Antihypertensive Effect of Boysenberry Seed Polyphenols on Spontaneously Hypertensive Rats and Identification of Orally Absorbable Proanthocyanidins with Vasorelaxant Activity

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The antihypertensive effect of a single oral administration of a boysenberry seed polyphenol extract to spontaneously hypertensive rats was evaluated at different doses (100 and 200 mg/kg), and a significant decrease in systolic blood pressure (SBP) was observed up to 6 h post administration. The extract was separated into proanthocyanidin-rich and ellagitannin fractions by solvent partition. A significant decrease in SBP was observed only after administering the proanthocyanidin-rich fraction, and this decrease was abolished by an 

\[ \text{N}^\text{G}-\text{nitro}-\text{l}-\text{arginine methyl ester (l-NAME) injection.} \]

An analysis of the orally absorbable components showed that intact dimeric and trimeric procyanidins and propelargonidins were detectable in the plasma with a maximal concentration 2 h post administration. The vasorelaxant activity of the extract was also confirmed by in vitro assay using rat aorta rings. These results suggest that proanthocyanidins (PAs) in boysenberry seeds may have played an important role in the observed antihypertensive effect.

Key words: antihypertensive effect; boysenberry seed; proanthocyanidin; absorption; vasorelaxation

Hypertension is one of the major risk factors for developing cardiovascular disease.2) This pathology is a common and usually progressive disorder, which if not effectively treated, has a high mortality rate. Epidemiological as well as experimental studies have demonstrated that lifestyle modification, including a change in dietary habits, was important for the prevention and therapy of hypertension. Daily consumption of a polyphenol-rich diet has been shown to be an important and useful dietary measure for reducing this risk factor.2,3) Among the various classes of polyphenols, proanthocyanidins (PAs) have been attracting increasing attention for not only their antioxidative effects, but also for their structural diversity in such plants as cacao, apple, grape, and berries which have significant roles in the human diet.4–6)

Pioneering studies on cacao7) and grape seeds8) have already shown that PAs endogenous to these plants favorably augmented endothelium-dependent vasorelaxation in vitro via the nitric oxide (NO)-cGMP pathway by increasing the NO level in rat aorta rings. These results are consistent with the improvement of flow-mediated dilation and with the blood pressure decrease in human subjects.8,9) PAs are flavanol polymers, and their structure, physiological activity, and bioavailability vary widely according to species, tissue, and methods of preparation. The degree of polymerization of PAs was found to be a key factor affecting the oral absorbability; for example, the distribution of the degree of polymerization in PAs from cacao and grape seed extracts was found to vary widely, from 2 to over 10. However, only the dimeric to trimeric oligomers of PAs have been detected in human plasma10,11) and in rat urine12) after an oral administration, although apple procyanidins ranging from dimers to octamers have been detected in rat plasma.13) These reports indicate that the absorbability depended on the PA structure and that short oligomeric PAs would probably be favorable for oral absorption. As the degree of polymerization of PAs ranges from 2 to over 10 in many plants,9) short oligomeric PAs have become an attractive target for the development of orally-active dietary compounds.

The polyphenol components from boysenberry seeds, part of the waste from processing boysenberry fruits for juice and puree, have been discussed in a previous study.14) These seeds are convertible to value-added products because they contain five classes of polyphenolics and are rich sources of PAs and ellagitannins (ETs). Their PA profiles included short oligomers with a degree of polymerization of 2 and 3 (Fig. 1), which could be expected to have good oral bioavailability. Other major components of these seeds included polyesters with a sugar moiety (or other non-aromatic polyhydroxy compounds) and hexahydroxydiphenic acid (HHDP). These compounds have also been reported to have vasorelaxant activity during in vitro rat aorta experiments,15,16) although their oral absorbability was reported to be very poor.17,18) PAs and ETs occur together in such edible fruits and nuts as boysenberry,14) raspberry,5) strawberry,19) and walnut.6) There has been only one recent report on the in vivo antihypertensive...
effect of polyphenols from a raspberry extract; however, the active components were not characterized. It is therefore important to distinguish whether PA or ET is absorbed and results in the observed antihypertensive effect after an oral administration.

The purpose of the present study was to assess the antihypertensive effect of boysenberry seed polyphenols after orally administering to spontaneously hypertensive rats (SHRs) and to investigate candidates for the active components by determining their oral absorbability in vivo and vasorelaxant activity in vitro.

Materials and Methods

Reagents and materials. Boysenberry seeds were obtained from Berryfruit Export New Zealand Ltd. (Nelson, New Zealand). A polyphenol fraction from the boysenberry seeds was prepared by using an XAD column, and the polyphenol components shown in Table 1 were identified by using reversed-phase HPLC with a diode array detector and mass spectrometry according to the method previously described. 

Flavanols, PA dimers, and PA trimers were respectively quantified by using authentic CA, procyanidin B2, and procyanidin C2 as reference compounds. Analytical standards for catechin (CA), epicatechin (EC), and epicatechin gallate (ECA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Indomethacin and 1H-[1,2,4]oxadiazolo[4,3-a]quinolinin-1-one (ODQ) were purchased from Wako Pure Chemicals (Osaka, Japan). All the other reagents and solvents used in this study were of the highest analytical or HPLC grade available.

Animals. Male SHRs (14–16 weeks old, 280–310 g) were purchased from Japan SLC (Shizuoka, Japan) and housed at 24 ± 1°C with a 12-h light:dark cycle and free access to commercial feed (Labo MR Stock, Nosan Co., Kanagawa, Japan) and distilled water for 1 week before the experiment. The study was conducted in accordance with the Animal Experimentation Guidelines of Niigata University of Pharmacy and Applied Life Sciences.

Blood pressure measurement following administration of the boysenberry seed polyphenols. Eighteen SHRs were assigned to three groups (n = 6). The polyphenol fraction [100 or 200 mg/kg of body weight in water (2 mL)], PA or ET fraction [50 mg/kg of body weight each in water (2 mL)], and only water (2 mL as a control), were administered by stomach tube. The systolic blood pressure (SBP) was measured 0, 2, 4, 6, 8, and 24 h after administration by the tail cuff method (BP-98A-L, Softron, Tokyo, Japan).

In a separate set of experiments, the PA fraction [50 mg/kg of body weight in water (1 mL)] and water only (1 mL) were similarly administered to six 14-week-old SHRs. After 4 h, the rats from both groups were injected intraperitoneally with L-NAME [30 mg/kg of body weight dissolved in water (1 mL)], as described by Quinones et al.

Analysis of plasma. The PA fraction (1,000 mg/kg of body weight) was administered to each SHR (n = 25) via a stomach tube. Five
animals were anesthetized with diethyl ether 0, 1, 2, 4, or 6 h post administration, and blood was collected from the abdominal aorta with heparin-moistened syringes. Plasma was obtained by centrifugation at 2,500 × g for 20 min at 4 °C, and 0.2 mL of ascorbic acid (10 mg/mL) and ethylenediaminetetra-acetic acid (2 mg/mL) were added to 1 mL of plasma. All samples were stored at −80 °C.

**Qualitative and quantitative analyses of the polyphenols.** PAs in the rat plasma were extracted as described by Serra et al.\(^{23}\) Samples of plasma (1 mL) were spiked with catechol (50 μL, 20 μg/mL in water) as an internal standard. After phosphoric acid (30 μL) had been added to the plasma to cleave protein from the proanthocyanidins, each plasma sample was charged into a cartridge of OASIS HLB (60 mg, Waters, Milford, MA, USA) that had been conditioned with methanol:water:acetic acid (99:0.8:0.2 v:v:v, 4 mL), and concentration of the eluate to dryness (70:29.5:0.5 v:v:v, 4 mL), and concentration of the eluate to dryness after which the eluted solution (100 μL) was reconstituted with methanol:water (10:90) and then passed through a polytetrafluoroethylene syringe filter (0.45 μm) to purify each analytical sample.

The intact polyphenol compounds and expected metabolites in the plasma were detected by reversed-phase HPLC-DAD-MS/MS as previously described.\(^{24}\) Data were measured with a Prominence UPLC system (Shimadzu Corp., Kyoto, Japan) equipped with a diode array detector (DAD) and an API 3200 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, CA, USA). Briefly, the polyphenols were separated by using an RP L-column\(^{2}\) column (150 × 2.1 mm i.d. 3 μm; Chemicals Evaluation and Research Institute, Tokyo, Japan) at a flow rate of 0.2 mL/min at 40 °C. The linear mobile phase gradient consisted of acetonitrile with 0.1% v/v formic acid and water containing ethylenediaminetetra-acetic acid (2 mg/mL) to purify each analytical sample.

**Statistical analysis.** A statistical analysis was performed by using ANOVA and subsequent Tukey’s test for pairwise comparisons. Data are expressed as the mean ± SD (standard deviation), differences being considered significant at p < 0.05.

**Results**

**Octanol-water partitioning and fractionation of PA and ET fractions with ethyl acetate and water**

The octanol/water partition ratios (log \(P_{ow}\)) of the main polyphenolic components in the polyphenol fraction were measured (Table 1). The values for short oligomeric PAs were between 0.10 and −1.0, and those for dimeric and trimeric ETs were between −2.0 and less than −3.0, indicating that the dimeric ETs were slightly more hydrophobic than the trimeric ETs. These results suggested that ethyl acetate with a dielectric constant of 0.73 might be suitable for fractionating PA and ET. Partitioning of the polyphenol fraction between ethyl acetate and subsequent analysis of the polyphenolic components revealed that the PA content had increased to 8.88% in the PA fraction, but that PAs were not detectable in the ET fraction (Table 2).

**Effect on blood pressure of a single oral administration of the boysenberry seed fractions**

The three fractions in Table 2 with different polyphenolic compositions were evaluated.

**Polyphenol fraction**

The respective contents of ETs and PAs were 50.4 and 43.6% (w/w). The effects of the oral administration of the polyphenol fraction on SBP in SHR s are shown in Fig. 2A. SBP with the 200 mg/kg dosage group showed respective apparent decreases of −15.7 ± 11.4, −16.5 ± 8.2, −17.3 ± 3.6, −9.8 ± 10.1, and 2.6 ± 5.7 mm of Hg 2, 4, 6, 8, and 24 h post administration, these values being significantly different from those of the control group at 2 and 6 h. Similar significant differences were

<table>
<thead>
<tr>
<th>Components</th>
<th>(\log P_{ow})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicatechin</td>
<td>0.26</td>
</tr>
<tr>
<td>Procyanidin</td>
<td></td>
</tr>
<tr>
<td>PC dimer B3</td>
<td>−0.64</td>
</tr>
<tr>
<td>PC dimer B4</td>
<td>−0.65</td>
</tr>
<tr>
<td>two PP dimers</td>
<td>0.10</td>
</tr>
<tr>
<td>PC trimer C2</td>
<td>−1.00</td>
</tr>
<tr>
<td>PC trimer 6</td>
<td>−0.57</td>
</tr>
<tr>
<td>Ellagitannins</td>
<td></td>
</tr>
<tr>
<td>monomeric</td>
<td>−0.89</td>
</tr>
<tr>
<td>dimeric</td>
<td>−2.01</td>
</tr>
<tr>
<td>trimer</td>
<td>−3.10</td>
</tr>
<tr>
<td>Cyanidin-3-glucoside</td>
<td>−0.94</td>
</tr>
<tr>
<td>Quercetin-3-arabinoside</td>
<td>0.48</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Refer to Fig. 1 for the constitutional units and linkages of proanthocyanidins.

PC, procyanidin; PP, propelargonidin.
Table 2. Polyphenolic Compositions on the Three Fractions (fr) Prepared from Boysenberry Seeds

<table>
<thead>
<tr>
<th>Components</th>
<th>Contents, % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPh fr</td>
</tr>
<tr>
<td>Flavan-3-ol</td>
<td>0.72</td>
</tr>
<tr>
<td>monomers</td>
<td></td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>4.36</td>
</tr>
<tr>
<td>PC dimers</td>
<td>0.94</td>
</tr>
<tr>
<td>PP dimers</td>
<td>0.59</td>
</tr>
<tr>
<td>PC trimers</td>
<td>1.14</td>
</tr>
<tr>
<td>PP trimers</td>
<td>1.68</td>
</tr>
<tr>
<td>Ellagitannins</td>
<td>50.43</td>
</tr>
<tr>
<td>monomeric</td>
<td>13.70</td>
</tr>
<tr>
<td>dimeric</td>
<td>36.47</td>
</tr>
<tr>
<td>trimers</td>
<td>0.90</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>1.85</td>
</tr>
<tr>
<td>Flavonol glycosides</td>
<td>1.43</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>2.11</td>
</tr>
</tbody>
</table>

Refer to Fig. 1 for the constitutional units and linkages of proanthocyanidins.

PPh fr, polyphenol fraction; PA fr, proanthocyanidin fraction; ET fr, ellagitannin fraction; PC, procyanidin; PP, propelargonidin

![Antihypertensive Effect of Oral Administration of the Boysenberry Seed Polyphenol Fractions on the Systolic Blood Pressure (SBP) in Spontaneously Hypertensive Rats.](image)

A. Dose-dependent effect of the polyphenol fraction (PPh fr); B. Effects of the proanthocyanidin fraction (PA fr) and ellagitannin fraction (ET fr) prepared by ethyl acetate/water partition. Values are expressed as the mean ± SD (n = 6). Level of statistical significance: *p < 0.05 with respect to the corresponding value for the water control.

Also apparent in the 100 mg/kg group at 6 h. The values for SBP after 24 h in both test groups returned to the initial values and displayed no significant differences compared with the controls.

Table 3. Changes in Systolic Blood Pressure (SBP) 6 h Post Administration with and without an Intraperitoneal Injection of L-NAME

<table>
<thead>
<tr>
<th>Group</th>
<th>Change in SBP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>−5.6 ± 17.6*</td>
</tr>
<tr>
<td>PA fr</td>
<td>−19.5 ± 5.0</td>
</tr>
<tr>
<td>Water + l-NAME</td>
<td>6.4 ± 9.7**</td>
</tr>
<tr>
<td>PA fr + l-NAME</td>
<td>7.2 ± 8.1**</td>
</tr>
</tbody>
</table>

Water and the PA fraction (50 mg/kg of body weight) were orally administered to the control and PA fr groups, respectively. Values are expressed as the mean ± SD (n = 6), showing changes in SBP at 6 h post administration. The two groups with + L-NAME were treated with an L-NAME injection (30 mg/kg of body weight) at 4 h. Differences between the PA fr and other groups were determined by one-way ANOVA and subsequent Tukey-Kramer test. *p < 0.05, **p < 0.01.

SYP, systolic blood pressure; PA fr, proanthocyanidin fraction; l-NAME, N²-nitro-l-arginine methyl ester

PA and ET fractions

The results of administering the same dosage of the PA fraction and ET fraction are shown in Fig. 2B. In the PA fraction group, SBP respectively decreased −2.8 ± 6.6, −12.9 ± 11.3, −19.5 ± 5.0, and −11.3 ± 5.9 mm of Hg 2, 4, 6, and 8 h post administration, and SBP after 6 h was significantly different from that of the control group. The SBP profile of the ET fraction group was similar to that of the control group; there was no significant difference between profiles up to 8 h post administration.

l-NAME injection after administering the PA fraction

The effect of an intraperitoneal injection with L-NAME (30 mg/kg of body weight) 4 h after administering the PA fraction (50 mg/kg of body weight) on the change in SBP was investigated. As summarized in Table 3, l-NAME injection abolished the significant antihypertensive effect (−19.5 ± 5.0 mm of Hg) of the PA fraction (7.2 ± 8.1 mm of Hg), and the difference became non-significant between the controls with (6.4 ± 9.7 mm of Hg) and without the l-NAME injection (−5.6 ± 17.6 mm of Hg).

Qualitative and quantitative analyses of polyphenols in the plasma by reversed-phase HPLC-DAD-MS/MS

Blood was collected 0, 1, 2, 4, and 6 h after orally administering the PA fraction (1,000 mg/kg of body weight) to male SHRs (n = 25). The flavanol monomers, procyanidin dimers, propelargonidin dimers, procyanidin trimers, and propelargonidin trimers in the plasma were respectively analyzed in the MRM mode by HPLC-MS/MS as m/z 289/245, 577/289, 561/289, 865/289, 849/289, 489/289, Fig. 3 showing the profiles of these compounds. The structures were shown by their constitutional units and linkages ([(E)CA, catechin or epicatechin; (E)AF, afzelechin or epiafzelechin] with reference to our previous data. The peaks at 12.3 and 16.5 min with m/z 289/245 were identified as CA and EC; the peaks at 11.2 and 13.9 min with m/z 577/289 were identified as procyanidin dimers B3 (CA-4α → 8-CA) and B4 (CA-4α → 8-EC); the peaks at 14.7 and 17.1 min with m/z 561/289 were identified as two propelargonidin dimers ([(E)AF-4 → 8-(E)CA]; the peaks at 12.0 and 15.3 min with m/z 865/289 were identified as procyanidin trimers C2 (CA-4α → 8-CA-4α → 8-CA) and 6 [(E)CA-4 → 8-(E)CA-4 → 8-
were found to decrease by 30-fold (\(849/289\)) and the peaks at 14.4, 15.7, and 17.9 min with maxima at 1–2 h (Fig. 4B). AUCs of the procyanidin dimers \(\text{B}3\) (CA-4\(\alpha\) \(\rightarrow\) 8-CA) and \(\text{B}4\) (CA-4\(\alpha\) \(\rightarrow\) 8-EC) were identified as three propelargonidin trimers \(\text{C}2\) (CA-4\(\alpha\) \(\rightarrow\) 8-CA-4\(\alpha\) \(\rightarrow\) 8-CA), and \(\text{B}4\) (CA-4\(\alpha\) \(\rightarrow\) 8-EC-4\(\alpha\) \(\rightarrow\) 8-EC). Two new \(m/z\) values of parent/product ions in the multiple reaction monitoring mode with negative ionization.

ACE activity in the plasma

The ACE activity in the plasma at 0, 1, 2, 4, and 6 h post administration was also measured and respectively found to be 42.8 \(\pm\) 0.1, 38.8 \(\pm\) 1.7, 37.4 \(\pm\) 2.7, 45.3 \(\pm\) 1.8, and 41.7 \(\pm\) 4.8 mU/mL/min. A statistical analysis indicated no significant difference between these measured values and the baseline value, although the \(p\) value reached a minimum (0.08) 1 h post administration.

In vitro vasorelaxant effect of the boysenberry seed polyphenol fractions

During the in vitro experiments using norepinephrine-treated rat aortic rings with intact endothelia, three boysenberry seed fractions, the polyphenol fraction, PA fraction, and ET fraction, induced dose-dependent vasorelaxation in a similar manner, with respective maximum relaxation of 66.1 \(\pm\) 4.0, 58.4 \(\pm\) 8.9, and 49.0 \(\pm\) 2.9% observed for 25 \(\mu\)g/mL of each fraction (Fig. 5A). The vasorelaxant effect of the polyphenol fraction was abolished in the rings without endothelia (data not shown), indicating that the PA fraction was able to induce endothelium-dependent relaxation. To further explore the possible mechanism involved, the

\((E)\text{CA}\); and the peaks at 14.4, 15.7, and 17.9 min with \(m/z\) 849/289 were identified as three propelargonidin trimers \(\{\text{two} (E)\text{CA}-4\alpha \rightarrow 8-(E)\text{CA}, \text{one} (E)\text{AF}-4\alpha \rightarrow 8-(E)\text{CA}, \text{and} \text{one} (E)\text{AF}-4\alpha \rightarrow 8-(E)\text{CA}\}\); \(E\), Three propelargonidin trimers of two \((E)\text{CA}-4\alpha \rightarrow 8-(E)\text{AF}-4\alpha \rightarrow 8-(E)\text{CA}\) and \((E)\text{AF}-4\alpha \rightarrow 8-(E)\text{CA}-4\alpha \rightarrow 8-(E)\text{CA}\). The \(m/z\) values of parent/product ions in the multiple reaction monitoring mode with negative ionization.

Changes in the intact and metabolite concentrations of the flavanol monomers and PA fractions in the plasma were monitored for 6 h after administration. Most flavanol monomers were detected as metabolites, and the concentrations, expressed as the area under the curve (AUC, 0.27 \(\pm\) 0.01 h\(\mu\)g/mL) of the intact monomers were found to decrease by 30-fold (8.07 \(\pm\) 0.62 h\(\mu\)g/mL) relative to the metabolite forms (Fig. 4A). Changes in the dimer and trimer profiles were similar, with maxima at 1–2 h (Fig. 4B). AUCs of the procyanidin and propelargonidin components were respectively calculated as 0.48 \(\pm\) 0.03 and 0.13 \(\pm\) 0.01 h\(\mu\)g/mL. These data show that each intact PA and its monomeric metabolite reached the respective maximal concentration in rat plasma 1–2 h after the oral administration.
effects of specific inhibitors on vasorelaxation were measured. l-NAME (100 μM) (Fig. 5B) and ODQ (10 μM) (data not shown), an inhibitor of sGC, inhibited the observed vasorelaxation. In contrast, indomethacin (10 μM) (Fig. 5B), a COX inhibitor, had no effect on the relaxation response. These results suggest that the vasorelaxation due to active components in the polyphenol fraction was endothelium-dependent through an NO/cGMP pathway.

Discussion

Our results demonstrate that an oral administration of the total polyphenol fraction from boysenberry seeds exerted an antihypertensive effect on SHRs. The absorbable components of these boysenberry extract fractions were detected as flavanol monomers and short oligomeric PAs, these being proposed to play an important role in the observed antihypertensive effect.

The polyphenol fraction contained five polyphenolic classes, 14 the major components being PAs (4.36%) and ETs (50.4%). PAs and ETs present in the polyphenol fraction were separated to directly assess their antihypertensive effects. These results suggest that the absorbability of the propelargonidin oligomers depended not only on their degree of polymerization, but also on their inter-flavan linkages. These findings are the first demonstration of propelargonidin absorption in rats. While the oral administration of almond polyphenols containing propelargonidins has recently been reported, 26 the absorption of propelargonidins was not described. In the present study, each PA peaked at 1 h post administration. Most flavanol monomers were converted to monoglucuronide and monomethyl-monoglucuronide metabolites, in agreement with the results previously reported. 26,27 Ellagitannins were not detectable in the plasma, consistent with reports from other authors. 17,18 We have previously reported a similar antihypertensive effect of proanthocyanidins from persimmon leaf tea without any identification of the absorbed components, 28 but in this study, we could prove that absorbed proanthocyanidin short-chain oligomers with vasorelaxant activity were the active antihypertensive components.

The possible mechanisms by which PAs may exert their antihypertensive effect are of interest, as previous studies have implicated the effect of the relationships between the endothelial function, angiotensin-convert-
ing enzyme, platelet adhesion, and nerve systems in modulating SBP. PAs from cacao and grape seeds have been reported to induce endothelium-dependent vasorelaxation through an endothelial NO-mediated pathway, although combination with hyperpolarization by multiple K+ channel activation has also been reported from different origins such as apple PAs.

To investigate whether boysenberry seed PAs also exerted an antihypertensive effect via vasorelaxation similar to grape seed PAs, the effect of L-NAME injection on SBP was examined after administering the PA fraction. The results show that the antihypertensive effect 6 h post administration was completely abolished by L-NAME injection (Table 3). Furthermore, the in vitro vasorelaxant activity of the boysenberry seed polyphenol fractions was confirmed by using norepinephrine-treated SHR aortic rings with intact endothelium. PAs and ETs have been reported to induce in vitro vasorelaxation mediated via the NO-cGMP pathway, and three fractions of polyphenol (4.36% PA content), PA (8.88% PA content), and ET (no detectable PA content) from boysenberry seeds exhibited in vitro vasorelaxant activity (Fig. 5A). However, in contrast to the antihypertensive effects of other components on SHRs, only the ET fraction had no effect, suggesting that the active antihypertensive components in this study were PAs absorbed into the blood. The fact that the antihypertensive effect of a PA fraction on SHRs was abolished by L-NAME injection (Table 3) is in good agreement with the observation in the in vitro experiment where the vasorelaxant activity of the polyphenol fraction was completely inhibited by L-NAME (Fig. 5B).

In addition to vasorelaxation, we monitored changes in the ACE activity in plasma for 6 h after administering the PA fraction. The ACE activity varied between 37.4 ± 2.7 (at 2 h) and 45.3 ± 1.8 (at 4 h), and no significant change was apparent, although it is possible that the ACE activities of other organs, including the aorta and kidneys, may have decreased. Our experimental results support the finding that boysenberry seed PAs with vasorelaxant activity had an important antihypertensive effect on SHRs. However, further experiments are needed to more accurately define the in vivo antihypertensive mechanisms of the polyphenol fraction.

In conclusion, this study has shown that SBP was significantly decreased in SHRs following the oral administration of a total polyphenol fraction from boysenberry seeds. Orally absorbable components were identified as flavonol monomers and PAs consisting of dimeric and trimeric procyanidins and propelargonidins with endothelium-dependent vasorelaxant activity. These findings show that short oligomeric PAs in boysenberry seeds have a potential use in the production of value-added food products or nutraceuticals with an antihypertensive effect by using seed remnants from the processing of boysenberry fruits for juice and puree. Furthermore, our findings indicate that oligomeric PAs are good candidates for dietary use, either solely or synergistically with known antihypertensive ingredients such as milk peptides and acetic acid respectively having different major mechanisms through the inhibition of the angiotensin I-converting enzyme and decrease of plasma renin activity.

Acknowledgment

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References


