Cardiovascular effects of resveratrol and atorvastatin treatments in an $H_2O_2$-induced stress model

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Abstract. Oxidative stress has been implicated in the pathophysiology of several types of cardiovascular disease (CVD). Statins are widely used to inhibit the progression of atherosclerosis and reduce the incidence of CVD. Certain over-the-counter products, including resveratrol, show similar effects to statins and may thus be used in conjunction with statins for the treatment of the majority of patients with CVD. The aim of the present study was to evaluate the effects of atorvastatin, resveratrol and resveratrol + atorvastatin (R+A) pretreatment on myocardial contractions and vascular endothelial functions in the presence of $H_2O_2$ as an experimental model of oxidative stress in rats. Four groups were established and referred to as the control, atorvastatin, resveratrol and R+A groups. Atorvastatin (40 mg/kg, per oral) and/or resveratrol (30 mg/kg, intraperitoneal) treatments were administered for 14 days. On the 15th day, the thoracic aortas and hearts of the rats were dissected and placed into isolated organ baths. Vascular responses to cumulative doses of $H_2O_2$ (1x10$^{-5}$-1x10$^{-4}$ M) with and without N-(G)-nitro-L-arginine methyl ester (L-NAME) incubation were measured. In addition, myocardial electrical stimulation (ES) responses to various $H_2O_2$ concentrations (1x10$^{-7}$-1x10$^{-5}$ M) were evaluated. In the control and atorvastatin groups, $H_2O_2$ application caused a significant dose-dependent decrease in the ES-induced contractions in the myocardial tissue of rats. In the resveratrol and R+A groups, $H_2O_2$ application did not significantly affect myocardial contraction at any dose. In all groups, incubation with L-NAME caused a significant augmentation in the $H_2O_2$ response, revealing that this effect was mediated via the vascular endothelium. In conclusion, pretreatment with R+A for CVD appears to be superior to pretreatment with either agent alone.

Introduction

Oxidative stress has been implicated in the pathophysiology of several types of cardiovascular disease (CVD), including ischemic stroke, myocardial ischemia, myocardial stunning, ischemia-reperfusion injury, hypertension and atherosclerosis. It is also considered to play a role in the progression of atherosclerosis (1-4). Previous studies have demonstrated that the majority of patients with CVD are likely to have chronic oxidative stress and that this is associated with their diagnosed disease state (5-7). $H_2O_2$ is an important byproduct of oxidative metabolism and is a major contributor to oxidative stress-induced functional and metabolic dysfunction.

The β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are widely used to inhibit the progression of atherosclerosis and reduce the incidence of CVD. As well as their cholesterol-lowering effects, statins improve endothelial function in normocholesterolemia (8,9). Certain over-the-counter (OTC) products, including resveratrol, also exhibit similar effects to statins. Resveratrol (trans-3,5,4-trihydroxy stilbene) is a polyphenol (phytoalexin) that naturally occurs in red wine and in a variety of therapeutic plants. In vitro experiments have revealed that the cardiovascular protective effects of resveratrol may occur through a number of mechanisms. Resveratrol inhibits the proliferation of smooth muscle cells, platelet aggregation and the oxidation of low-density lipoprotein cholesterol, and reduces the synthesis of lipids and eicosanoids, which promote inflammation and atherosclerosis (10). These multiple protective effects of resveratrol increase its demand as an OTC product, even for those undergoing treatment with statins.

A number of studies have demonstrated the aggravating effects of statins on oxidative stress in organisms (11,12). Such aggravating effects of statins on the myocardium have already been shown (13,14,15,16). The aim of the present study was to evaluate the effects of atorvastatin, resveratrol and resveratrol + atorvastatin (R+A) pretreatment on myocardial contractions and endothelial function in the presence of $H_2O_2$ as an experimental model of oxidative stress in rats.

Materials and methods

Animals and experimental procedure. A total of 28 male Wistar albino rats, aged 8 weeks and weighing 260-280 g, obtained from the Animal Care Facility of Meram Medical Faculty.
period, the thoracic rings were contracted with 80 mM KCl. After the 30-min wash-out period in which the tissues were repeatedly washed every 10 min with KHS, 1x10^{-8}, 1x10^{-7}, 1x10^{-6}, 1x10^{-5} and 1x10^{-4} M H_{2}O_{2} were cumulatively added to the organ bath. Once the contractions reached a plateau, the tissues were washed twice every 15 min and incubated with 1x10^{-4} M (G)-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (NO) formation, for 30 min to evaluate the effect of the vascular endothelium on the H_{2}O_{2} results. Following incubation, 1x10^{-8}, 1x10^{-7}, 1x10^{-6}, 1x10^{-5} and 1x10^{-4} M H_{2}O_{2} were cumulatively added to the organ bath once more. All results are expressed as a percentage of the previous contraction induced by 80 mM KCl.

Statistical analysis. Data are expressed as the mean ± standard error of the mean. The statistical significance of differences between the groups was analyzed by one-way analysis of variance or the Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Intragroup myocardial results. H_{2}O_{2} was applied to the organ bath at doses of 1x10^{-7}, 1x10^{-6} and 1x10^{-5} M. To observe the effects of increasing doses of H_{2}O_{2}, an intragroup comparison of the myocardial results was carried out for each group. In the control group, H_{2}O_{2} significantly reduced the contractions induced by ES at all doses (1x10^{-7} vs. 1x10^{-6} M and 1x10^{-6} vs. 1x10^{-5} M, P<0.01). The results were 94.16±5.94, 80.35±5.66 and 62.61±8.28% for doses of 1x10^{-7}, 1x10^{-6} and 1x10^{-5} M, respectively. In the rats treated with atorvastatin, H_{2}O_{2} caused a significant dose-dependent decrease in myocardial contractions (72.09±3.80, 66.59±3.14 and 48.96±8.93% for H_{2}O_{2} doses of 1x10^{-7}, 1x10^{-6} and 1x10^{-5} M, respectively; 1x10^{-5} vs. 1x10^{-7} M and 1x10^{-6} M, P<0.01). In the resveratrol group, no significant changes in contraction were observed following H_{2}O_{2} application at all doses (87.91±2.33, 89.66±14.91 and 79.77±17.33% for H_{2}O_{2} doses of 1x10^{-7}, 1x10^{-6} and 1x10^{-5} M, respectively; P>0.05). In the R+A group, the contraction results following H_{2}O_{2} application were 76.57±1.40, 66.34±5.91 and 66.55±11.10% for 1x10^{-7}, 1x10^{-6} and 1x10^{-5} M H_{2}O_{2}, respectively; H_{2}O_{2} application did not significantly decrease the contractions when its concentration was increased.

Intragroup myocardial results. Intergroup comparisons of the myocardial results were evaluated for all doses of H_{2}O_{2}. At 1x10^{-7} M H_{2}O_{2}, the atorvastatin group showed a significantly lower contraction percentage when compared with the control and resveratrol groups (P<0.01). The R+A group also demonstrated a significant decrease in contraction percentage when compared with the control group (P<0.01). However, no significant difference was observed between the contraction percentages of the resveratrol and control groups (Fig. 1A). Following a 20-min washing period, the organ bath was adjusted to 1x10^{-6} M H_{2}O_{2}. The myocardial contractions of the atorvastatin and R+A groups were significantly lower than those control group (P<0.05). The resveratrol group tissues showed a significantly higher percentage contraction than those of the atorvastatin and R+A groups (P<0.01). No...
significant difference was observed between the myocardial contractions in the resveratrol and control groups (Fig. 1B). At the final dose of $H_2O_2$ (1x10^-5 M), the atorvastatin group exhibited a significant decrease in contraction compared with all the other groups (atorvastatin versus control and R+A groups, P<0.05; atorvastatin versus resveratrol group; P<0.01). The results of the present study demonstrated that resveratrol treatment alone attenuated the decrease in contraction percentage and that this effect was significant compared with all groups at 1x10^-5 M $H_2O_2$ (P<0.01 vs. atorvastatin and control group; P<0.01 vs. R+A group; P<0.01 vs. resveratrol group). In the R+A group, the contraction percentages were higher than those in the atorvastatin group but significantly lower than those in the resveratrol group (P<0.05) (Fig. 1C).

**Results of thoracic aorta responses.** Fig. 2 shows the cumulative dose responses to $H_2O_2$ (between 1x10^-8 and 1x10^-4 M) in aortic segments for the control, resveratrol, atorvastatin and R+A groups, with and without 1x10^-4 M L-NAME incubation. In all groups, $H_2O_2$ caused vasoconstriction and the contraction responses increased in a concentration-dependent manner. The aortic rings reached their maximum contraction at 1x10^-4 M $H_2O_2$, and the maximum contraction responses were 16.14±1.09, 7.50±0.75, 6.82±1.33 and 5.58±1.37% for the control, atorvastatin, resveratrol and R+A groups, respectively. The $H_2O_2$ responses were significantly lower in the treatment groups than those in the control group at 1x10^-4 and 1x10^-5 M $H_2O_2$ (control versus atorvastatin and resveratrol groups, P<0.05; control versus R+A group, P<0.01). When the maximum vasoconstriction responses of the treatment groups were examined, no statistical differences were identified among the resveratrol, atorvastatin and R+A groups (Fig. 2A). Following incubation with 1x10^-4 M L-NAME for 30 min, the contraction responses of the tissues to $H_2O_2$ significantly increased when compared with their previous responses (Fig. 2B). In all groups, L-NAME incubation significantly augmented the $H_2O_2$ response at 1x10^-5 and 1x10^-4 M when compared with the response without L-NAME incubation (Fig. 3). The maximum responses were 25.56±3.32, 18.06±1.74, 26.19±3.17 and 23.24±3.24% for the control, atorvastatin, resveratrol and R+A groups, respectively.

The thoracic aorta responses demonstrated that treatment of rats with resveratrol, atorvastatin and R+A resulted in a significantly lower vascular contraction response to $H_2O_2$ at a concentration 1x10^-4 M when compared with the control group. However, this result was eliminated with 1x10^-4 M L-NAME incubation.
The cardiac results of the present study revealed that oral administration of 40 mg/kg atorvastatin for 14 days resulted in a more sensitive myocardial response to H$_2$O$_2$ in rats. Treatment with 30 mg/kg i.p. resveratrol showed a cardioprotective effect against atorvastatin-aggravated and H$_2$O$_2$-induced contractile dysfunction in the rat myocardium. Furthermore, the study revealed that atorvastatin and resveratrol exhibited a protective effect against H$_2$O$_2$-induced vasoconstriction and that this protective effect was mediated by NO. Vasoconstriction induced by cumulative concentrations of H$_2$O$_2$ was augmented with L-NAME incubation. These results indicated that the vasoconstriction elicited by high concentrations of H$_2$O$_2$ was negatively modulated by the endothelium. NO exerted a protective effect to counteract the oxidative effect of H$_2$O$_2$ in the groups without L-NAME incubation. The protective effect of NO on H$_2$O$_2$ in endothelial monolayer permeability has been previously demonstrated by McQuaid et al (18). In their study, it was concluded that, although lower levels of NO may only give a small amount of cytoprotection, the barrier dysfunction in the endothelium caused by H$_2$O$_2$ may be partially reversed by NO (13). It may be concluded from the results of the present study that the protective effect of resveratrol and/or atorvastatin treatment may be attributed to increased NO production in the vascular endothelium.

Under normal conditions, the H$_2$O$_2$ concentrations in human plasma, blood and vascular cells are likely to be in the lower micromolar ranges or below. However, in pathological states, including myocardial ischemia and heart failure, it has been demonstrated that H$_2$O$_2$ concentrations can increase to millimolar levels (19-21). Atorvastatin impairs cholesterol production by inhibiting the synthesis of mevalonate. In addition to cholesterol-lowering effects, statins inhibit the biosynthesis of the major natural antioxidants ubiquinone (ubiphenol) Q10 and glutathione peroxidase (22-24). As a result of this, statins may aggravate oxidative stress in the organism. Such aggravating effects of statins on the myocardium have previously been demonstrated (16). These changes may modulate myocardial contractility. A previous study revealed that an increase in reactive oxygen species (ROS) in the myocardium resulted in ischemia, reperfusion injury and myocardial damage (25).

Studies on the antioxidant effects of statins have been performed using oxidative stress markers (OSMs) in body fluids (26-29). Although OSMs are accepted to reflect the levels of oxidative stress within tissues, Argüelles et al (30) demonstrated that OSMs were not correlated with the tissue levels of oxidative stress; furthermore, they suggested that OSMs did not reflect the local oxidative stress status of individual organs. This is consistent with the results of the present study, in which atorvastatin treatment aggravated the H$_2$O$_2$ response in the myocardial tissue and showed a protective effect on H$_2$O$_2$-induced vascular contractions. Although the current study did not assess the antioxidant capacity of the myocardium, the diminished myocardial response may be attributed to decreased levels of antioxidant agents in the myocardium, including ubiquinone Q10, whose production is HMG-CoA reductase-dependent (31). The protective effect of resveratrol can be attributed to its inhibitory effect on ROS production in the myocardium (32).

Increased levels of pro-oxidants have been associated with vascular diseases and they are considered to be an important initial step in the development of vascular diseases, including atherosclerosis and hypertension (33). The present study demonstrated that atorvastatin treatment disrupted ES-induced myocardial function in the presence of H$_2$O$_2$, but that its co-treatment with resveratrol recovered this effect. R+A treatment also exhibited a protective effect on H$_2$O$_2$-induced vascular responses. From these results, resveratrol appears to...
be a promising treatment for the improvement of myocardial function in diseases associated with the development of oxidative stress. Resveratrol has been a popular choice in OTC products. Resveratrol is frequently used for the prevention of atherosclerosis; thus, the indications for its administration appear to be similar to those for the administration of statins. The combined treatment of R+T provides a superior treatment for CVD compared with treatment with either agent alone.

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