

Review Article

The effects of benfotiamine in attenuating hyperglycemia-induced cardiac pathology

Garson Kirsty-Lee, Mapanga R.F., Milne R and Essop M.F.*

Cardio-Metabolic Research Group (CMRG), Department of Physiological Sciences, Stellenbosch University, Stellenbosch, South Africa

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ABSTRACT

Type 2 diabetes is a major global health problem. It is also a risk factor for the onset of cardiovascular diseases, the current leading cause of global mortality. The first part of this mini-review describes hyperglycemia-induced cellular alterations and its effects on cardiac function. In particular, we emphasize the role of hyperglycemia-induced oxidative stress in the activation of non-oxidative glucose pathways (NOGPs), that may contribute to cardiac pathology. For the second part, we evaluate the utility of benfotiamine (a vitamin B1 derivative) in treating diabetes-related cardiac pathology. The focus is on its role in activating the pentose phosphate pathway, which may reduce flux through the NOGPs. A possible role for benfotiamine in activating pro-survival signaling and reducing cell death in the heart is also described. We also discuss benfotiamine's potential cardioprotective role in preventing the diabetic cardiomyopathy, treating myocardial infarction and maintaining the viability of cardiac progenitor cells. These findings warrant further investigation into the therapeutic potential of benfotiamine in treating diabetes-related cardiac complications.

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INTRODUCTION

In 2011 the global prevalence of diabetes mellitus was approximately 366 million, with this figure expected to rise to ~552 million by the year 2030 (Whiting et al. 2011). Diabetes and its complications place an immense financial burden upon individuals

and economies, hindering development in low- and middle-income countries. During 2010 ~11.6% of the total global healthcare expenditure was attributed to diabetes (Colagiuri 2010). Moreover, this condition is associated with a significantly higher incidence of cardiovascular diseases (Fox 2010), the leading cause of global mortality.

Type 2 diabetes comprises the majority of reported diabetes cases and elicits a plethora of metabolic perturbations that are well-described, including hyperinsulinemia, hyperlipidemia and hyperglycemia (Calles-Escandon et al. 1999). For the purposes of this mini-review, we will focus on the effects of hyperglycemia with particular emphasis on its impact on the heart. Moreover, the role of benfotiamine (BFT) in counteracting these effects will also be assessed.

*Address for correspondence:

Email: mfessop@sun.ac.za

Tel: +27 21 808 3146

Fax: +27 21 808 3145

Damaging effects of hyperglycemia on cardiac function

With type 2 diabetes, reduced responsiveness to insulin and greater plasma glucose concentrations lead to increased intracellular glucose levels in several cell types. The resulting increase in glycolysis and overproduction of NADH and FADH₂ (in citric acid cycle) lead to the enhanced generation of reactive oxygen species (ROS) - specifically superoxide - by the electron transport chain (Du et al. 2000). An emerging paradigm proposed by Brownlee (2001) describes mitochondrial superoxide overproduction as the cause of a number of hyperglycemia-induced alterations (Figure 1). Here superoxide is proposed to cause DNA damage and subsequent activation of poly-ADP-ribose polymerase (PARP) as a restorative mechanism (Graziani & Szabó 2005). However, PARP activation is associated with inhibition of the key glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Du et al. 2003). This in turn leads to the build-up of upstream glycolytic metabolites and increased diversion into non-oxidative glucose pathways (NOGPs), with maladaptive consequences (Brownlee 2005).

NOGP activation includes increased flux through the polyol pathway, an elevation of advanced glycation end-product (AGE) formation, activation of protein kinase C (PKC) isoforms, and greater flux through the polyol and hexosamine biosynthetic pathways (HBP). For example, we recently reported increased HBP activation in leukocytes isolated from pre-diabetic and diabetic patients (Springhorn et al. 2012) and also greater activation of all four NOGPs in the heart in response to acute hyperglycemia (Mapanga et al. 2014).

Excessive NOGP flux is associated with a range of harmful alterations that vary according to cell type. For example, hyperglycemia in cardiac cells and the resulting increase in NOGP activation are proposed to contribute to systolic and diastolic dysfunction (Poornima et al. 2006). Here increased HBP flux leads to post-translational modification of proteins that may impair relaxation of the heart (Clark et al. 2003) and cause cell death (Fiordaliso et al. 2001). This is supported by studies from our laboratory, demonstrating that cardiac cells cultured under hyperglycemic conditions display elevated oxidative stress, enhanced HBP activation, and increased cell death (Rajamani & Essop 2011). These findings were confirmed in heart tissues isolated from a rat model of diet-induced insulin resistance (Rajamani et al. 2011). Excessive AGE formation is associated with cross-linking of collagen (Candido et al. 2003) and ryanodine

receptors (Bidasee et al. 2003) that may reduce contractility and increase ventricular stiffness (Herrmann et al. 2003). Furthermore, high flux through the polyol pathway elevates oxidative stress (Tang et al. 2010) and promotes cardiomyocyte apoptosis (Galvez et al. 2003), while enhanced PKC activation can lead to cardiac hypertrophy, impaired relaxation and increased ventricular stiffness (Wakasaki et al. 1997; Scherer et al. 2006). Thus hyperglycemia-induced perturbations are associated with oxidative stress and NOGP induction that contribute to myocardial cell death and impaired heart function. Together such alterations can contribute to the diabetic cardiomyopathy, that refers to diabetes-related cardiac dysfunction in the absence of coronary artery disease or hypertension (Boudina & Abel 2007).

However, the effects of hyperglycemia on the heart are not limited to the development of the diabetic cardiomyopathy. By promoting atherosclerosis, hyperglycemia also increases the likelihood of myocardial infarction in diabetic patients (Fowler 2008). Moreover, hyperglycemia *per se* may exert direct effects on the heart. For instance, the presence of hyperglycemia during myocardial infarction is associated with increased mortality (Koracevic et al. 2006). This was confirmed by our recent work demonstrating coordinate induction of NOGPs in response to acute hyperglycemia and resulting in increased oxidative stress and cell death together with impaired cardiac function following ischemia-reperfusion (Mapanga et al. 2014). Our data therefore support a significant pathophysiologic role for such NOGP induction within the context of ischemia-reperfusion under hyperglycemic conditions.

A third context where the effects of hyperglycemia on the heart are visible is with cardiogenesis. For example, a recent study found decreased abundance and proliferation of cardiac progenitor cells in the hearts of diabetic mice as well as an increase in AGEs. Thus the damaging effects of hyperglycemia are compounded by its impairment of the heart's already-limited regenerative capacity (Katare et al. 2013). To summarize, hyperglycemia leads to NOGP activation and pathological alterations that can blunt the heart's function, induce myocardial damage and impair cardiac regeneration. This paradigm therefore allows for the evaluation of novel therapeutic approaches to limit NOGP activation under these conditions, e.g. by stimulation of the pentose phosphate pathway (PPP).

Therapeutic potential of the pentose phosphate pathway

The PPP is well-known for its role in producing NADPH and ribose 5-phosphate, essential for regulating intracellular redox balance and DNA repair. However, it plays important additional roles in preventing apoptosis and promoting angiogenesis, that are linked to glucose 6-phosphate dehydrogenase (G6PD) (Riganti et al. 2012).

G6PD is the first and rate-limiting enzyme of the PPP and catalyzes a reaction that generates NADPH (Figure

2). NADPH is a key regulator of intracellular redox balance, essential in generating anti-oxidant capacity as well producing ROS. The main components of the anti-oxidant machinery, namely the glutathione system, catalase and superoxide dismutase, require NADPH to function. However, NADPH is also needed for ROS production by NADPH-dependent enzymes such as

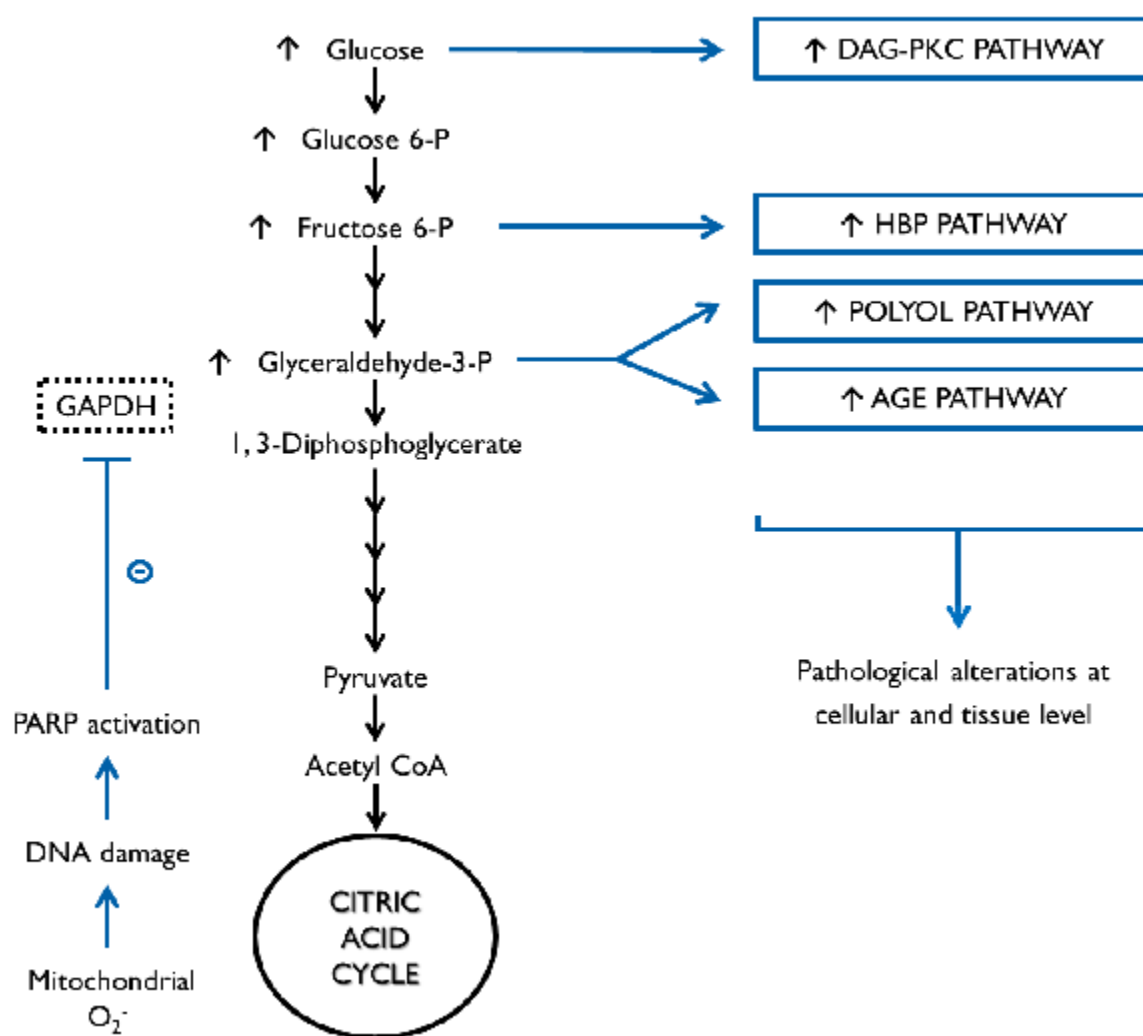


Fig. 1. **Unifying mechanism of hyperglycemia-induced cellular alterations.** Under hyperglycemic conditions, mitochondrial superoxide overproduction leads to DNA damage, PARP activation and GAPDH inhibition. This in turn causes a build-up of upstream glycolytic metabolites and excessive flux through non-oxidative glucose pathways. Increased flux through the PKC, HBP, polyol and AGE pathways causes a range of pathological alterations that vary according to cell type. AGE: advanced glycation endproducts, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, HBP: hexosamine biosynthetic pathway, O₂⁻: superoxide, PARP: poly(ADP-ribose) polymerase, PKC: protein kinase C.

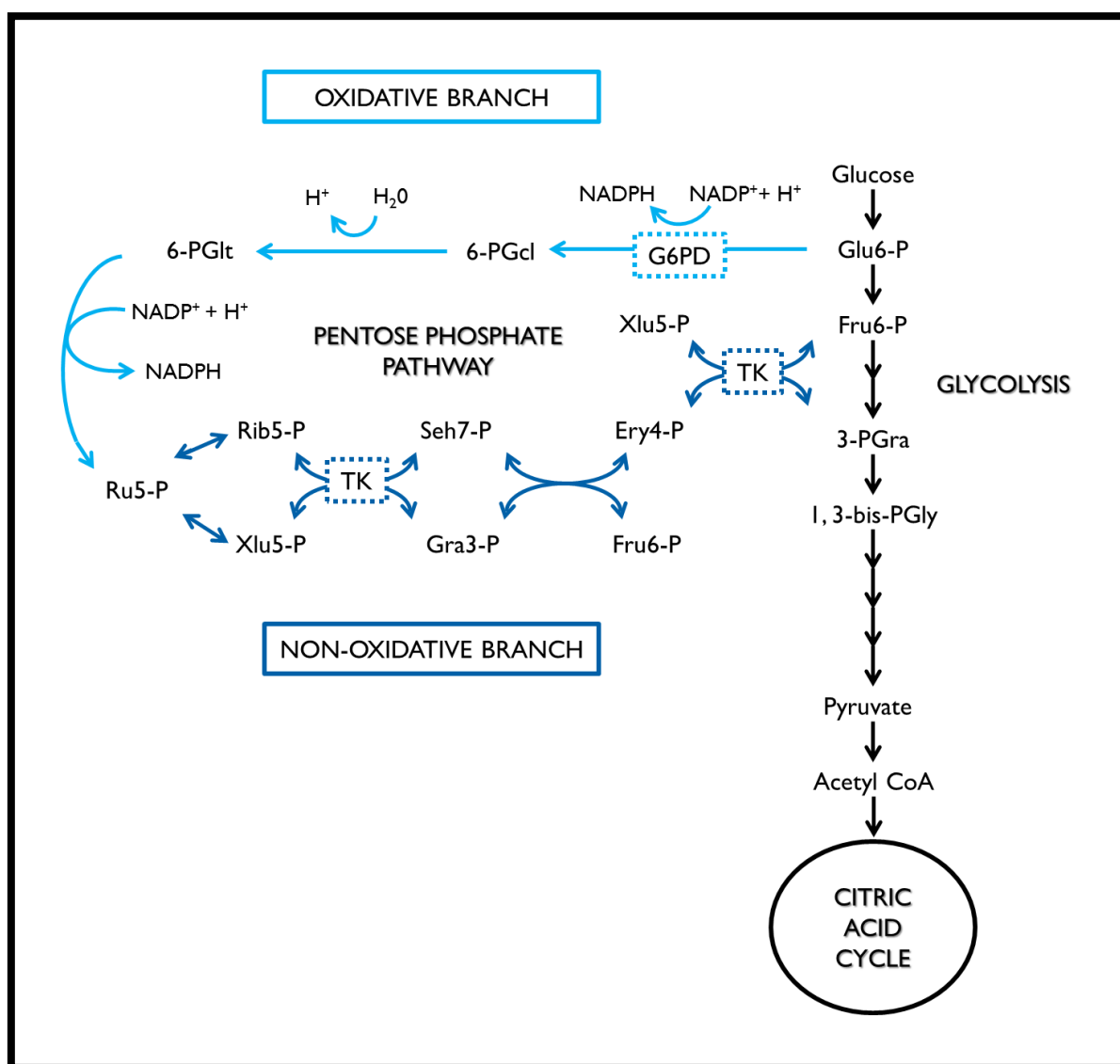


Figure 2: The pentose phosphate pathway in glucose metabolism. Benfotiamine administration leads to increased transketolase and glucose 6-phosphate dehydrogenase activity, thereby enhancing flux of glycolytic metabolites into the pentose phosphate pathway. This increases the production of ribose 5-phosphate and NADPH that are used in DNA repair and regulating redox balance. 1,3-bis-PGly: 1,3-diphosphoglycerate, 3-PGra: glyceraldehyde 3-P, 6-PGcl: 6-P gluconolactone, 6-PGlt: 6-P gluconate, α -KGDH: α -ketoglutarate dehydrogenase, Ery4-P: erythrosine 4-P, Fru6-P: fructose 6-P, G6PD: glucose 6-phosphate dehydrogenase, Glu6-P: glucose 6-P, Rib5-P: ribose 5-P, Ru5-P: ribose 5-P, Seh7-P: sedoheptulose 7-P, Gra3-P: glyceraldehyde 3-P, PDH: pyruvate dehydrogenase, TK: transketolase, Xlu5-P: xylulose 5-P.

NADPH-oxidase (NOX) (Zhao et al. 2012). Therefore the effects of increasing NADPH production in the heart via G6PD activation are controversial. For example, G6PD inhibition in rat cardiomyocytes increased oxidative stress and contractile dysfunction (Jain et al. 2003), suggesting that NADPH opposes oxidative stress. By contrast, upregulation of G6PD in a rat model of type 2 diabetes was associated with greater NADPH generation, increased NOX-catalyzed superoxide production and diminished anti-oxidant

capacity in the heart (Serpillon et al. 2009). This suggests that activating NADPH production in the heart as a protective strategy may represent a “double-edged sword” since this may also promote oxidative stress. Further investigations are therefore needed to better understand the specific circumstances under which NADPH fulfills each aspect this dual role in regulating redox balance.

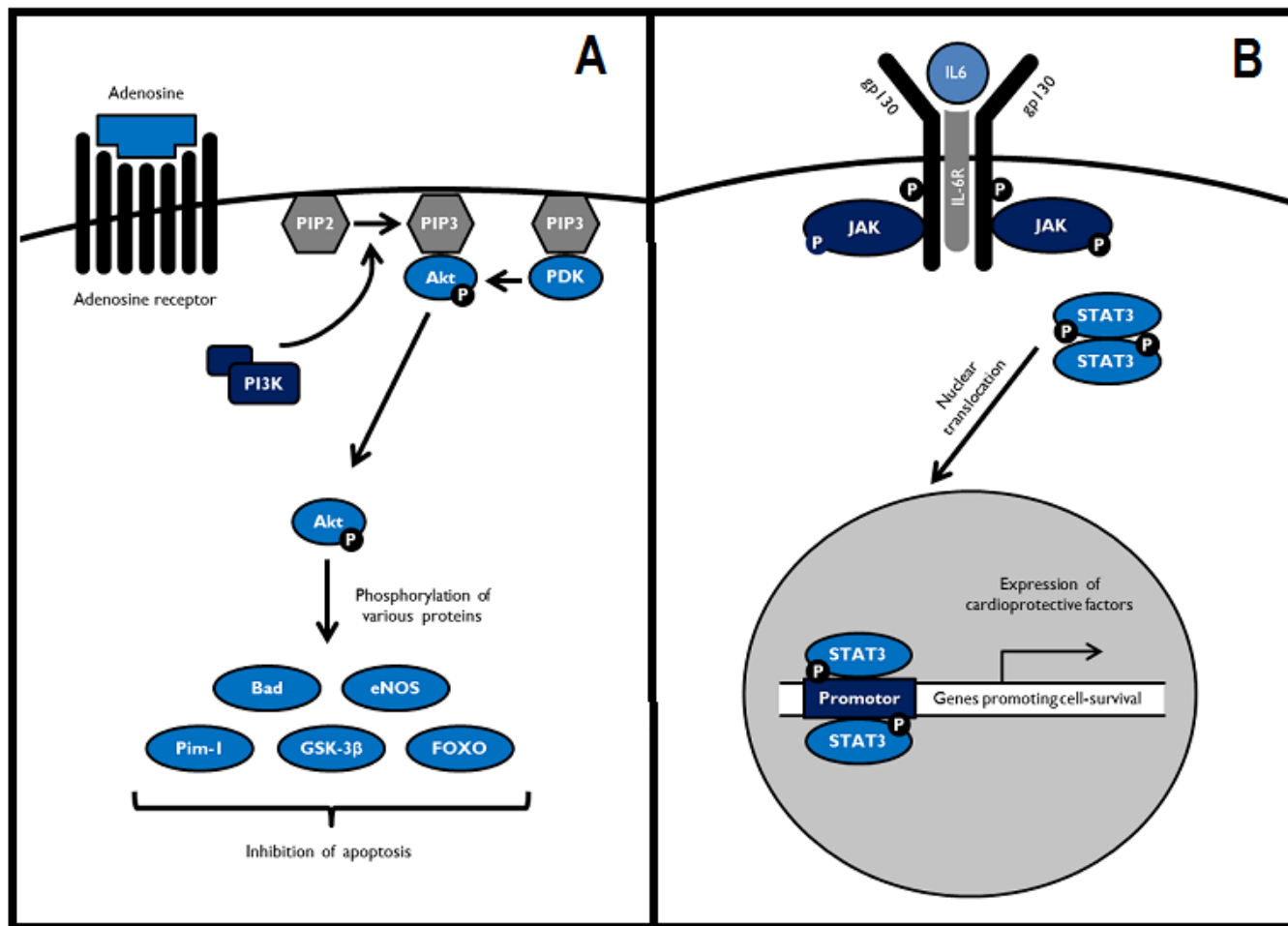


Fig. 3.

Two major pro-survival pathways in the heart. A) The PI3K/Akt signaling pathway can be activated by upstream regulators, e.g. adenosine. Adenosine binding to its receptor causes PI3K to phosphorylate PIP2 to form PIP3, followed by Akt and PDK recruitment to the plasma membrane – in close proximity. PDK phosphorylates and activates Akt that can prevent apoptosis by phosphorylating various downstream targets including Bad, eNOS, Pim-1, GSK-3 and FOXO. Note: VEGF2 and VEGFR2 may also potentially feed into this pathway. B) The JAK/STAT pathway can be triggered by an upstream modulator, e.g. IL-6. IL-6 binding to its receptor recruits two gp130 molecules with associated JAK proteins. JAK proteins can auto-phosphorylate itself and also the gp130 molecules, creating docking sites for STAT proteins to bind. Once bound, STATs become phosphorylated before translocating into the nucleus and regulating expression of cardioprotective factors. eNOS: endothelial nitric oxide synthase, FOXO: forkhead box O, GSK: glycogen synthase kinase, Gp130: glycoprotein 130, IL-6: interleukin 6, PDK: 3-phosphoinositide-dependent protein kinase, PI3K: phosphatidylinositol 3-kinase, STAT: signal transducer and activator of transcription, VEGF: vascular endothelial growth factor, VEGFR2: vascular endothelial growth factor receptor 2.

In addition to its role in regulating redox balance, G6PD activation is also associated with pro-survival signaling, e.g. to prevent apoptosis and promote angiogenesis by activation of vascular endothelial growth factor receptor 2 (VEGFR2) and Akt (Zhang et al. 2014). This notion is supported by several lines of evidence. Firstly, Jain et al. (2003) demonstrated that stimulation of G6PD activity in response to increased oxidative stress resulted in its translocation to the plasma membrane. Moreover, G6PD is also necessary for phosphorylation and activation of VEGFR2, Akt and endothelial nitric oxide synthase (eNOS), with

4). After allithiamine (a naturally-occurring thiamine derivative) was discovered in Japan (Fujiwara et al. 1954), researchers synthesized a group of additional thiamine derivatives with improved bioavailability. They subsequently proceeded to assess the value of such compounds in treating various diseases (Shimazono & Katsura 1965).

BFT, or S-benzoylthiamine O-monophosphate, has primarily been investigated as a treatment for diabetes-related cardiovascular disorders. By increasing

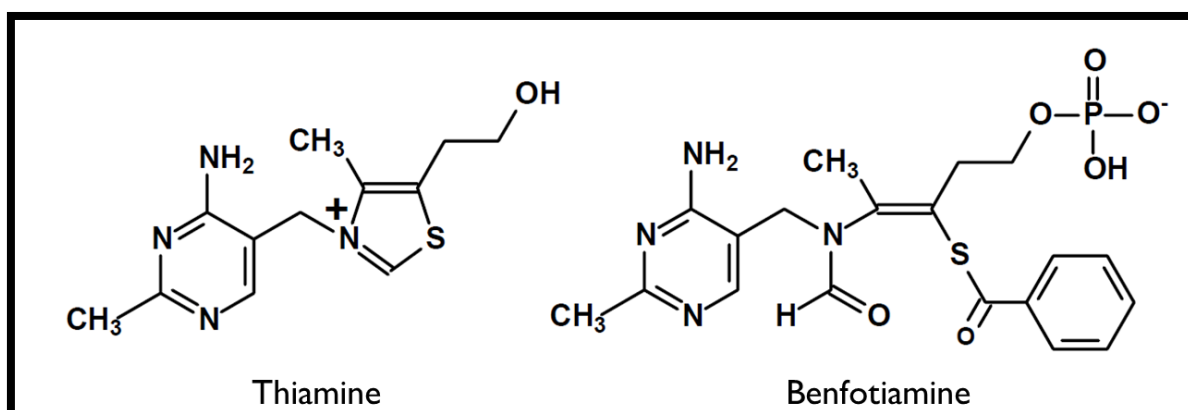


Fig. 4. The chemical structure of thiamine and benfotiamine. Benfotiamine is a benzoylated derivative of the phosphorylated version of thiamine.

VEGF2/Akt/eNOS signaling resulting in stimulation of angiogenesis (refer Figure 3A for some basic contextualization of these modulators) (Leopold et al. 2003). The activation of the VEGFR2/Akt signaling cascade is associated with increased levels of pAkt, Pim-1, pBad and Bcl-2 and reduced apoptosis (Katara et al. 2010). Further evidence for the involvement of G6PD in stimulating angiogenesis and preventing apoptosis was provided by partially G6PD-deficient mice that displayed impaired angiogenesis (Leopold et al. 2003) and increased myocardial dysfunction following ischemia-reperfusion (Jain et al. 2003). Thus increasing G6PD activity and promoting VEGFR2/Akt signaling may represent an effective cardioprotective strategy within the context of hyperglycemia.

Benfotiamine

BFT is one of a number of thiamine (vitamin B1) derivatives investigated for therapeutic use (Figure

PPP flux, it can shunt glycolytic metabolites away from the NOGPs and hence attenuate the negative downstream consequences previously discussed (Berrone et al. 2006; Du et al. 2008). BFT administration is linked to roles in preventing the diabetic cardiomyopathy, treating myocardial infarction, and maintaining the function and number of cardiac progenitor cells under hyperglycemic conditions.

Preventing diabetic cardiomyopathy

BFT's ability to treat the diabetic cardiomyopathy was tested in three studies. Here streptozotocin-induced diabetic mice treated with BFT for six weeks displayed attenuated collagen, methylglyoxal (an AGE pathway intermediate), AGE receptor and methylglyoxal-derived AGE formation in the heart compared to untreated diabetic rats (Ma et al. 2009). Such findings were complemented by experiments in isolated cardiomyocytes where methylglyoxal-derived AGEs were associated with cardiomyocyte dysfunction, secondary to mitochondrial

membrane potential depolarization and decreased glycogen synthase kinase (GSK-3 β) (Ma et al. 2009). In support, others evaluated the effects of two weeks of BFT treatment on diabetes-induced heart dysfunction and found that it attenuated oxidative stress and counteracted diabetes-induced contractile dysfunction (Ceylan-Isik et al. 2006).

In order to investigate the efficacy of long-term BFT supplementation in preventing diabetic cardiomyopathy, Katare et al. (2010A) employed two diabetic mouse models. Here streptozotocin-induced and leptin receptor-deficient db/db mice were supplemented with benfotiamine for 8 and 16 weeks, respectively. Hearts of untreated diabetic mice showed reduced transketolase (TK), G6PD and GAPDH activities, while ROS production and cardiomyocyte apoptosis were elevated. The progression of diabetic cardiomyopathy was also associated with decreased phosphorylation of signal transducer and activator of transcription 3 (STAT3), Akt, eNOS, Forkhead box O (FOXO) and Bad, in addition to lower levels of proviral integration site of Moloney murine leukemia virus (Pim-1) and Bcl-2. Phosphorylation of these proteins is associated with the prevention of apoptosis (refer Figure 3B for some basic contextualization of these modulators). Such perturbations were all prevented by BFT treatment. These findings suggest that, in addition to inducing protection by activating the PPP and reducing NOGP flux, BFT may also induce protection by activating pro-survival via Akt and Pim-1.

Treating myocardial infarction

Hyperglycemia is common during myocardial infarction. Chronic hyperglycemia is characteristic of diabetes, a condition that commonly manifests with myocardial infarction. Furthermore, acute hyperglycemia occurs during myocardial infarction in non-diabetic individuals as result of sympathetic nervous system activation in response to stress. Since the presence of hyperglycemia during myocardial infarction is associated with increased mortality (Koracevic et al. 2006), two recent studies investigated the role of BFT in treating myocardial infarction (with and without hyperglycemia).

A study by Katare et al. (2010B) investigated long-term BFT supplementation in streptozotocin-treated mice and found the diabetic mice displayed increased apoptosis, more oxidative stress, less reparative angiogenesis, impaired VEGFR2/Akt/Pim-1 signaling, and lower functional recovery and survival than their non-diabetic counterparts. BFT supplementation restored VEGFR2/Akt/Pim-1 signaling, increased levels of pAkt, Pim-1, pBad and

Bcl-2, decreased apoptosis, and counteracted the above-mentioned effects of diabetes. These findings were complemented by experiments in isolated cardiomyocytes where BFT treatment preserved VEGFR2/Akt/Pim-1 signaling under hypoxic and hyperglycemic conditions (Katare et al. 2010B).

Secondly, a study by our group found that acute BFT administration was beneficial in attenuating hyperglycemia-induced perturbations, preventing oxidative stress and attenuating cell death in the rat heart. Here BFT administration blunted hyperglycemia-mediated NOGP activation, thereby activating beneficial downstream effects, more specifically a reduction in infarct size and an improvement in functional recovery following ischemia and reperfusion (Mapanga et al. 2014). Our data also revealed that BFT provided cardioprotection under normoglycemic conditions and we propose that this may occur via the Akt/Pim-1 pro-survival signaling. However, further studies are required to confirm this interesting proposition.

In addition to preventing hyperglycemia-induced cellular alterations and protecting cardiac cells from apoptosis exacerbated by hyperglycemia, BFT can also restore impaired cardiac regeneration as a result of diabetes.

Maintaining cardiogenesis

A recent study demonstrated decreased abundance and proliferation of cardiac progenitor cells in diabetic mice (Katare et al. 2013). Here isolated cardiac progenitor cells exhibited impaired activity of TK and G6PD, increased levels of superoxide and AGEs, and attenuated Akt/Pim-1/Bcl-2 signaling. Human and mouse cardiac progenitor cells cultured in high glucose showed similar perturbations and such changes were associated with increased apoptosis. However, BFT administration restored PPP enzyme activity and cardiac progenitor cell availability and function *in vitro* and *in vivo* (Katare et al. 2013).

CONCLUSION

Hyperglycemia induces cellular alterations that contribute to a range of cardiac pathologies. Experimental studies suggest that BFT shows promise in counteracting these alterations and opens up possibilities for the treatment of the diabetic cardiomyopathy, myocardial infarction and impaired cardiogenesis typically found with diabetes. The therapeutic potential of BFT in treating diabetes-related cardiac complications therefore warrants further investigation.

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