Research-Article

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An anxiolytic drug buspirone ameliorates hyperglycemia and endothelial dysfunction in type 2 diabetic rat model

[Anksiyolitik bir ilaç olan buspiron tip 2 diyabet sıçan modelinde hiperglisemi ve endotelyal disfonksiyonu düzeltiyor]

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Abstract

Objectives: Type 2 diabetes is a common metabolic disease and anxiety disorders are very common among diabetics. Buspirone is used in the treatment of anxiety, also having blood glucose-lowering effects. The aim of the study was to investigate the effects of buspirone on the glucose and lipid metabolism as well as vascular function in type 2 diabetic rats.
Methods: A type 2-diabetic model was induced through a high-fat diet for eight weeks followed by the administration of low-dose streptozotocin (35 mg/kg, intraperitoneal) in rats. Buspirone was given at two different doses (1.5 mg/kg/d and 5 mg/kg/d) and combined with metformin (300 mg/kg/d). The fasting glucose and insulin levels, lipid profile were analyzed, and vascular response measured from the thoracic aorta was also evaluated.
Results: Both doses of buspirone caused a significant improvement in fasting blood glucose levels. In particular, the buspirone treatment, combined with metformin, improved endothelial dysfunction and was found to be correlated with decreased nitrate/nitrite levels.
Conclusions: Buspirone may be effective in the treatment of type 2 diabetes, either alone or in combination with other treatments, particularly in terms of endothelial dysfunction, inflammation and impaired blood glucose, and insulin levels.

Keywords: buspirone; endothelial dysfunction; lipids; type 2 diabetes; vascular responses.

Öz

Amaç: Tip 2 diyabet yaygın bir metabolik hastalıktır ve anksiyete bozuklukları diyabetikler arasında çok yaygındır. Buspiron, anksiyetenin tedavisinde kullanılır ve aynı zamanda kan şekerini düşürücü etkilere sahiptir. Bu çalışmanın amacı, tip 2 diyabetik sıçanlarda buspironun glikoz ve lipid metabolizması üzerindeki ve vasküler fonksiyonlara olan etkilerini araştırmaktır.
Gereç ve Yöntemler: Tip 2-diyabetik model, sekiz hafta boyunca yüksek yağlı bir diyet ve ardından sıçanlara düşük dozlu streptozotosin (35 mg/kg, intraperitoneal) verilerek oluşturuldu. Buspirone iki farklı dozda (1.5 mg/kg/gün ve 5 mg/kg/gün) verildi ve metformin (300 mg/kg/gün) ile

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combine edildi. Açlık glikoz ve insülin düzeyleri, lipid profili analiz edildi ve torasik aorttan ölçülen vasküler yanıtlar değerlendirildi.

Bulgular: Her iki buspirone dozu açlık kan şekeri seviyelerinde önemli bir düzelmeye neden oldu. Özellikle, metformin ile kombine uygulanan buspiron tedavisi, endotelyal disfonksiyonu düzeltti ve nitrat/nitrit seviyelerinin azalması ile ilişkili bulundu.

Sonuç: Buspiron, tip 2 diyabetin tedavisinde tek başına veya diğer tedavilerle kombinasyon halinde, özellikle endotelyal disfonksiyon, inflamasyon ve bozulmuş kan şekeri ve insülin seviyeleri açısından etkili olabilir.

Anahtar Kelimeler: Tip 2 diyabet; buspiron; endotel disfonksiyonu; lipitler; vasküler yanıtlar.

Introduction

Type 2 diabetes mellitus is a chronic metabolic disease that is characterized by hyperglycemia and insulin resistance [1, 2], with increasing prevalence all around the world [3, 4]. Neuropsychiatric diseases such as anxiety disorders, attention-deficit hyperactivity disorder (ADHD), neurodevelopmental disorders, and bipolar disorder are common in diabetic patients [5]. However anxiolytic drugs have the risk of increasing plasma glucose and insulin levels [6]. Buspirone is a sedative, hypnotic, and anxiolytic drug that is also a partial agonist of the serotonin (5-hydroxy tryptamine (5-HT)) 5-HT1A receptors. It is shown that pancreatic β cells to synthesize and store 5-HT and 5-HT receptors (5-HTR) [7]. It has been known that 5-HT is present in the same vesicle with insulin in pancreatic β-cell, and 5-HT has been associated with the regulation of the blood glucose concentration. Islet 5-HT acts in an autocrine/paracrine manner through the 5-HT3 receptor to increase the glucose responsiveness of β-cells and thereby increases the overall islet glucose-stimulated insulin secretion [8].

Many studies have identified that there is a strong relationship with diabetes and macrovascular complications such as coronary artery disease, peripheral arterial disease, stroke, and microvascular complications such as retinopathy, diabetic nephropathy and neuropathy. [1, 8, 9]. Endothelial dysfunction is an important contributor to the progression of atherosclerosis in type 2 diabetes mellitus [9, 10]. On the other hand, oxidative stress is a leading factor in the development of vascular dysfunction, as well as dyslipidemia [11]. In assessments of cardiovascular risk, endothelial response and metabolic parameters must be evaluated together. There are studies of buspirone on glycemic control and stress induced hyperglycemia are available. Raghunathan et al. [12] reported that buspirone therapy prevented metabolic abnormalities and cardiac complications in streptozotocin (STZ)-induced type 1 diabetic rats. However, till date, there is no data available on effect of buspirone on type 2 diabetes and vascular responses.

The present study investigates the effects of buspirone on the glucose and lipid metabolism, as well as vascular function, in a type 2 diabetes mellitus model involving rats induced with low dose STZ and a high fat diet (HFD).

Materials and methods

Thirty-six male Wistar rats [250–350 g] were used in the study. The rats were given access to laboratory food and water *ad libitum* and were housed in cages under standard laboratory conditions (12 h light–dark cycle at a room temperature (21 ± 2 °C), relative humidity of 55%). The study protocol was approved by the Ethics Committee of Manisa Celal Bayar University, Turkey [07.01.2015/77.637.435-06]. The rats were divided into six groups (n=6 per group):

Control group (C) treated with saline solution intraperitoneally (i.p.); STZ-induced diabetics (HFD + diabetes mellitus (DM)); diabetics treated with metformin 300 mg/kg/daily (MET); diabetics treated with buspirone 1.5 mg/kg/daily as (BUS 1.5); diabetics treated with buspirone 5 mg/kg/daily (BUS 5); and diabetics treated with metformin 300 mg/kg/daily + buspirone 1.5 mg/kg/daily (MET + BUS). Type 2 diabetic rat model was developed using an HFD plus multiple low doses of STZ is commonly used [13, 14]. The HFD which was contain 48% carbohydrate, 22% fat, and 20% protein and 44.3 kJ/kg calories. The control rats fed with regular chow contain of 53% carbohydrate, 5% fat, and 23% protein, and 25 kJ/kg total calorific value (Bilgen Lab. Istanbul, Turkey).

Following 8 weeks of dietary intervention, the diabetic group was injected i.p. with low doses of streptozotocin (Sigma, St. Louis, MO, USA, 35 mg/kg, dissolved in 0.1 M sodium citrate buffer, pH 4.5). One week later, blood samples were collected by tail cutting for fasting blood glucose measurements were measured by (Accu-Check Active – glucometer). Rats with fasting blood glucose were less than 300 mg/100 mL were injected i.p. with a low dose of STZ (30 mg/kg). Control rats were given vehicle citrate buffer (pH 4.4) in a matched volume (0.25 mL/kg) via intraperitoneal injection. Control group fed continued with regular chow, and other three groups were given high-fat diet for Four weeks than fasting blood glucose levels were measured again. Four weeks after the streptozotocin injection, the fasting blood glucose was measured again, and rats with a fasting blood glucose of ≥300 mg/100 mL considered diabetic [12].

The rats were allowed to continue to feed on their respective diets until the end of the study. Body weights and were measured at the beginning and at the end of the study. Metformin and buspirone given directly into the stomach by oral gavage daily for four weeks (dissolved in distillated water).

At the end of treatment, the animals were anesthetized by using an i.p. injection urethane (1000 mg/kg, 20%) after fasting 12 h. Thoracic aorta was removed, placed in cold Krebs–Henseleit solution, cleaned gently of adherent connective tissue and cut into rings (approximately 3 mm length). Blood samples from abdominal aorta

were collected, when rats were killed, for the measurement of glucose, insulin, nitric oxide (NO), lipid metabolic parameters, and some antioxidant enzyme levels.

The aortic rings were suspended in organ chambers (20 mL) oxygenated (5% CO_2, 95% O_2) and warmed (37 °C) Krebs solution (pH: 7.4), with the following composition [mM]: NaCl 118.0, KCl 4.7, $NaHCO_3$ 25.0, NaH_2PO_4 1.2, $CaCl_2$ 1.8, $mgSO_4$ 1.2, and dextrose 11.0. Aorta will be connected to isometric force transducers, and measured isometric contractions than record on computer using a transducer data acquisition system (MP 150 Biopac systems, Ankara, Turkey).

After 1 h washing and equilibration, contractile responses to phenylephrine were measured in the rings to evaluate the precontractile tone in the vessels. Phenylephrine (10^{-9}–10^{-6} M) was applied cumulatively to the bath, until a maximum response was achieved. Relaxation responses were induced by acetylcholine and sodium nitroprusside (SNP) obtained from aortic rings which are precontracted with a submaximal concentration of phenylephrine. Acetylcholine (10^{-9}–10^{-5} M) was used to investigate endothelium-dependent relaxations, to the precontracted rings by phenylephrine (10^{-9}–10^{-5} M). SNP used to analyze endothelium-independent relaxations. (10^{-9}–10^{-6} M).

Glucose oxidase method used to measure plasma glucose levels (Contour TS Bayer, Basel, Switzerland). Serum insulin concentrations were measured using a rat insulin ELISA kit (Millipore, Billerica, MA). Intraassay CV levels were 3.22% at 1.15 ng/mL, 1.33% at 2.32 ng/mL and 1.17% at 3.65 ng/mL. Triglyceride, very low-density lipoprotein (VLDL), high-density lipoprotein (HDL) and total cholesterol were determined with colorimetric method in an automatic immunoanalyzer (Beckman Unicell DxC 800). Friedewald equation was used to calculate LDL levels [15]. A mathematical formula has been used to assess the sensitivity of tissue to homeostasis (Homeostatic model assessment-insulin resistance(HOMA-IR)): HOMA-IR: (Fasting plasma insulin (x fasting plasma glucose/405 [16]. The C-reactive protein (CRP) activity and the plasma malondialdehyde (MDA) levels was measured using the CRP and MDA test kits, respectively (Shanghai, Çhina). MDA and CRP analyzes were performed with the Sunred Rat (MDA) Eliza kit (Shanghai, China) in serum.

Measurement of total nitrate/nitrite concentrations were measured by NO Cayman fluorometric assay kit (Ann Arbor, MI). Intraassay CV levels were 2.5% at 30 µmol/L, 1.4% at 77.2 µmol/L, and 1.6% at 138.0 µmol/L. Interassay CV levels were found to be 4.6% at 31.5 µmol/L, 3.6% at 79.9 µmol/L, 3.4% at 138.7 µmol/L. The minimum measurement limit is 0.25 µmol/L.

SPSS for Windows version 23.0 software was used for the statistical analyses (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in mean and standard error of the mean (SEM). The GLM-repeated measure post-hoc Dunnett test was used to analyze differences between the concentrations in isometric vascular responses. The differences among diabetic and non-diabetic groups were analyzed using a one-way analysis of variance [ANOVA] with a *post-hoc* Dunnett test. A p value of <0.05 was considered statistically significant.

Results

The body weights of the normal and the diabetic rats are presented in Table 1. The body weights of all animals had increased at the end of the study when compared to the baseline. When the weights of the different groups were compared at the end of the study, the diabetic group on an HFD was found to have gained significantly more weight than all of the other groups. Buspirone treatment resulted in no change in weight in the animals.

Biochemical analysis

In the untreated diabetic group, fasting blood glucose levels were significantly increased when compared to the control group, and significant reductions in blood glucose values were noted in all of the treated experimental groups (buspirone, metformin and combined group) (Table 2) ($p < 0.05$). Both low-and high-dose buspirone treatments reduced blood glucose levels ($p < 0.01$).

In the diabetic group, insulin levels increased significantly ($p < 0.05$), although the metformin, high-dose buspirone and combined treatment groups showed a statistically significant decrease when compared to the untreated diabetic group (MET and BUS 5 $p < 0.05$, combined treatment $p < 0.0001$). The HOMA-IR index increased in diabetic animals ($p < 0.0001$), while metformin, high-dose buspirone and combined treatment decreased when compared to the diabetic group (respectively, $p < 0.005$, $p < 0.01$, $p < 0.0001$).

The blood lipid profiles of all groups are shown in Table 3. LDL and total cholesterol levels increased significantly in the diabetic groups ($p < 0.001$), LDL levels were not changed all treatment groups than the diabetic group and also HDL cholesterol levels showed no difference among the groups. In the diabetic group, triglyceride and VLDL levels increased (both of, $p < 0.05$). Both triglyceride and VLDL levels decreased in all treatment groups.

A comparison of the serum nitrite/nitrate levels of the groups revealed a reduction in the BUS 5 and combined

Table 1: Body weight values of the animals.

Groups	Initial body weight (g)	Final body weight (g)
Control	221.14 ± 7.36	328.12 ± 9.43
DM	231.61 ± 12.06	387.50 ± 8.07[a]
MET	235.00 ± 7.94	303.57 ± 10.80
BUS 1.5	221.25 ± 9.62	320.76 ± 14.48
BUS 5	234.16 ± 8.02	316.12 ± 14.12
MET + BUS	225.00 ± 7.04	308.30 ± 10.81

DM, Diabetic group; MET, Diabetic group treated with metformin 300 mg/kg/d; BUS 1.5, Diabetic group treated with buspirone 1.5 mg/kg/d; BUS 5, Diabetic group treated with buspirone 5 mg/kg/d; MET + BUS, Diabetic group treated with metformin (300 mg/kg/d) + buspirone 1.5 mg/kg/d. The results are shown as Values ± SEM in all the groups.

[a]$p < 0.05$ compared with other groups.

Table 2: Fasting blood glucose, insulin levels, and homeostatic model assessment-insulin resistance (HOMA-IR).

Groups	Fasting blood glucose (mg/dL)	Insulin (μIU/mL)	HOMA-IR
Control	126 ± 3.073	7.80 ± 0.69	2.43 ± 0.24
DM	471 ± 43.89[a]	12.82 ± 0.80[a]	14.89 ± 1.66[a]
MET	259 ± 17.32[a,b]	7.49 ± 1.44[b]	7.40 ± 1.44[a,b]
BUS 1.5	245 ± 13.25[a,b]	11.47 ± 1.25[a]	13.07 ± 1.76
BUS 5	270 ± 29.91[a,b]	8.39 ± 1.07[b]	8.01 ± 0.87[a,b]
MET + BUS	260 ± 28.32[a,b]	3.56 ± 0.98[a,b,c]	5.15 ± 1.25[a,b,c]

DM, Diabetic group; MET, Diabetic group treated with metformin 300 mg/kg/d; BUS 1.5, Diabetic group treated with buspirone 1.5 mg/kg/d; BUS 5, Diabetic group treated with buspirone 5 mg/kg/d; MET + BUS, Diabetic group treated with metformin (300 mg/kg/d) + buspirone 1.5 mg/kg/d. The results are shown as Values ± SEM in all the groups.

[a]p < 0.05 compared with control group.

[b]p < 0.05 compared with diabetic group.

[c]p < 0.05 compared with MET, BUS 1.5, and BUS 5 groups.

Table 4: Serum nitrite/nitrate, malondialdehyde (MDA) and C-reactive protein (CRP) levels.

Groups	Nitrite/nitrate (μmol/L)	MDA (ng/mL)	CRP (ng/mL)
Control	16.64 ± 1.39	107.99 ± 9.74	0.94 ± 0.16
DM	15.20 ± 1.66	128.68 ± 7.21	2.47 ± 0.14[b]
MET	13.50 ± 0.94	134.82 ± 7.81	0.96 ± 0.49
BUS 1.5	11.54 ± 0.90	141.85 ± 20.16	0.99 ± 0.35
BUS 5	9.84 ± 0.90[a]	116.84 ± 26.26	0.99 ± 0.26
MET + BUS	9.40 ± 0.65[a]	133.07 ± 14.33	0.91 ± 0.17

DM, Diabetic group; MET, Diabetic group treated with metformin 300 mg/kg/d; BUS 1.5, Diabetic group treated with buspirone 1.5 mg/kg/d; BUS 5, Diabetic group treated with buspirone 5 mg/kg/d; MET + BUS, Diabetic group treated with metformin (300 mg/kg/d) + buspirone 1.5 mg/kg/d. The results are shown as Values ± SEM in all the groups.

[a]p < 0.05 compared with control and diabetic groups.

[b]p < 0.05 compared with other groups.

Table 3: Lipid profile.

Groups	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	Triglyceride (mg/dL)	VLDL cholesterol (mg/dL)
Control	49.0 ± 3.42	29.83 ± 2.63	1.67 ± 0.21	74.33 ± 12.70	14.67 ± 2.54
DM	76.6 ± 8.91[a]	33.2 ± 1.59	38 ± 1.22[a]	167.2 ± 55.32[a]	33.2 ± 11.08[a]
MET	72.4 ± 6.91[a]	39.2 ± 4.64	15.8 ± 0.97[a,b]	96.6 ± 19.92	19.4 ± 4.01
BUS 1.5	82.2 ± 9.08[a]	42 ± 6.78	24.4 ± 2.42[a,b]	119.2 ± 7.89	23.8 ± 5.09
BUS 5	80.0 ± 10.28[a]	38.4 ± 2.38	18.8 ± 2.35[a,b]	119.6 ± 6.77	23.8 ± 5.15
MET + BUS	80.8 ± 11.25[a]	36.2 ± 2.35	7.8 ± 0.37[a,b,c]	107.8 ± 5.30	20 ± 4.91

DM, Diabetic group; MET, Diabetic group treated with metformin 300 mg/kg/d; BUS 1.5, Diabetic group treated with buspirone 1.5 mg/kg/d; BUS 5, Diabetic group treated with buspirone 5 mg/kg/d; MET + BUS, Diabetic group treated with metformin (300 mg/kg/d) + buspirone 1.5 mg/kg/d. The results are shown as Values ± SEM in all the groups.

[a]p < 0.05 compared with control group.

[b]p < 0.05 compared with diabetic group.

[c]p < 0.05 compared with MET, BUS 1.5 and BUS 5 groups.

treatment groups, compared to the other groups (both $p < 0.05$). No significant difference was identified in the MDA levels of the groups ($p>0.05$). Blood CRP levels were increased in the diabetic group (all $p < 0.0001$) and decreased in all of the treatment groups (all $p < 0.0001$) (Table 4).

Vascular activity

As shown in Figure 1A, contractile responses to phenylephrine decreased in the untreated diabetic group, when compared to the other groups ($p < 0.05$). Phenylephrine-induced contractions increased in the diabetic aorta in patients treated with metformin and with a combined treatment (both $p < 0.05$). Acetylcholine-induced relaxation decreased significantly in the untreated diabetic rats when compared to the control rats ($p < 0.001$). Doses of buspirone, as well as metformin and combined treatment, improved diabetes-induced decrease in the relaxation response observed with an acetylcholine effect ($p < 0.0001$) (Figure B). SNP induced relaxation responses were also similar among the groups (Figure C).

All correlation analyses are shown in Table 5. We identified a strong negative correlation between insulin levels and phenylephrine-induced contraction responses (phenylephrine 10^{9} concentration, $r=-0.586$, $p=0.001$; phenylephrine 10^{8} concentration, $r=-0.534$, $p=0.002$), and a very strong negative correlation between CRP levels and acetylcholine-induced relaxation responses (acetylcholine

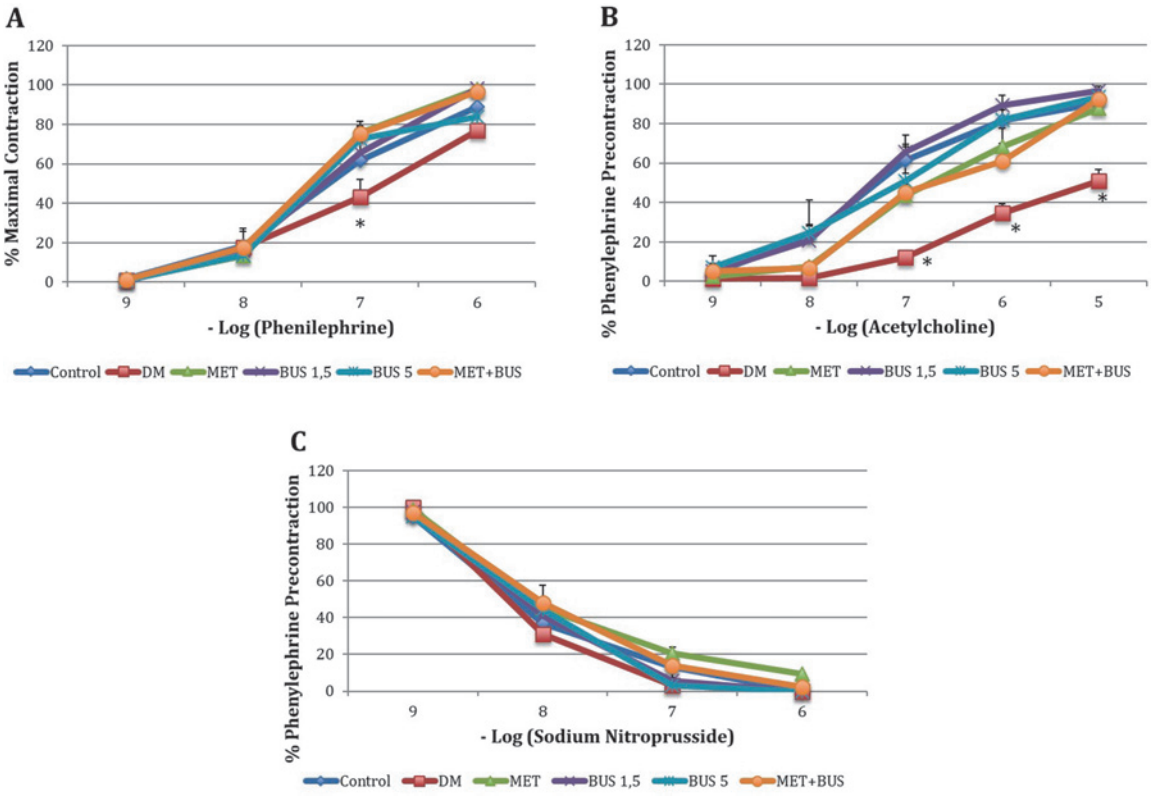


Figure 1: Vasocontractile responses. (A) Contraction responses obtained with phenylephrine in vascular strips. (B) Relaxation responses obtained with acetylcholine in vascular strips. (C) Relaxation responses obtained with sodium nitroprusside (SNP) in vascular strips. DM; Diabetic group, MET; Diabetic group treated with metformin 300 mg/kg/d, BUS 1.5; Diabetic group treated with buspirone 1.5 mg/kg/d, BUS 5; Diabetic group treated with buspirone 5 mg/kg/d, MET + BUS; Diabetic group treated with metformin (300 mg/kg/d) + buspirone 1.5 mg/kg/d. The results are shown as Values ± standard error of the mean (SEM) in all the groups. *p < 0.05 compared with other groups.

10^5 concentration, r=−0.752, p=0.0001) (Figure 2A). There is a moderate positive correlation between LDL and cholesterol levels (r=0.432, p=0.015).

Strong positive correlation was also found between LDL and HOMA-IR calculations (r=0.589, p=0.0001), and strong negative correlation was found between LDL and nitrate/nitrite levels. In addition, a strong positive correlation was noted between insulin levels and the HOMA-IR calculation (r=0.766, p=0.0001).

Table 5: Correlation analysis results.

	Phenilephrine	Acetylcholine	LDL
LDL	r=−0.463, p=0.009	r=−0.587, p=0.0001	1
Insulin	r=−0.586, p=0.0001	r=−0.315, p=0.084	r=0.629, p=0.0001
CRP	r=−0.388, p=0.034	r=−0.752, p=0.0001	r=0.690, p=0.0001
HOMA-IR	r=−0.382, p=0.034	r=−0.391, p=0.030	r=0.838, p=0.0001

Discussion

The present study showed that diabetes impaired the vascular response, decreasing phenylephrine-induced contraction and acetylcholine-induced relaxation. Diabetes also changed the lipid profile and increased CRP levels. Low- and high-dose buspirone treatment combined with metformin improved the vascular responses, while high-dose buspirone and combined treatment decreased insulin levels and decreased insulin resistance. LDL levels were increased in DM and were not decreased in all the treatment groups. Nitrate/nitrite levels decreased with both high-dose buspirone and combined treatment, and CRP levels decreased in all treatment groups. Endothelial dysfunction is a condition that precedes atherosclerosis and it is predictor of cardiovascular complications [17, 18]. Endothelial dysfunction insulin resistance, hyperglycemia, increased LDL levels and high levels of CRP were developed in untreated diabetic animals. Impaired lipid metabolism damages to the endothelium [19]. Chronic low-

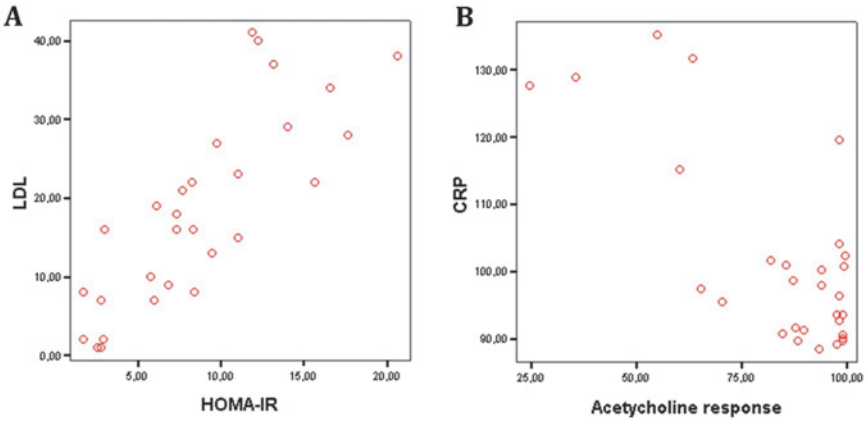


Figure 2: Correlation results. (A) Blood C-reactive protein (CRP) levels negative correlated with acetylcoline relaxation (10^{-5} M) response. (B) Blood LDL levels positively correlated with HOMA index. CRP; C – Reactive Protein, LDL; Low-Density Lipoprotein, HOMA-IR; Homeostatic Model Assessment for Insulin Resistance.

grade inflammation with production of high levels of inflammatory proteins such as CRP has been implicated in the development of type 2 diabetes. In many studies CRP was reported to be a strong indicator of diabetic cardiovascular diseases and an independent risk factor for predicting the development of type 2 diabetes mellitus [20, 21].

Acetylcholine stimulates the endothelium to release NO and cause vasodilatation [22]. When the NO production or bioactivity reduced decrease in vascular responses come out and it leads to endothelial dysfunction. Nitrite/nitrate are products of the NO metabolism and serve also as reservoirs in the formation of NO hence, measurement of nitrate and nitrite levels gives information of relaxation capability of the vessels [23, 24]. Nitrate/nitrite levels were decreased in the BUS 5 and combined treatment groups, and a moderate negative correlation was also found between the nitrate/nitrite levels and acetylcholine-induced relaxation. Based on these findings, it can be suggested that NO might have played a role in the relaxation response in these two groups. However, the association between hyperglycaemia and serum NO levels has not yet been fully clarified. There is no consensus regarding the effect of type 2 diabetes on nitrate/nitrite metabolism; both increased and decreased serum and urinary concentration of nitrate/nitrite were observed in type 2 diabetic patients [25].

In the present study there is also positive correlation between the HOMA-IR index and LDL levels. Insulin levels decreased, blood glucose levels improved, and HOMA-IR index values decreased in our treatment groups, although the values in the BUS 5 and combined treatment groups were lower than in the other groups. There are a number of studies evaluating the effect of buspirone on glycemic status in the literature [26, 27]. Buspirone decreased blood glucose levels in all studies, although its effects on insulin levels varied. Blood insulin levels increased in some studies, but remained unchanged in others. In many studies have shown that the nervous system can regulate insulin secretion from pancreatic β-cells [28]. In our study, buspirone reduced glucose levels, as well as insulin and HOMA-IR, although insulin and HOMA-IR decreased more in the combined treatment group. Metformin increases the insulin sensitivity of muscle and, then, decrease the glucose levels. Metformin is the most commonly used drug in the treatment of the type 2 diabetes by decreasing blood glucose and leads to a significant decrease in plasma fasting insulin levels [29]. This has led to its frequent use as a positive control.

In this study, inflammation decreased in all treatment groups. In previous studies, metformin was shown to reduce inflammation and to improve endothelial dysfunction by increasing NO bioavailability and reducing inflammation diabetic animal models [30]. The literature contains a few studies into the anti-inflammatory effects of buspirone. In one such article, intrathecal buspirone treatment was seen to decrease peripheral inflammation [31]. In another article, published more recently, IP buspirone treatment modulated inflammation via decreased inflammatory cytokines in a Parkinson rat model [32]. Finally, in our study, type 2 diabetic model did not lead to body weight loss in diabetic rats, compared to baseline body weights. In type 1 diabetes models, high-dose STZ injections have been shown to decrease body weight in rats. In type 2 diabetics, weight gain is a major problem, in our study, the body weights of diabetic rats increased, while no significant difference was observed between the other groups and the diabetic group at the end of the study.

As far as we know, this is the first study to show an improving effect of buspirone on vascular responses and metabolic parameters in type 2 diabetic rats. The lack of an experimental group that measures parameters in HFD-fed rats without the development of diabetes may be a limitation for our study. However, it is predicted that longer time is needed for lipid parameters and vascular changes in HFD-fed rats only without inducing diabetes.

Another limitation of this study is that the calculation of LDL with Friedwald formula may be misleading results in high-fat dietary rats.It was reported that in hypercholesterolemis rats the results clearly show that the Friedewald formula overestimates LDL levels [33]. It is thought that the in our study high LDL levels also related about this and we might be find the values higher than they are. Related to this, especially high fed diet groups our LDL results were higher than they expected in our study.

Based on these findings; clinical importance of this study is, buspirone appears to be effective in the treatment of type 2 diabetes, either alone or in combination with other treatments, particularly in terms of endothelial dysfunction, inflammation, and impaired blood glucose and insulin levels. Considering the number of patients with comorbidity of depression and diabetes buspirone may be considered as an appropriate treatment option in diabetes-related depression with additive effects of combination treatment. It is suggested that future studies will help to understand the underlying mechanism and pioneering clinical research that can be used as a treatment alternative in patients with diabetes and anxiety.

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