Short Communication

Effects of Aminoguanidine Schiff's Base on Biomarkers of the Oxidative Stress, 4-hydroxy-2-nonenal and Conjugated Dienes, in the Model Diabetes Mellitus

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Abstract. Diabetes mellitus evoked by streptozotocine in rats is associated with the oxidative stress. We examined the effect of Schiff's base 2,5-dihydroxybenzaldehyde with a well-known antidiabetic drug aminoguanidine, 2,5-dihydroxybenzilideneaminoguanidine (BAG) on the production of markers of oxidative stress such as 4-hydroxy-2-nonenal (4HNE) and conjugated dienes in diabetic rats. BAG administration did not affect glucose level in diabetic rats but significantly decreased the production of 4HNE and conjugated dienes. On the other hand, BAG caused the elevation of conjugated dienes and an insignificant increase of 4HNE levels in the control animals.

Key words: Diabetes mellitus — Oxidative stress — Antioxidant — 4-hydroxy-2-nonenal — 2,5-dihydroxybenzilideneaminoguanidine

Introduction

The imbalance between the production and removal of the oxygen and nitrogen reactive species followed by the damage of biomacromolecules is called oxidative stress. The oxidative stress plays an important role in the etiopathogenesis of many diseases and participates in the development of their chronic complications. For example significantly increased formation of products of the oxidative damage to lipids, proteins and DNA was detected in the blood and tissues of Diabetes mellitus (DM) patients. On the other hand these patients contain decreased levels of antioxidants (Muchová 1999; Čársky 1999; Zeman et al. 2000).

Oxidation of lipoproteins is a characteristic event in the oxidative stress caused by the oxidative damage to polyunsaturated fatty acids. Especially lipids and other

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lipoproteins of biological membranes are very sensitive to the lipoperoxidation depending on the content of unsaturated fatty acids by free radicals action. Various oxidative products are formed by the action of free radicals, especially aldehydes, as malonedialdehyde (MDA) or 4-hydroxy-2-nonenal (4HNE) and other hydroxyalkenals with various chain length. These aldehyde molecules are considered to be end mediators of toxic effects evoked by various oxidants. Relatively long-lived hydrophobic aldehydes are assumed to penetrate through cell membranes to distant places, in contrast to short-lived free radicals. These aldehydes act on intracellular and extracellular targets of different organs to elicit cell and tissue damage (Ďuračková 1998). Toxic messengers as 4HNE and other aldehydes mediate oxidative damage on the molecular level (Parola et al. 1999). They may react with glutathione, thiols of low-molecular weight and various macromolecules, e.g. proteins and nucleic acid. Reactions of 4HNE with certain aminoacid units of cell proteins may produce Schiff's base structures, cross-links and Michael's adducts. For example, 4HNE reacts with the sulphur in cysteine, ε-amino group of lysine and with nitrogen of the imidazole ring (Friguet et al. 1994; Nadkarni and Sayre 1995; Waeg et al. 1996; Parola et al. 1999). This aldehyde also binds to amino groups of phospholipids, (e.g. phosphatidylethanolamine) and changes their properties.

Aminoguanidine (AG) is a compound with a strong affinity to aldehydes, which prevents the change of apoprotein B elicited by LDL oxidation. Endothelial cells or copper ions may be involved in this oxidation. Aminoguanidine is assumed to act by an antioxidative mechanism, blocks reactive oxo-groups, or scavenges dicarbonyl intermediates produced in glycooxidative processes. This mechanism is characterised by the inhibition of reactions leading to formation of advanced glycation end products (AGEs). The therapeutic application of this compound is problematic because of aminoguanidine's toxic effects. Therefore various Schiff's bases of AG were synthesised to produce less toxic analogues. One of them, resorcilidene aminoguanidine (RAG) (Čársky et al. 1978), shows antidiabetic effects and inhibits the production of AGEs (Šikurová et al. 2000; Waczulíková et al. 2000). However, this derivative has also some prooxidative effects so another Schiff's base, 2,5-dihydroxybenzylideneaminoguanidine (BAG) was synthesised and tested.

The objective of this study was to determine effects of BAG on production of certain substances characteristic for the oxidative stress in streptozotocin induced DM in rats.

Materials and Methods

Animals

We used Wistar rats, males with weight of 280–350 g. They were fed with the standard Larsen diet. Animals had the free access to the food and drinking water. We divided rats into four groups.

Control group (C) – healthy animals with administered water into their stomach (5 ml/kg) by the sounder. The physiological solution was applied subcutaneously (0.5 ml/kg).

Control group (C+BAG) – healthy animals with administered BAG in physiological solution into their stomach (5 mg/kg) once a day during eight weeks.

Experimental group (DIA) – animals with evoked DM. Drinking water (5 ml/kg) was administered into their stomach.

Experimental group (DIA+BAG) – animals with DM. BAG in physiological solution (5 mg/kg) once a day during eight weeks was applied into their stomach by the sounder. DM was elicited by the single-dose administration of streptozotocine (60 mg/kg in 0.5 mol/dm^3 citrate buffer, pH 4.5) into tail's vein. Insulin MONO ID at the dosage 12 U/kg was injected subcutaneously to both diabetics groups every day during eight weeks.

During the rat handling the national rules and instructions for the rearing the laboratory animals were followed.

Chemicals

4-hydroxy-2-nonenal; steptozotocine (Sigma, Germany); HPLC-acetonitrile, hexane (Merck, Germany); HPLC-methanol (Fluka, Germany); 2,4-dinitrophenylhydrazine (Lachema, Czech republic), insulin MONO ID (Léčiva, Czech republic), heparin (Mercle, Germany). All other chemicals were obtained from Lachema (Brno, Czech republic) and were of analytical grade. Solutions were prepared in redistilled water. BAG was synthesised by the condensation of water-alcoholic solution of AG and 2,5-dihydroxybenzaldehyde.

Methods

We obtained plasma from heparinised blood (25 units/ml). Plasma samples were stored in the deep freezer at −80 ℃ (VXE 380, Jouan, Czech republic).

Oxidation of lipoproteins

We determined the oxidation of lipoproteins by the method of Schnitzer et al. (1998). Reaction mixture (2 ml) contained 20 μl of plasma, $CuCl_2 \cdot 2H_2O$ (5×10^{-6} mol/dm^3), 3.3×10^{-3}mol/dm^3 phosphate buffer (pH 7.4). The production of conjugated dienes was examined as an absorbance change at the wavelength 245 nm during 240 min at 37 ℃ (Biochrom 4060, Pharmacia, Finland).

4HNE determination

We determined 4HNE levels by the method of Kinter (1996). 250 μl physiological solution and 500 μl DNPH (5 mmol/dm^3) were added to 250 μl of plasma. After the intensive stirring, the reaction mixture stood in dark at room temperature for 1 hour and than was extracted 3 times into 1 ml of hexane. Pooled extracts were evaporated to dryness under the argon stream at 40 ℃. The extract was diluted in 70 % acetonitrile in distilled water and used for HPLC analyse. The flow rate was 1 ml/min, injected volume 20 μl, detection was performed by the UV detector (DeltaChrome UVD 200, Czech republic) at 355 nm. The 4HNE concentration was determined from the calibration curve.

We assessed the glucose concentration by the standard biochemical methods in the automatic analyzator Hitachi 911 (Roche, Switzerland). To evaluate the results statistically, Student's t-test was used. We present the values as the mean ± standard error of mean.

Results

Oxidability of plasma lipoproteins

We examined lipoprotein sensitivity to the oxidation by the determination of conjugated dienes levels. The kinetic of lipid oxidation is represented by a curve ($A = f(t)$, where: A – absorbance at 245 nm; t – time in minutes), which is characterised by various parameters (Nagyova et al. 1998, 1999, 2000). We used slope (maximal rate of accumulation of absorbing products – conjugated dienes, expressed in ΔA per second), which shows the rate of lipid oxidation and the rate of conjugated dienes production.

Average values of slope characterising the lipoprotein oxidation in rat's plasma are shown in Table 1.

The higher value of slope corresponds to the faster lipid oxidation. The differences in the maximal rate values of accumulation of absorbing products in different groups (C, DIA, C+BAG, DIA+BAG) expressed in percents are shown in Figure 1.

We found the statistically significant elevation of slope which characterises the faster oxidation of lipoproteins in plasma of diabetic rats (DIA) when compared to the control group (C) ($p = 0.0073$).

The slope in diabetic rats with BAG administration (DIA+BAG) compared to the diabetic rats (DIA) is significantly lower ($p = 0,0289$). It means that BAG decreases the rate of conjugated dienes production by 41.81 % in diabetic rats (Fig. 1). On the other hand, BAG administration to healthy animals (C) significantly elevated the lipoprotein oxidation by 34.94 % ($p = 0.026$) (Fig. 1).

4HNE determination

4HNE concentrations determined in rat plasma are shown in Table 1. In Figure 2 there are depicted 4HNE concentrations in rat's plasma of different experimental groups (C, DIA, C+BAG, DIA+BAG), expressed in percents.

Table 1. Parameters, which characterise the lipoprotein oxidation. Glucose was determined in blood and 4HNE was determined in plasma of rats. Slope characterises the rate of conjugated dienes formation. Values are expressed as mean ± S.E.M. ($n = 6$–8)

Groups	Glucose ($\mathrm{mmol \cdot dm^{-3}}$)	Slope (10^{-6} ΔA/s)	c(4HNE) ($\mu\mathrm{mol \cdot dm^{-3}}$)
Control	7.19 ± 0.36	8.30 ± 0.96	4.46 ± 0.42
DIA	20.97 ± 1.04	13.85 ± 1.35	5.36 ± 0.14
C+BAG	7.41 ± 0.35	11.20 ± 0.69	5.11 ± 0.41
DIA+BAG	19.21 ± 4.11	10.37 ± 0.68	4.60 ± 0.40

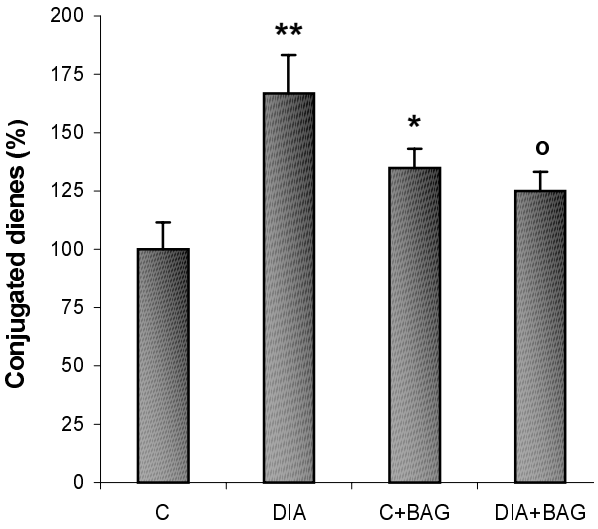

Figure 1. Slope (ΔA/s) characterises the kinetic of the lipoprotein oxidation in plasma of diabetic rats. The slope is 100% in the control group. $*$ – significance is compared with the control group (C) ($0.01 < p < 0.05$); $**$ – significance is compared with the control group (C) ($0.001 < p < 0.01$); $\circ$ – significance is compared with DIA group ($0.01 < p < 0.05$); $n = 6$–8.

We found significant increase of 4HNE levels in diabetic rats when compared with the control group ($p = 0,0464$). BAG markedly decreases 4HNE levels in diabetic rats (Fig. 2) ($p = 0.0768$). However, BAG administration to the control group elicits insignificant increase in 4HNE levels when compared to the controls without BAG.

Discussion

Lipoprotein peroxidation is one of the oxidative stress effects found in DM (Haffner et al. 1995; Mowri et al. 2000; Rašlová et al. 2000). LDL particles are very sensitive substrates for the oxidative damage, which can be structurally and functionally modified. Both lipid and protein components of LDL can undergo the oxidative damage. Lipid oxidation in unfractionated plasma is also effected by other plasma components such as hydrophilic and lipophilic antioxidants and relatively high concentration of albumin. Albumin acts as an antioxidant by binding ions of transmission elements, e.g. Cu^{2+} (Schnitzer et al. 1995) to prevent their participation in the free radical reactions. LDL oxidation also depends on the type of antioxidant, which is present during the oxidative stress. In case of lack of physiological antioxidants, exogenously administered compounds can substitute their insufficient levels, (Aruoma 1998; Muchová 1999) and could diminish the oxidative attack (Curcio and Ceriello 1992; Packer 1993; Cunningham et al. 1994; Holeček and Racek 1995).

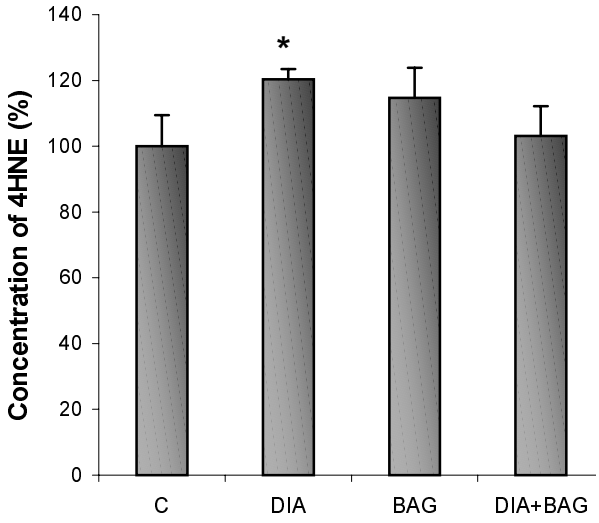

Figure 2. Content of 4HNE in rat plasma (%) compared to control (100%). ∗ – significance compared to control (C) ($0.01 < p < 0.05$); $n = 6$–8.

The effect of another AG derivative, RAG, was monitored in DM patients. RAG modified increased transmembrane potential of erythrocyte's membrane and their fluidity (Waczulíková et al. 2000), as well as the fluidity of myocard sarcolema (Ziegelhöffer et al. 1997). In model diabetes mellitus RAG decreased levels of TBARS. On the other hand RAG was shown to have prooxidative effects in control animals (Liptáková et al. 2002) and therefore other derivatives of AG (e.g. BAG) were studied.

The kinetic of the lipid oxidation can be characterised by various parameters. The slope represents the rate of conjugated dienes production. The lowers are levels, the later starts the lipid oxidation and the substrate is less sensitive to the oxidation.

The rate of the lipid oxidation in diabetic rats is significantly increased when compared to healthy animals. Our results clearly show that BAG administration to diabetic rats causes the significant decrease of the rate of the lipoprotein oxidation (Table 1). Compared to diabetic rats without BAG, fewer primary products of the lipoperoxidation (conjugated dienes) are produced.

BAG administration to diabetic rats significantly decreases their sensitivity to the lipoperoxidation and decreases the production of conjugated dienes in comparison with diabetic rats without BAG supplementation. On the other hand, BAG administration to healthy animals significantly increases the rate of the lipid oxidation and the production of conjugated dienes when compared to controls (Fig. 1).

Similar double-edged effect of BAG on control rats was detected during the monitoring of 4HNE (Fig. 2). Increased production of 4HNE was determined in

diabetic rats (Fig. 2). BAG treatment of diabetic rats inhibited 4HNE production equally to conjugated dienes formation. However BAG administration to the control group initiated insignificant increase in 4HNE formation.

Despite these facts the inhibition of conjugated dienes and 4HNE formation in Diabetes mellitus after BAG supplementation suggests that BAG is a potentially suitable inhibitory compound in oxidative stress. The elevated glucose level evokes oxidative stress in diabetic rats, even though the direct inhibitory effect of BAG on the glucose level has not been proved yet (unpublished results). It is not known whether the product of Schiff's bases hydrolysis has also antidiabetic effect when it reacts with toxic dicarbonyl metabolites formed in glycooxidative reactions. BAG degradation to 2,4-dihydroxybenzaldehyde and AG could lead to the increased oxidative stress, which was found in controls after BAG treatment. Aromatic diphenols could initiate the increased production of free radicals during their metabolic degradation. Although BAG supplementation to diabetic rats inhibited the formation of oxidative damage products, BAG's stimulatory effect on the production of 4HNE and conjugated dienes in controls predetermines this compound for further, especially toxicological investigations

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