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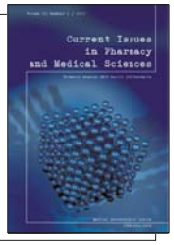

Two different markers of oxidative protein damage in plasma of alloxan-induced hyperglycemic rabbits: effects of pioglitazone and repaglinide

Oxidative protein damage and two antidiabetics

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ABSTRACT

Reactive oxygen and nitrogen species are known to contribute to different pathological alterations in hyperglycemic state. Especially proteins are major targets resulting from their rapid reactions with many oxidants. Therefore, the present study was undertaken to examine some common markers of oxidative/nitrosative stress in plasma of alloxan-induced hyperglycemic rabbits with special regard to protein oxidation. The second goal was to investigate which of two antidiabetics, pioglitazone from peroxisome proliferator-activated receptor gamma agonists, and repaglinide from non-sulfonylurea K_{ATP} channel blockers, could be more effective in ameliorating these oxidative/nitrosative changes. In our hyperglycemic animals glutathione decreased by 41% as compared to control group. Simultaneously, lipid peroxidation products increased by 62%, nitrites by 59%, protein carbonyl groups by 71% and advanced oxidized protein products by 53%. In hyperglycemic-treated animals pioglitazone increased glutathione above control values. In turn, lipid peroxidation was reduced by 29%, nitrites by 22%, advanced oxidized protein products by 37% and protein carbonyl groups by 42% in relation to hyperglycemic-non treated rabbits. After repaglinide treatment, lipid peroxidation decreased by 17% but the levels of glutathione, nitrites and both markers of oxidized proteins, were not affected. Our results confirmed that the action of pioglitazone is more comprehensive than that of repaglinide, independently of their action on hyperglycemia.

Keywords: Experimental hyperglycemia; Oxidative/nitrosative stress; Oxidized proteins; Antidiabetics

INTRODUCTION

It is well documented that hyperglycemia is responsible for overproduction of reactive oxygen/nitrogen species (ROS/RNS) and occurrence of oxidative/nitrosative stress [1]. Proteins are between major targets for these reactive species as a result of their abundance in plasma and other tissues, and their reactivity [2]. The attack of ROS/RNS modifies amino acids: lysine, arginine, proline, and histidine producing protein carbonyl groups (PCG). Additionally, protein carbonyls may be formed as ketoamine derivatives by enhanced glycation processes, thus generating new reactive radicals and perpetuating this vicious cycle [3]. Other products known as advanced oxidized protein products (AOPPs) are formed during oxidative stress by the action of chlorinated oxidants, mainly hypochlorous acid and chloramines produced by myeloperoxidase (MPO) in activated neutrophils. Structurally, they are described as dityrosine containing cross-linked protein products [4]. These both products (PCG and AOPPs) have been identified as early markers for oxidative stress and can be used as a measure of protein damage in different pathological situations. Among other things, the increased PCG and AOPPs can play role in the pathogenesis of diabetic nephropathy and vascular complications [3,5-7]. Therefore, our present study was undertaken to determine several markers of oxidative/nitrosative stress: glutathione (GSH), lipid peroxidation products (LPO) and total nitrites (NO_2), and then to explore some relationships between them and PCG or AOPPs in plasma of alloxan-induced hyperglycemic rabbits.

There is a strong theoretical basis to believe that therapeutic amelioration of oxidative/nitrosative stress would be beneficial in diabetic patients. Therefore, the second goal of the present study was to investigate which of two oral antidiabetic drugs, pioglitazone from peroxisome proliferator-activated receptor gamma (PPARγ) agonists, and repaglinide from non-sulfonylurea K_{ATP} channel blockers, could be more effective in ameliorating these oxidative/nitrosative changes. Pioglitazone belongs to 2,4-thiazolidinediones (TZDs) family and acts in diabetes by decreasing insulin resistance at the level of the muscle and by increasing glucose uptake. To a lesser extent, it decreases insulin resistance in liver and hepatic glucose production [8]. On the other hand, pioglitazone has been shown to be potent antioxidant in dif-

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ferent pathological situations connected with oxidative/nitrosative stress including diabetes [9-13]. The second drug, repaglinide, is a hypoglycemic agent from a chemical class of drugs with a unique molecular structure and mechanism of action belonging to the group of prandial glucose regulators. It is a benzoic acid derivative that lacks a sulfa group. Additionally, some results from the literature have previously demonstrated that it may have positive effects on parameters of oxidative/nitrosative stress [14,15].

MATERIAL AND METHODS

Animals and chemicals. White male New Zealand rabbits (the mean weight 3.1kg) were housed in a controlled environment with 12h light and dark cycles. They were provided with standard diet and water ad libitum. Animal care was in accordance with the Guidelines of Medical University of Lublin Animal Ethics Committee. The rabbits were divided into six groups with 5 animals in each: normal control (Group C), control treated with pioglitazone (Group CP), control treated with repaglinide (Group CR), hyperglycemic control (Group H), hyperglycemic treated with pioglitazone (Group HP) and hyperglycemic treated with repaglinide (Group HR). Hyperglycemia was induced by intravenous injection of 80 mg/kg of alloxan. Two weeks after the injection, administration of pioglitazone at a dose of 1 mg/kg and repaglinide at a dose of 0.3 mg/kg was started and continued for 4 weeks. The drugs were given directly to oral cavity, before the morning feeding. Concentration of glucose was estimated with a glucometer Precision QID from Abbott UK Ltd. (UK). At the end the animals were sacrificed with pentobarbital sodium (60 mg/kg). Blood was collected from a jugular vein, immediately centrifuged at 4°C and stored at -70°C until analysis was made.

GSH and LPO were determined using Bioxytech® GSH-400™ and LPO-586™ kits from OxisResearch (USA), respectively. Total NO_2 were estimated using a Nitrate/Nitrite Assay Kit from Fluka Chemicals (UK). Nitrates were reduced to nitrites by incubation of each sample for 120 min in the presence of nitrate reductase and NADPH. Total nitrites were then assayed by adding of Griess reagent and measuring the absorbance at 540 nm. PCG and AOPPs were determined using respective ELISA or colorimetric kits from Immundiagnostik AG (Germany). Protein content was determined by the method of Lowry et al. [16] using bovine serum albumin as standard. Two spectrophotometers, a UV-Vis CE-6000 from CECIL Instruments (UK) and a Microplate Reader PowerWave™ XS from Bio-Tek Instruments Inc. (USA) were used.

Statistical analysis. All numerical data are presented as the mean with the respective standard error (SEM). The significance of differences was determined with Kruskal-Wallis and Mann-Whitney's U tests. Probability p less than 0.05 was considered significant. For all statistical evaluation, Statistica software (version 9.0) was used.

RESULTS

In plasma of our hyperglycemic animals there was a decrease in GSH level by 41% as compared to control group. Simultaneously, there were increases in LPO by 62%, in NO_2 by 59%, in PCG and AOPPs by 71% and 53%. In hyperglycemic-treated animals pioglitazone increased GSH level above the control values. In turn, LPO level was reduced by 29%, NO_2 by 22%, PCG by 41% and AOPPs by 37% in relation to hyperglycemic-non treated rabbits. After repaglinide treatment LPO level decreased by 17% in relation to hyperglycemic-non treated animals. However, this drug did not affect the levels of GSH, NO_2, PCG, and AOPPs (Tab. 1).

Table 1. Effect of pioglitazone and repaglinide on glucose, insulin, and oxidative/nitrosative stress parameters in plasma of control and hyperglycemic rabbits

	Group C	Group CP	Group CR	Group H	Group HP	Group HR
Glucose (mmol/l)	5.7±0.3	5.9±0.3	4.0±0.3[a]	24.9±2.8[a]	23.9±1.8[a]	24.0±2.3[a]
Insulin (mU/l)	13.3±1.12	14.1±0.98[b]	20.0 ±1.42[a,b,]	2.79±0.79[a]	2.01±0.34[a]	2.02±0.04[a]
GSH (nmol/ml)	172.0±6.0	204.6±7.2[a]	164.7±9.3	100.8±4.6[a]	229.4 ±7.4[a,b]	127.0±6.6[a]
NO_2 (nmol/ml)	47.42±3.39	60.21±3.82[a]	68.92±6.36[a]	116.3±4.09[a]	91.03 ±6.15[a,b]	113.6±5.48[a]
LPO (nmol/ml)	1.54±0.16	1.82±0.05	1.52±0.06	4.01±0.26[a]	2.85 ±0.20[a,b]	3.33 ±0.10[a,b]
PCG (nmol/mg protein)	1.94±0.22	2.17±0.11	3.18±0.16[a]	6.62±0.82[a]	3.88 ±0.28[a,b]	6.29±0.42[a]
AOPPs (nmol/mg protein)	3.13±0.28	3.47±0.12	3.59±0.17	6.61±0.41[a]	4.17 ±0.13[a,b]	6.59±0.48[a]

Values are mean ± SEM (n=5).GSH-glutathione, NO_2-total nitrites, PCG-protein carbonyls, AOPPs-advanced oxidized protein products. Group C-control rabbits, CP-control rabbits treated with pioglitazone, CR-control rabbits treated with repaglinide, H-hyperglycemic rabbits, HP-hyperglycemic rabbits treated with pioglitazone, HR-hyperglycemic rabbits treated with repaglinide. [a]p.05 versus Group C. [b]p.05 versus Group H.

Some correlations were observed when respective pairs of parameters were taken into account. In hyperglycemic group we observed a negative correlation between AOPPs and GSH (r^2=0.8459 at p=0.0270) and positive correlation in a pair AOPPs-NO_2 (r^2=0.9665 at p=0.0026). In the case of PCG positive correlations occurred in pairs: PCG-LPO (r^2=0.9075 at p=0.0123) and PCG-NO_2 (r^2=0.7874 at p=0.0446). In hyperglycemic-pioglitazone treated group positive correlations in the pairs AOPPs-NO_2 (r^2=0.9898 at p=0.0000) and PCG-NO_2 (r^2=0.9087, p=0.0120) were observed. Because repaglinide affected neither of the markers of protein oxidation, any possible correlations were not taken into account (Fig. 1).

DISCUSSION

Over the past few years lipid peroxidation generated by free radicals in diabetic patients has been extensively investigated. However, a fewer number of studies concerning protein damage have been available so far [3,5-7,17-21]. Meanwhile, damage to protein may be even more important than damage in lipids. Oxidized proteins are usually poorly removed and this may contribute to their accumulation in tissues. Oxidative damage to amino acids residues and/or to the

peptides backbone of proteins produced PCG. On the other hand, upon formation of transient radical species such as chloramines produced by MPO and nitrogen radicals, dityrosine containing cross-linked protein products known as AOPPs are formed [17-19].

A wide spectrum of reliable methods for the determination of PCG like colorimetry, ELISA, immunoblotting and immunochemistry, are available. For AOPPs assay, the method described by Witko-Sarsat et al. [4] as well as different colorimetric kits are now available and even easier to perform in typical laboratory practice.

Previously [20,21], PCG was significantly increased in our alloxan-induced hyperglycemic animals when a classic colorimetric method with 2,4-dinitrophenylhydrazine [22] was used. In the present study, the occurrence of protein oxidative damage in plasma was confirmed by PCG-ELISA method and by AOPPs assay. Similarly to our results, in three other studies the elevated PCG and AOPPs were also found [5,18,19]. Interestingly, in one previous study, plasma proteins were found to be oxidized largely when micro vascular complications were present. In addition, antioxidant status was decreased more in these patients indicating close relationship between oxidative stress and subsequent development of micro vascular complications [5]. In the study of Piwowar et al. [19], more marked increase in the level of AOPPs compared with PCG level was found. It was explained by lower susceptibility of AOPPs cross-linked proteins to proteolysis and their subsequent accumulation in plasma. Other authors even supposed that AOPPs might represent an additive marker of phagocyte derived oxidative stress confirming the dysregulation in the balance between pro-inflammatory cytokines and their inhibitors [23].

Our results confirmed the usefulness of both markers and both methods, PCG-ELISA and colorimetric AOPPs assay. Although the correlations obtained between them and other markers of oxidative/nitrosative stress seem to be higher in the case of AOPPs, itcan be stated that they both show similar trends, especially for correlation with NO_2 level. Interestingly, similar correlations were also present in our pioglitazone treated animals (Fig. 1).

Under physiological conditions, the antioxidant defense system within the body can handle the amount of free radicals. The first line of antioxidant defense contains low molecular antioxidants like ascorbic acid (AA) and GSH. This line is able to scavenge ROS/RNS and thus less reactive compounds are formed. AA participates, among other things, in a variety of enzymatic reactions as an electron donor and is maintained in its reduced form by GSH. In turn, total antioxidant status (TAS) reflects the overall antioxidant properties because of the interactions among these various antioxidant components.

Previously, AA, TAS and GSH had significantly decreased levels in many studies concerning diabetic patients and animals [7,15,24-26]. In the present study, the diminished level of GSH has also been confirmed in our hyperglycemic group. This may be due to its increased use in order to combat the ROS/RNS overproduction. Some reports suggest that intracellular GSH level may be decreased by RNS produced in access by inducible nitric oxide synthase (iNOS) [27]. Our data concerning the enhanced concentration of NO_2 in plasma of our hyperglycemic animals seem to confirm this. It is also consistent with other studies reporting that active hyperglycemia is directly related to increased iNOS activity, which would enhance NO_2 formation [17,28].

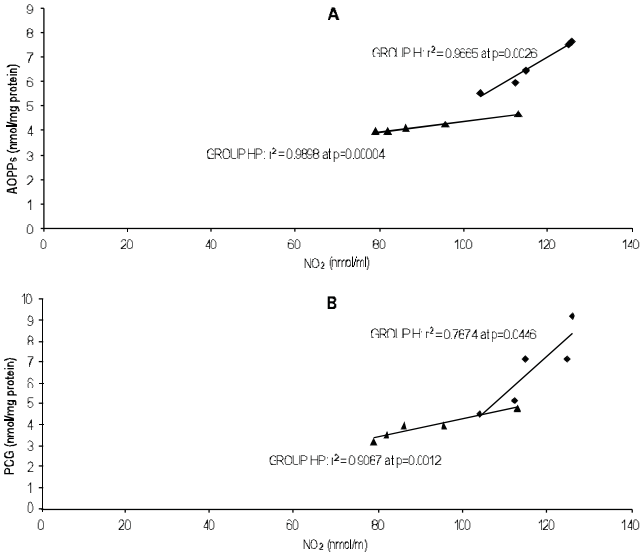

Fig. 1. The correlations between NO_2 and respective protein oxidation marker, AOPPs (A) or PCG (B), in hyperglycemic group (Group H) and hyperglycemic pioglitazone-treated group (Group HP)

Data from the literature have shown that beneficial effects of antioxidants against the development of oxidative/nitrosative changes in diabetes may involve the inhibition of nuclear factor κB (NF-κB) activation. On the other hand, it was observed that the activation of NF-κB and its related gene products was blocked by PPARγ ligands from TZDs family, including pioglitazone [8,29]. PPARγ agonists also participated in the control of inflammation, especially in modulating the production of inflammatory mediators such as tumor necrosis factor α, interleukin 1α, interleukin 6 (IL-6), iNOS and MPO [8,9,12,13,20,21,30,31]. Additionally, pioglitazone decreased lipid peroxidation as well as nitrite overproduction and restored GSH level in the studies concerning both diabetic patients and animals [10,12,13,32]. Resultantly, amelioration of both oxidative/nitrosative stress and inflammatory response was clearly noted and the present effect of the drug on AOPPs in aqueous humor of hyperglycemic animals may be, at least in part, a consequence of the above ability. Previous data concerning the decreased levels of NO_2 and MPO after pioglitazone in lung and testis of our hyperglycemic animals, confirm this [33,34].

In the literature also K_{ATP} channels are supposed to be involved in the regulation of a number of physiological processes. The possibility that these channels may be in-

volved, inter alia, in neutrophils functions, is discussed. What is more, they are supposed to provide some new ways for the treatment of inflammatory disorders [35].

In plasma of type 2 diabetic patients treated with a non-sulfonylurea K_{ATP} channel blocker mitiglinide, inhibition of lipid peroxidation and nitrosative stress as well as some pro-inflammatory factors including IL-6 was observed [36]. After administration of repaglinide, being another drug from this family, total serum antioxidant capacity [15] and lipid peroxidation [14,37] were also ameliorated. However, all these effects were observed in type 2 diabetes where they could be probable by improved controlling of postprandial hyperglycemia and thus by the cluster of oxidative/nitrosative stress. Additionally, it was stated that insulin per se reduces the level of NF-κB. Because NF-κB regulates the expression of proinflammatory cytokines and enzymes involved in ROS/RNS generation, insulin can modulate the mechanisms involved in oxidative/nitrosative stress and inflam- mation [35].

In our previous studies, some parameters of oxidative stress such as AA and TAS were ameliorated by both pioglitazone and repaglinide while the controversy existed in the range of PCG and IL-6 [20,21]. Therefore, we wanted to compare once more the action of these two drugs on oxidative/nitrosative stress with special regard to protein damage. As a result we stated that pioglitazone increased GSH level above the control values and significantly reduced LPO, NO_2, and especially PCG and AOPPs. After treatment with repaglinide, LPO levels decreased. However, this drug did not affect the levels of GSH, NO_2, PCG, and AOPPs. Similar differences, to some extent, were observed when rosiglitazone from TZDs family and glyburide from sulfonylureas K_{ATP} blockers were studied [38]. Importantly, in the present study pioglitazone and repaglinide did not significantly affect glucose concentration in our hyperglycemic animals. After alloxan injection, these animals preserved insulin secretion but its amount was very low because of destruction of many B cells by alloxan. Therefore, repaglinide could not stimulate its secretion in sufficient way and pioglitazone failed to affect its sensitivity. This lack of antihyperglycemic activity was expected and used by us to differentiate, at least in part, some direct antioxidative/antinitrosative effects of the drugs from the effects mediated via increased insulin action. When animals with type 2 diabetes were used, these two kinds of effects could not be separated.

In conclusion, we stated that the role of oxidative/nitrosative stress in diabetes is still an area of interesting investigation. In the range of protein oxidation, both of the markers, PCG and AOPPs could be helpful. However, one may assume that AOPPs as a marker connected with the activated neutrophils, may more clearly show the possible coincidence of oxidative/nitrosative stress and inflammation.

Therapeutic use of protective agents, acting early to prevent protein oxidation, may offer interesting therapeutic options aimed at reducing long-term complications in diabetic patients. We confirmed that pioglitazone and repaglinide differ significantly in their ability to ameliorate the examined parameters like NO_2, PCG, and AOPPs. It cannot be excluded that K_{ATP} channel blockers like repaglinide may affect oxidative/nitrosative stress but PPARγ agonists like pioglitazone seem to act more comprehensively, which was confirmed independently on their action on hyperglycemia. However, it is desirable to advance further our understanding on mechanisms of these alterations, especially in clinical studies in diabetic patients.

REFERENCES

1. Stadler K., Bonini M.G., Dallas S., Jiang J., Radi R.: Involvement of inducible nitric oxide synthase in hydroxyl radical mediated lipids peroxidation in streptozotocin-induced diabetes. *Free Radic. Biol. Med.* 45, 866, 2008.
2. Breusing N., Grune T.: Biomarkers of protein oxidation from a chemical, biological and medical point of view. *Exp. Gerontol.* 45, 733, 2010.
3. Pandey K.B., Mishra N., Rizvi S.I.: Protein oxidation biomarkers in plasma of type 2 diabetic patients. *Clin. Biochem.* 43, 508, 2010.
4. Witko-Sarsat V.M., Friendlander C., Capeillere-Blandin C., Nguyen-Khoa T., Nguen A.T., Zingraff J., Jungers P., Descamps-Latscha B.: Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int.* 49, 1304, 1996.
5. Martin-Gallan P., Carrascosa A., Gussinye M., Dominguez C.: Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. *Free Radic. Biol. Med.* 34, 1563, 2003.
6. Chakraborty A., Chowdhury S., Bhattacharyya M.: Effect of metformin on oxidative stress, nitrosative stress and inflammatory biomarkers in type 2 diabetes. *Diabetes Res. Clin. Prac*t. 93, 56, 2011.
7. Telci A., Cakatay U., Salman S., Satman I., Sivas A.: Oxidative protein damage in early stage type 1 diabetic patients. *Diabetes Res. Clin. Pract.* 50, 213, 2000.
8. Gross B., Staels B.: PPAR agonists: multimodal drugs for the treatment of type 2 diabetes. *Best Pract. Res. Clin. Endocrinol. Metab.* 2, 687, 2007.
9. Inoue I., Goto S., Matsunaga T., Nakajima T., Awata T., Hokari S., Komoda T., Katayama S.: The ligands/activators for peroxisome proliferator-activated receptor γ (PPARγ) and PPARă increase Cu^{+2},Zn^{+2}-superoxide dismutase and decrease $p22^{phox}$ message expression in primary endothelial cells. *Metabolism* 50, 3, 2001.
10. Vinik A.I., Ullm J., Parson H.K., Barlow P.M., Casellini C.M.: Pioglitazone treatment improves nitrosative stress in type 2 diabetes. *Diabetes Care* 29, 869, 2006.
11. King D.E., King M., Player M.S., Everett C.J.: The impact of pioglitazone on ADMA and oxidative stress markers in patients with type 2 diabetes. Prim. Care Diabetes 2011; doi: 10.1016/j.pcd.2011.06.002
12. Kumar A., Vashist A., Kumar P.: Potential role of pioglitazone, caffeic acid and their combination against fatigue syndrome-induced behavioral, biochemical and mitochondrial alterations in mice. *Inflammopharmacology* 18, 241, 2010.
13. Majithija J.B., Paramar A.N., Balaraman R.: Pioglitazone, a PPARγ agonist, restores endothelial function in aorta of streptozotocin-induced diabetic rats. *Cardiovasc. Res.* 66, 150, 2005.
14. Manzella D., Abbatecola A.M., Grella R., Paolisso G.: Repaglinide administration improves brachial reactivity in type 2 diabetic patients. *Diabetes Care* 28, 366, 2005.
15. Tankova T., Koev D., Dakovska L., Kirilov G.: The effect of repaglinide on insulin secretion and oxidative stress in type 2 diabetic patients. *Diabetes Res. Clin. Pract.* 59, 43, 2003.

16. Lowry O.H., Rosenbrough N.J., Farr A.L., Randall R.J.: Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193, 265, 1951.
17. Bhatia S., Bhatia R., Shukla S., Venkata Madhu S., Kaur Gambhir J., Madhava Prabhu K.: Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. *Clin. Biochem.* 36, 557, 2003.
18. Kalousova M., Skrha J., Zima T.: Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. *Physiol. Res.* 51, 597, 2002.
19. Piwowar A., Knapik-Kordecka M., Warwas M.: AOPP and its relations with selected marker of oxidative/antioxidative system in type 2 diabetes mellitus. *Diabetes Res. Clin. Pract.* 77, 188, 2007.
20. Gumieniczek A., Hopkała H., Roliński J., Bojarska-Junak A.: Antioxidative and anti-inflammatory effects of repaglinide in plasma of diabetic animals. *Pharmacol. Res.* 52, 162, 2005.
21. Gumieniczek A., Hopkała H., Roliński J., Bojarska-Junak A.: Interleukin-6 and oxidative stress in plasma of aloxan-induced diabetic rabbits after pioglitazone treatment. *Immunopharmacology* 28, 81, 2006.
22. Levine R.L., Williams J.A., Stadtman E.R., Shacter E.: Carbonyl assays for determination of oxidatively modified proteins. *Method Enzymol.* 233, 346, 1994.
23. Witko-Sarsat V.M., Gausson B., Descamps-Latscha B.: Are advanced oxidation protein products potential uremic toxins. *Kidney Int.* 63, S11-S14, 2003.
24. Ananthan R., Baskar C., NarmathaBai V., Pari L., Latha M., Ramkumar K.M. Antidiabetic effect of Gymnema montanum leaves: effect on lipid peroxidation induced oxidative stress in experimental diabetes. *Pharmacol. Res.* 48, 551, 2003.
25. Skrha J., Prazny M., Hilgertova J., Weiserova H.: Serum a-tocopherol and ascorbic acid concentrations in type 1 and type 2 diabetic patients with and without angiopathy. *Clin. Chim. Acta* 329, 103, 2003.
26. De Matia G., Laurenti O., Fava D.: Diabetic endothelial dysfunction. Effect of free radical scavenging in type 2 diabetic patients. *J. Diabetes Complicat.* 17, 30, 2003.
27. Livingstone C. Davis J.: Targeting therapeutics against glutathione depletion in diabetes and its complications. *Br. J. Diabetes Vasc.* 7, 258, 2007.
28. Pereira E.C., Ferderbar S., Bertolami M.C., Faludi A.A., Monte O., Xavier H.T., Pereira T.V., SaesParra Abdalla D.: Biomarkers of oxidative stress and endothelial dysfunction in glucose intolerance and diabetes mellitus. *Clin. Biochem.* 41, 1454, 2008.
29. Aljada A., Garg R., Ghanim H., Mohanty P., Hamouda W., Assian E., Dantona P.: Nuclear factor-κB suppressive and inhibitor-κB stimulatory effects of troglitazone in obese patients with type 2 diabetes: evidence of an anti-inflammatory action. *J. Clin. Endocrinol. Metab.* 2001; 86: 3250-3256
30. Villegas I., Ramon-Martin A., Toma W., Alarcon de la Lastra C.: Rosiglitazone, an agonist of peroxisome proliferator-activated receptor gamma, protects against gastric ischemia-reperfusion damage in rats: role of oxygen free radicals generation. *Eur. J. Pharmacol.* 505, 195, 2004.
31. Sung B., Park S., Yu B.P., Hung H.Y.: Amelioration of age-related inflammation and oxidative stress by PPARγ activator: Suppression of NF-κB by 2,4-thiazolidinedione. *Exp. Gerontol.* 41, 590, 2006.
32. Tao L., Liu H., Gao E., Teng Z., Lopez B.L., Christopher T.A., Ma X., Batinic-Haberle I., Willette R.N., Ohlstein E.H., Yue T.: Antioxidative, antinitrative and vasculoprotective effects of a peroxisome proliferator-activated receptor γ agonist in hypercholesterolemia. Circulation 108, 2805, 2003.
33. Gumieniczek A., Komsta Ł., Rachid Chehab M.: Effects of two oral antidiabetics, pioglitazone and repaglinide, on aconitase inactivation, inflammation and oxidative/nitrosative stress in tissues under alloxan-induced hyperglycemia. *Eur. J. Pharmacol.* 659, 89, 2011.
34. Gumieniczek A., Krzywdzińska M., Nowak M.: Modulation of nitrosative/oxidative stress in the lung of hyperglycemic rabbits by two antidiabetics, pioglitazone and repaglinide. *Exp. Lung Res.* 35, 371, 2009.
35. Da Silva-Santos J.E., Santos-Silva M.C., Cunha F., Assreuy J.: The role of ATP-sensitive Potassium channels in neutrophil migration and plasma exudation. *J. Pharmacol. Exp. Ther.* 300, 946, 2002.
36. Assaloni R., Da Ros R., Quagliaro L., Piconi L., Maier A., Zuodar G., Motz E., Ceriello A.: Effects of S21403 (mitiglinide) on postprandial generation of oxidative stress and inflammation in type 2 diabetic patients. *Diabetologia* 48, 1919, 2005.
37. Li Y., Xu L., Shen J., Ran J., Zhang Y., Wang M., Yan L., Cheng H., Fu Z.: Effect of short-term therapy with different insulin secretagogue on glucose metabolism, lipid parameters and oxidative stress in newly diagnosed type 2 diabetes mellitus. *Diabetes Res. Clinical Pract.* 88, 42, 2010.
38. Pop-Busui R., Oral E, Raffel D., Byun J., Bajirovis V., VivekanandanGiri A., Kellogg A., Pennathur S., Stevens MJ.: Impact of rosiglitazone and glyburide on nitrosative stress and myocardial blood flow regulation in type 2 diabetes mellitus. *Metab. Clin. Exp.* 58, 989, 2009.