doi: 10.4149/gpb_2015025

Short communication

Rosmarinic acid mitigates signs of systemic oxidative stress in streptozotocin-induced diabetes in rats

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Abstract. The aim of the work was to study the effect of rosmarinic acid (RA) on markers of oxidative stress in rats with diabetes. Diabetes was induced by streptozotocin (STZ), RA was administered orally for ten weeks. Water consumption was measured daily. Ten weeks after the first RA administration, urine was collected over 15 hours. N-acetyl-β-D-glucosaminidase (NAGA) activity, levels of thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) were determined in the pancreas, kidney, and plasma. RA administration to diabetic rats ameliorated markers of oxidative stress, as well as water consumption and urination. We assume that RA may mitigate STZ-induced diabetic manifestations by protecting rat tissues against damaging effect of free radicals.

Key words: Rosmarinic acid — Experimental diabetes in rats — Oxidative stress

Overproduction of reactive oxygen species (ROS) is considered to be a cause of tissue injury in several human diseases and degenerative processes. Oxidative stress contributes significantly also to the development of complications under conditions of diabetes, e.g. vasculopathies. Patients with diabetes mellitus were reported to be exposed to increased oxidative stress, which can be reduced by antioxidants (Fallahzadeh et al. 2012). Natural polyphenols are strong antioxidants suggesting a possible therapeutic potential in some human disorders, including cancer, diabetes, etc. Rosmarinic acid (RA) is a phenol carboxylic acid, a secondary metabolite found in many *Lamiaceae* herbs. Chemically, it is an ester of caffeic acid with 3,4-dihydroxyphenyl lactic acid. RA has multiple biological activities based on inhibition of the inflammatory processes and on scavenging of ROS (e.g. Osakabe et al. 2004; Peng et al. 2007). The antioxidant properties of rosmarinic acid *in vitro* and *ex vivo* have been repeatedly confirmed by our observations (e.g. Nosáľová et al. 2010; Brosková et al. 2013) and by studies of other authors (e.g. Meng et al. 2008).

In our previous work, we reported that RA improved vascular endothelial function of diabetic rats, presumably by its antiinflammatory effect (Sotnikova et al. 2013). To find assumed antioxidant properties, the aim of the present work was to study the effects of RA administration on markers of systemic oxidative stress in streptozotocin (STZ)-induced diabetes in rats.

Male Wistar rats from the Breeding Facility of the Institute of Experimental Pharmacology and Toxicology (IEPT SASc) Dobrá Voda (Slovak Republic), weighing 250–300 g, were used. The investigation was conforming to the Guide for the Care and Use of Laboratory Animals. The experiments were approved by the State Veterinary and Food Administration of the Slovak Republic. Experimental diabetes was induced by a repetitive *i.p.* dose of STZ (30 mg/kg; Sigma-Aldrich, USA) for three consecutive days. STZ was dissolved in 0.1 mol/l citrate buffer, pH 4.5. Control animals received 0.1 mol/l citrate buffer without STZ. After verification of diabetes, the animals were divided into four groups of 7 animals each: control rats (C), control rats treated with RA (R), diabetic rats (D), diabetic rats treated with RA (DR). The freshly prepared solution of RA (Sigma-Aldrich, Germany) was administered orally by intragastric gavage in the dose of 50 mg/kg daily for 10 weeks. Water consumption was measured daily. At the beginning and end of the experiment, plasma glucose levels were measured using commercial Glucose (Trinder) kit (Sigma, USA). Ten weeks after the first RA administration, urine was collected over 15 hours. The rats were then anesthetized, blood was

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collected from the vena cava and samples of pancreas and kidney were taken for biochemical studies. In a separate group of animals, insulin levels were determined in blood plasma using ELISA commertial test (Insulin rat/mouse ELISA, DRG, Germany) according to instructions of the manufacturer.

In the kidney, pancreas and plasma, thiobarbituric acid reactive substances (TBARS), markers of lipid peroxidation, were determined by measurement of the coloured product formed upon reaction with thiobarbituric acid (Esterbauer 1993). Glutathione (GSH) content was determined by the method of Tietze (1969). The specific activity of lysosomal N-acetyl-β-D-glucosaminidase (NAGA), a marker of cellular injury, was assayed in the tissues studied and in urine according to standard methods as described previously in Navarova et al. (1994).

Statistical analyses were performed by using ANOVA with Bonferroni posttest. Statistical significance was indicated at $p < 0.05$.

STZ induced changes characteristic for diabetes: decreased body weight gain, increased daily water consumption and urination. Postprandial plasma glucose concentrations increased from 5.8 ± 0.3 to 28.2 ± 0.2 mmol/l ($p < 0.01$). Insulin levels in plasma were not completely reduced, but they decreased from 0.7 ± 0.1 ng/ml to 0.3 ± 0.1 ng/ml ($p < 0.05$). RA did not influence the body weight gain and blood glucose levels either of control or diabetic rats.

The results obtained in the present study confirmed the STZ-diabetes-induced oxidative stress in experimental animals, as manifested by changes in the biochemical markers studied (Table 1). We observed increased levels of a marker of oxidative burst – TBARS – in plasma, pancreas and kidney. Our results are in accordance with findings of other authors (e.g. Kaushik et al. 2013). Moreover, as expected, levels of the native antioxidant GSH were decreased in diabetic tissues and plasma. The enzyme NAGA belongs to the group of lysosomal glycosidases. Its activity was found to be increased in plasma and several organs injured by pathological processes, such as ischemia-reperfusion (Nosáľová et al. 2010). Increased activity of NAGA in diabetic patients has been described (Mandic and Filipovic 1998). The importance of evaluating NAGA activity in pathological situations was supported by findings of increased urinary NAGA activity in hypertensive patients (Lisowska-Myjak et al. 2011). These authors have suggested using urinary NAGA activity as a marker of early renal impairment. In our experiments, we found elevated NAGA activity in all samples taken from diabetic animals – kidney, pancreas, plasma, and urine – confirming injury of the given tissues. These results provide evidence that STZ-diabetes induced global oxidative stress and tissue impairment in rats. Administration of RA to healthy control rats did not influence the biochemical parameters studied.

In our previous work (Sotnikova et al. 2013), RA was found to suppress manifestations of inflammatory damage of vessels induced by STZ-diabetes in rats. Moreover, depression of the increased TBARS plasma levels by RA suggested its antioxidant effect in the model of diabetes. We have thus assumed that RA should mitigate also systemic oxidative stress accompanying diabetes. Indeed, RA administration depressed lipid peroxidation, manifested by decreased concentrations of TBARS in the kidney and pancreas. Conversely, levels of GSH, decreased by diabetes, tended to return to control values (Table 1).

It is known that ROS are major effectors of β-cell death and consequently of pancreatic tissue impairment. In our experiments, the antioxidant RA administered to diabetic animals decreased lipid peroxidation and dumpend NAGA

Table 1. Effect of diabetes and rosmarinic acid on biochemical parameters

Parameter	Sample	Group			
		C	R	D	DR
TBARS (nmol/mg protein)	Plasma	3.25 ± 0.24	3.19 ± 0.18	4.53 ± 0.14**	3.78 ± 0.17^{x}
	Pancreas	1.65 ± 0.14	1.88 ± 0.09	4.58 ± 0.52**	2.02 ± 0.10^{x}
	Kidney	5.22 ± 0.32	4.86 ± 0.28	7.16 ± 0.50**	5.88 ± 0.17^{x}
GSH (μg/mg protein)	Plasma	0.097 ± 0.006	0.086 ± 0.005	0.069 ± 0.007*	0.089 ± 0.010
	Pancreas	41.90 ± 1.22	37.36 ± 1.00	30.54 ± 2.08**	32.40 ± 1.61^{x}
	Kidney	57.82 ± 2.80	52.88 ± 3.08	49.85 ± 2.74*	52.92 ± 3.32
NAGA (μg 4NP/min/mg protein)	Plasma	0.048 ± 0.003	0.070 ± 0.011	0.157 ± 0.016***	0.138 ± 0.003
	Pancreas	0.541 ± 0.039	0.648 ± 0.033	0.897 ± 0.068***	0.544 ± 0.032^{xxx}
	Kidney	24.56 ± 1.34	23.46 ± 1.19	31.33 ± 1.22**	23.26 ± 1.75^{xx}
	Urine	188.97 ± 35.53	160.41 ± 18.25	844.59 ± 125.02***	628.42 ± 33.20^{xxx}

Data are means ± S.E.M., $n = 7$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* C group, ^{x} $p < 0.05$, ^{xx} $p < 0.01$ ^{xxx} $p < 0.001$ *vs.* D group. 4NP, 4-nitrophenol; C, control group; R, control group treated with rosmarinic acid (50 mg/kg *per* day); D, diabetic group; DR, diabetic group treated with rosmarinic acid (50 mg/kg *per* day).

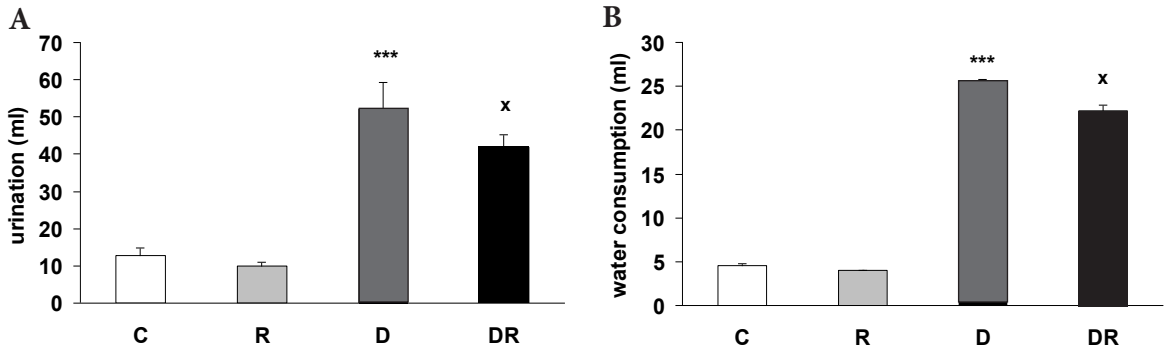

Figure 1. Effect of diabetes and rosmarinic acid on urination (ml/24 h; **A**) and water consumption (ml/24 h; **B**). Data are means ± S.E.M., $n = 7$. *** $p < 0.001$ *vs.* C group, ^{x} $p < 0.05$ *vs.* D group. C, control group; R, control group treated with rosmarinic acid (50 mg/kg *per* day); D, diabetic group; DR, diabetic group treated with rosmarinic acid (50 mg/kg *per* day).

activity in the pancreas (Table 1). We thus assume that RA may mitigate the STZ-induced diabetic manifestations by protecting the pancreas against the damaging effect of free radicals.

Oxidative stress, activated by hyperglycemia, has been considered a pathogenic factor for diabetic nephropathy which belongs to the most serious complications of diabetes. Thus improvement of the redox situation and alleviation of NAGA activity found in our experiments due to RA administration (Table 1) seems to reflect protection of the kidney against diabetic injury. Moreover, the beneficial effect of RA on the kidney results presumably, at least partly, in slightly reduced water consumption and urination reported in our previous work (Sotnikova et al. 2013) and confirmed in the present experiments (Fig. 1).

In summary, 10 weeks lasting experimental diabetes in rats was accompanied with global oxidative stress which was reduced by administration of the natural polyphenol – RA (50 mg/kg *per* day). Our results provide further evidence that polyphenol substances may have a potential to contribute to human health benefits.

Acknowledgement. The work was supported by The Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic for the Structural Funds of EU, OP R&D of ERDF by realization of the Project „Evaluation of natural substances and their selection for prevention and treatment of lifestyle diseases (ITMS 26240220040) and VEGA 2/0054/15.

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Received: May 20, 2015
Final version accepted: June 9, 2015
First published online: September 16, 2015