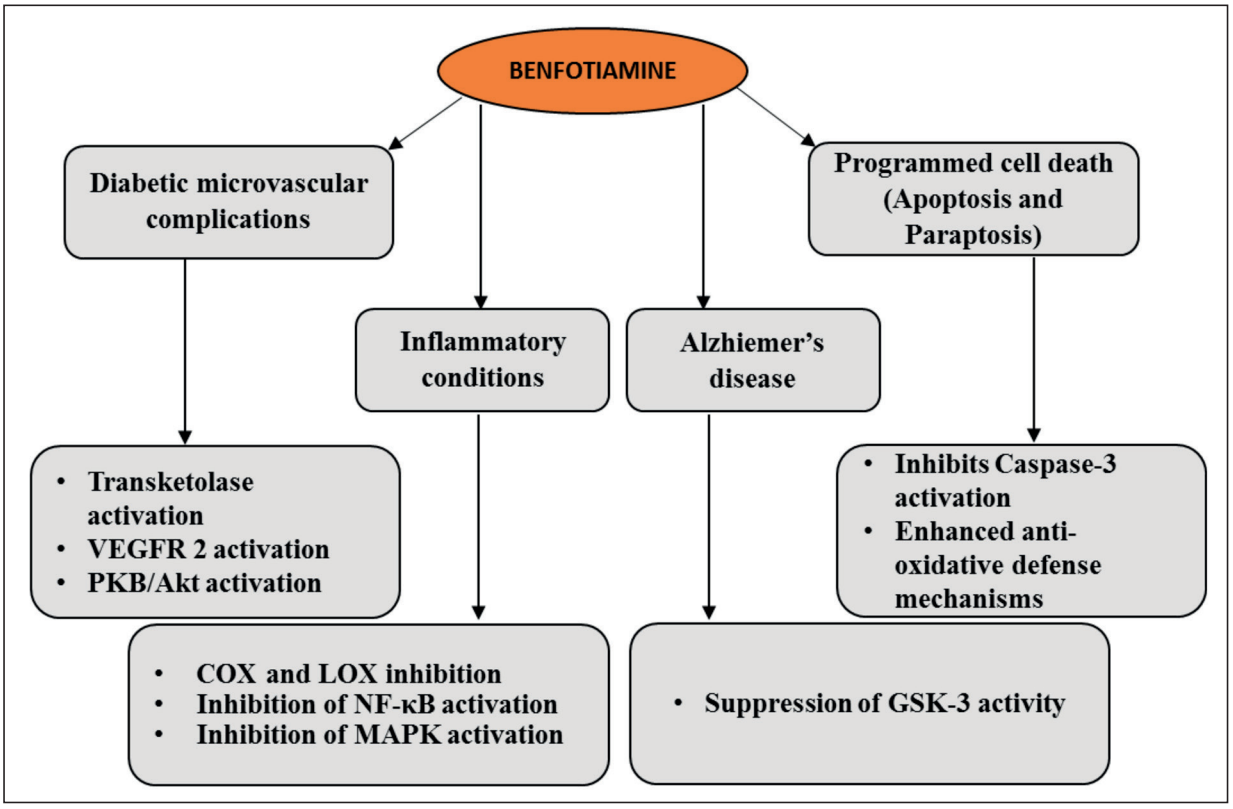

Figure 6. Role of benfotiamine in different conditions and its molecular targets.

dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), that are involved in antioxidant defense and showed free radical scavenging properties that are associated with downregulation of inducible nitric oxide synthase (iNOS)[32]. Furthermore, BFT is shown to be protective against diabetes-associated oxidative DNA, histone damage and suppression of p300 upregulation[72]. All these factors contribute to the anti-apoptotic action of BFT.

Apoptosis is initiated through intrinsic or extrinsic pathways, the process of apoptosis is regulated mainly by caspases which belong to the family of cysteine proteases. Among the many isoforms, the role of caspase-3 is pivotal in mediating apoptosis[69]. BFT is shown to inhibit caspase-3 activation in ischemic muscles and prevented apoptosis. The anti-apoptotic effect was achieved through post-translational modifications of caspase-3 and enhanced expression of the anti-apoptotic proteins such as survivin and Bcl-2[19,73]. BFT also protects against LPS-induced macrophagic cell death. The inflammatory signals induced by LPS involved activation of caspase-3, which in turn leads to poly ADP ribose polymerase (PARP) cleavage and the release of cytochrome-c, and other apoptotic intermediaries. All these processes are inhibited by BFT[74,21]. These observations clearly demonstrate that BFT plays a significant role in controlling cell death by regulating apoptosis[20,75].

BFT also plays a central role in attenuating genomic damage and raised transketolase activity and associated decrease in AGE production[76]. This is correlated with the accelerated pentose phosphate pathway by transketolase that eventually results in reduced production of nicotinamide adenine dinucleotide phosphate (NADPH) and activation of the antioxidant defense mechanisms. The anti-apoptotic action of BFT is mediated by improved antioxidant defense that further reduces the genomic damage in patients.

Paraptosis

According to a recent study, BFT is able to produce G1 phase cell cycle arrest by down-regulating cyclin-dependent kinase 3 (CDK3)[57]. Paraptosis is a type of programmed cell death that is caspase activation independent and mor-

phologically different from apoptosis and necrosis. Paraptosis may be associated with an imbalance in internal potassium ion homeostasis. However, the exact mechanisms are yet to be elucidated. A study in leukemia cells revealed that the antitumor activity of BFT is mediated by induction of paraptosis and not mediated by apoptosis, necrosis or autophagy. Big potassium channel (BK) inhibitor and cycloheximide prevented the vacuoles induced by BFT. Even though activation of both the MAPK/ERK and JNK pathways are observed in paraptosis, JNK1/2 activation is the only critical factor in BFT induced paraptosis[57]. BFT-induced paraptosis requires further investigation in order to clarify the exact mechanisms involved in the process.

Conclusions

High-calorie malnutrition is on the rise in the developed countries; subclinical thiamine deficiency is undoubtedly widespread as opposed to overt thiamine deficiency. Thiamine deficiency is commonly associated with neurological symptoms and is a major contributing factor for many vascular complications of diabetes. In clinical settings, thiamine supplements used diabetic and non-diabetic conditions have proven to be beneficial. Since, water-soluble thiamine absorption is mediated through cell membrane transporters, which limits its rate of absorption and bioavailability. However, this limitation was overcome by introducing lipid-soluble thiamine derivative, BFT, which is increasingly used in clinical settings with improved bioavailability. As a result, the therapeutic dose of BFT is much lower compared to water-soluble thiamine. A few clinical studies using BFT have shown to be beneficial in offering protection against diabetes-related complications including neuropathy, nephropathy, cardiomyopathy, and retinopathy via the activation of transketolase enzyme. Recent studies have shown, BFT is capable of modulating various signaling pathways that are involved in different disease and physiological processes. In this review, we have made an attempt to summarize all the molecular targets of BFT based on the most recent up to date scientific literature to shed some light on its multifaceted actions and therapeutic potential.

Author Contributions

SBS and SO conceptualized and edited the review. SBS and VR searched the literature, drafted, edited, and prepared the figures and final version of the manuscript. CH helped in editing the manuscript and reviewing of the literature. PB drew the chemical structure and also helped in editing the manuscript.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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