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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.