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Influence of pyridoxylidene aminoguanidine on biomarkers of the oxidative stress and selected metabolic parameters of rats with diabetes mellitus

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Abstract. Oxidative damage is considered to play an important role in the pathogenesis of several diseases, such as diabetes mellitus (DM), atherosclerosis, cardiovascular complications and chronic renal failure. DM is associated with the oxidative stress and formation of advanced glycation end products (AGEs). Different drugs inhibit oxidative stress and formation of advanced glycation end products. Aminoguanidine (AG) has been proposed as a drug of potential benefit in prophylaxis of the complications of DM. Recent reports show a pro-oxidant activity of AG. Therefore we examined the effect of structural analogue of AG, its Schiff base with pyridoxal – pyridoxylidene aminoguanidine (PAG) on the level of selected markers of oxidative stress. We found that PAG decreased total damage to DNA in controls as well as in diabetic group of rats. However, we also found that PAG supplementation increases susceptibility of lipoproteins to oxidation and formation of conjugated dienes in both, diabetic as well as control animals. Its administration to diabetic rats decreases antioxidant capacity of plasma. Therefore, it is necessary to search for other structural modifications of AG that would combine its higher anti-diabetic activity with less toxicity.

Key words: Oxidative stress — Free radicals — Diabetes mellitus — Pyridoxylidene aminoguanidine

Introduction

Diabetes mellitus (DM) is a chronical disease characterized with lack of insulin or its significantly decreased effect. In the pathogenesis of many diseases, including DM, oxidative stress plays important role (Aruoma 1998; Ďuračková 1998; Halliwell and Gutteridge 1999; Simmons 2006). Increased oxidative stress is related to free radicals formation, non-enzymatic glycation of proteins, auto-oxidation of glucose (glycoxidation processes), the changes in lipid metabolism and in antioxidative protection of the organism (Muchová 1999; Aliciguzel et al. 2003; Kalousová et al. 2005) and it is proposed to be connected with the progression of later chronical diabetic complications.

There is no doubt about the role of oxidative stress in pathogenesis of DM. The only questions remain if oxidative stress primarily plays role in development of diabetic complications, or if it occurs in early stages of DM or it is only the consequence of tissue damage thus reflecting appearance of diabetic complications (Lyons and Jenkins 1997; Baynes and Thorpe 1999). Many studies confirm hyperglycaemia to cause increased production of superoxide ($O_2^{\cdot -}$), which is the origin of all pathways in pathogenesis of diabetic complications. $O_2^{\cdot -}$ can react with nitroxide ($NO^{\cdot}$, hereafter NO) and form strong oxidant peroxinitrite ($ONOO^-$). These processes result in acute endothelium dysfunctions and activation of inflam-

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matory processes in blood vessels of patients with DM and they are the factors contributing to development of diabetic complications (Niedowicz and Daleke 2005; Cerielo 2006; Shahab 2006). The most known complications are cardiovascular diseases, impairment of vascular system (Liptáková 1999; Jay et al. 2006), peripheral nerves, kidneys, epidermis, retina, lens, neurodegenerative diseases like Alzheimer or Parkinson diseases and others (El-Atat et al. 2004; Vasavada and Agarwal 2005). High concentration of glucose and associated oxidative stress and glycoxidation processes affect adversely pancreatic β-cells (Robertson and Harmon 2006; Miyazaki et al. 2007). Oxidative stress unfavourably affects already children and teenagers and it is manifested also in paediatric diseases (Tsukahara 2007).

Glucose can non-enzymatically condense with $-NH_2$ groups of proteins and form Schiff base (glycation), which can be changed to Amadori product in rearrangement reaction. The Amadori products are changed over time and they provide another reactive compounds which can react with proteins and form cross-links as well as brown coloured and fluorescent molecules called Maillard products (advanced glycation end products – AGEs) (Wolff 1996; Kalousová et al. 2005; Monnier et al. 2008). AGEs are represented by a heterogeneous group of compounds, e.g. pentosidine, carboxymethyllysine, imidazolene, etc.

AGEs inactivate NO thus increase blood pressure and they stimulate cytokines which results in increased amount of free radicals in polymorphonuclear leukocytes. Glycation of lipoproteins facilitates their lipoperoxidation, so it is possible to conclude that glycation together with glycoxidation increase oxidative modification of lipoproteins in DM which can be related to vascular diseases development (Jenkins et al. 2004; Davi et al. 2005; Castelao and Gago-Dominguez 2008).

Involvement of glycoxidation and formation of AGEs in pathogenesis of diabetic complications raises the question of possibility of therapeutic affection of these complex processes. The compounds are searched for, which could prevent the formation AGEs or eliminate their effect (Vasan et al. 2003; Thomas et al. 2005; Machado et al. 2006; Coughlan et al. 2007; Tanimoto et al. 2007; Muellenbach et al. 2008). One of these compounds is aminoguanidine (AG), which was, thanks to its effect, proposed for the prevention of diabetic complications (Giardino et al. 1998; Gül et al. 2008; Wu et al. 2008). AG is assumed to work by anti-oxidative mechanism as well as by blocking reactive oxo-groups or scavenging dicarbonyl intermediates, respectively, which are formed in glycoxidation processes. AG binds to Amadori product to form triazene (eventually dihydrazone) so AGEs and cross-links cannot be formed. It can directly react with malondialdehyde (MDA) and destruct it. AG significantly has decreased formation of AGE-hemoglobine and it is effective in inhibition of inducible form of NO-synthase. AG prevents circulating AGEs to accumulate in tissues (Holeček and Racek 1995; Stoppa et al. 2006).

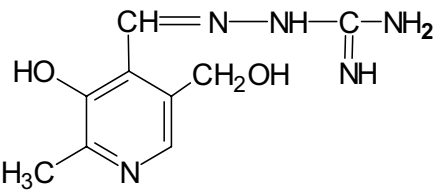

Figure 1. Formula of PAG.

However, it was found that AG irreversibly inhibits catalase. It takes part in H_2O_2 formation in processes catalyzed with transition metal ions. H_2O_2 can cause hydrolysis of AG to semicarbazide and hydrazine. Administration of AG in therapy is necessary to consider carefully because of its possible toxic and pro-oxidative effects as well as it is necessary to regard its long time administration (Ou and Wolff 1993; Skamarauskas et al. 1996). That is the reason why the possibilities of AG derivatives are investigated recently. One of them is Schiff base of AG with pyridoxal – pyridoxylidene aminoguanidine (PAG), (Fig. 1). The aim of our study was to find the effect of PAG administration to rats with streptozotocine-induced DM on levels of oxidative damage markers, anti-oxidative capacity of plasma and selected biochemical parameters.

Materials and Methods

Chemicals

Hexane, thiobarbituric acid, HPLC-acetonitrile (Merck, Germany); HPLC-methanol (Fluka, Switzerland); streptozotocine, luminol, 1,1,3,3-tetraethoxypropane, 4-hydroxy-2-nonenal (Sigma, USA); trolox (Aldrich, Germany); ACW (antioxidant capacity of water soluble substances) set from F.A.T. (Germany); heparin (Mercle, Germany); insulin MONO ID (Léčiva, Czech Republic). PAG was synthesized in the laboratory of prof. Čársky. All other used chemicals were from Lachema (Brno, Czech Republic). Solutions were prepared in redistilled water.

Experimental animals

Male Wistar rats with weights of 280–350 g were used in the experiment. The animals were fed with standard Larsen food and had *ad-libitum* access to food and fresh water. The rats were divided into four groups:

1. Control group (C) – healthy animals, to whom fresh water was administered into stomach by a probe (10 ml/kg), and physiological solution (0.5 ml/kg) subcutaneously.

2. Diabetic group (D) – animals with induced DM, to whom fresh water was administered into stomach by a probe (10 ml/kg).
3. Control group treated with PAG (P) – healthy animals, to whom PAG in physiological solution at doses of 10 mg/kg was administered into stomach by a probe, once daily during 8 weeks.
4. Diabetic group treated with PAG (D + P) – animals with induced DM, to whom PAG in physiological solution at doses of 10 mg/kg was administered into stomach by a probe, once daily during 8 weeks.

DM was induced by administration of a single dose of streptozotocin (45 mg/kg in 0.5 mol/l citrate buffer, pH 4.5) into the tail vein. Insulin MONO ID at doses of 6 U/kg was administered to both diabetic groups (D and D + P) subcutaneously, daily during 8 weeks.

National regulations for breeding and use of laboratory animals were respected in handling of the rats.

Methods

Plasma was prepared from heparinized blood (25 U/ml) from abdominal aorta and frozen at –80°C prior to use.

Oxidation of plasma lipoproteins

The oxidation of plasma lipoproteins was determined by the method of Schnitzer et al. (1998). Reaction mixure (2 ml) contained 20 µl of plasma, $CuCl_2 \cdot 2H_2O$ (100 µmol/l), phosphate buffer (3.3×10^{-3} mol/l, pH 7.4). The production of conjugated dienes was examined as an absorbance change at the wavelength 245 nm during 240 min at 37°C (Biochrom 4060, Pharmacia, Finland).

MDA determination

MDA was determined with the HPLC method (Watrex) with UV detection (DeltaChrome UV200, Czech Republic) according to Brown and Kelly (1996). 1,1,3,3-tetraethoxypropane was used as a standard. To 50 µl volume of sample or standard 250 µl of phosphoric acid (1.22 mol/l), 450 µl of water and 250 µl of thiobarbituric acid (0.046 mol/l) were added and reaction mixture was incubated at 95°C in the thermoblock for 60 min. After the reaction mixture was cooled, 360 µl of HPLC-grade methanol and 40 µl of NaOH (1 mol/l) were added to 200 µl of sample or standard reaction mixture and centrifuged for 5 min at 9500 × *g*. 10 µl of supernatant was used for further HPLC analysis. Mobile phase: 40% (v/v) methanol in 25 mmol/l phosphate buffer, pH 6.5; flow rate: 0.8 ml/min; column 250 × 4 mm Nucleosil 120-5 C18 (Watrex, Czech Republic); fluorescence detector FP Jasco 1520 (Ex 532 nm, Em 553 nm with gain × 1000). The MDA concentration was determined from the calibration curve and expressed in µmol of MDA *per* liter of plasma.

4-hydroxy-2-nonenal (HNE) determination

We determined HNE levels with the method of Kinter (1996). 250 µl of physiological solution and 500 µl of 2,4-dinitrophenylhydrazine (5 mmol/l) were added to 250 µl of plasma. After the intensive stirring, the reaction mixture stood in dark at room temperature for 1 hour and then was extracted 3 times into 1 ml of hexane. Pooled extracts were evaporated to dryness under the argon stream at 40°C. The extract was diluted with 70% acetonitrile in redistilled water and used for HPLC analyse. Column 250 × 4 mm Nucleosil 120-5 C18 was used; flow rate: 1 ml/min, injected volume 20 µl, detection was performed by the UV detector (DeltaChrome UV200, Watrex, Czech Republic) at 355 nm. The HNE concentration was determined from the calibration curve and expressed in µmol of HNE *per* liter of plasma.

Detection of DNA damage (comet assay)

Damage of DNA was determined with single cell gel electrophoresis (comet assay) according to Collins et al. (1997). DNA damage was expressed as total damage (TD) that was calculated for each gel by using the following formula in which i is a class of damage and N is the number of cells in each class:

$$\mathrm{TD} = \sum_{I=0}^{4} iN_i$$

Determination of antioxidant capacity of water-soluble substances of plasma (ACW)

ACW was determined by a photochemiluminescence method according to Popov and Lewin (1994). For determinations the ACW set, standard Trolox and the PHOTOCHEM photochemiluminometer (F.A.T., Berlin, Germany) were used. The levels of ACW were expressed in µmol of trolox *per* liter of plasma.

Plasma levels of glucose, cholesterol, triacylglycerols (TAG) and uric acid were determined by standard biochemical procedures, using the sets (Roche, Switzerland) and Hitachi 911 automatic analyzer. Glycated hemoglobin was determined according to Flückinger and Winterhalter (1976), and fructosamine was determined according to Johnson et al. (1982).

Statistical analysis

Statistical significance of observed effects was assessed using the Student's *t*-test. Values are expressed as mean ± S.E.M.

Results

Susceptibility of lipoproteins to oxidation

Susceptibility of lipoproteins to oxidation was determined by means of determination of conjugated dienes formation. Kinetics of oxidation is expressed by the curve of dependence of absorbance on time (A = f(t), where A is absorbance at 245 nm, t is time in minutes), which is characterized by different parameters: a) slope of curve tangent, which represents rate of lipids oxidation and rate of conjugated dienes formation expressed as ΔA/min or b) start time of lipoprotein oxidation indicating susceptibility to oxidation (Fig. 2). Susceptibility of lipoproteins to oxidation was evaluated by means of parameter slope of curve tangent. A higher value of slope of curve tangent represents faster oxidation of lipids and larger formation of conjugated dienes. Formation of conjugated dienes in diabetic rats was found increased by 50% compared to controls. However, in control rats, treatment with PAG caused increased formation of conjugated dienes by 84% and in diabetic rats by 38% compared to non-treated diabetic rats (Fig. 3).

MDA

In group of diabetic rats average concentration of MDA was 0.262 μmol/l and it was increased by 13% compared to controls. This increase was not statistically significant. Administration of PAG to control and diabetic rats did not influence formation of MDA significantly.

HNE

The average HNE concentration in diabetic rats was 18.27 μmol/l and it was not significantly higher compared to control rats (9%). Administration of PAG to control and diabetic rats did not influence formation of HNE significantly.

DNA damage

The DNA damage was determined by comet assay and expressed as total damage of DNA. In diabetic rats, the damage to DNA was found, which was increased by 112% of the control group. PAG decreases sum of DNA damage in control rats by 48% and in diabetic rats by 76% (Fig. 4).

Antioxidant capacity of water-soluble substances of plasma

ACW is decreased in diabetic rats by 26% compared to control rats. Administration of PAG decreases ACW level in control group by 19% as well as in diabetic group by 10% (Fig. 5).

Biochemical parameters

In DM, as consequence of lack of insulin or insulin resistance, blood glucose utilisation is decreased. In diabetic rats we con-

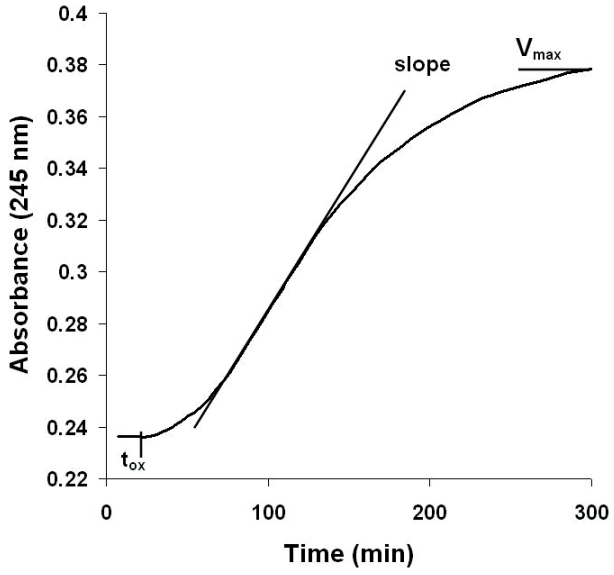

Figure 2. Lipoprotein oxidation kinetics expressed by the curve of dependence of absorbance on time. Slope of the curve indicates lipoprotein oxidation rate and rate of conjugated dienes production. Start time of lipoprotein oxidation (t_{ox}) indicates susceptibility of sample to oxidation, V_{max} indicates maximal rate of lipoprotein oxidation. Formation of conjugated dienes is expressed as change of absorbance at 245 nm dependent on time.

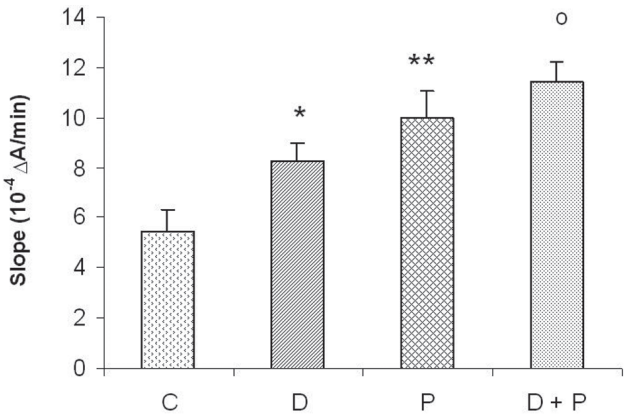

Figure 3. Effect of PAG treatment (in dose 10 mg/kg) on susceptibility of plasma lipoproteins to oxidation expressed by the slope (ΔA/min) of the curve of dependence of absorbance on time. C, control group; D, diabetic group; P, control group treated with PAG; D + P, animals with induced DM treated with PAG. DM was induced with single dose of streptozotocin (45 mg/kg in 0.5 mol/l citrate buffer, pH 4.5) into the tail vein. Significant difference compared to controls: * $p < 0.05$, ** $p = 0.01$. Significant difference compared to diabetic group: ^{o} $p < 0.05$. Values are expressed as means ± S.E.M. (n = 7–10).

firmed increased glycaemia ($p < 0.001$). In diabetic rats administration of PAG caused only non-significant decrease of glucose in plasma. The levels of glucose, glycated hemoglobin and fructosamine in plasma are indicators of metabolic status in DM. Administration of PAG did not influence levels of mentioned parameters in controls as well as in diabetic rats (Tab. 1).

Under conditions of our experiments in group of diabetic rats changes in lipid profile were observed such as increased cholesterol and TAG concentrations in plasma (Tab. 1). Administration of PAG increased cholesterol concentration in control as well as in diabetic rats and decreased TAG concentration in controls ($p < 0.05$). The influence on TAG concentration in diabetic rats was not observed. Administration of PAG did not significantly influence the uric acid concentration in controls but in diabetics this concentration was decreased ($p < 0.05$).

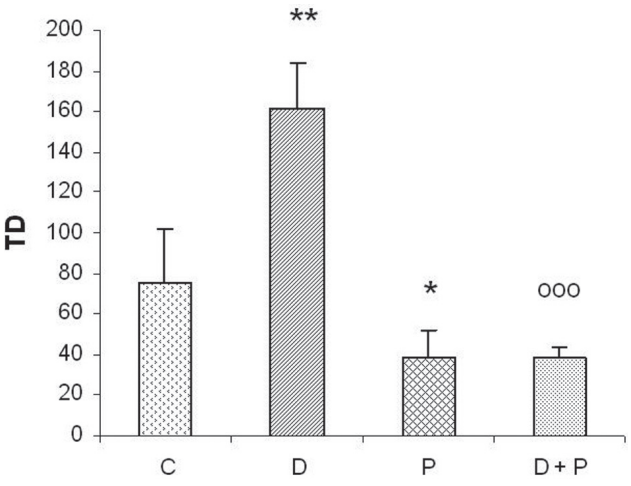

Figure 4. Effect of PAG treatment (in dose 10 mg/kg) on damage to DNA determined with single cell gel electrophoresis (comet assay) according to Collins et al. (1997) and expressed as TD (total damage), which is calculated according to the number of cells in certain class of DNA damage. C, control group; D, diabetic group; P, control group treated with PAG; D + P, animals with induced DM treated with PAG. DM was induced with single dose of streptozotocin (45 mg/kg in 0.5 mol/l citrate buffer, pH 4.5) into the tail vein. Significant difference compared to controls: * $p < 0,05$, ** $p = 0.01$. Significant difference compared to diabetic group: ^{ooo} $p < 0.001$. Values are expressed as means ± S.E.M. ($n = 7–10$).

Discussion

Several diseases are associated with oxidative and carbonyl stress including micro-inflammation and eventually autoimmune reaction. Oxidative and carbonyl stress cause damage to important biological structures – proteins, carbohydrates, lipids and nucleic acids and may enhance inflammatory

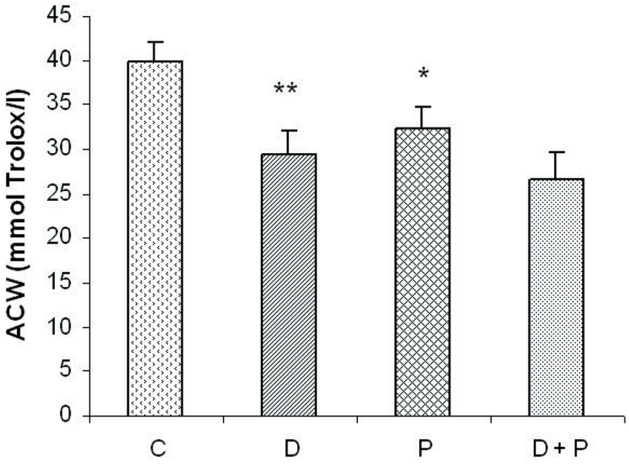

Figure 5. Effect of PAG treatment (in dose 10 mg/kg) on the levels of antioxidant capacity of water-soluble substances of plasma (ACW) expressed in μmol of trolox *per* liter of plasma. C, control group; D, diabetic group; P, control group treated with PAG; D + P, animals with induced DM treated with PAG. DM was induced with single dose of streptozotocin (45 mg/kg in 0.5 mol/l citrate buffer, pH 4.5) into the tail vein. Significant difference compared to controls: * $p < 0.05$, ** $p = 0.01$. Values are expressed as means ± S.E.M. ($n = 8$).

Table 1. Effect of PAG treatment (in dose 10 mg/kg) on selected biochemical parameters in the rat plasma

Parameter	C	D	P	D + P
Glucose (mmol/l)	7.61 ± 0.42	28.58 ± 2.82***	8.18 ± 0.24	26.72 ± 2.18
Cholesterol (mmol/l)	1.14 ± 0.08	1.42 ± 0.11	1.30 ± 0.05	1.77 ± 0.17
Triacylglycerols (mmol/l)	1.17 ± 0.25	1.24 ± 0.15	0.68 ± 0.05*	1.23 ± 0.18
Uric acid (μmol/l)	42.72 ± 2.34	44.05 ± 2.14	40.68 ± 2.11	36.62 ± 2.17^{o}
Glycated Hb (%)	4.53 ± 0.21	6.35 ± 0.345**	5.21 ± 0.39	6.39 ± 0.40
Fructosamine (mmol/l)	0.91 ± 0.04	1.24 ± 0.09*	0.89 ± 0.02	1.28 ± 0.14

C, control; D, diabetic group; P, control group treated with PAG; D + P, animals with streptozotocine-induced DM treated with PAG. DM was induced with single dose of streptozotocin (45 mg/kg in 0.5 mol/l citrate buffer, pH 4.5) into the tail vein. Significant difference compared to controls: * $p < 0.05$, ** $p = 0.01$, *** $p = 0.001$. Significant difference compared to diabetic group: ^{o} $p < 0.05$. Values represent means ± S.E.M. ($n = 7–10$).

response. New compounds and modified structures are formed, among them AGEs, advanced oxidation protein products and advanced lipoperoxidation end products (Kalousová et al. 2005).

Increased peroxidation of lipids is one of the manifestations of oxidative stress which was demonstrated in DM. Oxidation of lipoproteins expressed in formation of conjugated dienes was increased in diabetic rats (Fig. 3) compared to control rats ($p < 0.05$). Similarly, Elangovan et al. (2000) found increased levels of conjugated dienes in plasma as well as in heart, kidneys and brain of diabetic rats. However, we found that the administration of PAG increased susceptibility of lipids to oxidation both in control ($p < 0.01$) and diabetic ($p < 0.05$) rats.

Formation of MDA in plasma of diabetic rats was moderately increased compared to control group. Administration of PAG did not influence level of MDA in plasma of control and diabetic rats. Similar results were found in our laboratory after administration of different derivative of AG namely 2,5-dihydroxybenzylidene aminoguanidine (BAG) (unpublished results). However, it was found that administration of resorcylidene aminoguanidine (RAG) decreased formation of MDA by 31% in diabetic rats compared to diabetic rats treated with RAG (Liptáková et al. 2002).

The HNE level in group of diabetic rats was not significantly increased compared to controls. Administration of PAG had no effect on HNE formation in neither control group nor group of diabetic rats. Similar results were found also in our study with BAG administration to rats (Korytár et al. 2003).

Formation of HNE was studied also by Traverso et al. (2002) in liver microsomes and mitochondria of rats with DM. These authors found increased formation of MDA and HNE in the group of diabetic rats compared to control group and increased rate of lipoperoxidation in membranes of diabetic rats. They found that accumulation of HNE in liver of diabetic rats can be related to two mechanisms: increased lipoperoxidation and decreased enzymatic displacement (by the help of glutathione-S-transferase, aldehyde dehydrogenase and alcohol dehydrogenase).

It is known that under the conditions of DM the chronic oxidative stress occurs and functional integrity of cells is damaged. DNA is dangerously damaged mainly because of effect of hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$) but also NO and reactive nitrogen species. Several markers of DNA damage are formed, from which the most often 8-oxo-2'-deoxyguanosine (8-oxodG) is determined. Increased levels of 8-oxodG was found in urine, mononuclear cells and skeletal muscles of diabetic patients (Hinokio et al. 1999; Suziki et al. 1999; Wu et al. 2004). In rats with streptozotocine-induced DM increased concentrations of 8-oxodG were found, for example in plasma, liver, kidneys and β-cells of pancreas (Ihara et al 1999; Park et al 2001).

Although there is no doubt hyperglycaemia is important factor in oxidative stress in DM, the associations between increased glycaemia and 8-oxodG level in plasma, liver and kidney of rats with streptozotocine-induced DM was not found (Park et al. 2001). Shin et al. (2001) found no significant correlation between glycated haemoglobin and 8-oxodG level in plasma. Leinonen et al. (1997) found significantly increased concentration of 8-hydroxydeoxyguanosine (8-OHdG) in urine of patients with DM. Wu et al. (2004) also found increased 8-OHdG level in urine of patients with DM compared to control group as well as correlation of 8-OHdG level with incidence of diabetic nephropathy and retinopathy in that diabetic patients. These authors also found that 8-OHdG level is dependent on gender and the normal values of females and males are significantly different (32% higher in females).

Andican and Burcak (2005) determined in rats with streptozotocine-induced DM correlation between concentration of 8-oxodG in liver and glycaemia and glycated haemoglobin in plasma and the measured values were compared with those in control rats. They found increased concentrations of glucose and glycated haemoglobin in diabetic rats compared to control rats as well as significantly larger extent of DNA damage in liver of diabetic rats compared to controls. There was found also weaker correlation between 8-oxodG and glycated haemoglobin in diabetic group compared to control group.

Moreover our results show significant DNA damage under the conditions of DM ($p = 0.01$) (Fig. 4). Administration of PAG positively influenced the control group as well as diabetic rats. PAG decreased DNA damage in control group ($p < 0.05$) and mainly in diabetic group ($p < 0.001$).

Under the conditions of DM also other factors may influence damage of DNA, for example, insulin level, lipid peroxidation and products of advanced lipoperoxidation. In addition, the transition metal ions released from metaloproteins can directly or indirectly bind to DNA as glycochelates and change redox state of cells.

Oxidative stress is differently influenced by hydrophilic and lipophilic antioxidants. In the group of diabetic rats, the significantly decreased level of hydrophilic antioxidants was found (Fig. 5) ($p < 0.001$). PAG decreased level of hydrophilic antioxidants in control group ($p < 0.05$), however, in diabetic group there was found no significant effect. Similar results were observed in studies with other derivatives of AG administration, with RAG (Liptáková et al. 2002) and with BAG (Korytár et al. 2003). From our results follows that used PAG concentration did not modulate significantly antioxidative status. Elangovan et al. (2000) have found the decreased capacity of hydrophilic and lipophilic antioxidants in plasma, heart, kidney and brain of diabetic rats; only in liver a significantly increased lipophilic antioxidants level was found.

Administration of PAG did not significantly influence the uric acid concentration in controls but in diabetics this concentration was decreased ($p < 0.05$). Similar results were found in the case of administration of RAG (Liptáková et al. 2002) and BAG (Korytár et al. 2003) to control and diabetic rats. The role of uric acid in diabetic rats is not sufficiently understood up to now. It is necessary to prove or disprove in another studies whether uric acid confers anti-oxidative or pro-oxidative properties.

Chen et al. (2003) compared the effects of AG and its Schiff base with pyridoxal (AG-PL) in prevention of nephropathy in diabetic mice. AG-PL was more effective compared to AG, pyridoxal and pyridoxamine in effects on H_2O_2, $^{\cdot}OH$, $O_2^{\cdot -}$, in inhibition of ascorbic acid auto-oxidation and in oxidation of LDL lipoproteins. They found that anti-oxidative activity of AG-PL is related to chelating ability of transition metal ions and direct scavenging of free radicals. AG-PL shows also inhibitory activity in formation of AGEs and it is more efficient than AG in prevention of nephropathy in streptozotocine-induced DM in mice.

Vojtaššák et al. (2006) studied cytotoxic effect of PAG *in vitro*. They found that PAG in certain concentrations has inhibitory effect on proliferative activity of exposed cells but it has no cytotoxic effect on B-HEF-2 cells.

At present, PAG is in the phase of basic research. Its effects on levels of other parameters have to be tested, to focus on its function in organism from the viewpoint of overall metabolism, to compare advantages and disadvantages of its effect, to determine its pharmacokinetic and toxic properties. Our present results show that also in the case of control rats there is increased oxidability of lipids, formation of carbonyls (unpublished results), glucose and cholesterol concentrations or decreased level of hydrophilic antioxidants. That is the reason of the effort in searching for another derivatives of AG, which would not have negative influence on healthy individuals.

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