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Red wine counteracts nitrative stress and PARP-1 overactivation in the retina of diabetic rats

Czerwone wino przeciwdziała stresowi nitracyjnemu i nadaktywności PARP-1 w siatkówce szczurów z cukrzycą

INTRODUCTION

Diabetes mellitus has reached epidemic proportions – 3–6 percent of the population of the developed countries are suffering from the disease. 80% of diabetic patients with duration of disease over 10 years may develop retinopathy. Diabetic retinopathy results from a complex interplay between multiple pathogenetic processes, developing in both retinal vasculature and neural retina [6]. The most important pathogenetic mechanisms such as increased aldose reductase activity, non-enzymatic glycation, activation of protein kinase C, oxidative-nitrative stress, and poly(ADP-ribose) polymerase (PARP) activation, have been identified in experimental studies in diabetes- and diabetes-like models of early and advanced retinopathy in rats [3] and dogs [8].

Polyphenolic compounds, i.e. anthocyanins, resveratrol, galic acid, catechin, myricetin, quercetin, etc., abundant in red wines, could play a major role in enhancing the antioxidant system, since they behave as reactive oxygen species (ROS) scavengers, metal chelators and enzyme modulators [7]. Recent studies showed that polyphenols due to their chelating properties could enhance antioxidant capacity of plasma in humans by avoiding the free radical formation through the Haber-Weiss/Fenton reactions participating in the regulation of vascular tone and inhibiting the platelet aggregation [12].

The present study was aimed at evaluating the effect of red wine on nitrative stress and PARP activation in retinae of streptozotocin-diabetic rat. The possible physiological significance of these findings is discussed.

MATERIAL AND METHODS

Male Wistar rats (120–150 g), were fed a standard rat chow and had access to water ad libitum. Animals were separated into four groups of seven animals each: Group 1 – normal untreated control; Group 2 – red wine treated; Group 3 – STZ treated; and Group 4 – red wine and STZ treated. The STZ treatment was a single i.p. injection of 50 mg/kg body weight. The red wine treatment was an oral dose (300 ml/ 70 kg body weight/day) administered daily for two weeks prior to the STZ injection and daily for four weeks after the STZ injection. Group 2 received red wine for six weeks.

Western blot analyses of nitrated proteins individual retinas (one retina from each rat) were performed as described previously [9]. All sections were processed by a single investigator and evaluated blindly. Poly(ADP-ribose) and glial fibrillar acidic protein (GFAP) immunoreactivity was assessed as described [4].

The results are expressed as means ± standard deviations. Where overall significance ($p<0.05$) was attained, individual between-group comparisons were made using the unpaired two-tailed Student's t-test or Mann-Whitney rank sum test where appropriate. Significance was defined at $p \leq 0.05$.

RESULTS

Initial (after STZ injection) blood glucose concentrations were 133% higher in diabetic rats compared with controls (Table 1). Red wine consumption did not affect blood glucose concentrations in control or diabetic rats. Initial body weights were similar in control and diabetic rats. The final body weights was lower by 36% in the diabetic group compared with control (Table 1). This increase was slightly, but significantly, increased by the consumption of red wine ($p < 0.05$).

Table 1. Initial and final body weights and blood glucose concentrations in control and diabetic rats with or without red wine consumptions (M ± m. n = 5–7)

	Body weight (g)		Blood glucose (mmol/l)	
	Initial	Final	Initial	Final
Control	136,6 ± 15,5	208,8 ± 18,9	6,2 ± 0,9	6,1 ± 1,3
Control + RW	138,8 ± 12,4	203,4 ± 18,4	6,4 ± 0,7	6,5 ± 0,9
Diabetic	141,0 ± 11,7	133,4 ± 8,2**	14,5 ± 1,4**	23,2 ± 2,4 **
Diabetic + RW	144,0 ± 13,9	171,8 ± 10,9*,#	14,1 ± 1,7**	24,4 ± 4,8**

Data are means ± SEM, n = 5–7; *, ** $p < 0.05$ and < 0.01 vs controls; # $p < 0,05$ vs diabetic rats without wine consumption

Retinae nitrotyrosine (NT) modified proteins abundance assessed by Western blot analysis was increased by 38% in the untreated diabetic group compared with the control group (Fig. 1, A, B), and such increase was prevented in diabetic rats treated with red wine.

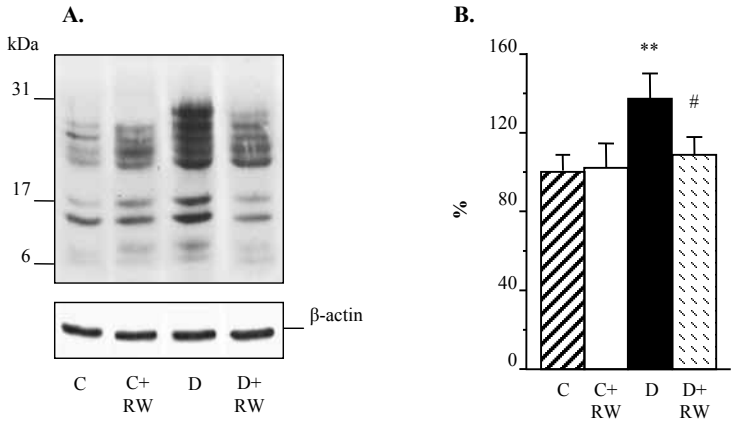

Fig. 1. Representative Western blot analyses of nitrotyrosine-modified proteins in the retinae (**A**) of control and diabetic rats with and without wine consumption; total nitrotyrosine context (**B**) (data for the control rats is taken as 100%); C – control groups; D – diabetic groups; RW – red wine. n = 5–7. ** $p < 0.01$ vs controls; # $p < 0.05$ vs diabetic rats without wine consumption

Poly(ADP-ribose) and GFAP immunohistochemistry scores using a percentage scoring method, was increased in retinae by 34.5% and 30%, correspondingly, in the diabetic rats compared with nondiabetic controls, and those increases were markedly reduced by red wine treatment (Fig. 2, A–D). Poly(ADP-ribose) positive nuclei were localized primarily in the ganglion cell layer but were also detectable in other parts of the retina.

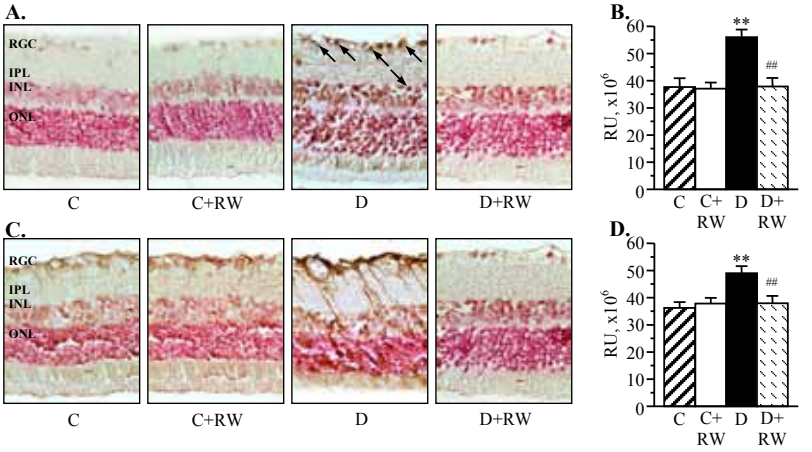

Fig. 2. Representative microphotographs of light microscopy immunohistochemical staining of poly(ADP-ribose) (**A**), and glial fibrillar acidic protein (**C**) in the retinae of control and diabetic rats with and without wine consumption. Magn. x40. Total poly(ADP-ribose) (**B**) and GFAP (**D**) content (relative units per image) in the retinae. Arrowed examples of poly(ADP-ribosyl)ated proteins of retinae cells. RGC – retinal ganglion cells, IPL – inner plexiform layer, ONL – outer nuclear layer, INL – inner nuclear layer, n = 10–15 per group, C – control rats, D – diabetic rats, RW – red wine. n = 10-15. ** $p < 0.01$ vs controls; ## $p < 0.01$ vs diabetic rats without wine consumption.

DISCUSSION

Excess glucose production and low glucose utilization in the body raises levels of blood glucose (hyperglycemia), which leads to increased osmotic diuresis quickly followed by fluid loss and electrolytes and, ultimately, dehydration and they lead to a decrease in body weight and other diabetes complications [5]. Our findings demonstrate that treatment with red wine increases the body weight of diabetic rats. The effect of red wine (weight loss alleviation) may be due to its positive effect on kidney homeostatic functions and/or protection from ketoacids, but further studies are required to validate this assumption.

One of the well-recognized fundamental mechanisms in the pathogenesis of chronic diabetes complications is an oxidative-nitrative (also known as nitrosative) stress, resulting from increased production of reactive oxygen species [10]. In diabetic and hyperglycemic conditions, superoxide, as a primary free radical, is rapidly converted to several other ROS, i.e. hydrogen peroxide, hydroxyl radicals and peroxynitrite. Peroxynitrite causes numerous cytotoxic effects (nitrative stress), i.e. protein nitration with resultant changes in cell signaling: activation of mitogen-activated protein kinases; upregulation of transforming growth factor-β; DNA single-strand breakage; overactivation of PARP with resultant NAD+ depletion and energy failure, with concomitant activation of several major pathogenetic mechanisms implicated in diabetic chronic complications [2, 10].

Our results provide evidence of clearly manifest nitrative stress in the retina under diabetic conditions. Furthermore, accumulation of NT has recently been documented in vascular endothelium, myocardium, retina, kidneys, and peripheral nervous system of diabetic rodents [4, 9] as well as in the human subjects with diabetes, i.e. circulation, vasculature, kidneys, skin and myocardium accumulate NT [11]. These data indicate that peroxynitrite-induced injury is presented on early and late stages of Type 1 and Type 2 diabetes mellitus and during the prediabetes stage.

Our results suggest that red wine treatment has a substantial effect on lowering NT content in the retina of diabetic rats. This may be due to red wine mediated decrease in oxidative stress. Free radicals react with phenolic compounds much faster than with proteins, lipids or DNA. Therefore, phenols protect proteins, lipids and DNA from oxidative damage.

The present study also revealed clearly manifest poly(ADP-ribose) accumulation in the retina. Moreover, red wine reduced the level of poly(ADP-ribosyl)ated proteins in diabetic animals to the control level. It was also shown that red wine counteracted diabetes-induced retinal glial activation manifest in GFAP accumulation, the early marker of diabetic angiopathy. Oxidative-nitrosative stress and PARP overactivation are interrelated with several other hyperglycemia-initiated mechanisms. Induced by hyperglycemia, the accumulation of sorbitol and fructose leads to the formation of AGEs, which in turn generate free radicals during the interaction with their receptors. Peroxynitrite-induced DNA breakage overactivates PARP leading to poly-(ADP-ribosyl)ation of nuclear proteins and resulting inhibition of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase, accumulation of methylglyoxal, sorbitol and fructose, with concomitant activation of several major pathogenetic mechanisms, i.e. nonenzymatic glycation, activations of PKC, and hexosamine pathway [1]. Activations of PKC and increased AGEs formations can trigger various inflammatory processes which can lead to the glial changes and damages of the retinae blood vessels. All of these

data findings support those found in our study and suggest the protective action of red wine and its "polyphenolic aid".

CONCLUSIONS

Our results provide the rationale for further studies of detailed mechanisms of anti-diabetic effects of red wine. Understanding the mechanism involved in red wine action can have potentially profound clinical implications.

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SUMMARY

Increased accumulation of nitrotyrosine and poly(ADP-ribosyl)ated proteins in the tissues of diabetics are associated with diabetes complications (diabetes neuropathy, nephropathy and retinopathy). The present study showed that red wine decreases the level of nitrotyrosine modified and

poly(ADP-ribosyl)ated proteins in the retinae of diabetic rats to the control level. This data allows us to assume an important role of red wine and its polyphenols preparation in the prevention and treatment of diabetic complications.

Keywords: diabetes retinopathy, polyphenols, oxidative-nitrative stress, poly(ADP-ribose) polymerase

STRESZCZENIE

Zwiększona akumulacja nitrotyrozyny i Poli-ADP-ribozylowanych białek w tkankach chorych z cukrzycą jest związana z powikłaniami cukrzycy (neuropatią cukrzycową, nefropatią i retinopatią). Badania wykazały, że czerwone wino zmniejsza poziom modyfikowanych nitrotyrozyną i i poli-ADP-ribozylowanych białek w siatkówce szczurów z cukrzycą w porównaniu z grupą kontrolną. Te dane pozwalają wnioskować, iż czerwone wino i jego preparaty polifenolowe odgrywają istotną rolę w prewencji i leczeniu powikłań cukrzycy.

Słowa kluczowe: retinopatia cukrzycowa, polifenole, stres oksydacyjno-nitracyjny, polimeraza Poli(ADP-rybozy)