Note

Attenuation of Renal Ischemia-Reperfusion Injury by Proanthocyanidin-Rich Extract from Grape Seeds

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Summary The effects of proanthocyanidin-rich extract in rats subjected to renal ischemia-reperfusion were examined. Proanthocyanidin-rich extract, which is prepared from grape seeds (Vitis vinifera L.), was given orally at doses of 5 and 10 mg/kg body weight/d for 20 consecutive days prior to ischemia-reperfusion. Administration of proanthocyanidin-rich extract attenuated renal dysfunction, as indicated by serum urea nitrogen and creatinine levels. Additionally, in the ischemic-reperfused kidneys, increased levels of thiobarbituric acid (TBA)-reactive substance and alterations of antioxidant enzyme activities such as superoxide dismutase, catalase and glutathione peroxidase (GSH-Px) were observed. Proanthocyanidin-rich extract-treated groups showed significantly reduced renal TBA-reactive substance levels and enhanced catalase and GSH-Px activities. These results suggest that proanthocyanidin-rich extract has protective effects against ischemia-reperfusion-induced renal damage associated with oxidative stress.

Key Words proanthocyanidin-rich extract, ischemia-reperfusion, thiobarbituric acid-reactive substance, catalase, glutathione peroxidase

Ischemia-reperfusion injury is frequently encountered in vascular surgery, organ procurement and transplantation, and can cause functional and structural damage to tissues. In the kidney, ischemia-reperfusion injury is a factor that provokes acute renal failure, which has an average mortality rate of 50% (1). Recently, the events occurring during ischemia-reperfusion have been examined, and various mechanisms have been proposed to explain the origins of tissue injury. Among these, it has been widely accepted that reactive oxygen species participate in the pathogenesis of ischemic acute renal failure (2, 3). Therefore, investigations of amelioration or prevention of cellular injury after renal ischemia through manipulation of free radical production have received much attention and indicated that antioxidants have therapeutic potential (4, 5).

Recently, polyphenol-rich foods, including vegetables, fruits, nuts, seeds and beverages such as tea and red wine, have attracted scientific interest as dietary sources of antioxidants that are valuable for human health. Grape seeds contain large amounts of proanthocyanidins, which are oligomers or polymers of polyhydroxyl flavan-3-ol units, and have been reported to have remarkable antioxidative and reactive oxygen species-scavenging activities (6–8). In this study, we examined the effects of proanthocyanidin-rich extract prepared from grape seeds in a model of renal ischemia-reperfusion injury.

Materials and Methods

Preparation of proanthocyanidin-rich extract from grape seeds. Grape seeds (Vitis vinifera L.) were washed with water at 60°C for 2 h and then extracted with water at 90°C for 2 h. The aqueous extract was freeze-dried to obtain the proanthocyanidin-rich extract, which was composed of 73.4% proanthocyanidins, 5.6% monomeric flavanols, 6.4% organic acids, 3.9% ash, 3.7% protein, 3.0% moisture, and 1.7% carbohydrate. A representative structure of procyanidins, the main components of proanthocyanidins, is shown in Fig. 1.

Animals and treatments

(1) Animal preparation: The “Guidelines for Animal Experimentation,” approved by Toyama Medical and Pharmaceutical University, were followed in these experiments. Male Wistar rats with a body weight of 150–160 g were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were kept in wire-bottomed cages under a conventional lighting regimen with a dark night. The room temperature (about 25°C) and humidity (about 60%) were controlled automatically.

The rats were allowed access to laboratory pellet chow (CLEA Japan Inc., Tokyo, Japan, comprising 24.0% protein, 3.5% lipid and 60.5% carbohydrate) and water ad libitum. Following several days' adaptation, the animals were divided into 4 groups of 7 rats, avoiding intergroup differences in body weight. Two groups were given the proanthocyanidin-rich extract (5 or 10 mg/kg body weight) and two groups (vehicle and sham) were given equivalent volumes per kg of water. The proanthocyanidin-rich extract was dissolved in water and administered orally by stomach tube every day for
20 consecutive days, after which the proanthocyanidin-rich extract-treated and vehicle rats were subjected to renal-ischemia reperfusion as follows. Anesthesia was induced with intraperitoneally administered sodium pentobarbital (50 mg/kg body weight), bilateral flank incisions were made and the renal arteries were exposed. Bilateral renal artery occlusion was carried out for 60 min using a non-traumatic vascular clamp. Following release of occlusion, the abdomen was sutured, the animal was returned to its cage, and 6 h later, blood samples were obtained by cardiac puncture under anesthesia and the serum was separated immediately by centrifugation. Then, the kidneys were perfused through the renal arteries with ice-cold physiological saline, extirpated from each rat, quickly frozen and kept at −80°C until analysis.

(2) Determination of serum urea nitrogen and creatinine (Cr) levels: Urea nitrogen and Cr levels were determined using the commercial reagents BUN Kainos and CRE-EN Kainos (Kainos Laboratories, Tokyo, Japan), respectively.

(3) Determination of thiobarbituric acid (TBA)-reactive substance levels: TBA-reactive substance levels of serum were measured using the method of Naito and Yamanaka (9), and those of kidney tissue were assayed according to the method of Mihara and Uchiyama (10).

(4) Determination of enzyme activities: Renal tissue was homogenized with a 9-fold volume of ice-cold physiological saline and the activities of enzymes in the homogenate were determined. Superoxide dismutase (SOD) activity was determined according to the nitrous acid method described by Elstner and Heupel (11) and Oyanagui (12), which is based on the inhibition of nitrite formation by hydroxylamine in the presence of a superoxide (O₂⁻) generator. Catalase activity was evaluated by following the decomposition of hydrogen peroxide (H₂O₂) directly by monitoring the decrease in extinction at 240 nm (13). Glutathione peroxidase (GSH-Px) activity was measured by a colorimetric assay that determined the concentration of 2-nitro-5-thiobenzoic acid, a compound produced by the reaction between glutathione and 5,5'-dithiobis(2-nitrobenzoic acid) (14). Protein levels were determined by the microbiuret method with bovine serum albumin as the standard (15).

Statistics. The results are presented as means ±SE. Differences among groups were analyzed by Dunnett’s test, and those at p<0.05 were considered significant.

Results and Discussion

In this study, ischemia-reperfusion produced significant increases in serum urea nitrogen and Cr levels compared with those of normal rats subjected to the sham operation (Table 1). In contrast, the serum urea nitrogen levels of rats given the 5-mg and 10-mg dose regimens of proanthocyanidin-rich extract decreased from 26.8 to 24.0 mg/dL (a 10% change, p<0.001) and from 26.8 to 21.7 mg/dL (a 19% change, p<0.001), respectively. Similarly, the serum Cr levels of both proanthocyanidin-rich extract-treated groups decreased significantly compared with that of the vehicle rats, as shown in Table 1. These results show that the renal dysfunction (in terms of increased levels of serum urea nitrogen and Cr) improved after administration of proanthocyanidin-rich extract for 20 consecutive days prior to ischemia-reperfusion injury.

Although the pathogenesis of the underlying renal dysfunction during ischemia-reperfusion is complicated, free radicals and oxidative stress have been extensively implicated in the pathogenesis of ischemia-reperfusion injury (2, 3, 16). Free radicals are highly reactive and injure lipids, proteins and nucleic acids, resulting in structural and functional impairment. Paller and Neumann (17) observed that subjecting renal proximal tubule cells to hypoxia and reoxygenation increased the rates at which they generated O₂⁻, H₂O₂ and hydroxyl radicals (·OH), which then increased lipid peroxidation and cellular permeability. Several studies have demonstrated that ischemia-reperfusion is associated with lipid peroxidation leading to oxidative destruction of cellular membranes (18) and hence decreased renal function. In this study, to examine the effect of proanthocy-
Table 2. Effect of proanthocyanidin-rich extract on TBA-reactive substance.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum (nmol/mL)</th>
<th>Kidney (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic and reperfused</td>
<td>1.90±0.09</td>
<td>0.086±0.004</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.89±0.24</td>
<td>0.172±0.008</td>
</tr>
<tr>
<td>Proanthocyanidin-rich extract (5 mg/kg B.W./d)</td>
<td>2.40±0.21</td>
<td>0.148±0.011</td>
</tr>
<tr>
<td>Proanthocyanidin-rich extract (10 mg/kg B.W./d)</td>
<td>2.03±0.13</td>
<td>0.123±0.008</td>
</tr>
</tbody>
</table>

Statistical significance: a p<0.01, b p<0.001 vs. sham operation values; c p<0.01, d p<0.001 vs. vehicle values with ischemia-reperfusion.

Table 3. Effect of proanthocyanidin-rich extract on radical scavenging enzyme activities in renal tissue.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/mg protein)</th>
<th>Catalase (U/mg protein)</th>
<th>GSH-Px (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>15.61±0.55</td>
<td>198.0±4.7</td>
<td>133.6±4.4</td>
</tr>
<tr>
<td>Ischemic and reperfused</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>12.52±0.55</td>
<td>157.1±12.5</td>
<td>85.1±2.9</td>
</tr>
<tr>
<td>Proanthocyanidin-rich extract (5 mg/kg B.W./d)</td>
<td>13.11±0.65</td>
<td>156.4±5.3</td>
<td>91.2±1.8</td>
</tr>
<tr>
<td>Proanthocyanidin-rich extract (10 mg/kg B.W./d)</td>
<td>13.67±0.83</td>
<td>185.8±9.4</td>
<td>94.0±1.5</td>
</tr>
</tbody>
</table>

Statistical significance: a p<0.001 vs. sham operation values; b p<0.05, c p<0.001 vs. vehicle values with ischemia-reperfusion.

Results suggest that the attenuation of oxidative stress through correcting the deterioration of enzymatic radical scavenger system activity caused by ischemia-reperfusion is involved in the beneficial effects of proanthocyanidin-rich extract.

In the fields of nutrition and preventive medicine, the use of dietary polyphenolic compounds with antioxidant properties is attracting increasing attention in response to the recognition of the importance of oxidative damage in the pathogenesis of many diseases. Our present study provides information that explains, at least in part, the health benefits of grape seed-derived proanthocyanidin-rich extract in the kidney, the functional status of which this extract preserved following ischemia-reperfusion induced injury associated with oxidative stress. However, further research is needed to establish the detailed mechanisms whereby this extract exerts its protective effects.

REFERENCES


