

EFFECT OF BENFOTIAMINE ON THE CORRECTED QT INTERVAL, QT DISPERSION AND SPATIAL QRS-T ANGLE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND CARDIAC AUTONOMIC NEUROPATHYVictoria A. Serhiyenko^{1*}, Galina I. Suslyk¹, Ludmila M. Serhiyenko² and Alexandr A. Serhiyenko¹¹Department of Endocrinology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.²Department of Medical Biology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.***Corresponding Author: Dr. Victoria A. Serhiyenko**

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ABSTRACT

Background: The significance of cardiac autonomic neuropathy (CAN) has not been fully appreciated and there is no unified treatment algorithm. Aim: To investigate the effects of benfotiamine (BFT) on the corrected QT interval (QTc), QT dispersion (QTd) and spatial QRS-T angle in patients with type 2 diabetes mellitus (T2DM) and CAN. **Patients and Methods:** 32 patients with T2DM and definite stage of CAN were allocated to two treatment groups: control (n = 15) received standard antihyperglycaemic therapy; group 2 (n = 17) - in addition BFT 300 mg/d for three months. The QTc interval, QTd and spatial QRS-T angle parameters were analyzed. **Results:** It was found out that BFT contributed to decrease of the QTc, QTd and QRS-T angle. **Conclusions:** The positive influences of BFT suggests the feasibility of its administration to patients with T2DM and definite stage of CAN. Obtained results suggest that the efficacy of BFT is the result of a direct effect of the BFT on the investigated indexes.

KEYWORDS: Type 2 diabetes mellitus, Cardiac autonomic neuropathy, Benfotiamine, Corrected QT interval, Spatial QRS-T angle.

INTRODUCTION

Cardiac autonomic neuropathy (CAN) is a serious complication of diabetes mellitus (DM) that is strongly associated with approximately five-fold increased risk of cardiovascular mortality. CAN manifests in a spectrum of things, ranging from resting tachycardia and fixed heart rate (HR) to development of "silent" myocardial infarction.^[1-3] The development of CAN is associated with the lesion of the autonomic nervous system, and may be accompanied by coronary vessels ischemia, arrhythmias, "silent" myocardial infarction (MI), severe orthostatic hypotension (OH) and sudden death syndrome.^[1,4-5]

Although it is common complication, the significance of CAN has not been fully appreciated and there are no unified treatment algorithms for today. Treatment is based on early diagnosis, life style changes, optimization of glycaemic control and management of cardiovascular risk factors. Pathogenetic treatment of CAN includes: balanced diet and physical activity; optimization of glycaemic control; treatment of dyslipidaemia; antioxidants, first of all α -lipoic acid, aldose reductase inhibitors, acetyl-L-carnitine; vitamins, first of all fat-soluble vitamin B1 (benfotiamine); correction of vascular endothelial dysfunction; prevention and

treatment of thrombosis; in severe cases-treatment of OH.^[6-9]

Prolongation of QT_i has been defined as a QT_c (corrected QT for HR).^[10] Hyperinsulinaemia can induce reversible prolongation of QT_c in healthy subjects, hyperglycaemia and acute hypoglycaemia can induce the prolongation of QT_c in both healthy and diabetic patients.^[10-12] In patients with DM prolongation of QT_c was found out during overnight hypoglycaemia and support an arrhythmic basis for the "dead in bed" syndrome.^[13]

The QRS-T angle, defined as the angle between the mean QRS and T vectors, indicates the main orientation of electrical heart activity during ventricular depolarization and repolarization, and has recently become an area of research interest. A wider QRS-T angle reflects an abnormal arrangement of ventricular repolarization and has been considered as a strong and independent risk indicator for cardiac morbidity and mortality compared to other traditional cardiovascular risk factors and electrocardiographic (ECG) risk indicators such as the length of the QT interval.^[14-16]

The spatial QRS-T angle has recently been shown to be a strong and independent predictor of cardiac mortality for

various patient groups such as coronary artery disease (CAD)^[17], heart failure^[18], type 2 diabetes mellitus (T2DM)^[19], elderly subjects.^[20,16]

Thus, we aimed to evaluate the effects of benfotiamine (a lipid-soluble thiamine derivative with higher bioavailability than thiamine) on the QTc, QT dispersion (QTd), spatial QRS-T angle in patients with T2DM and advanced stage of CAN.

MATERIALS AND METHODS

To explore the effectiveness of some above mentioned compounds we examined 32 patients with T2DM and definite stage of CAN, patients were aged between 50-59 years with disease duration 1-6 years and median glycosylated hemoglobin A1c (HbA1c) $7.1\% \pm 0.4\%$. Clinical characteristics of studied patients with T2DM and definite stage of CAN are given in Table 1.

Table 1: Baseline characteristics of patients included in this study.

Parameter	Patients with T2DM and definite stage of CAN (n = 32)	
	Control (n = 15)	Benfotiamine (n = 17)
	Group 1	Group 2
Age (years)	55.33 ± 0.95	54.12 ± 0.65
Gender		
Male (%)	8/53.3%	10/58.8%
Female (%)	7/46.7%	7/41.2%
Diabetes duration (years)	3.6 ± 0.42	4.06 ± 0.36
BMI (kg/m ²)	28.89 ± 0.16	26.66 ± 0.32
Medications		
ACE inhibitors (%)	12/80%	14/82.4%
β-blockers (%)	3/20%	4/23.5%
Metformin (%)	11/73.3%	11/64.7%
Sulfonylurea (%)	1/6.7%	1/5.9%
Combined hypoglycaemic therapy (%)	3/20%	5/29.4%
Hypertension (%)	12/80%	16/94.12%

T2DM: type 2 diabetes mellitus; CAN: cardiac autonomic neuropathy; BMI: body mass index; ACE: angiotensin-converting enzyme.

CAN was diagnosed according to previously proposed criteria.^[1] The work was done according to the principles of the Helsinki Declaration II and was approved by the medical ethics committee of Danylo Halytsky Lviv National Medical University. All participants signed an informed consent prior to their inclusion in the study.

Patients with T2DM and definite stage of CAN were allocated to two treatment groups: first group received traditional antihyperglycaemic therapy (n = 15, control group); patients in group 2 (n = 17) received in addition to standard treatment-benfotiamine (BFT) 300 mg/d. The duration of the treatment was three months.

The concentration of glucose in the blood was determined by the glucose oxidase method while HbA1c level was assessed by using a highly sensitive method of ion exchange liquid chromatography with D-10 analyzer and BIO-RAD reagents (United States).

Resting 12-lead surface ECG with a paper speed of 25 mm/s and a signal size of 10 mm/mV was recorded in the morning period. QTc was calculated by dividing the QT interval by the square root of the preceding normal-to-normal (NN) interval time series (Bazett's formula: $QTc = QT/\sqrt{NN}$).^[21] QTd was calculated as the difference between the maximum and minimum QTc. ECG-derived

measure of the difference in mean vectors of depolarization and repolarization (QRS-T angle). The absolute difference between the frontal QRS wave axis and T-wave axis was defined as frontal planar QRS-T angle. If such a difference exceeded 180°, the difference was calculated by subtracting from 180°.^[22] We performed resting ECG analysis included measurement of following parameters: heart rhythm, HR, conduction intervals and Holter-ECG [(ECG "EC-3H" ("Labtech," Hungary)] analysis included measurement of 24 hours ECG, circadian indexes and heart rate variability (HRV) parameters.^[23]

Statistical analysis

Statistical analysis was based on the variational method using statistical parametric t-test, nonparametric Wilcoxon t-test and Fisher's Pearson correlation coefficient. Data are presented as mean ± standard error of the mean (SEM). All tests were performed using the ANOVA (MicroCal Origin v. 8.0) software. Statistical significance was set at $p < 0.05$.

RESULTS

We found out that the HbA1c of patients with T2DM and advanced stage of CAN was not statistically significant influenced by the treatment ($p > 0.05$).

The features of the QTc, QTd and spatial QRS-T angle parameters in patients with T2DM and advanced stage of CAN after treatment with BFT are given in Table 2.

Table 2: Changes of the QTc, QTd and QRS-T parameters in patients with T2DM and CAN after 3-months of benfotiamine therapy ($\Delta\%$, Mean \pm SEM).

Parameter	Patients with T2DM and advanced stage of CAN (n = 32)			
	Groups	Baseline	After treatment	% change from baseline
QTc (ms)	Control group	433.4 \pm 6.45	427.8 \pm 4.72	-1.1% \pm 1.44%
	Benfotiamine	423.1 \pm 5.76	392.4 \pm 7.74^a	-7.3% \pm 1.36%
QTd (ms)	Control group	50.3 \pm 4.53	46.0 \pm 4.98	-5.6% \pm 6.97%
	Benfotiamine	58.1 \pm 3.94	39.4 \pm 4.42^a	-27.7% \pm 9.0%
QRS-T angle ($^{\circ}$)	Control group	78.0 \pm 6.44	69.7 \pm 4.27	-6.1% \pm 5.52%
	Benfotiamine	88.6 \pm 6.4	59.3 \pm 5.15^a	-24.4% \pm 10.2%

The results are presented as absolute values and as % change from baseline, ($\Delta\%$, Mean \pm SEM); ^ap < 0.01, compared to baseline. T2DM: type 2 diabetes mellitus; CAN: cardiac autonomic neuropathy; QTc: corrected QT interval; QTd: QT interval dispersion; QRS-T angle: spatial QRS-T angle.

Obtained results of this study could prove that prescription of BFT is accompanied by more significant decrease of QTc, QTd and QRS-T angle parameters compared to patients in control group (Table 2). As a result of our studies, it was found out that treatment with BFT contributed to a decrease in resting tachycardia [110 to 96 beats/min (p < 0.05)], improvement of subjective feeling and increase in tolerance of exercise loading. In addition in the majority of the patients with diabetic polyneuropathies (DPN) we observed the decrease and/or disappearance of pain, paresthesia, frequency of muscle cramps, improvement and/or restoration of tactile, vibration and temperature sensitivity.

DISCUSSION

In our previously investigations we have found out that in patients with T2DM and definite stage of CAN the QRS-T [78,3 \pm 1,95⁰ (p < 0.001)]; QTc [431.4 \pm 2.94 ms (p < 0.001)] and QTd [53.7 \pm 1.49 ms (p < 0.01)] were prolonged compared to patients without CAN.^[5] An association between CAN and QT interval prolongation was demonstrated in many studies and it may predispose to sudden death in DM.^[24,25] Increased QTd was also suggested as a marker of diabetic autonomic neuropathy.^[26] Most of the data regarding QT interval and diabetic CAN are in T1DM with only few studies in T2DM.^[27,28]

The pathogenesis of QTc prolongation is multifactorial and includes imbalance in cardiac sympathetic innervation, intrinsic metabolic and electrolytic myocardial changes, left ventricular (LV) hypertrophy, CAD, and genetic factors could lead to QTc prolongation.^[11] The day-night modulation of the QT/relative risk relation-on 24-h ECG recordings-was altered in CAN patients free of CAD, LV dysfunction, or hypertrophy, with a reversed day-night pattern and an increased nocturnal QT rate dependence.^[29] Reversible QTc prolongation may be induced by hyperinsulinaemia in healthy subjects, by hyperglycaemia and by acute hypoglycaemia in both healthy and diabetic subjects.^[11,29,12] In T1DM patients, prolonged QTc was

shown to occur frequently during overnight hypoglycaemia and was associated with cardiac rate/rhythm disturbances. These findings support an arrhythmic basis for the “dead in bed” syndrome and possibly a provocative role of hypoglycaemia-induced sympathetic activation in cardiovascular events.^[1,13,5]

Professor *Valensi P. et al.* demonstrated that changes in QTc can be considered as markers of cardiovascular autonomic dysfunction and considered as an important component in the potential prognostic value of the risk of arrhythmias.^[29] Preserving the function of the parasympathetic nervous system in patients with T2DM with CAN performs a protective function, and the predominance of the sympathetic nervous system or the imbalance of LF/HF [low- (LF) and high-frequency (HF) bands in HRV the ratio of the powers in those frequency bands, the so called LF-HF ratio (LF/HF)] is harmful to the electrophysiological activity of the myocardium and may lead to changes in QRS-T.^[14,15,30]

The spatial QRS-T angle was independently associated with glycaemic control, dyslipidaemia, and LV myocardial performance in the diabetic subjects.^[14] In the Rotterdam Study, spatial QRS-T angle values $\geq 105^{\circ}$ were found in 20% of patients with T2DM and were associated with increased risk of cardiovascular mortality and sudden cardiac death. A spatial QRS-T angle < 75 $^{\circ}$ was also significantly associated with increased risk for all clinical outcomes.^[31]

One recent study demonstrated that the spatial QRS-T angle is significantly wider in subjects with T2DM and CAN.^[15] Moreover, presence and severity of CAN were the strongest predictors of the spatial QRS-T angle values. HRV parameters were significantly and independently associated with the spatial QRS-T angle, and explained almost 50% of its variability, suggesting the presence of a common pathophysiological ground linking the structural, functional and electrical myocardial disturbances in DM. Additionally, from the clinical point of view, a wider spatial QRS-T angle in uncomplicated subjects with T2DM may point out to the presence of CAN, which is often underdiagnosed.^[14]

Diabetes might be considered as thiamine deficiency (TD) state, if not in absolute terms at least relative to the increased requirements deriving from accelerated and amplified glucose metabolism in non-insulin dependent

tissues that, like the vessel wall, are prone to complications.^[32-34] Some oxidized thiamine metabolites could also play role in pathogenesis of diabetes complications. It is reported that plasma thiamine levels are decreased by 75% in T2DM patients.^[32] The TD in clinical DM may increase the fragility of vascular cells to the adverse effects of hyperglycaemia and there by the increase of the risk of developing microvascular complications.^[33-36]

Thiamine and its derivatives have been demonstrated to prevent the activation of the biochemical pathways [increased flux through the polyol pathway, formation of advanced glycation end products (AGE's), activation of protein kinase C (PKC), and increased flux through the hexosamine biosynthesis pathway (HBP)] induced by hyperglycaemia in DM. TD plays a role in the diabetic endothelial vascular diseases (micro- and macroangiopathy), lipid profile, retinopathy, nephropathy, cardiopathy, and neuropathy.^[37] Thiamine acts as a coenzyme for transketolase (TKT) and for the pyruvate dehydrogenase and α -ketoglutarate dehydrogenase complexes, enzymes which play a fundamental role in intracellular glucose metabolism. TKT and glucose-6-phosphate dehydrogenase, the rate-limiting enzymes of the pentose-phosphates pathway, are inhibited in the diabetic heart under basal conditions.^[34] There is sufficient evidence to indicate that thiamine transporters and TKT activity are suppressed in DM. Restoring TKT activity via BFT or thiamin supplementation can increase the flux of glucose into hexose monophosphate (HMP) shunt, and also increase flux of glyceraldehyde 3-phosphate, and fructose 6-phosphate into HMP shunt and away from hyperglycaemia-induced pathways that lead to vascular damage.^[34,37]

Experiences from cardiology indicate that long-term increases in HRV and reduction in sudden cardiac death have only been shown with lipophilic agents that readily penetrate the blood nerve/blood brain barrier. In accordance with these observations experimental data indicate a preventive effect of BFT on the development of diabetic CAN.^[38,39] Thiamine supplementation can prevent hyperglycaemia-driven reductions in cell replication and proliferation as well as decreasing AGE's formation. BFT has been shown to prevent increased markers of HBP activity, intracellular AGE's formation, intracellular PKC activity and the nuclear factor kappa B (NF- κ B) activation seen with *in vitro* hyperglycaemic damage.^[40] Oral BFT in combination with the antioxidant alpha-lipoic acid treatment normalizes production of angiotensin-2, a marker of increased intracellular methylglyoxals in endothelial cells which contribute to AGE's formation, and N-acetylglucose modified protein, a marker of HBP activity.^[39] Treatment with BFT has been shown to reduce activation of the polyol pathway of glucose metabolism and to increase TKT expression in the presence of hyperglycaemia.^[40] Activation of AGE receptors in DM, found on

cardiomyocytes, pericytes, and podocytes, stimulates postreceptor signaling, intracellular reactive oxygen species formation, and altered gene expression, leading to vascular damage.^[34]

BFT significantly decreased production of pro-inflammatory mediators such as inducible form of nitric oxide (NO) synthase and NO; cyclooxygenase-2, heat-shock protein 70, tumor necrosis factor- α , interleukin (IL)-6, whereas it increased anti-inflammatory IL-10 production in lipopolysaccharide-stimulated BV-2 microglia. Moreover, BFT suppressed the phosphorylation of extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinases (JNK) and a serine/threonine protein kinase (Akt/PKB). Treatment with specific inhibitors revealed that BFT-mediated suppression of NO production was *via* JNK1/2 and Akt pathway, while the cytokine suppression includes ERK1/2, JNK1/2 and Akt pathways. Finally, the potentially protective effect is mediated by the suppression of translocation of NF- κ B in the nucleus. These findings suggest that thiamine/BFT may plays a role in modulating the inflammatory process.^[41] The explanations about positive thiamine effects were confined to hydrophobic thiamine metabolites that fulfill an important function under oxidative stress (OS) and nitrosyl stress.^[42]

A decrease in the blood supply to the heart caused by atherosclerosis or thrombosis is known to induce MI.^[37] The results of our study revealed that the appointment of BFT to patients with T2DM and advances stage of CAN were accompanied by a decrease of the thromboxane B2 (TxB2) concentration and TxB2/6-keto-prostaglandin F1alpha ratio, which may contribute to the improvement of the functional state of the prostacyclin I2-TxA2 system.^[5,43]

Benfotiamine supplementation for 14 wks (100 mg/kg/d) to streptozotocin-induced diabetic mice completely corrected hyperglycaemia-induced disruptions in Ca²⁺ homeostasis and mechanical functioning of cardiomyocytes.^[34] Cardiac OS is involved in heart failure that is induced by thiamine deprivation in rats. These findings suggest that thiamine modulates OS.^[37] Endothelial NO synthase (eNOS) and NO may play an important role in attenuating cardiac remodeling and apoptosis. BFT reduces OS and activates eNOS to enhance the generation and bioavailability of NO, and it subsequently improves the integrity of vascular endothelium to prevent sodium arsenite-induced experimental vascular endothelial dysfunction.^[34]

The identification of the association of polymorphisms related to the genes of thiamine and TKT with diabetic polyneuropathies (DPN) might be a first step in defining a DPN genetic risk profile with potential therapeutic repercussions. There is moderate evidence from preclinical experimental models that high-dose thiamine and BFT (1) inhibit the HMP, AGE's formation, and

diacylglycerol-PKC through the TKT activation; (2) target at various surrogate markers of hyperglycaemia-induced pathological processes and (3) can delay the progression of microangiopathic complications.^[36,44]

Benfotiamine treatment counters diabetes-induced cardiac mechanical dysfunction at the cellular level, associated with reduction in OS but not AGE's formation or cardiac protein carbonyl formation. This apparent discrepancy in BFT-elicited action on AGE's formation and OS (the oxidized/reduced glutathione ratio) seems to indicate that other mechanism(s) may predominantly contribute to diabetes-induced OS and cardiac contractile dysfunction in current experimental setting. Possible candidates may include alteration in glucose metabolism and PKC activation, although further study is warranted to verify involvement of these signaling pathways and beneficial effects of BFT against diabetic complications.^[45,46]

In patients with diabetic CAN, QTc prolongation should be avoided due to the risk of inducing severe ventricular arrhythmias. The relationship between diabetic CAN and CAD is intricate, as HRV is decreased in patients with CAD and decreased HRV has been shown to be a powerful predictor of cardiac mortality after MI.^[47,44]

The results of our study showed that the appointment of BFT in the treatment of patients with T2DM and advances stage of CAN for 3 months contributed to a decrease in the QTc, QTd and QRS-T angle parameters. Therefore, BFT may have therapeutic potential for neurological diseases by inhibiting inflammatory mediators and enhancing anti-inflammatory factor production.^[48,37,49]

CONCLUSION

Benfotiamine supplementation may provide benefits in the prevention of other diabetes-related vascular and neuronal comorbidities. The mechanism of BFT influence on diabetic angio, neuropathies pathogenesis is not well-known. Thus, further investigations aimed to understand the mechanism of action and for confirmation of the beneficial effect of BFT on biochemical parameters, dynamics of independent cardiovascular tests, daily monitoring of ECG, arterial wall stiffness parameters among patients with T2DM, diabetic angio-, neuropathies and its associated comorbidities may be needed to validate this clinical findings.

In conclusion, the positive influences of BFT on decrease of the QTc, QTd, QRS-T angle by us are partly confirmed by its neurotropic, cardioprotective and angioprotective properties; suggests the feasibility of its usage in the complex treatment of patients with T2DM and advances stage of CAN.

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