THE EFFECTS OF DPP4 INHIBITORS ON LIPID STATUS AND BLOOD PRESSURE IN RATS WITH DIABETES MELLITUS TYPE 2

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EFEKTI INHIBITORA DPP4 NA LIPIDNI STATUS I KRVNI PRITISAK PACOVA OBOLELIH OD DIABETES MELLITUS-A TIP 2

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ABSTRACT

The aim of the present study was to examine, evaluate and compare the effects of administered dipeptidyl peptidase -4 (DPP4) inhibitors saxagliptin and sitagliptin on lipid status parameters and blood pressure in rats with streptozotocine induced diabetes mellitus type 2. Forty-eight Wistar albino rats were divided randomly into 4 groups: 1. group I: control healthy group; 2. group II: rats with diabetes mellitus type 2; 3. group III: rats with diabetes mellitus type 2+ treated with 0.6 mg/kg of sitagliptin; 4. group IV: rats with diabetes mellitus type 2 treated with 0.45 mg/kg of saxagliptin. The rats from experimental groups were fed with a high-fat diet for 4 weeks and after 6–8 h of starvation received one dose of streptozotocin (STZ) intraperitoneally (25 mg/kg body weight) to induce type 2 diabetes mellitus (T2DM). Animals with fasting glucose above 7 mmol/L and insulin over 6 mmol/L were included in the study as rats with T2DM. Upon completion of the experiments, the blood was collected from the anesthetized animals and serum triglyceride (TG), total cholesterol (TCH), high density lipoprotein (HDL), and low density lipoprotein (LDL) were measured using spectrophotometry and commercial kits. At the beginning of the study and the day before sacrificing animals, the blood pressure and heart rate were measured by a tail-cuff noninvasive method. DPP4 inhibitors, as glucagon-like peptide-1 (GLP-1) agonists, were associated with modest reductions in DBP, LDL-C, TCH, and TGL and significant improvement in HDL, SBP and HR.

Keywords: *DPP4 inhibitors, lipid status, blood pressure, diabetes mellitus type 2, rats.*

SAŽETAK

Cilj ove studije je bio da ispita, proceni i uporedi efekte administriranih dipeptidil peptidaza-4 (DPP4) inhibitora saksagliptina i sitagliptina na parametre lipidnog statusa i krvnog pritiska kod pacova sa dijabetes mellitusom tipa 2 izazvanim streptozotocinom. Četrdeset osam Wistar albino pacova je svrstano u 4 grupe: 1. grupa I: kontrolna grupa zdravih pacova; 2. grupa II: pacovi sa diabetes mellitus tipom 2; 3. grupa III: pacovi sa diabetes mellitus tipom 2 tretirani sa 0,6 mg/kg sitagliptina; 4. grupa IV: pacovi sa diabetes mellitus tipom 2 tretirani sa 0.45 mg/kg saksagliptina. Pacovi iz eksperimentalnih grupa su hranjeni hranom sa visokim sadržajem masti 4 nedelje i nakon 6-8 sati gladovanja primili su jednu dozu streptozotocina (STZ) intraperitonealno (25 mg/kg telesne težine) radi izazivanja dijabetes melitusa tipa 2 (T2DM). Životinje sa glukozom natašte iznad 7 mmol/L i insulinom preko 6 mmol/L uključene su u studiju kao pacovi sa T2DM. Po završetku eksperimentalnog perioda, krv je sakupljena od anesteziranih životinja i serumski trigliceridi (TG), ukupni holesterol (TCH), lipoproteini visoke gustine (HDL) i lipoproteini niske gustine (LDL) su određivani spektrofotometrijski i korišćenjem komercijalnih kitova. Na početku studije i dan pre žrtvovanja životinja, krvni pritisak i srčana frekvenca su mereni neinvazivnom metodom repne pletizmografije. Inhibitori DPP4, kao agonisti glucagonu-sličnog peptida-1 (GLP-1), bili su povezani sa blagim redukcijama DBP, LDL-C, TCH i TGL i pozitivno su uticali na HDL, SBP i HR.

Ključne reči: *DPP4 inhibitori, lipidni status, krvni pritisak, diabetes melitus tip 2, pacovi.*

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INTRODUCTION

Cardiovascular diseases, such as coronary heart disease, cerebrovascular disease, and peripheral artery disease have remained the leading cause of morbidity and mortality worldwide. Across Europe and the USA, myocardial infarction represents direct cause of death in 40–50% of patients with diabetes, and the number of patients with diabetes that die as a direct result of ischemic heart disease is reported to be increasing (1). It is well known and explained that existing atherosclerotic vascular lesions and diabetes mellitus are with no doubt the strongest risk factors for further life-threatening vascular diseases. Hyperlipidemia and diabetes mellitus increase cardiovascular events via increasing atherosclerosis. What is more, collateral artery growth (arteriogenesis), which function is to compensate the loss of an artery due to atherosclerosis, is compromised in diabetes mellitus, a disease which is almost always associated with dyslipidemia (2). According to novel investigation, there is a high need for effective drugs that show positive effects in patients with diabetes, even with insulin resistance, but also express cardioprotective effects and therefore postpone or decrease the risk of cardiovascular complications in diabetic patients. Particularly, these drugs should have the capacity to promote arteriogenesis, which is a tissue-, and even life-saving, process. Dipeptidyl-peptidase 4 (DPP4/CD26) inhibitors might present such drugs.

Dipeptidyl peptidase-4 (DPP4) is a widely expressed protease that cleaves the N-terminal of peptides containing a penultimate alanine or proline, such as the incretins, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (or gastric inhibitory polypeptide GIP) (1). Drugs that inhibit DPP4 exhibit a decrease in degradation of these hormones, which improves glycemic control through increased glucose-mediated insulin secretion, decreased glucagon release and delayed gastric emptying. These hypoglycemic drugs of newer generation have shown to be effective, well tolerated and therefore, increasingly prescribed. In addition, recent research put the focus on potential cardioprotective and antihyperlipidemic effects of these drugs, beyond glycemic control, thus, making them an interesting and attractive therapeutic strategy, alone or in combination with other hypoglycemic drugs (2, 3). Not all representatives of this group of drugs express the same pharmacodynamics. For example, *in vitro* study has shown stronger inhibition of DPP4 and slower rate of dissociation from its active site of saxagliptine in comparison with sitagliptin and vildagliptin (4).

Besides outstanding glucose-lowering effects, DPP4 inhibitors have also shown other beneficial effects that are not of metabolic nature. This refers to anti-inflammatory effect and cardioprotective effect especially via blood pressure (BP) regulation. Recent findings suggest that sitagliptin could decrease systolic blood pressure (SBP), independently of glucose-lowering effect (5). Additionally, it was shown that sitagliptin exerts BP reduction effect both in diabetic and non-diabetic patients (6-9). Data provided from other studies have also confirmed hypotensive effects of DPP-4 inhibitors in animal models and supported this phenomenon (10-13). On the other hand, there is a growing number of evidence claiming the opposite - that no changes in BP were measured when compared to control group (14-17). Furthermore, some research groups have come to conclusion that DPP-4 inhibitors might even lead to the increase in BP when combined with ACE (angiotensin-converting enzyme) inhibitors (18).

Taken into consideration all mentioned above, the aim of the present study was to examine, evaluate and compare the effects of administered DPP4 inhibitors saxagliptin and sitagliptin on lipid status parameters and blood pressure in rats with streptozotocine induced diabetes mellitus type 2.

MATERIALS AND METHODS

Ethical approval

This research was carried out in the laboratory for cardiovascular physiology of the Faculty of Medical Sciences, University of Kragujevac, Serbia. The protocol of the current study was approved by the Ethical Committee for the experimental animals' well-being of the Faculty of Medical Sciences, University of Kragujevac, Serbia. All experiments were performed according to EU Directive for welfare of laboratory animals (86/609/EEC) and principles of Good Laboratory Practice (GLP).

Animals and design of the study

Forty-eight Wistar albino rats (males, six weeks old, body weight 200 ± 20 g, at the beginning of experiments) were included in the study. They were housed in a room with a 12/12-hour light/dark cycle, an ambient temperature of 22 ± 2°C. The rats had free access to food and water - *ad libitum*. Rats were divided randomly into 4 groups (12 animals per group):

1. Group I: Control healthy group (n=12);
2. Group II: Rats with diabetes mellitus type 2 (n=12);
3. Group III: Rats with diabetes mellitus type 2 treated with 0.6 mg/kg of sitagliptin (n=12);
4. Group IV: Rats with diabetes mellitus type 2 treated with 0.45 mg/kg of saxagliptin (n=12).

Except for the control healthy group, the rats were fed with a high-fat diet for 4 weeks and after 6–8 h of starvation received one dose of STZ intraperitoneally to induce T2DM. STZ was prepared *ex tempore* by dissolving in citrate buffer and, depending on the body weight, it was administered in a dose of 25 mg/kg (18). Three days after STZ injection and 12 h after starvation fasting glucose and insulin level as well as blood pressure were measured. Animals with fasting glucose level above 7 mmol/L and insulin level over 6 mmol/L were included in the study and were used in the study as rats with T2DM. The T2DM rats were then randomly divided into three groups: T2DM rats (n = 12), T2DM rats treated with 0.6 mg/kg body weight of sitagliptin (n=12) and T2DM treated with 0.45 mg/kg body weight of saxagliptin (n=12). Sitagliptin and saxagliptin were applied intraperitoneally once a day for three weeks.

Drugs

Streptozotocin (MW= 265,221), sitagliptin (MW= 523,32) and saxagliptin (MW= 315,41) were purchased from Sigma-Aldrich Chemie GmbH Eschen str. 5, 82024 Taufkirchen, Germany.

Evaluation of blood pressure and heart rate

At the beginning of the study and the day before sacrificing animals, the blood pressure and heart rate were measured by a tail-cuff noninvasive method BP system (Rat Tail Cuff Method Blood Pressure Systems (MRBP-R), IITC Life Science Inc., Los Angeles, CA, USA) (19). At least ten determinations were made in each session, with mean values taken.

Lipid profile

Upon completion of the experiments, the blood was collected from the anesthetized animals into blood collection tubes after an overnight fast (12 h). After standing for 30 min, the serum was prepared by centrifugation of blood at 1000 × g for 10 min at 4°C and stored at −80°C until analysis.

Serum triglyceride (TG), total cholesterol (TCH), high density lipoprotein (HDL), low density lipoprotein (LDL) were measured in the serum using spectrophotometry and commercial kits from Siemens Healthcare Diagnostics (Frimley, Camberley, Surrey, UK) and according to the manufacturer's instructions on the programmed analisator (Dimension Xpand, Siemens, IL, USA).

Statistical analysis

We used traditional parameters of descriptive statistics: average value±standard deviation (SD), and minimal and maximal values. Normality of the parameter distribution was evaluated with the Shapiro–Wilk and Kolmogorov-Smirnov tests. Additionally, data was analyzed using a one-way analysis of variance (ANOVA) and the post hoc Bonferroni test for multiple comparisons. The statistical significance was based on $p<0.05$. Complete statistical evaluation was performed with SPSS Statistics 22 (SPSS, Chicago, IL).

RESULTS

Lipid profile

Level of triglycerides (TGL), total cholesterol (TCH) and low-density lipoprotein (LDL) were significantly increased in experimental groups (rats with T2DM) compared to the control group, while the level of high-density lipoprotein (HDL) was decreased. Sitagliptin and saxagliptin have significantly decreased the level of TGL, TCH, and LDL compared to the T2DM group. There was no significant change in the level of HDL (Figure 1).

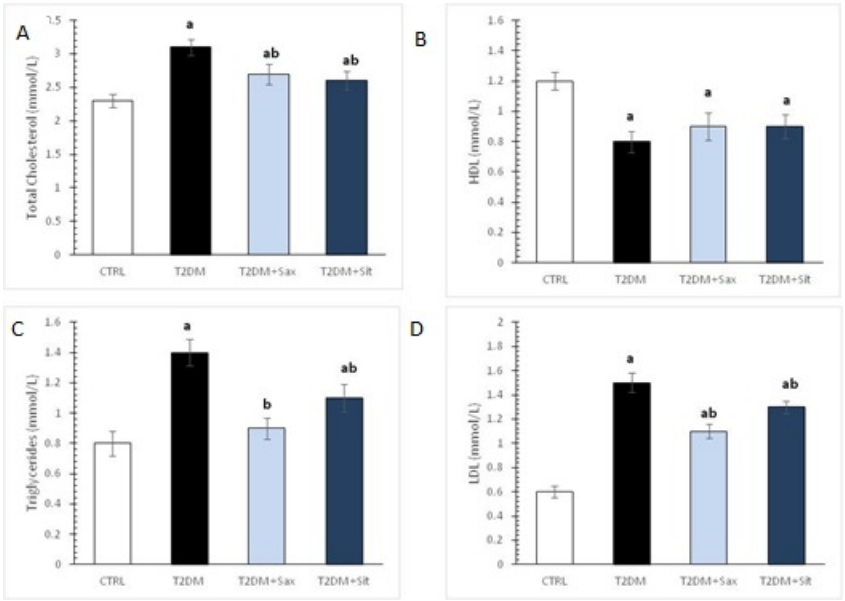

Figure 1: Changes in lipid profile in healthy and rats with T2DM:
(**A**) Total Cholesterol (TCL, mmol/l); (**B**) high-density lipoprotein (HDL, mmol/l);
(**C**) Triglycerides (TGL, mmol/l); (**D**) Low-density lipoprotein (LDL, mmol/l).

Values are expressed as mean ± standard deviation (SD) for 12 animals, for each group. Values p < 0.05 were considered statistically significant. a - statistical significance in relation to control (CTRL) group; b - statistical significance in relation to T2DM group.

Blood pressure and heart rate

Systolic and diastolic blood pressures were significantly increased in the T2DM group compared to the control group. Diastolic pressure was significantly decreased in groups with sitagliptin and saxagliptin compared to the T2DM group. There was no significant change in the heart rate (Figure 2).

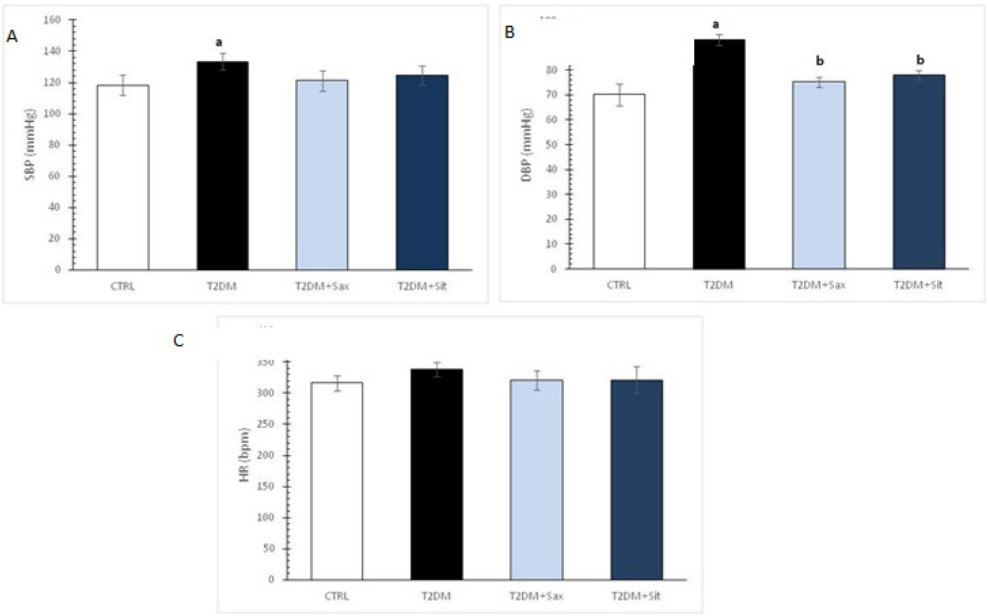

Figure 2: Changes in blood pressure and heart rate in healthy and rats with T2DM: (**A**) systolic blood pressure (SBP, mmHg); (**B**) diastolic blood pressure (DBP, mmHg); (**C**) heart rate (HR, bpm). Values are expressed as mean ± standard deviation for 12 animals, for each group.

Values $p < 0.05$ were considered statistically significant. a - statistical significance in relation to control (CTRL) group; b - statistical significance in relation to T2DM group.

DISCUSSION

The prevalence of hypertension in individuals with T2DM is estimated to be twice higher compared to non-diabetic individuals (20, 21). Possible reasons for this include diabetes related metabolic disorders, such as chronic hyperglycemia and hyperlipidemia along with low grade inflammation and oxidative stress. Blood pressure responses to DPP-4 inhibitor therapy are shown to be either neutral or modestly reduced (22-25). In addition, by measuring the blood pressure, we have confirmed the existence of a model diabetes mellitus type 2, which is quite obvious by noticing a statistically significant difference in the systolic and diastolic pressure in the T2DM group compared to the control. Using this parameter, we have managed to show that the drugs from the group of DPP-4 inhibitors lower diastolic blood pressure in comparison with T2DM group (Figure 2).

Recent studies highlighted that sitagliptin might induce changes in blood pressure in different ways, but this depends primarily on the presence of coronary disease, antihypertensive co-therapy (mostly ACE inhibitors and AT blockers), and thus, in the case of healthy animals (without coronary disease) this medicine decreased diastolic and systolic pressure, but not in the case of spontaneously hypertensive rats (17, 26). On the other hand, when dealing specifically with patients with T2DM, the study indicates a statistically significant decrease in systolic pressure after intravenous treatment with DPP-4 inhibitors (vildagliptin and sitagliptin). Pathophysiological mechanism responsible for BP reduction of DPP4 inhibitors are well explained by many authors. Firstly, DPP4 inhibition surely increases incretin level (GLP-1), which is associated with cardiac friendly lipid status in recent meta-analyses (27). There are DPP-4 non-incretin substrates that are involved in inflammation, immunity and cardiovascular system and its expression on endothelial surface suggests that its inhibition might reduce the vascular tone. Furthermore, animal studies have shown NO- dependent or independent arterial relaxation induced by GLP-1 (28). These vasodilator properties might also be mediated through GLP-1 metabolites and independently of the GLP-1 receptor, acting instead through an NO/cGMP-dependent mechanism (29). It is explained in the literature that

immune systems, both innate and adaptive might contribute to low-grade inflammation, which is connected with the development and progression of hypertension. DPP4 (CD26), besides being included in glucose and lipid metabolism, also participates in non-specific inflammation by regulating the activity and chemotaxis of macrophages, monocytes, NK and T cells. These cells secrete inflammatory mediators that can disturb the function of vascular endothelium (reactive oxygen species, cytokines, chemokines, adhesion molecules) in the form of increased proliferation of smooth muscle cells and vascular remodelling. Recent studies provided evidence about DPP4 inhibitors treatment inhibiting production of cytokines controlling the proliferation of T lymphocites and therefore express hypotensive effect via reduction of inflammation (28, 30, 31). Other research groups claimed the opposite since no effect on BP was noticed with DPP4 inhibitors treatment. There is also smaller amount of evidence regarding possible increase in BP by using ACE inhibitors and DPP4 inhibitors co-therapy (14, 15, 17). As for heart rate, values did not vary between groups and no statistically significant differences were noticed. DPP-4 inhibitors have not shown any effect on this parameter in our research (Figure 2). These results are consistent with various studies, where the DPP4 inhibitors therapy did not ameliorate cardiovascular outcomes in T2DM patients.

Lipid profile is a strong determinant of cardiovascular risk in T2DM. Current guidelines recommend an accurate control of hypercholesterolemia in order to reduce macrovascular complications. The fact that type 2 diabetic patients are more likely to be dyslipidemic than the general population is well known for decades. Lipid abnormalities associated with T2DM refer to high serum triglyceride levels, a high proportion of small dense low-density lipoprotein (LDL) particles, higher triglyceride-enriched, verylow-density lipoprotein (VLDL) particles, and lower protective high-density lipoprotein cholesterol (HDL) levels, together with glycation of apolipoproteins and increased LDL oxidation, all of which contribute to genesis of foam cell in atherosclerosis (32, 33). According to Derosa et al, the addition of sitagliptin to existing hypoglycemic therapy might lead to a better and durable (over 7 years of therapy) improvement of lipid profile. This beneficial effect is supposed to be due to delayed gastric emptying (34). Our study results elucidated that sitagliptin and saxagliptin improve lipid status in T2DM rats, via significant reduction of TCH, LDL and TGL (Figure 1). In line with these results are also the results of other research groups (35, 36). Possible explanation for beneficial lipid effects of DPP4 inhibitors may be connected to its stimulating effect on the activated proteine-kinase pathway, which leads to increase in glucose and lipid catabolism (37). On the other hand, no improvement in HDL parameters was achieved in our study, which is in correlation with the findings of Saad et al. (36). What is more, GIP can purify chylomicron in the circulation by stimulating the lipoproteine lipase from adipose tissue, while GLP-1 could decrease the postprandial secretion of triacylglycerol after meal (38-40). Since DPP inhibitors potentiate the GLP-1 and GIP function, it is clear why it improves lipid profile. Clinical trials have also confirmed beneficial effects of sitagliptin in diabetic patients, referring to amelioration of lipoprotein and lipid profile, which is explained by decrease in atherogenic remnant lipoproteins (RemL-C) (41).

CONCLUSION

DPP4 inhibitors, as GLP-1 agonists, were associated with modest reductions in DBP, LDL-C, TCH, and TGL and significant improvement in HDL, SBP and HR. These drugs might be helpful in achieving homeostasis in lipid status and blood pressure in T2DM patients. Hence, further evidence is needed to determine if improvements in lipid profile and BP might translate into reductions and amelioration of cardiovascular outcomes.

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