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Effects of two oral antidiabetics, pioglitazone and repaglinide, on aconitase inactivation, inflammation and oxidative/nitrosative stress in tissues under alloxan-induced hyperglycemia

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ABSTRACT

Pathological changes identified in different tissues in hyperglycemic state are undoubtedly connected with increased oxidative/nitrosative stress and inflammation. In our study myeloperoxidase (MPO), nitrotyrosine and lipid peroxidation were enhanced in the heart and lung of alloxan-treated hyperglycemic animals. Additionally, pulmonary aconitase was inhibited. In the testis the changes occurred as an increase of MPO and lipid peroxidation, and as a decrease of aconitase. The effects of two different antidiabetics, the peroxisome proliferator activated receptor gamma (PPARγ) agonist, pioglitazone, and a short acting insulin secretagogue, repaglinide, on the mentioned parameters, were investigated and compared. The insulin deficient alloxan-induced hyperglycemic animals were used to differentiate a direct anti-oxidative effect of the drugs from secondary effects mediated via increased insulin sensitivity or secretion. Pioglitazone acted by normalization of pulmonary and testicular aconitase, normalization of pulmonary and cardiac nitrotyrosine, reduction of pulmonary and testicular MPO, and by reduction of lipid peroxidation in all tissues examined. Repaglinide prevented oxidative changes by normalization of aconitase activity in the lung and testis, and by reduction of lipid peroxidation and nitrotyrosine in the heart and lung. At the same time, no effect of this drug on MPO was observed. Finally, principal component analysis was performed to explore and visualize similarities and differences of the results obtained for the both drugs.

1. Introduction

Aconitase is an enzyme that catalyzes reversible isomerization of citrate and isocitrate via *cis*-aconitate in the Krebs cycle and also regulates intracellular iron levels. Both isoforms of aconitase, mitochondrial and cytosolic, are inactivated by reactive oxygen species like superoxide anion, mainly due to oxidation of the Fe-center. Studies from the literature have revealed that several proteins including aconitase are also nitrated and that this nitration is promoted by reactive nitrogen species like peroxynitrite (Tórtora et al., 2007). Such an increased oxidative/nitrosative stress has been demonstrated in many previous experiments (Lin et al., 2009; Muralidhara, 2007; Nicolaie et al., 2003).

Also inflammatory response, predominantly mediated by activated neutrophils, monocytes and macrophages, is connected with enhanced formation of reactive oxygen and nitrogen species. The next enzyme, myeloperoxidase (MPO), is present in granules of inflammatory cells and released in response to inflammatory and infectious stimuli. It uses hydrogen peroxide to create several potent oxidants, including hypochlorous acid, hydroxyl radical, nitrogen dioxide and peroxynitrite. These byproducts of MPO subsequently modify lipids as well as tyrosine residues in protein to create oxidized lipids and nitrotyrosine (Turkyilmaz et al., 2008). An overproduction of reactive oxygen/nitrogen species was clearly showed in plasma of types 1 and 2 diabetic subjects (Ceriello et al., 2001; Marfella et al., 2001). This overproduction as well as inflammation were also present in other tissues from diabetic subjects, including cardiovascular system (El-Alfy et al., 2005; Pacher et al., 2005), lung (Ricardiollo et al., 2006) and testis (Muralidhara, 2007).

Taking into account the above questions, the first goal of the present study was to shed light on the aconitase, MPO and oxidative/nitrosative stress in the heart, lung and testis under alloxan-induced hyperglycemia.

Oral antidiabetic drug pioglitazone is a synthetic ligand of peroxisome proliferator activated receptor gamma (PPARγ) which improves glycemic control in type 2 diabetes by enhancing insulin sensitivity (Inoue et al. 2001). PPARγ ligands have been also described as regulators of cellular proliferation, apoptosis and inflammation (Staels and Fruchart, 2005). In addition, previous studies have showed that lipid peroxidation and nitrotyrosine formation are suppressed by such a treatment (Gumieniczek et al., 2009; Shiojiri et al., 2002).

The present study was undertaken to determine whether pioglitazone was able also to modulate aconitase and MPO levels. The effect of pioglitazone was compared to that of repaglinide, an

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antidiabetic agent with different molecular structure and mechanism of action. Repaglinide is a short acting insulin secretagogue that binds to an ATP-sensitive K^+ channel on the cell membrane of pancreatic β cells (Wolffenbuttel, 1999). Additionally, the previous results have demonstrated that it has positive effects on oxidative/nitrosative stress (Gumieniczek et al., 2009; Manzella et al., 2005).

To the best of our knowledge, the present report it is the first as far as concerns the action of pioglitazone and repaglinide on the mentioned parameters taken as a whole. In our study, the insulin deficient alloxan-induced hyperglycemic animals were used to differentiate a direct anti-oxidative effect of the drugs from secondary effects mediated via increased insulin sensitivity or secretion.

2. Materials and methods

2.1. Animals

The study was performed according to the protocol described earlier (Gumieniczek, 2005; Gumieniczek et al., 2009). Male New Zealand rabbits were housed in a controlled environment with 12 h light–dark cycles. They were fed once daily in the morning with 150–200 g of standard rabbit chow containing 14–16% protein, 1.5–2% fat and 50–60% carbohydrate (Cargill, Poland) and with 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: control, control-pioglitazone treated, control-repaglinide treated, hyperglycemic, hyperglycemic-pioglitazone treated and hyperglycemic-repaglinide treated.

Hyperglycemia was induced by intravenous (by marginal ear vein) injection of 80 mg/kg of alloxan in sterile saline. Control animals were injected with sterile saline alone. Two weeks after the alloxan injection (the start of experiment), 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 end of experiment). The drugs were administered orally once daily in the morning. At the start and the end of experiment, body weight, glucose and insulin concentrations were measured. Additionally, concentration of glucose was controlled once a week with a glucometer Precision QID from Abbott UK Ltd. At the end of experimental period, the animals were sacrificed with intravenous injection of pentobarbital sodium (60 mg/kg) and immediately opened surgically.

2.2. Biochemical measurements

The hearts, lungs end testes were removed and processed by homogenization procedure in phosphate buffer at pH 7.5. The homogenates (25% w/v) were centrifuged at 20,000×*g* for 20 min at 4 °C. The supernatants were stored at −70 °C until analysis.

Protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard. For aconitase, lipid peroxidation and nitrotyrosine, Bioxytech® Aconitase-340™, Bioxytech® LPO-586™ and Bioxytech® Nitrotyrosine-EIA kits from Oxis Research™ USA were used. MPO was determined using MPO ELISA kit from Immundiagnostik AG Germany. Insulin concentration was estimated using Ultrasensitive Insulin ELISA kit from DRG Diagnostic Germany. Bio-Tek ELX800 absorbance microplate reader from Bio-Tek Instruments Inc. USA was used for all ELISA readings.

2.3. Statistical and chemometric analysis

All values in Table 1 were expressed as mean ± S.E.M. The significance of differences between experimental groups was determined with H Kruskal–Wallis and Mann–Whitney U tests. Differences with a *P* value less than 0.05 were considered significant. Additionally, principal component analysis was performed on the data matrix obtained for the both drugs, using GNU R computational environment.

3. Results

3.1. Body weight, blood glucose and plasma insulin concentrations

Generally, there was a moderate increase in the body weight of control animals while hyperglycemic rabbits showed a moderate decrease. Values for all groups are presented as means ± S.D. at the start and at the end of our experiment: control 3.1 ± 0.1 and 3.3 ± 0.1, control-pioglitazone treated 2.9 ± 0.0 and 3.1 ± 0.0, control-repaglinide treated 3.4 ± 0.1 and 3.5 + 0.1, hyperglycemic 3.1 ± 0.2 and 2.8 ± 0.4, hyperglycemic-pioglitazone treated 3.0 ± 0.1 and 2.6 ± 0.1, hyperglycemic-repaglinide treated 3.0 ± 0.1 and 2.6 ± 0.1 kg.

At the start and the end of our experiment the respective values for glucose concentration were: control group 6.2 ± 0.1 and 5.7 ± 0.3, control-pioglitazone treated 6.6 ± 0.6 and 5.9 ± 0.3, control-repaglinide treated 6.3 ± 0.2 and 4.0 + 0.3 (significant at $P<0.05$), hyperglycemic 26.3 ± 2.3 and 24.9 ± 2.8, hyperglycemic-pioglitazone treated 27.2 ± 0.2 and 23.9 ± 1.8, hyperglycemic-repaglinide treated 26.4 ± 1.2 and 24.0 ± 2.3 mmol/l.

The respective values of plasma insulin concentrations were: control group 13.2 ± 1.3 and 13.3 ± 1.1, control-pioglitazone treated 11.7 ± 0.7 and 14.1 ± 1.0, control-repaglinide treated 12.8 ± 1.0 and 20.0 ± 1.4 (significant at $P<0.05$), hyperglycemic 3.2 ± 0.6 and 2.8 ± 0.8, hyperglycemic-pioglitazone treated 2.3 ± 0.3 and 2.0 ± 0.3, hyperglycemic-repaglinide treated 2.3 ± 0.2 and 2.0 ± 0.0 mU/l.

3.2. Alterations in the heart

Hyperglycemia increased lipid peroxidation, MPO and nitrotyrosine by 52, 17 and 156% as compared to control animals, while aconitase was not affected. Pioglitazone normalized nitrotyrosine and decreased lipid peroxidation by 53%. Repaglinide decreased lipid peroxidation by 17% and nitrotyrosine by 29% as compared to hyperglycemic non-treated animals (Table 1).

3.3. Alterations in the lung

Chronic hyperglycemia caused an increase of lipid peroxidation by 115% and nitrotyrosine by 190% as compared to a control group. The same animals showed elevation of MPO by 77% while aconitase was diminished by 76%. Pioglitazone ameliorated lipid peroxidation and nitrotyrosine as well as aconitase to control values and nearly normalized MPO. Repaglinide did not affect MPO whereas aconitase was increased by 182%. However, lipid peroxidation and nitrotyrosine were diminished, respectively by 30 and 52% as compared to hyperglycemic non-treated animals (Table 1).

3.4. Alterations in the testis

In hyperglycemic animals, there were increases of lipid peroxidation and MPO by 56 and 64%, and a decrease of aconitase by 66% as compared to respective controls. At the same time, nitrotyrosine was not affected. With pioglitazone treatment, all hyperglycemia affected parameters were changed to control values. With repaglinide treatment, aconitase was normalized while lipid peroxidation was reduced by 15% as compared to hyperglycemic non-treated rabbits (Table 1).

3.5. Principal component analysis

In Figs. 1–3, the principal component 1 versus principal component 2 scores plots are explanatory to 87.7, 94.8 and 78.7% of the total variation in the data set obtained for the heart, lung and testis of hyperglycemic, hyperglycemic pioglitazone-treated and hyperglycemic repaglinide-treated groups, respectively.

Table 1
Effects of pioglitazone and repaglinide on the examined markers in the tissues of control and hyperglycemic groups.

	Group C	Group CP	Group CR	Group H	Group HP	Group HR
Heart						
Aconitase (mU/mg protein)	0.44 ± 0.04	0.36 ± 0.03	0.47 ± 0.02	0.46 ± 0.05	0.72 ± 0.07[a,b]	0.53 ± 0.03
MPO (ng/g tissue)	3.12 ± 0.08	2.81 ± 0.22	2.53 ± 0.17[a,b]	3.64 ± 0.16[a]	3.81 ± 0.18[a]	3.59 ± 0.16[a]
Nitrotyrosine (nmol/mg protein)	8.79 ± 0.43	5.52 ± 0.30[a,b]	8.59 ± 0.13[b]	22.45 ± 0.99[a]	7.76 ± 0.71[a,b]	15.90 ± 0.69[a,b]
Lipid peroxidation products (nmol/g tissue)	42.7 ± 1.8	46.9 ± 1.9[b]	46.5 ± 1.6[b]	64.7 ± 2.9[a]	30.4 ± 1.9[a,b]	53.7 ± 2.9[a,b]
Lung						
Aconitase (mU/mg protein)	0.45 ± 0.04	0.41 ± 0.04[b]	0.49 ± 0.05[b]	0.11 ± 0.03[a]	0.57 ± 0.05[b]	0.31 ± 0.03[a,b]
MPO (ng/g tissue)	3.27 ± 0.11	3.52 ± 0.10	3.80 ± 0.21	5.78 ± 0.46[a]	4.32 ± 0.06[a,b]	5.15 ± 0.14[a]
Nitrotyrosine (nmol/mg protein)	8.15 ± 0.03	7.91 ± 0.09[b]	8.29 ± 0.06[b]	23.62 ± 0.08[a]	8.38 ± 0.08[b]	11.35 ± 0.14[a,b]
Lipid peroxidation products (ng/g tissue)	73.3 ± 3.8	76.1 ± 3.9[b]	74.5 ± 3.2[b]	157.7 ± 4.8[a]	81.6 ± 3.8[b]	110.1 ± 5.9[a,b]
Testis						
Aconitase (mU/mg protein)	1.63 ± 0.16	1.47 ± 0.13[b]	1.67 ± 0.27[b]	0.56 ± 0.12[a]	2.20 ± 0.30[a,b]	2.57 ± 0.13[a,b]
MPO (ng/g tissue)	2.72 ± 0.09	3.46 ± 0.16[a,b]	3.63 ± 0.18[a,b]	4.46 ± 0.01[a]	3.05 ± 0.28[b]	4.18 ± 0.21[a]
Nitrotyrosine (nmol/mg protein)	7.13 ± 0.40	5.82 ± 0.14[a,b]	6.88 ± 0.35	7.25 ± 0.28	7.08 ± 0.35	7.87 ± 0.21[a]
Lipid peroxidation products (ng/g tissue)	39.8 ± 0.5	40.5 ± 0.6[b]	41.8 ± 0.8[b]	61.9 ± 4.4[a]	39.1 ± 2.5[b]	52.6 ± 0.1[a,b]

Values are mean ± S.E.M (n = 5).
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.
MPO—myeloperoxidase.
[a] $P<0.05$ versus Group C.
[b] $P<0.05$ versus Group H.

4. Discussion

In the present study, the alloxan-induced hyperglycemia was chosen as a model of oxidative/nitrosative stress and inflammation. It is well known that chronically elevated glucose level causes such a toxicity in blood and other tissues (Choi et al., 2008). It is also well known that various pathological effects under these conditions depend on target molecules and on the extent of changes (Pérez-Matute et al., 2009). Therefore we supposed that unique oxidants and inflammation were generated in the heart, lung and testis of our alloxan-induced hyperglycemic rabbits.

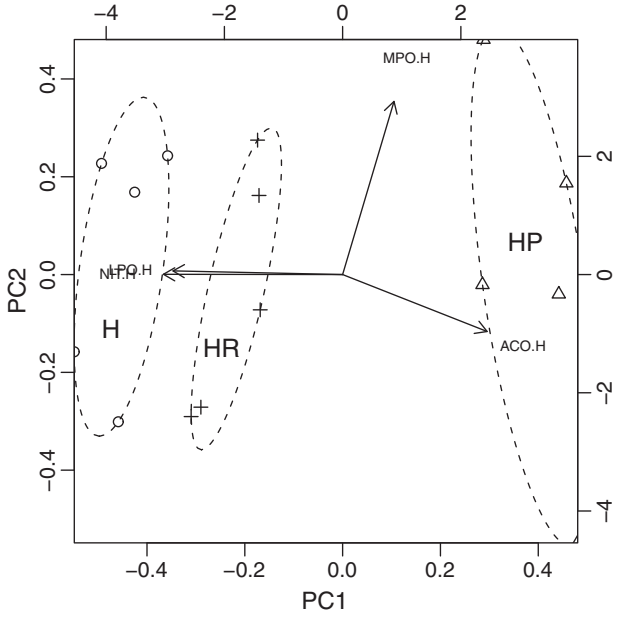

Fig. 1. The principal component 1 (PC1) versus principal component 2 (PC2) scores plot of the data obtained for the heart of hyperglycemic (H), hyperglycemic pioglitazone-treated (HP) and hyperglycemic-repaglinide treated (HR) animals (ACO.H—aconitase, LPO.H—lipid peroxidation products, MPO.H—myeloperoxidase, NIT.H—nitrotyrosine).

In previously published papers, the data on the enhanced lipid peroxidation and nitration in diabetic state were reported (Aydin et al., 2001; Hoeldtke, 2003; Gumieniczek et al., 2009; Pop-Busui et al., 2009; Ricardiollo et al., 2006). Hyperglycemia led also to increased inflammatory response when activated neutrophils induced tissue injuries through the release of new reactive metabolites like MPO (Şener et al., 2002). It was observed in serum of type 2 diabetic patients with a confirmed coronary artery disease (Jornayvaz et al., 2009). On the other hand, this enzyme was observed to be reduced in leukocytes of type 1 and type 2 diabetic patients (Uchimura et al., 1999).

In the literature, aconitase has been established a sensitive target of reactive oxygen/nitrogen species, however, the consequences of its inactivation still remain not fully understood (Tórtora et al., 2007). Because the increased oxidative/nitrosative stress together with alterations in mitochondria have been demonstrated in diabetes, it is probably that the activity of mitochondrial aconitase is then affected (Lin et al., 2009). From this point of view, aconitase inactivation in diabetes may reflect the intracellular or mitochondrial formation of reactive oxygen/nitrogen species. Therefore it appears logical for us to examine MPO, aconitase, nitrotyrosine and lipid peroxidation as a whole in order to discover potential relationships between inflammation and oxidative/nitrosative stress.

Taking together all our results, we stated that hyperglycemia enhanced MPO and nitrotyrosine as well as lipid peroxidation in the heart and lung of our hyperglycemic rabbits. In the testis, the

significant changes occurred as an increase of MPO and lipid peroxidation. It confirms an overproduction in reactive oxygen/nitrogen species, and additionally, the enhanced inflammatory response. However, a decrease of aconitase was incidentally observed in the pulmonary and testicular tissues, not in the heart. Previously, aconitase activity was unchanged in skeletal muscles of the animals subjected to extensive exercises (Zhang et al., 2007). It was supposed that either level of reactive species was not high enough to inactivate aconitase or that some mechanisms acted to maintain this activity unchanged. The activity of aconitase was also unaffected in the hearts subjected to ischemia/reperfusion (Lin et al., 2009). These authors proposed the association of aconitase with iron binding proteins as a potential mechanism for the enzyme protection.

It is generally accepted that therapies interrupting oxidative and nitrosative pathways in target tissues may be beneficial in preventing many diabetic complications.

In our study, pioglitazone and repaglinide had no effect on blood glucose and insulin concentrations in hyperglycemic animals (the significant effect was only observed for control animals receiving repaglinide). The reason for this is that the alloxan-induced hyperglycemic animals were not insulin resistant but insulin deficient. In this type of diabetes, the pancreatic β cells deteriorate to such an extent that repaglinide cannot stimulate insulin secretion and pioglitazone cannot improve insulin action sufficiently.

However, our present and previous results (Gumieniczek et al., 2009) confirm that pioglitazone acted by normalization of pulmonary and testicular aconitase, normalization of pulmonary and cardiac nitrotyrosine, reduction of pulmonary and testicular MPO, and by reduction of lipid peroxidation in all tissues examined. In one other study, the protective effects of pioglitazone on the ischemia/reperfusion-dependent lung injuries were presented as reduction of MPO, lipid peroxidation and tumor necrosis factor-α (Ito et al., 2004). Also, other PPARγ agonist, rosiglitazone, decreased nitrotyrosine in endothelial cells in hypercholesterolemia (Tao et al., 2003), in acute inflammation in the lungs (Cuzzocrea et al., 2004) and in plasma of type 2 diabetic patients (Jornayvaz et al., 2009). The authors supposed that these actions were due to preventing the activation of some transcription factors, e.g. nuclear factor-κB and finally due to reducing expression of inflammatory cytokines and oxidative stress (Aljada et al., 2001). Other investigators have demonstrated that PPARγ agonists reduced oxidative stress via modulation many related enzymes and independently by the anti-inflammatory mechanisms (Lee et al., 2006). There is also evidence that PPARγ agonists exert direct and rapid effects on mitochondrial respiration inhibiting complex I and complex III. Then, the both electron transport and reactive oxygen species generation in mitochondria are affected (Collino et al., 2006). Taken together, these drugs are suggested to reduce overproduction of reactive oxygen/nitrogen species directly and/or indirectly through inhibition of inflammation. In our study, it is confirmed by reduction of lipid peroxidation, decreased levels of nitrotyrosine and MPO as well as by normalization of aconitase.

The second drug being examined, repaglinide, prevented oxidative changes by normalization of aconitase activity in the lung and testis, and by reduction of lipid peroxidation and nitrotyrosine in the heart and lung. At the same time, no effect of this drug on MPO was observed. In one previous study, after the treatment with mitiglinide, an insulin secretagogue similar to repaglinide, lipid peroxidation and nitrotyrosine were significantly diminished in plasma of type 2 diabetic patients (Assaloni et al., 2005). As far as repaglinide concerns, it was observed to decrease lipid peroxidation and to increase total anti-oxidative capacity in type 2 diabetic patients (Li et al., 2010; Manzella et al., 2005). In type 2 diabetes, however, these effects of the drug were attributed, at least in part, to improved glycemic control. On the other hand, repaglinide is a highly lipophilic benzoic acid derivative. In the literature, the data about a direct anti-oxidative activity such compounds, especially appearing in the presence of hydroxyl radical, were reported (Haseloff et al., 1990). Our data about the decreased lipid peroxidation and nitrotyrosine (Gumieniczek et al., 2009) together with normalization of aconitase in the present study confirm this.

Finally, principal component analysis was performed to better show potential relationships between aconitase alterations, oxidative/nitrosative stress and inflammation in our hyperglycemic and hyperglycemic-treated animals. For the heart and lung (Figs. 1–2), the plots are composed of positive scores of lipid peroxidation and nitrotyrosine. For these two tissues, a negative score of aconitase is also clearly seen. From these plots it is concluded that positive effects leading to control values after the treatments, i.e. reduction of nitrotyrosine and lipid peroxidation as well as normalization of aconitase, are correlated. On the other hand, the reduction of MPO after pioglitazone seems to be uncorrelated with other changes. Similarly, for testicular tissue (Fig. 3), a positive score of lipid peroxidation and a negative score of aconitase are present. However, the scores of testicular nitrotyrosine and MPO are oriented differently than these for the heart and lung. Therefore, we can conclude that the observed correlations may depend on the parameter being examined and/or on the specificity of the examined tissue.

Principal component analysis was also performed to better show similarities and differences between the both drugs. From Figs. 1–3 it is clearly seen that location of pioglitazone-treated and repaglinide-treated groups in relation to hyperglycemia-non treated animals differs from each other. Therefore, we can state that the changes after pioglitazone are higher than these after repaglinide, especially for the heart and lung.

In conclusion, we can state that the observed anti-oxidative effects of the both drugs are not secondary to their action on hyperglycemia. Next, pioglitazone prevents tyrosine nitration and lipid peroxidation better than repaglinide, to much extent affects aconitase and, as opposed to repaglinide, affects MPO level. In this aspect, the specific action of pioglitazone as PPARγ agonist is probably essential.

We realize that further work is needed to closely examine these effects of the both drugs. However, in the literature it is also supposed that some drugs are better at minimizing glucose toxicity than others, even though markers of glycemic control are similar (Choi et al., 2008).

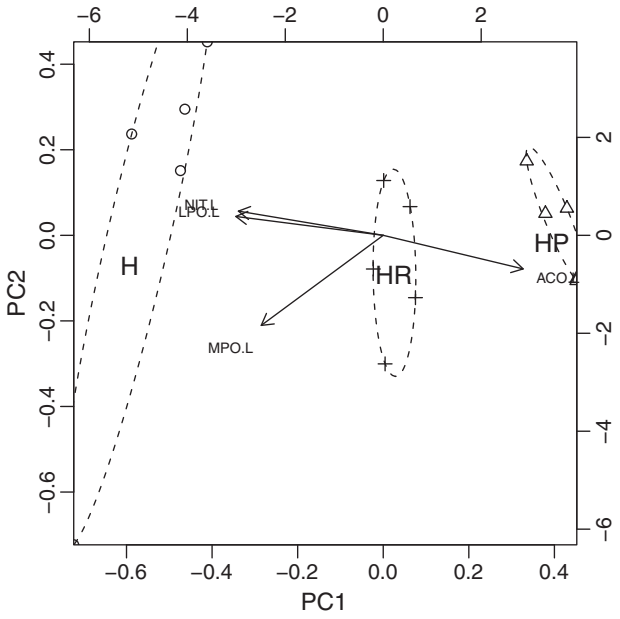

Fig. 2. The principal component 1 (PC1) versus principal component 2 (PC2) scores plot of the data obtained for the lung of hyperglycemic (H), hyperglycemic pioglitazone-treated (HP) and hyperglycemic-repaglinide treated (HR) animals (ACO.L—aconitase, LPO.L—lipid peroxidation products, MPO.L—myeloperoxidase, NIT.L—nitrotyrosine).

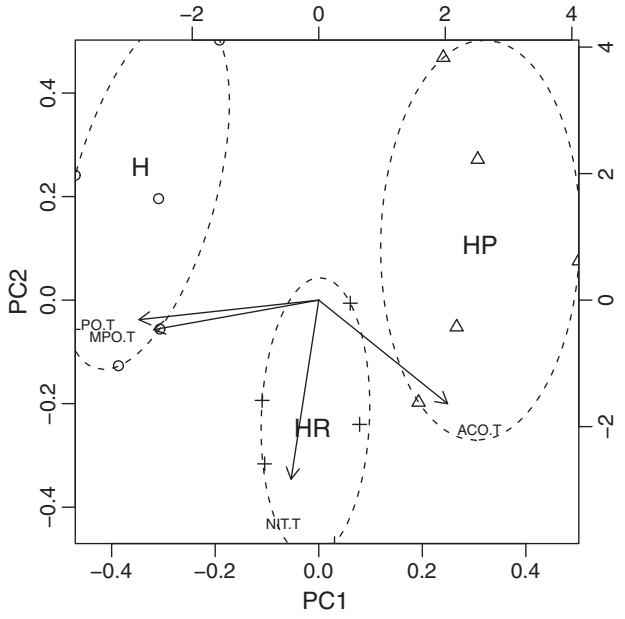

Fig. 3. The principal component 1 (PC1) versus principal component 2 (PC2) scores plot of the data obtained for the testis of hyperglycemic (H), hyperglycemic pioglitazone-treated (HP) and hyperglycemic-repaglinide treated (HR) animals (ACO.T—aconitase, LPO.T—lipid peroxidation products, MPO.T—myeloperoxidase, NIT.T—nitrotyrosine).

The present results are worthy to discuss because of very limited data presented previously. To the best of our knowledge it is the first report trying to shed light on potential relationships between aconitase inactivation and possible mitochondrial dysfunctions, oxidative/nitrosative stress and inflammatory response in hyperglycemic state. It is also the first report concerning the action of pioglitazone and repaglinide on the mentioned parameters *in vivo* experiment.

References

Aljada, A., Garg, R., Ghanim, H., Mohanty, P., Kumbkarni, Y., Assian, E., Haouda, W., Dandona, P., 2001. Suppression of nuclear factor-κB and stimulation of inhibitor κB by troglitazone: evidence for an anti-inflammatory effect and a potential anti-atherosclerotic effect in the obese. J. Clin. Endocrinol. Metab. 8, 1306–1312.

Assaloni, R., Da Ros, R., Quagliaro, L., Piconi, L., Maier, A., Zuodar, G., Motz, E., Ceriello, A., 2005. Effects of S21403 (mitiglinide) on postprandial generation of oxidative stress and inflammation in type 2 diabetic patients. Diabetologia 48, 1919–1924.

Aydin, A., Orhan, H., Sayal, A., Ozata, M., Sahin, G., Isimer, A., 2001. Oxidative stress and nitric oxide parameters in type II diabetes mellitus: effect of glycemic control. Clin. Biochem. 34, 265–270.

Ceriello, A., Mercuri, F., Quagliaro, L., Assaloni, R., Motz, E., Tonutti, L., Taboga, C., 2001. Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. Diabetologia 44, 834–838.

Choi, S., Benzie, I.F.F., Ma, F., Strain, J.J., Hannigan, B.M., 2008. Acute hyperglycemia and oxidative stress: direct cause and effect? Free Radic. Biol. Med. 44, 1217–1231.

Collino, M., Aragno, M., Mastrocola, R., Galicchio, M., Rosa, A.C., Dianzani, C., Thiemermann, C., Fantozzi, R., 2006. Modulation of the oxidative stress and inflammatory response by PPAR-γ agonists in the hippocampus of rats exposed to cerebal ischemia/reperfusion. Eur. J. Pharmacol. 539, 70–80.

Cuzzocrea, S., Pisano, B., Dugo, L., Ianaro, A., Maffia, P., Patel, N., Di Paola, R., Ialenti, A., Genovese, T., Chatterjee, P., Di Rosa, M., Caputi, A., Thiemermann, C., 2004. Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-γ, reduces acute inflammation. Eur. J. Pharmacol. 483, 79–93.

El-Alfy, A.T., Ahmed, A.A.E., Fatani, A.J., 2005. Protective effect of red grape seeds proanthocyanidins against induction of diabetes by alloxan in rats. Pharmacol. Res. 252, 264–270.

Gumieniczek, A., 2005. Effects of repaglinide on oxidative stress in tissues of diabetic rabbits. Diab. Res. Clin. Pract. 68, 89–95.

Gumieniczek, A., Krzywdzińska, M., Nowak, M., 2009. Modulation of nitrosative/oxidative stress in the lung of hyperglycemic rabbits by two antidiabetics, pioglitazone and repaglinide. Exp. Lung Res. 35, 371–379.

Haseloff, R.F., Blasig, I.E., Meffert, H., Ebert, B., 1990. Hydroxyl radical scavenging and antipsoratic activity of benzoic acid derivatives. Free Radic. Biol. Med. 9, 111–115.

Hoeldtke, R.D., 2003. Nitrosative stress in early type 1 diabetes. Clin. Auton. Res. 13, 406–421.

Inoue, I., Goto, S., Matsunaga, T., Nakajima, T., Awata, T., Hokari, S., Komoda, T., Katayama, S., 2001. The ligands/activators for peroxisome proliferator-activated receptor α (PPARα) and PPARγ increase Cu + 2, Zn + 2-superoxide dismutase and decrease p22phox message expression in primary endothelial cells. Metabolism 50, 3–11.

Ito, K., Shimada, J., Kato, D., Toda, S., Takagi, T., Naito, Y., Yoshikawa, T., Kitamura, N., 2004. Protective effects of preischemic treatment with pioglitazone, a peroxisome proliferator-activated receptor-γ ligand, on lung ischemia–reperfusion injury in rats. Eur. J. Cardiothorac. Surg. 25, 530–536.

Jornayvaz, F., Brulhart-Meynet, B., James, R.W., 2009. Myeloperoxidase and paraoxonase-1 in type 2 diabetic patients. Nutr. Metab. Cardiovasc. Dis. 19, 613–619.

Lee, K.S., Kim, S.R., Park, S.J., park, H.S., Min, K.H., Jin, S.M., Lee, M.K., Kim, U.H., Lee, Y.C., 2006. Peroxisome proliferator activated receptor-γ modulates reactive oxygen species generation and activation of nuclear factor κB and hypoxia-inducible factor 1α in allergic airway disease in mice. J. Allergy Clin. Immunol. 118, 120–127.

Li, Y., Xu, L., Shen, J., Ran, J., Zhang, Y., Wang, M., Yan, L., Cheng, H., Fu, Z., 2010. Effects of short-term therapy with different insulin secretagogues on glucose metabolism, lipid parameters and oxidative stress in newly diagnosed type 2 diabetes mellitus. Diab. Res. Clin. Pract. 88, 42–47.

Lin, G., Brownsey, R.W., MacLeod, K.M., 2009. Regulation of mitochondrial aconitase by phosphorylation in diabetic rat heart. Cell. Mol. Life Sci. 66, 919–932.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265–267.

Manzella, D., Abbatecola, A.M., Grella, R., Paolisso, G., 2005. Repaglinide administration improves brachial reactivity in type 2 diabetic patients. Diab. Care 28, 366–371.

Marfella, R., Quagliaro, L., Nappo, F., Ceriello, A., Giugliano, D., 2001. Acute hyperglycemia induces an oxidative stress in healthy subjects. J. Clin. Invest. 108, 635–636.

Muralidhara, B.S., 2007. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: its progression and genotoxic consequences. Reprod. Toxicol. 23, 578–587.

Nicolaie, T., Zavoianu, C., Nuta, P., 2003. Pulmonary involvement in diabetes mellitus. Rom. J. Intern. Med. 41, 365–374.

Pacher, P., Schultz, R., Liaudet, L., Szabó, C., 2005. Nitrosative stress and pharmacological modulation of heart failure. Trends Pharmacol. Sci. 26, 302–310.

Pérez-Matute, P., Zulet, M.A., Martinez, J.A., 2009. Reactive species and diabetes: counteracting oxidative stress to improve health. Curr. Opin. Pharmacol. 9, 771–779.

Pop-Busui, R., Oral, E., Raffel, D., Byun, J., Bajirovic, V., Vivekanandanpgiri, A., Kellogg, A., Pennathur, S., Stevens, M.J., 2009. Impact of rosiglitazone and glyburide on nitrosative stress and myocardial blood flow regulation in type 2 diabetes mellitus. Metab. Clin. Exp. 58, 989–994.

Ricardiollo, F., Di Stefano, A., Sabatini, F., Folkerts, G., 2006. Reactive nitrogen species in the respiratory tract. Eur. J. Pharmacol. 533, 240–252.

Shiojiri, T., Wada, K., Nakajima, A., Katayama, K., Shibuya, A., Kudo, C., Kadowaki, T., Mayumi, T., Yura, Y., Kamisaki, Y., 2002. PPARγ ligands inhibit nitrotyrosine formation and inflammatory mediator expressions in adjuvant-induced rheumatoid arthritis mice. Eur. J. Pharmacol. 448, 231–238.

Staels, B., Fruchart, J.C., 2005. Therapeutic roles of peroxisome proliferator-activated receptor agonists. Diabetes 54, 2460–2470.

Şener, G., Kübra, P., Şehirli, A.Ö., Dülger, A.G., Alican, I., 2002. The effects of melatonin on ischemia–reperfusion induced changes in rat corpus cavernosum. J. Urol. 167, 2624–2627.

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., 2003. Anti-oxidative, antinitrative, and vasculoprotective effects of a peroxisome proliferators-activated receptor-γ agonist in hypercholesterolemia. Circulation 108, 2805–2811.

Tórtora, V., Quijano, C., Freeman, B., Radi, R., Castro, L., 2007. Mitochondrial aconitase reaction with nitric oxide, S-nitrosoglutathione and peroxynitrite: mechanisms and relative contributions to aconitase inactivation. Free Radic. Biol. Med. 4, 1075–1088.

Turkyilmaz, S., Alhan, E., Ercin, C., Vanizor, B.K., Kaklikkaya, N., Ates, B., Erdogan, S., Topaloglu, S., 2008. Effects of caffeic acid phenethyl ester on pancreatitis in rats. J. Surg. Res. 145, 19–24.

Uchimura, K., Nagasaka, A., Hayashi, R., Makino, M., Nagata, M., Kakizawa, H., Kobayashi, T., Fujiwara, K., Kato, T., Iwase, K., Shinohara, R., Kato, K., Itoh, M., 1999. Changes in superoxide dismutase activities and concentrations and myeloperoxidase activities in leukocytes from patients with diabetes mellitus. J. Diab. Complications 13, 264–270.

Wolffenbuttel, B.H.R., 1999. Repaglinide—a new compound for the treatment of patients with type 2 diabetes. Neth. J. Med. 55, 229–234.

Zhang, S.J., Sandstrom, M.E., Lanner, J.T., Thorell, A., Westerblad, H., Katz, A., 2007. Activation of aconitase in mouse fast-twitch skeletal muscle during contraction-mediated oxidative stress. Am. J. Physiol. Cell Physiol. 293, C1154–C1159.