A Red Wine Vinegar Beverage can Inhibit the Renin-Angiotensin System: Experimental in Vivo

Sachiko HONSHO, Atsushi SUGIYAMA,* Akira TAKAHARA, Yoshioki SATOH, Yuji NAKAMURA, and Keitaro HASHIMOTO

Department of Pharmacology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi; Tamaho-cho, Nakakoma-gun, Yamanashi 409–3898, Japan.

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A new beverage made of red wine vinegar and grape juice (Budo-no-megumi™) was developed for people who wish to take effective amount of both polyphenols and vinegar. Since the beverage was recently demonstrated to exert hypotensive effect in rats, we analyzed its underlying mechanisms in this study. Sprague-Dawley rats were anesthetized with pentobarbital, and the blood pressure and lead II ECG were continuously monitored (n=6). The effects of recommended volume of the beverage (3 ml/kg, p.o.) on the renin-angiotensin system were assessed in vivo. At the basal control state, the increase in the mean blood pressure induced by the angiotensin I (1 μg/kg, i.v.) and norepinephrine (0.3—3 μg/kg, i.v.) were +57±2 and +36±8 mmHg, respectively. Sixty minutes after the administration of the beverage, the angiotensin I-induced pressor response decreased to +45±7 mmHg at 60 min (p<0.05), whereas no significant change was detected in the norepinephrine-induced pressor response. In another parallel series of the experiment using Sprague-Dawley rats (n=6), the serum angiotensin-converting enzyme activity was 39.4±1.2 IU/l at basal control state, which was slightly but significantly decreased to 37.0±1.4 IU/l at 60 min after the administration of the beverage (p<0.01). These results suggest that previously described hypotensive action of the beverage may be partly induced by the inhibition of angiotensin-converting enzyme.

Key words: grape; polyphenol; vinegar; hypotensive effect; angiotensin I-converting enzyme

Since hypertension is one of the major independent risk factors for arteriosclerosis, stroke, myocardial infarction and end-stage renal disease, attempts have been extensively made in the field of food science and technology to discover food-stuffs that may improve the self-regulating mechanism of the blood pressure levels. A new beverage made of red wine vinegar and grape juice (Budo-no-megumi™) was developed for people who wish to get enough grape polyphenols and vinegar for their health. A recent study has indicated that a single oral administration of the beverage (3 ml/kg) decreases the blood pressure and heart rate of the pentobarbital-anesthetized rats. Since information regarding the mechanism of hypotensive action of the beverage is still limited, we analyzed the in vivo effects of the beverage on the renin-angiotensin system in this study.

MATERIALS AND METHODS

Animals were obtained through Animal Laboratory for Research of University of Yamanashi. All experiments were performed in accordance with Guidelines for Animal Experiments of University of Yamanashi.

Effects on the Angiotensin I-Induced and Norepinephrine-Induced Pressor Responses Male Sprague-Dawley rats weighing 200—300 g were anesthetized with 50 mg/kg of intra-peritoneal injection of pentobarbital sodium. After a tracheal cannula was inserted, the animals were artificially ventilated with room air (Shinano, SN-480-7, Tokyo, Japan). The tidal volume and respiratory rate were set at 10 ml/kg and 60 strokes/min, respectively. The right femoral artery and vein were cannulated with heparinized catheters for the blood pressure measurement and the drug administration, respectively. The surface lead II electrocardiogram (ECG) was obtained from the limb electrodes. The ECG and systemic blood pressure were continuously monitored using a polygraph system (Nihon-Kohden, RM-6200, Tokyo, Japan) and analyzed with a real time full automatic data analysis system (MP/VAS 3 for Macintosh, Physio-Tech, Tokyo, Japan). After confirming the stability of the cardiovascular variables, angiotensin I (1 μg/kg) and norepinephrine (0.3—3 μg/kg) were intravenously injected with a recovery interval of >5 min, and the increase in the mean blood pressure were assessed. After confirming the reproducibility of each pressor response, a recommended dose of the beverage (3 ml/kg, n=6) or distilled water (3 ml/kg, n=6) was administered directly into the stomach via a gastric tube, whereas a clinically available angiotensin I-converting enzyme (ACE) inhibitor temocapril hydrochloride (0.04 mg/kg, n=6) was intravenously injected. Then, the same doses of angiotensin I and norepinephrine were injected 60 min after the administration of the beverage and distilled water, whereas those were done 10 min after the administration of temocapril, based on the previous report. 3) Effects on the Serum ACE Activity In another parallel series of the experiment using male Sprague-Dawley rats weighing 170—240 g, the effects of the beverage and temocapril on the serum ACE activity were analyzed. Under anesthesia with ether inhalation, blood was collected from the jugular vein before and 60 min after the administration of the beverage (3 ml/kg, p.o., n=6), whereas that was performed before and 10 min after the injection of temocapril (0.04 mg/kg, i.v., n=6), respectively. The serum ACE activity was assayed with the method by Kasahara and Ashihara. 5) Subjects and Drugs 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 manufactu-
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 polyphenols as gallic acid units. Pentobarbital sodium (Tokyo Kasei, Tokyo, Japan), heparin calcium (Mitsui, Tokyo, Japan), angiotensin I (Peptide Institute, Osaka, Japan), norepinephrine (Sigma, St. Louis, MO, U.S.A.) and temocapril hydrochloride (Sankyo, Tokyo, Japan) were purchased.

Statistics The data are presented as the mean±S.E. The statistical comparisons of mean values within a group were performed using paired t-test, whereas those between the groups were assessed using unpaired t-test or one-way, factorial ANOVA. A p-value less than 0.05 was considered significant.

RESULTS

Effects on the Angiotensin I-Induced or Norepinephrine-Induced Pressor Responses The basal heart rate and mean blood pressure values were 283±38 beats/min and 91±7 mmHg in the beverage group, 378±32 beats/min and 112±5 mmHg in the distilled water group, and 327±25 beats/min and 99±7 mmHg in the temocapril group, respectively. No significant difference was detected in the respective basal control values between the groups. The heart rate and mean blood pressure values of each group tended to decrease during the experimental period, but they did not achieve the statistical significance.

The effects of the beverage, distilled water and temocapril on the angiotensin I- and norepinephrine-induced pressor responses are summarized in Fig. 1. The control pressor responses by the angiotensin I and norepinephrine were +57±2 and +36±8 mmHg in the beverage group, +54±3 and +42±4 mmHg in the temocapril group, and +51±2 and +49±2 mmHg in the distilled water group, respectively. No significant difference was detected in the respective basal responses between the groups. The beverage and temocapril decreased the angiotensin I-induced pressor response to +45±7 mmHg at 60 min (p<0.05) and +28±2 mmHg at 10 min (p<0.01), respectively, whereas the distilled water hardly affected them (Fig. 1B). On the other hand, norepinephrine-induced pressor response was not modified by the administration of either the beverage, temocapril or distilled water (Fig. 1C).

Effects on the Serum ACE Activity Figure 2 summarizes the effects of the beverage and temocapril on the serum ACE activity. The basal serum ACE activities were 39.4±1.2 IU/l in the beverage group (n=6) and 36.9±1.4 IU/l in the temocapril group (n=6). No significant difference was detected in the respective basal values between the groups. The beverage slightly but significantly decreased the ACE activity to 37.0±1.4 IU/l at 60 min (p<0.01), whereas temocapril suppressed it to 1.7±0.1 IU/l at 10 min (p<0.01).

DISCUSSION

A new beverage made of red wine vinegar and grape juice was recently demonstrated to activate the endothelial nitric oxide synthase to exert vasodilation, resulting in the decrease of the blood pressure. In order to explore other potential hypotensive mechanisms, in this study we analyzed the effects of the beverage on the renin-angiotensin system in vivo. As clearly shown in the functional as well as enzymatic analysis, the p.o. administration of the beverage inhibited the ACE activity of rats.

Of high interest in this study would be an identification of potential mechanism related to the ACE inhibition by the beverage. In previous studies, vinegar has been shown to suppress the renin activity of spontaneously hypertensive rats, to inhibit the ACE activity of the mouse pulmonary tissue in vitro, and to reduce the blood pressure of the spontaneously hypertensive rats. On the other hand, ACE inhibitory effects have been identified in several polyphenols derived from plant, such as tannin and procyanidins, which are also present in grapes. Since currently analyzed beverage has been shown to contain certain amount of vinegar and grape polyphenols, these previous knowledge may support the idea that multiple components of the beverage may have cooperatively suppressed the ACE activity in the in vivo rats.

The difference of the extent of serum ACE inhibition be-
tween the beverage and temocapril also deserve a comment. As shown in the results, the beverage decreased the angiotensin I-induced pressor response by 21%; however, the extent of the inhibition of the serum ACE activity was only 7%. On the other hand, temocapril decreased the pressor response by 48% and the extent of the inhibition of the serum ACE activity was 95%. These results suggest that serum ACE inhibition may not fully explain the change in the angiotensin I-induced pressor response in vivo. Indeed, some drugs have been shown to decrease the blood pressure chiefly through the ACE inhibition at target tissue like the blood vessel and kidney.\textsuperscript{10} Similar profile has been reported for the ACE inhibitory factors in the foodstuffs. For example, the development of hypertension in spontaneously hypertensive rats was inhibited by sour milk feeding, of which ACE activity in the aorta was significantly reduced, but that in the serum was hardly affected.\textsuperscript{21} Therefore, one can speculate that similar mechanism may have operated for the currently analyzed beverage to suppress the angiotensin I-induced pressor response.

In conclusion, the p.o. administration of the new beverage suppresses the ACE activity in vivo, which may partly explain the previously demonstrated hypotensive action of the beverage. More importantly, the beverage may be useful for the prevention of the various cardiovascular diseases, including the atherosclerosis, ventricular remodeling following myocardial infarction, diabetic nephropathy, and hypertension, of which ACE may play important roles for the onset.

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\textbf{REFERENCES}