Plasma Lipid Profile of Experimentally Induced Hyperlipidemic New Zealand White Rabbits Is Not Affected by Resveratrol

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ABSTRACT

Hyperlipidemia is well recognized as an important risk factor in the development of atherosclerosis. Low-density lipoproteins (LDL) are components of cholesterol that are highly associated to an increased risk of cardiovascular diseases. Hypercholesterolemia induces proteolytic and oxidative changes in vasculature, leading to a local inflammatory response. Since dietary antioxidants have attracted considerable attention as preventive and therapeutic agents, the polyphenolic compound resveratrol seems to play an important role in prevention of human atherosclerosis. Researches show that resveratrol inhibits LDL oxidation and platelet aggregation, as well as vascular proliferation of smooth muscle cells. However, recent findings in animal models reveal conflicting results regarding its effects on plasma lipid levels. The aim of the present study was to evaluate the effect of resveratrol on plasma biochemistry profile in New Zealand white rabbits submitted to a hypercholesterolemic diet. Twenty healthy, male, adult New Zealand white rabbits were fed with ordinary diet for one week before being divided into four treatment groups, containing five animals each. Group CT received maintenance diet; group R received maintenance diet and resveratrol (3mg/kg/day) given orally; group CL received maintenance diet enriched with 1.5% cholesterol; and group CR received maintenance diet enriched with 1.5% cholesterol and resveratrol (3mg/kg/day) given orally. During the experiment, from each animal, samples of 3mL venous blood were collected in

heparin twice monthly for measurements of total cholesterol, triglycerides, and low- and high-density lipoproteins. The data analysis revealed that resveratrol did not have a hypolipidemic effect in experimentally induced hypercholesterolemic New Zealand white rabbits.

INTRODUCTION

Presently, cardiovascular diseases are the main causes of morbidity and mortality, being considered the most significant health issue in THE adult populationS of developed countries.[13] Atherosclerosis is the pathological condition that precedes the majority of cardiovascular episodes, namely myocardial infarcts and brain vascular accidents.[4] Hyperlipidemia is recognized as an important risk factor to atherosclerosis, and most evidence focuses specifically on hypercholesterolemia. LDL, its main component, is most universally associated to an increased risk of cardiovascular diseases.[4]

Dietary antioxidants have attracted considerable attention as preventive and therapeutic agents. *In vivo* and *in vitro* studies using animal models show that the consumption of antioxidants can prevent and delay atherosclerosis progression. Resveratrol seems to play an important role in atherosclerosis prevention in human subjects through its inhibitory effect on LDL oxidation, platelet aggregation, and vascular proliferation of smooth muscle cells.[1,6] The hypocholesterolemic action of resveratrol is attributed, at least in part, to an increased excretion of neutral sterols and bile acids into feces. However, there are conflicting results regarding its effects on plasma lipid levels, since some studies have failed to show a reduction in plasma lipid levels induced by such a substance.[20]

The aim of the present study was to evaluate plasma LDL, high-density lipoproteins (HDL), triglycerides, and total cholesterol in experimentally hypercholesterolemic New Zealand white rabbits treated with resveratrol.

MATERIALS AND METHODS

Twenty healthy male adult New Zealand white rabbits whose mean weight was 2.5 kg were used on the study, which was conducted from March 15 to May 15, 2007. The animals were previously fed with regular rabbit ration during one week. Afterwards the animals were divided into four experimental groups containing five animals each, with the following feeding protocol: blank control group (CT) received maintenance diet[a]; group R received maintenance diet with simultaneous oral administration of resveratrol[b] (3 mg/kg/day)[a] ; group CL received 1.5% cholesterol[c] added to the maintenance diet; and group CR received 1.5% cholesterol added to the maintenance diet and simultaneous oral administration of resveratrol[b] (3 mg/kg/day). For the preparation of hypercholesterolemic diet, a cholesterol powder[b] was added to the previously grounded maintenance diet, mixed, pelleted, and stored at refrigerated units. The dose of resveratrol used was based in previous studies.[19] During the experiment, each animal received daily water *ad libitum* and 100 g of diet. The experiment was performed under approval of Animal Welfare and Ethics Committee of Universidade Estadual Paulista, Campus de Jaboticabal – UNESP.

Samples of 3 mL of heparinized blood were collected twice monthly from the central ear artery, with the animals kept in restraint cages. Those blood samples were used for plasma level determinations of triglycerides, total cholesterol, HDL, and LDL, through enzymatic methods[c] , accomplished in Department of Veterinary Clinics and Surgery Laboratory, Campus de Jaboticabal, UNESP. HDL levels were measured through selective inhibition

Table 1—Lipid profile (mg/dl) in rabbit fed a normal (CT and R) or high cholesterol diet (CL and CR) during 60 days, with or without supplementation of resveratrol.

	Total Chol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Tg (mg/dL)
Groups	M SD	M SD	M SD	M SD
CT	47.03[b] ± 15.84	14.88[b] ± 20.12	21.12[b] ± 5.37	55.30[b] ± 15.55
R	49.51[b] ± 17.09	12.99[b] ± 17.38	26.30[ab] ± 4.08	51.01[b] ± 10.49
CL	950.94[a] ± 662.90	872.74[a] ± 639.57	39.89[a]±17.43	190.57[ab] ± 116.35
CR	1508.50[a] ± 889.71	1407.26[a] ± 850.93	40.46[a]± 17.00	302.43[a] ± 175.29

Chol= cholesterol; Tg= triglycerides; M= means; SD=standard deviation. Duncan's test, P<0.05. CT= control nornolipemic; R= resveratrol normolipemic; CL= control hyperlipemic; CR= resveratrol hyperlipemic.

technique, while total cholesterol, triglycerides, and LDL through enzymatic / Trinder method. All analytic procedures were made in triplicates. During the experimental period, the animals were weighed on a weekly basis for the adjustments of doses of resveratrol.

Statistical analysis was performed using Statistical Analysis Software (SAS)[d]. The results were analyzed through analysis of variance (ANOVA). A Duncan's test was employed to compare means. A $P<0.05$ value was considered significant.

RESULTS

Mean values ± standard deviation of plasma lipid profile obtained in this study are expressed in the Table 1. The values of HDL, LDL, triglycerides, and total cholesterol indicate that the control group was different ($P<0.05$) from CL and CR groups, but not from R group.

The results obtained in lipid measurements on the first day of the experimental period were similar to all groups. No alterations in plasma lipid profile of the animals fed with regular diet were detected when the values obtained in the beginning and in the end of the experiment were compared, not even in CR group, which showed similar values to CT group in the end of the experiment. Nevertheless, there was a significant increase in total cholesterol, triglycerides, LDL, and HDL in those animals submitted to a hypercholesterolemic diet.

DISCUSSION

Epidemiological studies show a decreased risk of cardiovascular diseases with the intake of high amounts of polyphenolic substances. Thus, resveratrol contributes with the antioxidant potential and plays an important role in cardiovascular disease prevention in human subjects. The protective effects of resveratrol have been attributed to a number of mechanisms such as antioxidant, anti-inflammatory, anti-proliferative, and anti-thrombotic activities, as well as its hypolipidemic properties. Therefore, considering its antioxidant effects, early studies have proven that polyphenols from red wine were able to reduce plasma oxidation of LDL.[18] A fat-enriched meal prejudices endothelial function for more than four hours, being more severe in coronary disease patients.[22] Such a dysfunction is mainly attributable to an increase in endothelial oxidative stress, which is inhibited by wine antioxidants.

When the *in-vitro* effects of trans-resveratrol, some wine-derived phenol compounds, and some antioxidants were studied, a blockage in platelet aggregation and eicosanoid synthesis in human cells was observed, contributing to the cardioprotective effect of resveratrol,

mainly against atherosclerosis in coronary disease patients.[2] The beneficial effect of resveratrol on endothelial integrity through an inhibition in VCAM-1 (Vascular Cell Adhesion Molecule 1), ICAM-1 (Intracellular Adhesion Molecule 1), and transcription factor NF- Í, (Nuclear Factor Kappa ?) expression, the latter being responsible for pro-inflammatory modulatory mechanisms, is also documented.[5,9,21]

In the present experiment, groups CL and CR shared similarities to all variables studied, although differing from groups CT and R, which were similar. HDL values remained in the species reference range in all groups, only a slight increase being observed in CL and CR groups. LDL, triglycerides, and cholesterol, as expected, reached high levels in hypercholesterolemic groups, but remained in the reference range in CT and R groups, without differing among them.

The analysis of the findings allowed to infer that the use of resveratrol during the experimental period did not affect LDL, HDL, triglycerides, and cholesterol levels, similar to early studies.[17,19,20] Opposite to this evidence, some researchers detected changes in lipid profile induced by different flavonoids[3,8,10,11,16] and vitamin E[7,15] in various species. Furthermore, a synergic effect between statins and resveratrol was observed in hypercholesterolemic rats, leading to a significative reduction in total plasma cholesterol, triglycerides, and LDL.[12,14] The use of an analogue of resveratrol (pterostilbene) in hypercholesterolemic hamsters showed a 29% reduction in plasma LDL and a 7% increase in plasma HDL.

In conclusion, resveratrol at 3 mg/kg/day did not show hypolipidemic activity in rabbits experimentally hypercholesterolemic, used as experimental model in atherosclerosis.

Sources of Manufacturers

a. Purina Paulínica, São Paulo, Brazil.
b. Vetec Duque de Caxias, Rio de Janeiro, Brazil.
c. LABTEST DIAGNÓSTICA S.A, Minas Gerais, Brazil.
d. SAS, Statistics Software v2, version 6.08, Cary, NC.

REFERENCES

1. Araim O, Ballantyne J, Waterhouse AL, Sumpio BE: 2002, Inhibition of vascular smooth muscle cell proliferation with red wine and red wine polyphenols. *J Vasc Surg* 35:1226-1232.
2. Asciak CR, Hahn S, Diamandis EP, et al.: 1995, The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Int J Clin Chem* 235:207-219.
3. Auger C, Teissedre PL, Gerain P, et al.: 2005, Dietary wine phenolics catequin, quercetin, and resveratrol efficiently protect hypercholesterolemic hamsters against aortic fatty streak accumulation. *J Agric Food Chem* 23:2015-2021.
4. Cotran RS, Schoen F. Vasos Sanguíneos [Blood Vessels]. In: Cotran RS, Kumar V, Collins T: 2000, *Patologia Estrutural e Funcional* [Functional and Structural Pathology], 6th ed., pp. 441-460. Guanabara Koogan, São Paulo, Brazil.
5. Ferrero ME, Bertelli AE, Fulgenzi A, et al.: 1998, Activity in vitro of resveratrol on granulocyte and monocyte adhesion to endothelium. *Am J Clin Nutr* 68:1208-1214.
6. Fremont L: 2000, Minireview: Biological Effects of Resveratrol. Life Sci 66:663-673.
7. Hidiroglou N, Gilani GS, Long L, et al.: 2004, The influence of dietary vitamin E, fat, and methionine in blood cholesterol profile, homocysteine levels, and oxidizability of low density lipoprotein in the gerbil. *J Nutr Biochem* 15:730-740.
8. Juzwiak S, Wojcicki J, Morkrzycki K, et al.: 2005, Effect of quercetin on experimental hyperlipidemia and atherosclerosis in rabbits. *Pharmacol Rep* 57:604-609.
9. Manna SK, Mukhopadhyay A, Aggarwal BB: 2000, Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-Íb, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J Immunol* 164:6509-6519.

10. Miura D, Miura Y, Yagasaki K: 2003, Hypolipidemic action of dietary resveratrol, a phytoalexin in grapes and red wine, in hepatoma bearing rats. *Life Sci* 73:1393-400.

11. Oliveira TT, Nagem TJ, Lopes RM, et al.: 2004, Efeito de diferentes doses de rutina sobre lipídeos no soro de coelhos machos e fêmeas [Effect of different dosis of rutin on serum lipids in male and female rabbits]. *Rev Bras Anal Clin* 36:213-215.

12. Penumathsa SV, Thirunavukkarasu M, Konery S, et al.: 2007, Statin and resveratrol in combination induces cardioprotection against myocardial infarction in hypercholesterolemic rat. *J Mol Cell Cardiol* 3:508-516.

13. Perez G, Pena A, Sala J, et al.: 1998, Acute myocardial infarction case fatality, incidence and mortality rates in a population registry in Gerona, Spain, 1990-1992. *Int J Epidemiol* 27: 599-604.

14. Rimando AM, Nagmani R, Feller DR, Yokoyama W: 2005, Pterostilbene, a new agonist for the peroxisome proliferator-activated receptor ·-isoform, lowers plasma lipoproteins and cholesterol in hypercholesterolemic hamsters. *Agric Food Chem* 53:3403- 3407.

15. Schaefer EJ: 2002, Lipoprotein, nutrition, and heart disease. *Am J Clin Nutr* 75:191-212.

16. Shanmuganayagam D, Warner TF, Krueger CG, et al.: 2006, Concord grape juice attenuates platelet aggregation, serum cholesterol and development of atheroma in hypercholesterolemic rabbits. *Atherosclerosis* 190:135–142.

17. Turrens JF, Lariccia J, Nair MG: 1997, Resveratrol has no effect on lipoprotein profile and does not prevent peroxidation of serum lipids in normal rats. *Free Radic Res* 27:557- 562.

18. Wakabayashi Y: 1999, Effect of red wine consumption on low-density lipoprotein oxidation and atherosclerosis in aorta and coronary artery in Watanabe heritable hyperlipidemic rabbits. *J Agric Food Chem* 46:4724-4730.

19. Wang Z, Zou J, Cao K, et al.: 2005, Dealcoholized red wine containing know amounts of resveratrol supresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels. *Int J Molec Med* 16:533-540.

20. Wilson T, Knight TJ, Beitz DC, et al.: 1996, resveratrol promotes atherosclerosis in hypercholesterolemic rabbits. Life Sci 59:l15-121.

21. Wung BS, Hsu MC, Wu CC, Hsieh CW: 2005, Resveratrol suppresses IL-6-induced ICAM-1 gene expression in endothelial cells: effects on the inhibition of STAT3 phosphorylaton. *Life Sci* 12:389-397.

22. Zhao SP, Liu L, Gao M, et al.: 2001, Impairment of endothelial function after a hight-fat meal in patients with coronary artery disease. *Coron Artery Dis* 12:561-565.