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ε-Viniferin Is More Effective Than Its Monomer Resveratrol in Improving the Functions of Vascular Endothelial Cells and the Heart

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The present study compared the effects of resveratrol and its dimer ε-viniferin on vascular endothelial cells (VECs) functions, and on the blood pressure and cardiac mass of spontaneously hypertensive rats (SHRs). Treatment of VECs with these compounds enhanced cell proliferation *via* nitric oxide generation and protected the cells from oxidative stress by suppressing increases in intracellular oxygen species. ε-Viniferin was more potent than resveratrol in most of these effects. ε-Viniferin, but not resveratrol inhibited angiotensin-converting enzyme activity *in vitro*. Three weeks of ε-viniferin treatment (5 mg/kg) reduced the systolic blood pressure and improved the whole cardiac mass and left ventricle mass indexes in SHRs. In contrast, resveratrol administration (2.5 mg/kg) failed to lower the blood pressure and significantly improve these mass indexes. These data suggest that ε-viniferin as well as resveratrol may be involved in protecting the functions of VECs and the heart.

Key words: angiotensin-converting enzyme; ε-viniferin; resveratrol; spontaneously hypertensive rat; vascular endothelial cell

Hypertension is the major risk factor for coronary artery disease and a leading cause of mortality worldwide. The renin-angiotensin system (RAS) plays an important role in the pathogenesis of hypertension and cardiac hypertrophy.[1)] These phenomena are attributable in part to excessive production of reactive oxygen species (ROS), particularly superoxide and hydrogen peroxide. ROS formation impairs nitric oxide (NO) generation and contributes to hypertension.[2)]

Polyphenols from grapes and wines are known to decrease the incidence of coronary heart disease. Diebolt *et al.*[3)] found that short-term oral administration of red wine polyphenolic compounds decreased blood pressure in normotensive rats and enhanced endothelium-dependent relaxation. Mizutani *et al.*[4)] also indicated that extracts of wine phenolics attenuated the elevation of blood pressure in stroke-prone spontaneously hypertensive rats (SHRs). Stilbenes are the most important polyphenolic subclass occurring naturally in grapes and related products. Among them, resveratrol (3,4′,5-trihydroxystilbene) has been the most widely studied for its role in human health (Fig. 1A). The cardioprotection effect of resveratrol has been documented in different experimental settings of pressure overload.[5,6)] In SHRs, a well-established genetic model of hypertension and subsequent cardiac hypertrophy, resveratrol attenuated the pathological cardiac hypertrophy without lowering blood pressure.[7,8)] In contrast, it reduced hypertension in male Sprague-Dawely rats[9)] and obese Zucker rats.[10)] In contrast to many reports describing the beneficial effects of resveratrol on the vasculature and heart, there are very few reports concerning the beneficial functions of ε-viniferin, a natural dehydrodimer of resveratrol. ε-Viniferin content is comparable to or greater than resveratrol content, depending on the type of red wine and the stage of noble rot development of grapes.[11,12)] Tran *et al.*[13)] indicated that ε-viniferin has enhanced antioxidant activity as compared to resveratrol. Mi *et al.*[14)] demonstrated that ε-viniferin was more potent than resveratrol in inducing the vasorelaxation of isolated thoracic aortas. We recently found that ε-viniferin is more effective than resveratrol in inhibiting platelet-derived growth factor (PDGF)-dependent cell proliferation and migration of cultured vascular smooth muscle cells by reducing the increased intracellular ROS levels induced by PDGF.[15)] These data suggest that ε-viniferin as well as resveratrol may contribute to preventing the pathogenesis of atherosclerosis.

Angiotensin-converting enzyme (ACE) in RAS catalyzes the formation of angiotensin II from angiotensin I, and also hydrolyses and inactivates the vasodilator peptide bradykinin. ACE inhibition is an important therapeutic approach in the treatment of hypertension. ACE inhibitors not only lower the blood pressure but also ameliorate heart failure.[16,17)] Indeed, a number of plant-derived compounds have been found to posses *in vitro* ACE inhibitory activity, including procyanidins, flavanols,[18,19)] tannins,[20)] and peptides.[21,22)]

The present study was designed to compare the effects of resveratrol with those of ε-viniferin on vascular endothelial cells (VECs) and ACE *in vitro*, and on blood pressure and cardiac mass in SHRs.

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Abbreviations: ACE, angitonsin-converting enzyme; DAF-2DA, diaminofluorescein-2 diacetate; DBP, diastolic blood pressure; DCF-DA, 2′,7′-dichlorofluorescein diacetate; EGC, epigallocatechin; GPx, glutathione peroxidase; L-NAME, NG-nitro-L-arginine methyl ester; LPA, lysophosphatidic acid; LV, left ventricle; NO, nitric oxide; ROS, reactive oxygen species; SBP, systolic blood pressure; SHR, spontaneously hypertensive rat; VEC, vascular endothelial cell; WKY, Wistar-Kyoto rat

Materials and Methods

Materials. *Trans*-resveratrol and lysophosphatidic acid (LPA) were purchased from Cayman Chemical (Tokyo). Epigallocatechin (EGC) was obtained from Mitsui Norin (Tokyo). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin, Hanks' balanced salt solution (HBSS), 2′,7′-dichlorofluorescein diacetate (DCF-DA), L-arginine, glutathione reductase (GR), trypsin-EDTA, and corn oil were purchased from Sigma Chemical (St. Louis, MO). Diaminofluorescein-2 diacetate (DAF-2DA) was from Daiichi Pure Chemicals (Tokyo). Glutathione reduced (GSH) was purchased from MP Biomedicals (Illkrich). NG-nitro-L-arginine methyl ester (L-NAME) was obtained from Wako Pure Chemical Industries (Osaka, Japan). Anti-eNOS antibody was from Cell Signaling Technology (Beverly, MA).

Purification of ε-viniferin. Polyphenolic compounds containing resveratrol and ε-viniferin derived from grapes were kindly provided by Sun Bright (Tokyo), and ε-viniferin was purified by the Gilson Auto-Preparative HPLC System (Gilson, Middleton, WI) with a purity of about 98%. Separation was achieved on a Mightysil RP-18 column (10 mm i.d. × 250 mm, Kanto Chemical, Tokyo).

Cell culture. VECs were isolated from porcine pulmonary arteries, obtained at a local abattoir (Tsuchiura, Japan). The cells were cultured in DMEM containing low amounts of glucose supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified 5% CO_2 atmosphere.[23] The responses of VECs to the compounds were analyzed between passages 8 and 10 after 16-h incubation in DMEM containing 1% FBS. Resveratrol and ε-viniferin dissolved in dimethyl sulfoxide (DMSO) were added to the medium at a final DMSO concentration of 1%. The same final concentration of DMSO was used in place of these reagents in control treatments.

Wound repair. Serum-starved VECs (3×10^5 cells/well) plated onto 35-mm dishes were pretreated with various concentrations of resveratrol or ε-viniferin for 30 min. Then VEC monolayers were manually displaced by scratching using a sterile 200 μL pipette tip to generate a clean area of constant diameter.[23] The cells were incubated with LPA, resveratrol, or ε-viniferin at 37 °C for 24 h. Immediately after injury (0 h) and at 8 h and 24 h, the wounds were photographed with an inverted microscope attached to a digital camera (Leica, Wetzlar) and the cells that had migrated into the cell-free area were counted. The number of these cells was determined in three randomly selected microscopic fields.

Cell proliferation assay. Serum-starved VECs (2×10^5 cells/well) plated onto 35-mm dishes were treated with and without L-NAME for 1 h before adding various concentrations of resveratrol or ε-viniferin for 24 h. Cell proliferation was then determined by counting trypan blue negative cells (viable cells) by trypan blue dye exclusion using a hemacytometer after trypsinization.[23]

Cell viability. Hydrogen peroxide (H_2O_2)-induced cytotoxicity as to VECs was evaluated in preliminary experiments by incubating the cells with increasing concentrations (0–700 μM) of H_2O_2 for 24 h. Because about 50% of cell viability was lost at 500 μM, this concentration was used in all subsequent cell viability investigations. Serum-starved VECs (2×10^5 cells/well) plated onto 35-mm dishes were pretreated with various concentrations of resveratrol or ε-viniferin for 24 h and then H_2O_2 was added for another 24 h. Cell viability responses were determined by counting the number of viable cells per total cells by trypan blue dye exclusion.[23]

Measurement of NO production. The fluorescence reagent DAF-2DA was used to measure NO production.[24] Briefly, VECs were loaded with 10 μM DAF-2DA (Daiichi Pure Chemicals, Tokyo) for 30 min. Thereafter, the cells were co-incubated with 100 μM L-arginine (Sigma, St. Louis, MO) and various concentrations of resveratrol or ε-viniferin for 2, 4, 6, and 8 h. Production of NO was estimated by recording the fluorescence at 485 nm as excitation and 530 nm as emission using a luminescence spectrophotometer (PerkinElmer, Waltham, MA).

Western blotting. After incubation with reagents, the cells were lysed with ice-cold lysis buffer consisting of 20 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 10% glycerol (vol/vol), 100 mM NaF, 10 mM sodium pyrophosphate, 1% TritonX-100, 1 mM Na_3VO_4, 1 mM PMSF, 10 μg/mL antipain, 10 μg/mL leupeptin, 10 μg/mL aprotinin, and centrifuged at 14,000 rpm at 4 °C for 15 min. The supernatant was collected and used for subsequent analyses. The protein concentration of the cell lysates was determined using BCA reagents (Pierce, Rockford, IL). After SDS–PAGE, the proteins were transferred to polyvinylidene difluoride membranes. After blocking, the membranes were incubated with an anti-phospho-eNOS antibody and then with peroxidase-conjugated secondary antibody. Blots were developed with a chemiluminescence kit according to the manufacturer's instructions (Nacalai Tesque, Kyoto, Japan).

Determination of intracellular ROS. The generation of intracellular ROS was monitored spectrofluorometrically using the oxidant-sensitive probe DCF-DA (Sigma) as described previously.[25] Fluorescence was monitored by confocal laser scanning microscopy (TCPSP2, Leica) at an excitation wavelength of 488 nm and an emission wavelength of 500–630 nm. Data were expressed as changes relative to initial fluorescence.

Catalase activity assay. VECs were treated with resveratrol or ε-viniferin for 12, 24, and 48 h. After these time periods, the cells were harvested in RIPA buffer and lysed by sonication. The sonicated cells were centrifuged and the total protein concentration in the supernatant was determined using BCA reagents (Pierce). H_2O_2 at 10 mM (80 μL) was incubated with 10 μL of 10 mM Tris–HCl (pH 7.4)/1 mM EDTA at 37 °C for 2 min, and mixed with 10 μL of cell lysates. The absorbance was measured at 240 nm at 25 °C for 5 min; the changes in absorbance over time are proportional to the H_2O_2 decomposition. Catalase activity was expressed as micromoles of H_2O_2 consumed per min per mg protein.

Glutathione peroxidase (GPx) activity assay. VECs and lysates were prepared as described for the catalase activity assay. Thereafter, 50 μL of lysates was added to a reaction mixture containing 50 μL of 1 × TE, 290 μL H_2O, 10 μL of 100 mM GSH, 50 μL of 10 U/mL glutathione reductase, and 50 μL of 2 mM NADPH, and incubated at 37 °C for 2 min. Then 10 μL of 7 mM tert-butyl hydroperoxide was added and NADPH oxidation was measured at 340 nm. Nonenzymatic oxidation of NADPH at 340 nm was measured by adding RIPA buffer instead of the lysates. Enzymatic consumption of NADPH was calculated by subtracting the nonenzymatic consumption of NADPH from the total. GPx activity was expressed as nmol of NADPH consumed per min per mg of protein.

In vitro *assay for inhibition of ACE activity.* ACE inhibitory activity was measured spectrofluorometrycally using ACE Kit-WST Detection (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions.

Animal experiments. Fourteen-week-old male SHRs and normotensive Wistar-Kyoto rats (WKYs) (Charles River Laboratories, Japan) were maintained in accordance with the Guidelines for the Proper Conduct of Animal Experiments, Science Council of Japan, and the protocol approved by the Animal Care and Use Committee of the University of Tsukuba. They were housed under an animal facility with a 12-h light/dark cycle and were fed the usual rat chow and tap water. An adaptation period of one week for vehicle administration and blood pressure measurements was allowed before the initiation of experimental protocols. Six rats were randomly assigned to each group and body weight was measured every week. The SHRs were administered 2.5 mg/kg body weight resveratrol or 5 mg/kg body weight ε-viniferin daily by oral gavage for three weeks. The dose of resveratrol was chosen according to previous experimental models using hypertension in SHRs.[7,8] In order to give the same dose of the molecules, the dose of ε-viniferin used was the double of that of the monomer. The untreated WKYs and SHRs were administered vehicle orally by gavage (corn oil, Sigma) for the same period.

Measurement of blood pressure. Systolic and diastolic blood pressure (SBP and DBP) was measured once a week by tail-cuff

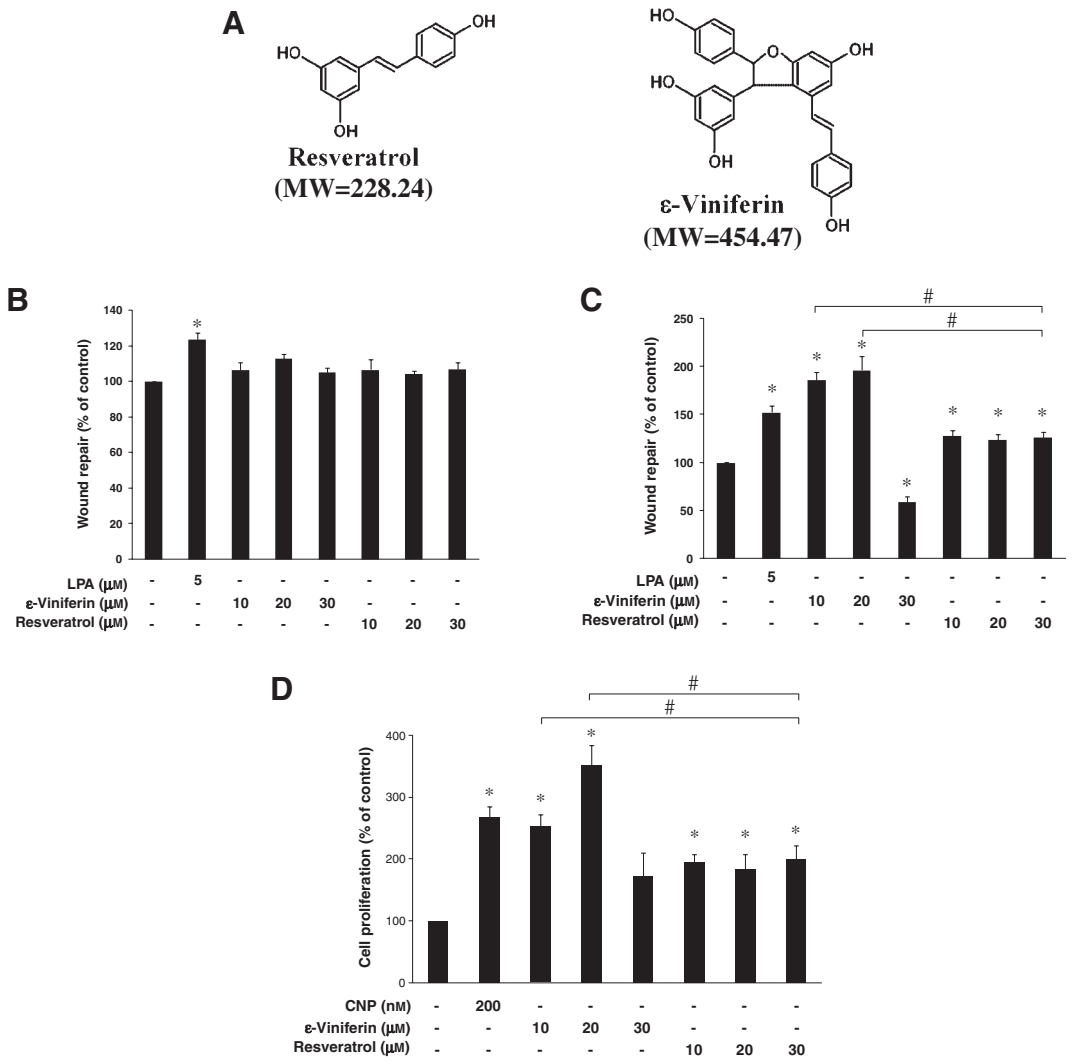

Fig. 1. *ε*-Viniferin as Well as Resveratrol Promotes Wound Repair and Proliferation of VECs.

A, Chemical structures of resveratrol and *ε*-viniferin. B and C, Serum-starved VECs monolayers were wounded with a pipette tip, then treated with 5 μM LPA or the indicated concentrations of resveratrol or *ε*-viniferin for 8 h (B) or 24 h (C). The number of cells that migrated into the cell-free area represents wound repair as described in "Materials and Methods." D, Serum-starved VECs were treated with 200 nM CNP or the indicated concentrations of resveratrol or *ε*-viniferin for 24 h. Cell proliferation was assessed by trypan blue dye as described in "Materials and Methods." Data are mean ± SE of three separate experiments for B and C, and four separate experiments for D. $^{*}p < 0.05$ *vs.* untreated cells. $^{\#}p < 0.05$.

plethysmography (BP-98A; Softron, Tokyo) just prior to administration of the compounds. The final SBP and DBP are presented as means of five measurements.

Cardiac weight. At the end of the experiment the rats were scarified, and the hearts were excised immediately, cleaned, and weighed. The atria were discarded and the free wall of the right ventricle was carefully removed, leaving the left ventricle (LV) free wall and the intraventricular septum as total LV. They were weighed separately. The heart and ventricular weights were normalized to body weight and were expressed as heart and LV mass index (mg/g).

Statistical analysis. Data represent mean ± SE. ANOVA was used to assess significant differences between treatment groups. When treatment was found to have a significant effect, Duncan's multiple range test was used to compare multiple group means. $p < 0.05$ was considered statistically significant.

Results

ε-Viniferin stimulates the wound repair of VECs more effectively than resveratrol by inducing cell proliferation

Initially we investigated the wound repair effects of *ε*-viniferin and resveratrol on cultured VECs by incubating the cells for 8 and 24 h. Treatment with each of the compounds at 10, 20, and 30 μM for 24 h significantly enhanced the wound healing except for 30 μM *ε*-viniferin (Fig. 1C) as LPA, a stimulator of wound repair in VECs.[26] In contrast, an 8-h incubation had no effect (Fig. 1B). These data suggest that both compounds enhance the wound repair by inducing cell proliferation, but not cell migration. In addition, it should be noted that *ε*-viniferin at 10 and 20 μM more efficiently promoted the healing than resveratrol at 30 μM. We confirmed the cell proliferation effect of the compounds by trypan blue exclusion assay (Fig. 1D). Again, *ε*-viniferin at 10 and 20 μM was more effective than resveratrol at 30 μM. Therefore, we concluded that *ε*-viniferin is more potent than resveratrol for the protection of VECs.

NO production is involved in the proliferation effect of ε-viniferin and resveratrol on VECs

Because NO plays an important role in promoting VEC proliferation and migration,[27] we examined to

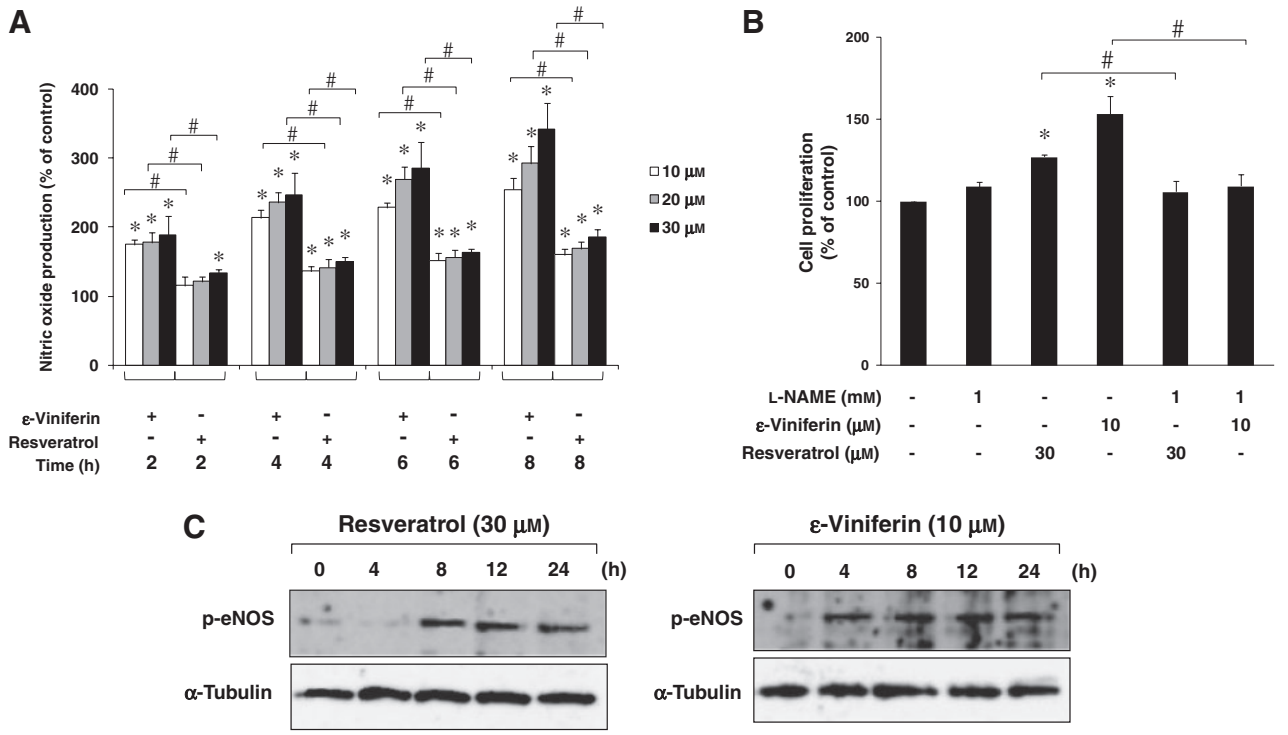

Fig. 2. ε-Viniferin and Resveratrol Promote NO Production in VECs, Leading to Cell Proliferation.

A, ε-Viniferin induces NO production more effectively than resveratrol. Serum-starved cells were treated with the indicated concentrations of resveratrol or ε-viniferin for 2, 4, 6, and 8 h, and then NO production was measured using DAF-2DA as described in "Materials and Methods." B, L-NAME, an NOS inhibitor, suppresses the proliferative effect of resveratrol and ε-viniferin on VECs. Serum-starved cells were treated with 30 μM resveratrol or 10 μM ε-viniferin in the presence or absence of 1 mM L-NAME, and the proliferation was assessed using trypan blue dye as described in "Materials and Methods." Data are mean ± SE of four separate experiments for A and B. C, Resveratrol and ε-viniferin induce the phosphorylation of eNOS. Serum-starved cells were treated with 30 μM resveratrol and 10 μM ε-viniferin, respectively, for 4, 8, 12, and 24 h. Thereafter, cells were lysed and western blotted using anti-phospho-eNOS antibody. Representative data are shown from three independent experiments. $^{*}p < 0.05$ *vs.* untreated cells. $^{\#}p < 0.05$.

determine whether ε-viniferin and resveratrol produce NO. As we expected, both compounds significantly increased NO generation in a time-dependent manner (Fig. 2A). The NO production rate of ε-viniferin at each concentration was significantly higher than that of resveratrol at the same concentration, which agrees well with the data on the wound repair and cell proliferation (Fig. 1C and D). We used 10 μM ε-viniferin and 30 μM resveratrol in all subsequent experiments. The proliferative effects of ε-viniferin and resveratrol were inhibited by the addition of L-NAME, a general inhibitor of NO synthase (NOS) (Fig. 2B). These data suggest that ε-viniferin and resveratrol induce VEC proliferation through NO production.

We investigated to determine whether both compounds phosphorylate eNOS since eNOS is activated by phosphorylation.[28] As Fig. 2C indicates, ε-viniferin and resveratrol induced sustained phosphorylation of the enzyme, which is consistent with the sustained NO generation induced by these compounds.

ε-Viniferin and resveratrol protect VECs from oxidative stress

Since oxidative stress is known to be one of the main causes of endothelial injury and increasing risk of hypertension,[29] we examined the cytoprotective properties of ε-viniferin and resveratrol against H_2O_2-induced cell damage. As Fig. 3A indicates, ε-viniferin as well as resveratrol significantly protected the cells from the cytotoxic effect of H_2O_2. Both compounds reduced intracellular ROS levels in VECs exposed to H_2O_2 (Fig. 3B). That is, the increased intracellular ROS was significantly decreased by the 24-h pretreatment of the cells with ε-viniferin and resveratrol. Because phase II detoxifying enzymes are essential components of the cellular antioxidant defense system detoxifying ROS, we examined the activity of catalase and GPx enzymes in the presence and the absence of each compound. Both compounds upregulated catalase and GPx activities in a time-dependent manner (Fig. 3C). Therefore, we concluded that ε-viniferin as well as resveratrol attenuates intracellular ROS generation in VECs through the increased activities of antioxidant enzymes, which confers resistance to oxidative stress.

ε-Viniferin, but not resveratrol, inhibits ACE

Because ACE inhibitors are extremely effective in reducing blood pressure, we investigated the ACE inhibitory effect of ε-viniferin and resveratrol *in vitro*. ACE was incubated in the presence of ε-viniferin, resveratrol, epigallocatechin (EGC), or EDTA (Fig. 4). ε-Viniferin exhibited a potent ACE inhibitory activity whereas resveratrol did not. Notably, the inhibitory effect of ε-viniferin at 100 μM was more pronounced than that of EGC, an established food-derived ACE inhibitor.[18] Together with the data on NO generation shown in Fig. 2A, these data suggest the possibility that ε-viniferin, but not resveratrol, reduces blood pressure.

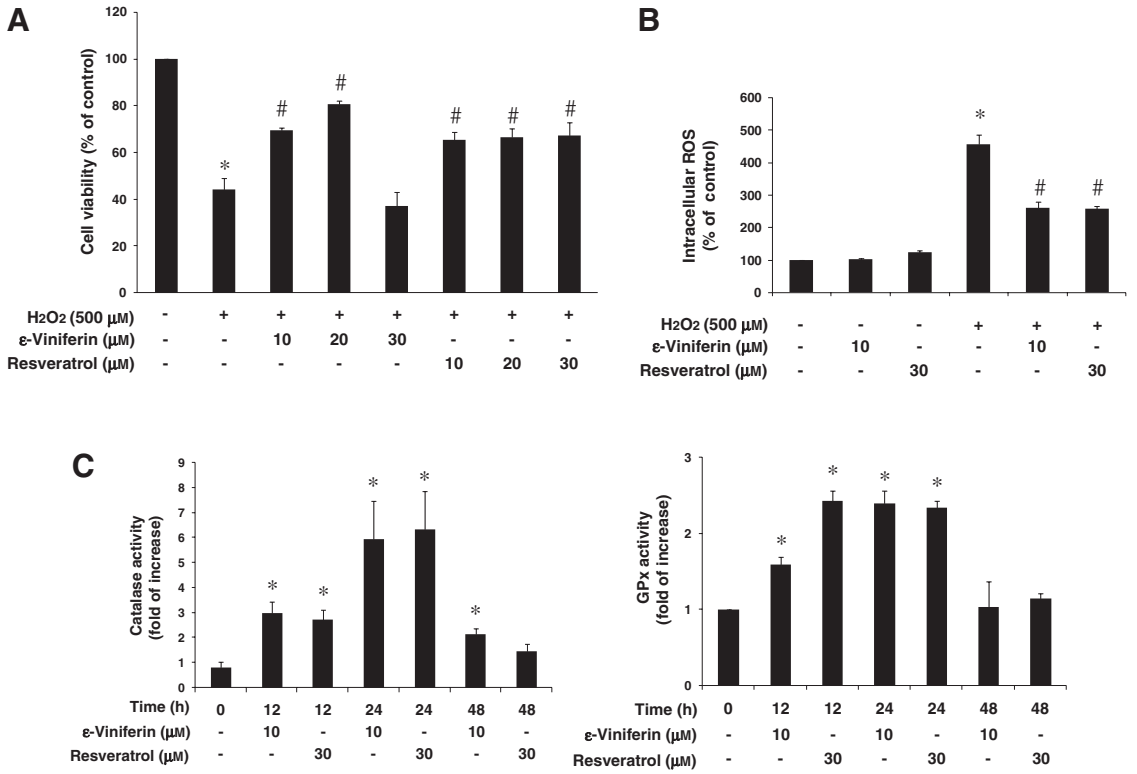

Fig. 3. ε-Viniferin and Resveratrol Protect VECs from Oxidative Stress and Increase the Activities of Catalase and GPx.

A, ε-Viniferin and resveratrol protect VECs from H_2O_2-induced cell death. Serum-starved cells were treated with the indicated concentrations of resveratrol or ε-viniferin for 24 h, washed, and incubated with 500 μM H_2O_2 for another 24 h. Cell viability was assessed using trypan blue dye as described in "Materials and Methods." B, ε-Viniferin and resveratrol suppress increased intracellular ROS levels in VECs exposed to H_2O_2. Serum-starved cells were treated with 30 μM resveratrol or 10 μM ε-viniferin for 24 h followed by incubation with 500 μM H_2O_2 for 15 min. Intracellular ROS accumulation was determined by measuring DCF-derived fluorescence as described in "Materials and Methods." Data are mean ± SE of four separate experiments for A and B. $^{*}p < 0.05$ *vs.* untreated cells. $^{\#}p < 0.05$ *vs.* H_2O_2-treated cells without stimuli. C, ε-Viniferin and resveratrol upregulate catalase and GPx activities. Serum-starved cells were incubated with 30 μM resveratrol or 10 μM ε-viniferin for 12, 24 or 48 h, and enzymatic activities were assessed as described in "Materials and Methods." Data are mean ± SE of four separate experiments. $^{*}p < 0.05$ *vs.* untreated cells.

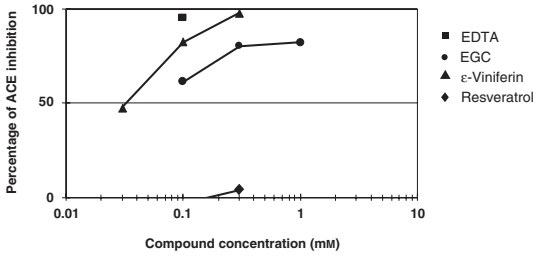

Fig. 4. ε-Viniferin, but Not Resveratrol, Inhibits ACE Activity *in Vitro*.

The percentage of ACE inhibition was assessed using ACE Kit-WST Detection according to the manufacturer's instructions. Data are mean of a single experiment performed in triplicate.

ε-Viniferin, but not resveratrol, loweres blood pressure in SHRs

We investigated the effects of ε-viniferin and resveratrol administration on blood pressure in SHRs (Fig. 5). As expected, SBP in the ε-viniferin-treated SHRs was significantly decreased at all time points as compared to age-matched vehicle-treated SHRs. In contrast, DBP was unaltered by ε-viniferin administration. Three-week resveratrol treatment did not lower SBP or DBP. Rather, resveratrol tended to increase the blood pressure.

ε-Viniferin is more effective than resveratrol in improving cardiac mass in SHRs

Because a reduction in cardiac mass with antihypertensive agents has been reported in experimental as well as human hypertension,[30,31] we examined the effects of the administration of each of the compounds on cardiac mass (Table 1). The heart and LV mass indexes in the vehicle-treated SHRs were significantly higher than those in the WKYs. These parameters were significantly reduced in the ε-viniferin-treated SHRs as compared to the vehicle-treated SHRs. In contrast, there was no significant difference in heart and LV mass indexes between the vehicle-treated SHRs and the resveratrol-treated SHRs (2.5 mg/kg). Therefore, the reduction in cardiac mass indexes in the SHRs treated with ε-viniferin correlated with the observed reduction in SBP. These data strongly indicate that ε-viniferin more effectively improves cardiac mass than resveratrol in hypertensive rats.

Discussion

The present study is the first to describe the direct effect of ε-viniferin on cultured VECs and on SHRs. Our major findings are as follows: First, ε-viniferin is more effective than resveratrol in inducing the proliferation and wound repair in VECs *via* NO production and in protecting VECs from oxidative stress-induced cell death. Second, ε-viniferin, but not resveratrol, inhibits ACE activity *in vitro*. Third, ε-viniferin, but not resveratrol, reduces blood pressure and improves cardiac mass in SHRs.

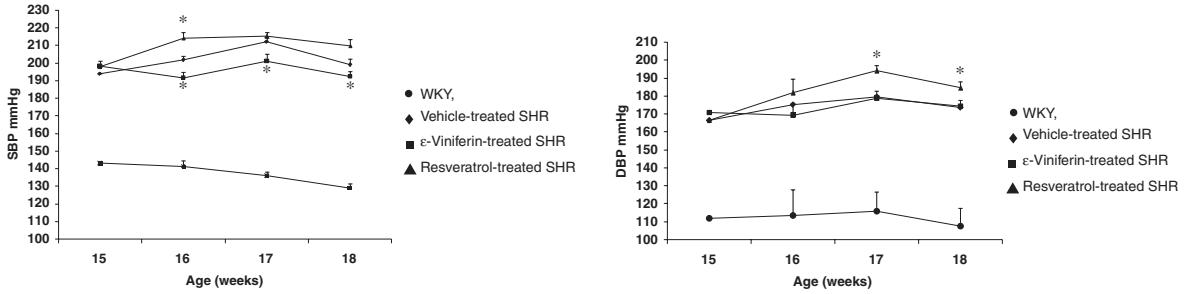

Fig. 5. ε-Viniferin, but Not Resveratrol Attenuates the Elevation of Blood Pressure in SHRs.
Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by tail-cuff plethysmography as described in "Materials and Methods." Data are mean ± SE of six rats per treatment. $^{*}p < 0.05$ *vs.* vehicle-treated SHRs.

Table 1. Body Weight and Cardiac Mass Indexes after 3 Weeks of Treatment with ε-Viniferin or Resveratrol in SHRs
HMI, heart mass index; LVMI, left ventricular mass index. Data are means ± SE of six rats per treatment. $^{*}p < 0.05$ *vs.* WKYs, $^{\#}p < 0.05$ *vs.* vehicle-treated SHRs.

	WKYs	Vehicle-treated SHRs	ε-Viniferin-treated SHRs	Resveratrol-treated SHRs
Heart weight (g)	1.26 ± 0.03	1.42 ± 0.03*	1.23 ± 0.02#	1.37 ± 0.06
Body weight (g)	364.33 ± 1.4	357.12 ± 3*	342.83 ± 5.2#	358.60 ± 7.2
HMI (mg/g)	3.45 ± 0.07	3.98 ± 0.12*	3.60 ± 0.04#	3.82 ± 0.11
LVMI (mg/g)	2.55 ± 0.05	3.01 ± 0.05*	2.85 ± 0.02#	2.97 ± 0.06

Our data indicate that ε-viniferin, but not resveratrol, reduces blood pressure in SHRs. This distinct effect is probably due to the two points of difference between the two compounds. The first is that ε-viniferin generates NO more effectively than resveratrol. NO is a strong vasodilator that lowers the blood pressure. The second is that ε-viniferin, but not resveratrol, inhibits ACE activity. Our data are in good agreement with a recent report indicating that ε-viniferin has a more potent vasorelaxant effect than resveratrol in the isolated rat thoracic aorta.[14] The fact of no ACE inhibitory effect of resveratrol in the present study is also consistent with past reports.[19,32] It should be noted that ACE inhibition promotes the release of NO.[31] ACE not only produces the vasoconstrictor angiotensin II, but also degrades the vasodilator bradykinin. Bradykinin is known to stimulate endothelial NO production. Thus, there is a possibility that ε-viniferin enhances NO generation by inhibiting ACE activity which is followed by a blockade of bradykinin inactivation. There has been no report indicating the plasma concentrations of ε-viniferin after oral administration of it to rats. Marier *et al.*[33] found that oral administration of resveratrol to rats at a dose of 50 mg/kg body weight exhibited over 100 μM of total plasma concentration of the aglycone and glucuronide forms of resveratrol. Also, Abd El-Mohsen *et al.*[34] identified the presence of resveratrol metabolites not only in the plasma, but also in the kidney, heart, and brain that are closely associated with the blood pressure. Although further investigation is necessary for the concentrations of ε-viniferin absorbed *in vivo*, our data together with past reports suggest the possibility that the ACE inhibitory effect of ε-viniferin is involved in the ε-viniferin-dependent reduction in blood pressure.

We found that ε-viniferin and resveratrol protect VECs from H_2O_2-induced cell death by suppressing intracellular ROS accumulation. Our findings support the view that both compounds themselves exert antioxidant effects due to the redox properties of their phenolic hydroxyl groups.[18] Direct scavenging of H_2O_2 may reflect the ability of the two compounds to have the cytoprotective effects against H_2O_2. In addition to this, ε-viniferin and resveratrol may promote the vascular expression of enzymes involved in scavenging H_2O_2, such as catalase and GPx. Our thought is supported by Ungvari *et al.*[35] who demonstrated that *in vitro* treatment with resveratrol increased oxidative stress resistance in VECs through both direct scavenging of H_2O_2 and upregulation of antioxidant enzymes. In our experiments, 30 μM ε-viniferin inhibited the wound healing and exhibited a weaker effect on the cell proliferation than 10 and 20 μM ε-viniferin. We cannot exclude the possibility that high concentrations of ε-viniferin are toxic to VECs; however, these toxic effects observed in our *in vitro* experiments may have been due to the serum-starved condition (1% FBS) to clearly show the functions of the compound. Further study is necessary concerning this issue.

The three-week resveratrol treatment failed to alter the blood pressure in SHRs, consistent with previous studies.[7,8] Although the compound tended to improve the whole cardiac and LV mass indexes, the effects were not significant. In contrast, ε-viniferin significantly reduced SBP and cardiac mass in SHRs. Caristrom *et al.*[36] demonstrated that dietary inorganic nitrate, which is converted *in vivo* to NO and other bioactive nitrogen oxides, prevented cardiac hypertrophy in rats subjected to the unilateral nephrectomy and a chronically high-salt diet. Matsuoka *et al.*[37] also indicated that chronic L-arginine administration, which induces NO generation, attenuated cardiac hypertrophy in SHRs. Similar mechanism may mediate ε-viniferin-dependent cardioprotective effect because this compound produces NO in VECs.

To the best of our knowledge this is the first report on ACE inhibitory, antihypertensive, and cardioprotective

effects of ε-viniferin. Further studies, including identification of target molecules for ε-viniferin and resveratrol in the cardiovascular system, are necessary to clarify the mechanisms underlying these beneficial effects.

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