The Endothelium-Dependent Vasodilator Action of a New Beverage Made of Red Wine Vinegar and Grape Juice

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A new non-alcoholic beverage made of red wine vinegar and grape juice (Budo-no-megumi) has been recently demonstrated to lower the blood pressure of human as well as rats. In this study, we pharmacologically analyzed the mechanism of its hypotensive action. The thoracic aorta with intact endothelium was isolated from Sprague-Dawley rats, and incubated with a Tyrode’s solution. The beverage in concentrations of 0.25 to 2% relaxed the pre-contracted aorta with an α-adrenoceptor agonist phenylephrine in a concentration-dependent manner, 2% of which induced 59% relaxation. In contrast, the vasodilator response disappeared in the aorta without endothelium. l-Nitro-arginine methyl ester (l-NAME), an inhibitor of nitric oxide synthase, significantly reduced the vasodilator effect of the beverage, whereas indomethacin, an inhibitor of cyclooxygenase, hardly affected it. These results suggest that the beverage can activate the nitric oxide synthase activity to exert vasodilation, which may partly explain its previously reported hypotensive action.

Key words grape; polyphenol; vinegar; vascular endothelium; nitric oxide

In addition to antioxidant/antiradical activity, polyphenols have been known to decrease the platelet aggregation, oxidation of low-density lipoprotein cholesterol and endothelin synthesis and promote nitric oxide production.1—5) Indeed, red wine and/or grape juice have been demonstrated to prevent coronary heart disease in the epidemiological and clinical studies.6,7) Based on the information, a new beverage made of red wine vinegar and grape juice (Budo-no-megumi) has been recently developed for people who wish to get significant amount of grape polyphenols without taking alcohol. A previous study indicated that single oral administration of the beverage (3 ml/kg) exerts hypotensive and bradycardiac effects in human as well as the in vivo rat model.8) However, there is no information regarding the mechanism of hypotensive action by the beverage itself. In this study, we assessed the effects of the beverage on the isolated thoracic aorta of rats using the pharmacological method to clarify the mechanism.

METHODS AND MATERIALS

All experiments were performed in accordance with Guidelines for Animal Experiments of University of Yamanashi. The experiments were carried out essentially according to a previous report.9) The thoracic aorta was isolated from male Sprague-Dawley rats weighting 200—300 g (Charles River, Yokohama, Japan) and incubated with the Tyrode’s solution with the following composition (mM): NaCl 137, KCl 5.4, CaCl₂ 2.0, MgCl₂ 1.0, NaHCO₃ 11.9, NaH₂PO₄ 0.4, and glucose 5.6, which was oxygenated with a mixture gases of 95% O₂ and 5% CO₂ and maintained at 37°C. Tension of the aortic ring was isometrically measured under a resting tension of 1 g. After the contraction was obtained by an α₁-adrenoceptor agonist phenylephrine in a concentration of 0.1 μM (Sigma, St. Louis, MO, U.S.A.), acetylcholine (Sigma) in a concentration of 10 μM was added. The aortic ring exhibiting >80% relaxation by acetylcholine was judged to have intact endothelium in this study.

The beverage made of red wine vinegar and grape juice (Budo-no-megumi) was generously provided by Asaya Foods Co., Ltd. (Yamanashi, Japan). The beverage consists of 25% (v/v) of red wine vinegar containing 4.5% acetic acid, 5% (w/v) of originally manufactured 100% grape juice, and appropriate amount of honey, oligosaccharides, vitamin C, citrate, glucose and calcium lactate. The beverage has been shown to contain >1.5 mg/ml of polyphenol as gallic acid units.8) The beverage was diluted with distilled water in preparing each concentration of reaction mix. The pH of Tyrode’s solution was not affected by the addition of currently used concentrations of the beverage. Although precise bioavailability data of the beverage cannot be available, a concentration of 2% in plasma can be attained after administration of 3 ml/kg, p.o. at bioavailability of 25% when the effective circulatory plasma volume is 4% of body weight.

The data are presented as the mean±S.E. The statistical comparisons of mean values within a group were carried out using one-way repeated measures ANOVA followed by Contrast for statistical analysis between basal values and others, whereas those between the groups were assessed using two-way repeated measures ANOVA. A p-value <0.05 was considered to be significant.

RESULTS

Effects of the Beverage on the Aortic Ring Preparation in the Presence and Absence of the Endothelium Figure 1A shows a typical trace of the vasodilator action of the beverage in a concentration of 2% (v/v) on the pre-contracted aortic ring preparation with intact endothelium. Figure 1B summarizes the effects of the beverage in concentrations of 0.25 to 2% on the pre-contracted aortic ring preparation with or without endothelium. Phenylephrine in a concentration of 0.1 μM increased the tension by 1.39±0.04 g in the presence of the endothelium (n=10) and 1.81±0.20 g (n=4) in the
absence of the endothelium. In the aortic ring preparation with endothelium, the beverage exerted vasodilator actions in a concentration-dependent manner, in which 59±9% relaxation was observed by a concentration of 2% \((n=5)\). On the other hand, in the aortic ring preparation without endothelium, the vasodilator action of the beverage was attenuated, in which 8±2% relaxation was observed by a concentration of 2% \((n=4)\). The application of distilled water did not affect the tension of the aortic ring preparation with intact endothelium \((n=5)\).

**Pharmacological Analysis of the Beverage-Induced Vasodilator Action of the Aortic Ring Preparations in the Presence of the Endothelium**  
Figure 2A shows a typical trace of the effect of a nitric oxide synthase inhibitor L-nitro-arginine methyl ester (L-NAME, Sigma) on the beverage-induced vasodilator action of the pre-contracted aortic ring preparation with intact endothelium, indicating that the vasodilator action of the beverage disappeared after the treatment with 100 \(\mu M\) of L-NAME. Figure 2B summarizes the effect of L-NAME and a cyclooxygenase inhibitor indomethacin (Sigma) on the beverage-induced vasodilator action of the aortic ring preparations with endothelium. In the L-NAME (100 \(\mu M\))- and indomethacin (10 \(\mu M\))-treated aortic ring preparations, phenylephrine in a concentration of 0.1 \(\mu M\) increased the tension by 1.85±0.15 g \((n=5)\) and 1.30±0.06 g \((n=5)\), respectively. The beverage-induced vasodilator action disappeared in the L-NAME-treated aortic ring preparations, whereas the same extent of the action as that of non-treated aortic ring preparations (control) was detected in the indomethacin-treated aortic ring preparations.

**DISCUSSION**

Generally, polyphenols as contained in red wine have been shown to facilitate the release of nitric oxide from the vascular endothelial cells. However, this principle does not necessarily apply to all wines, since the content and/or type of polyphenols in each wine are different among the grape variety, area of cultivation and vinification methods. In this study, we investigated the vascular effect of a new non-alcoholic beverage made of red wine vinegar and grape juice, the former of which was made from domestic red wine in Yamanashi prefectural area of Japan.8)

As clearly shown in the results, 0.25 to 2% of the beverage relaxed the rat aorta in the presence of the intact endothelium, which is in good accordance with a previous report that showed the endothelium-dependent vasodilator activity of various grape products. More importantly, these concentrations can be attained after oral administration of hypotensive dose of 3 ml/kg when the bioavailability of the beverage is 3 to 25%. Since removal of the endothelium significantly attenuated the vasodilator effect of the beverage, its vasodilator action can be considered to depend on vasoactive mediators from the endothelial cells. Also, a slight vasodilator action was observed in the rat aorta in the absence of the endothelium, suggesting that the beverage can modestly but directly act on the vascular smooth muscle cells to potentiate the endothelium-dependent vasodilation.

A nitric oxide synthase inhibitor L-NAME completely suppressed the vasodilator action of the beverage, whereas a cyclooxygenase inhibitor indomethacin did not inhibit it significantly. These results suggest that the constituents of the beverage may enhance nitric oxide production, leading to vasodilatation through the increase of cyclic guanosine monophosphate (cGMP) in the vascular smooth muscle cells, but hardly affects prostaglandin synthesis. One can speculate that the constituents of the beverage polyphenols
might play important role in the vasodilator action, since polyphenols in red wine have been shown to stimulate the nitric oxide synthase.10 In addition, another constituent of the beverage vinegar may have facilitated the blood pressure lowering effect of the beverage,8 since a previous study13 as well as our preliminary experiment has confirmed that vinegar can suppress the renin-angiotensin system. Therefore, the beverage-induced hypotensive action in vivo8 may be associated with effects of multiple constituents besides polyphenols in the beverage.

In conclusion, the beverage can exert vasodilator action via the nitric oxide synthase activation of the rat aorta, which may be one of the mechanisms of the blood pressure lowering action of the beverage in the in vivo animals and human.8)

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REFERENCES