Effects of red wine and wine polyphenol resveratrol on platelet aggregation in vivo and in vitro

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Received October 22, 2001; Accepted November 12, 2001

Abstract. Low to moderate consumption of red wine reportedly has a relatively greater benefit than other alcoholic beverages in the prevention of atherosclerosis and coronary heart disease (CHD). This beneficial effect is increasingly attributed to the polyphenol resveratrol, present in red wine. In the present study, we investigated the effects of resveratrol and red wine on aggregation of platelets isolated from healthy, normotensive male volunteers and in rabbits with experimental hypercholesterolemia. Platelet aggregation rate (PAR) was measured using Born's method. The results showed that aggregation of platelets from healthy subjects induced in vitro by collagen (5 µg/ml), thrombin (0.33 units/ml), and ADP (4 µM) was significantly inhibited by 10-1000 µM resveratrol, in a concentration-dependent manner. Hypercholesterolemic rabbits showed enhanced ADP-induced platelet aggregation; the average PAR increased from 39.5±5.9% in normal animals to 61.0±7.0% in the high-cholesterol fed group (n=8, p<0.001). Resveratrol (4 mg/kg/day) inhibited ADP-induced platelet aggregation in vivo by maintaining the PAR at 35.7±6.3% (vs. 39.5±5.9% for control rabbits, n=8, p=0.228), but had no effect on serum lipid levels. Similarly platelet aggregation in hypercholesterolemic rabbits was also inhibited when animals received intragastrically Chinese red wine (with or without alcohol, 4 ml/kg/day). These results suggest that resveratrol can inhibit platelet aggregation both in vitro and in vivo, which conceivably could be one of the mechanisms by which this red wine polyphenol exerts its cardioprotective effects.

Introduction

CHD and acute myocardial infarction are multifactorial diseases, whose etiology is related to dysfunctional platelets.

Platelet adhesion and aggregation triggered by ADP/TXA2, and PDGF release may attribute to the development of atherosclerosis, proliferation of smooth muscle cell, and acute thrombosis (1). Effective anti-platelet treatment can reduce the risk of thrombo-embolism.

Extensive investigations on the basis of the French Paradox in the 90’s suggested that consumption of red wine could reduce the mortality and morbidity of CHD. Further studies showed that resveratrol, a polyphenolic compound present in red wines, was the major contributor for the decreased incidence of CHD (2,3). Recent studies showed that resveratrol inhibited platelet aggregation and secretion in response to thrombin, ADP, and collagen in vitro (4,5). Whether resveratrol administered orally could affect platelet aggregation and modulate platelet aggregation accompanying hyperlipidemia is not known. The aim of this study was to investigate whether resveratrol and red wine produced in China could affect blood lipid levels and platelet aggregation in vivo in hypercholesterolemic rabbits. The effect of resveratrol on aggregation of platelets isolated from normotensive, healthy male volunteers in vitro was also studied.

Materials and methods

Materials. New Zealand rabbits (body weights 2.4±0.3 kg) were provided by the Animal Facilities of Nanking Medical University. ADP and resveratrol were purchased from Sigma Chemical Company (USA). Red wine (resveratrol concentration, 3.98 mg/l; alcohol content, 12% v/v) and de-alcohol red wine (resveratrol concentration, 3.26 mg/l) were provided by Wangfu winery Co. Ltd., Lianyungang, China. Cholesterol (analytical grade) was purchased from Shanghai Xinxing Chemical Engineering Reagent Research Institute. TYXN-91 platelet aggreganometer was provided by the Shanghai Tongyong Machino-electric Technology Institute.

Methods

Effect of resveratrol on platelet aggregation in vitro in humans. Blood was obtained by venous-puncture from 6 healthy male volunteers fasted for at least 12 h. Blood (9 parts) was put into plastics tube containing sodium citrate (1 part) as anticoagulant. Platelet-rich plasma (PRP) was obtained by centrifugation at 500 rpm for 5 min at room temperature. Platelet-poor plasma (PPP) was obtained by centrifugation at 3000 rpm for 15 min.
Density of platelets in PRP was adjusted with PPP to $3 \times 10^8$/ml. PRPs (200 µl) were mixed with resveratrol to yield final concentrations of 10, 100 and 1000 µM, respectively. The control, solvent control and positive control samples received saline, dimethyl sulfoxide (final concentration 0.75% v/v), and acetylsalicylic acid (final concentration 100 µM). Following 5 min incubation at 37°C, inducers containing respectively 0.33 units thrombin, 4 µM ADP, and 5 µg/ml collagen were added, and platelet aggregation was measured in 5 min in order to calculate the aggregation inhibitory rate (AIR).

**Effect of resveratrol on platelet aggregation in vivo in rabbits.** Forty male New Zealand rabbits were randomly divided into 5 groups: control, fed normal forage; hypercholesterolemic, forage containing 1.5% cholesterol; resveratrol treated, forage containing 1.5% cholesterol and 4 mg/kg/day resveratrol introduced intragastrically; red wine treated, forage containing 1.5% cholesterol and red wine 4 ml/kg/day fed intragastrically; de-alcohol red wine treated group, forage containing 1.5% cholesterol and de-alcohol red wine 4 ml/kg/day intragastrically. Prior to and 12 weeks post-treatment, blood (9 parts) was drawn from each animal following a 12 h fast, mixed with sodium citrate (1 part), and rate of platelet aggregation was measured, using 10 µM ADP as the inducer. Total cholesterol, triglyceride and high-density lipoprotein (HDL) in serum were measured using Olympus AU800 biochemical analysis apparatus.

**Statistical analysis.** All data are expressed as means ± SD. Significance of differences was analyzed using a two-tailed Student’s t-test with p < 0.05 as significant.

**Results**

**Changes in lipid levels in rabbits fed high-cholesterol diets.** Compared to rabbits fed with normal forage, the total, HDL- and LDL-cholesterol levels in groups fed the high-cholesterol diet were increased significantly, irrespective of whether animals also had concurrent treatment with resveratrol, red wine or de-alcohol red wine. The levels of triglycerides were unaffected by any of the treatments (Table I).

**Effect of resveratrol on platelet aggregation in vivo.** At 12 weeks post-treatment, venous blood was drawn for the determination of platelet aggregation. The average PAR in normal rabbits was 39.5 ± 5.9%. The PAR in the high-cholesterol diet group increased significantly (61.0 ± 7.0% vs. 39.5 ± 5.9%, n=8, p<0.001), which, with resveratrol treatment, returned to levels of the control animals (35.7 ± 6.3% vs. 39.5 ± 5.9%, n=8, p=0.228). A significant difference in PAR was observed between the high-cholesterol fed animals and those given high-cholesterol together with resveratrol supplement (35.7 ± 6.3% vs. 61.0 ± 7.0%, n=8, p<0.001). Similar PARs were found in groups of animals additionally given red wine or de-alcohol red wine (45.1 ± 8.9% and 43.4 ± 7.6%, n=8, p<0.05).

### Table I. Changes in serum lipids in rabbits fed high-cholesterol diet, X ± SD, n=8.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Normal control Before</th>
<th>Normal control After</th>
<th>High-cholesterol diet Before</th>
<th>High-cholesterol diet + RES After</th>
<th>High-cholesterol diet + red wine Before</th>
<th>High-cholesterol diet + de-alcohol red wine Before</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>2.1±0.5</td>
<td>2.3±0.7</td>
<td>2.7±0.8</td>
<td>23.7±49.7</td>
<td>2.0±0.8</td>
<td>20.5±10.0</td>
</tr>
<tr>
<td>TG</td>
<td>1.9±0.9</td>
<td>1.3±0.6</td>
<td>2.1±0.9</td>
<td>1.5±0.6</td>
<td>1.1±0.3</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.8±0.2</td>
<td>1.1±0.3</td>
<td>1.1±0.2</td>
<td>2.4±0.8</td>
<td>0.8±0.1</td>
<td>2.0±0.9</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.4±0.3</td>
<td>0.6±0.4</td>
<td>0.7±0.7</td>
<td>20.6±8.5</td>
<td>0.6±0.3</td>
<td>17.5±7.3</td>
</tr>
</tbody>
</table>

*Paired Student’s t-test p<0.01.

### Table II. Effect of resveratrol on platelet aggregation in healthy subjects, X ± SD, n=6.

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Platelet aggregation (%)</th>
<th>Control</th>
<th>DMSO</th>
<th>Resveratrol (10 µM)</th>
<th>Resveratrol (100 µM)</th>
<th>Resveratrol (1000 µM)</th>
<th>ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin</td>
<td>82.9±12.5</td>
<td>74.4±8.4</td>
<td>64.0±13.9</td>
<td>43.6±23.3</td>
<td>30.6±5.2</td>
<td>52.5±4.6</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>64.8±5.1</td>
<td>61.3±3.7</td>
<td>55.2±6.2</td>
<td>46.2±9.3</td>
<td>15.5±2.3</td>
<td>48.8±6.3</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>74.0±4.0</td>
<td>72.2±4.5</td>
<td>60.6±4.8</td>
<td>47.2±7.1</td>
<td>26.9±4.3</td>
<td>43.9±4.7</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05, †p<0.01, compared with control group.
The PARs in these two groups were also significantly lower than that in high-cholesterol diet group (n=8, p=0.004 and p=0.001, respectively).

**Effect of resveratrol on platelet aggregation in vitro in healthy subjects.** The effect of resveratrol on platelet aggregation in vitro in healthy subjects is shown in Table II. Resveratrol at various concentrations inhibited platelet aggregation induced by collagen (5 µg/ml), thrombin (0.33 units/ml), and ADP (4 µM) in a concentration-dependent manner. The aggregation inhibitory rate (AIR) of resveratrol at concentrations of 10, 100, and 1000 µM was 22.5, 46.9, and 62.9%, respectively for platelet aggregation induced by thrombin, 14.6, 28.7, and 74.7% for that induced by ADP, and 18.2, 36.4, and 63.7% for that induced by collagen.

**Discussion**

In the early 1990s, epidemiological investigations showed that the incidence of myocardial infarction of the French is only one-third of that in the United States, despite presence of equally prevalent cardiovascular risk factors, exemplified by high fat intake, lack of exercise, and heavy cigarette smoking. This phenomenon, commonly referred to as the ‘French paradox’ (2), has been attributed to the regular consumption of red wine at meals by the French and further implies that mild or moderate consumption of red wine could reduce the mortality and morbidity of CHD (6). More recent studies suggest that the cardioprotective effects of red wine may largely derive from the wine polyphenol resveratrol (3). The exact manner by which resveratrol affects atherosclerosis has not been elucidated.

Results of this study show that in rabbits, hypercholesterolemia increases the propensity of platelets to undergo aggregation and this effect can be negated by resveratrol, in a lipid-independent manner. As red wine and de-alcohol red wine showed similar effects, it may be concluded that the active agent is not alcohol.

Previous studies have suggested that hyperlipidemia may be causally involved in vascular endothelial cell injury. Presence of LDL in the sub-endothelial lining, leading to engulfment by mononuclear-macrophages, ultimately gives rise to foam cells, as part of initiation of atherosclerosis. Hyperlipidemia also promotes thrombosis. For example, LDL, particularly the oxidized form of LDL, has been reported to enhance platelet aggregation induced by various inducers. Our results showed that rabbits fed a high-cholesterol diet had elevated serum cholesterol and LDL-cholesterol simultaneously with an increase in platelet aggregation. When animals received oral administration of resveratrol, platelet aggregation induced by hypercholesterolemia was markedly inhibited, although the level of serum LDL was not affected. These results may be explained by the anti-oxidative property of resveratrol, which previously has been reported to inhibit LDL oxidation (7). A related explanation may be the inhibition of Ca²⁺ influx by resveratrol in platelets (8); an increase in free Ca²⁺ in platelets is essential for platelet aggregation.

Similar to that reported previously (5), we observed that resveratrol inhibited platelet aggregation in vitro induced by collagen, thrombin, and ADP. These results support earlier published observations showing the ability of red wine or purple grape juice, and not white wine, grapefruit juice or orange juice, to inhibit platelet aggregation in human subjects, as attributable to resveratrol in the red wine (9-12). Recent studies found that beverages containing resveratrol without alcohol also have beneficial effect on human health (12,13). Interestingly, the inhibitory effect of resveratrol was more potent than that of aspirin. Since thrombosis is known to play a very important role in the development of CHD and acute myocardial infarction, and is intimately associated with platelet aggregation, it is tempting to speculate that resveratrol, as a phytoalexin present in numerous natural plants and in high concentrations in red wine, may offer a novel means of preventing or treating atherosclerosis. Answers to this possibility must await future studies since resveratrol is presently not approved for clinical use in humans.

**Acknowledgments**

This research was supported in part by the Vivian Wu-Au Memorial Cancer Research Fund.

**References**