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Resveratrol and its dimers ε-viniferin and δ-viniferin in red wine protect vascular endothelial cells by a similar mechanism with different potency and efficacy

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Abstract

Red wine compounds have been reported to reduce the rate of atherosclerosis by inducing nitric oxide (NO) production and antioxidant enzyme expression in vascular endothelial cells (VECs). The present study compared the effects of the three red wine compounds resveratrol and its dimers, ε-viniferin and δ-viniferin, on VECs function for the first time. Both 5 μM ε-viniferin and δ-viniferin, but not 5 μM resveratrol, significantly stimulated wound repair of VECs. Increased levels of wound repair induced by 10 and 20 μM ε-viniferin were significantly higher than those stimulated by 10 and 20 μM resveratrol, respectively. These stimulatory effects of the three compounds were suppressed by the NO synthase inhibitor L-NAME. When VECs were exposed to each compound, endothelial NO synthase was activated and the expression of sirtuin 1 (SIRT1) and HO-1 was induced. Addition of the SIRT1 and HO-1 inhibitors EX527 and ZnPPiX, respectively, suppressed wound repair stimulated by the three compounds, demonstrating that SIRT1 and HO-1 are involved in these wound repair processes. Furthermore, each compound induced the suppression of H_2O_2-dependent reduction of cell viability as well as the expression of the antioxidant enzyme catalase. These data suggest that not only resveratrol, but also its dimers, ε-viniferin and δ-viniferin, may be effective in preventing atherosclerosis by a similar molecular mechanism with different potency and efficacy.

KEYWORDS
nitric oxide (NO), resveratrol, SIRT1, δ-Viniferin, ε-Viniferin

1 | INTRODUCTION

Atherosclerosis is a chronic inflammatory process associated with endothelial dysfunction and oxidative stress.[1] Nitric oxide (NO) production and antioxidant enzyme expression are induced to protect against atherosclerosis at an early stage.[1,2] Epidemiological studies have demonstrated that red wine phenolic compounds protect the vascular system and reduce the risk of cardiovascular disease (CVD).[3–5]

Resveratrol is a common red wine polyphenol. Many studies have indicated that resveratrol increases lifespan in vivo by decreasing the incidence of coronary heart disease and preventing cancers.[6] The best-known function of resveratrol is its protective role in the

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cardiovascular system, which is mediated by several mechanisms, such as increased endothelial function through the induction of NO production.[7, 8] Resveratrol inhibits the aggregation of platelets via the activation of guanylyl cyclase, followed by the production of cyclic guanosine monophosphate, which suppresses reactive oxygen species (ROS) by antioxidant enzymes and decreases vascular inflammation by inhibiting NFκB activity.[9]

ε-Viniferin and δ-viniferin are dehydrodimers of resveratrol.[10, 11] Both viniferins are polyphenols that occur naturally in grape skins, grapevine leaves, grape canes, and are rich content in red wine. The average content of δ-viniferin, ε-viniferin, and cis-resveratrol in the commercially-available red wines are 6.4, 2, and 10 mg/L, respectively.[12, 13] Additionally, they can be produced from resveratrol by horseradish peroxidase.[14] ε-Viniferin and δ-viniferin are also known antioxidants. ε-Viniferin has previously been shown to have many biological functions, such as inhibiting human cytochrome P450 activity and inducing apoptosis in leukemia B-cells via inducible NO synthase (iNOS).[15, 16] In addition, it has been demonstrated to prevent carbon tetrachloride-induced hepatic injury in mice and act as an antidepressant by inhibiting noradrenaline and 5-hydroxytryptamine uptake in the rat brain.[17, 18] δ-viniferin has been reported to suppress apoptosis induced by the high glucose-induced generation of ROS in human umbilical vein endothelial cells (HUVECs) by increasing sirtuin 1 (SIRT1) expression.[19] However, comparative study of the effects of resveratrol, ε-viniferin, and δ-viniferin on the functions of the vascular system has not yet been performed.

SIRT1, a member of the sirtuin family of proteins, has been reported to be regulated by resveratrol. SIRT1 mediates a large variety of physiological events. In mammals, SIRT1 protects the function of the circulatory system, decreases coronary heart disease risk, and prevents endothelial dysfunction at early stages of the pathogenesis of atherosclerosis.[20–22] SIRT1 exerts atheroprotective effects by activating endothelial NOS (eNOS) production in endothelial cells and diminishes NFκB activity in macrophages.[23, 24] SIRT1 also suppresses ROS by inducing catalase and superoxide dismutase activity in astrocytes and endothelial cells.[25, 26]

Our previous study has demonstrated that resveratrol and ε-viniferin induce wound repair, increase NO production in vascular endothelial cells (VECs) and that ε-viniferin, but not resveratrol, reduces blood pressure and suppresses cardiac hypertrophy in a spontaneously hypertensive rat model.[8] We have also shown that resveratrol and ε-viniferin raise the activity of the potent antioxidant enzymes catalase and glutathione peroxidase, reduce intracellular ROS levels,[8] and suppress platelet-derived growth factor-induced cell proliferation in vascular smooth muscle cells.[27]

In contrast to the large number of reports regarding the biological functions of resveratrol, relatively few reports have described the functions of resveratrol dimers, ε-viniferin, and δ-viniferin even they are rich content in commercially available red wine.[13] Especially, there has been only one report describing the effect of δ-viniferin on VECs.[19] Therefore, the present study compared the biological functions of resveratrol, δ-viniferin, and ε-viniferin, focusing on their effects on VECs.

2 | MATERIALS AND METHODS

2.1 | Materials

Trans-resveratrol was purchased from Cayman Chemical Co. (Tokyo, Japan) for cell experiments and from Tokyo Chemical Industry Co. (Tokyo, Japan) for the preparation of δ-viniferin, respectively. ε-Viniferin and L-NAME were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, Hanks' balanced salt solution (HBSS), SIRT1 inhibitor III (EX527), and peroxidase (type VII-A), and anti-heme oxygenase-1, anti-catalase, and anti-β-actin antibodies were from Sigma Chemical Co. (Saint Louis, Missouri, USA). Diaminofluorescein-2 diacetate (DAF-2DA) was obtained from Sekisui Medical Co. (Tokyo, Japan). Anti-SIRT1, anti-mouse IgG, and anti-rabbit IgG antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, California). Anti-α-tubulin and zinc protoporphyrin-9 (ZnPPIX) were obtained from Calbiochem.

2.2 | Preparation of δ-viniferin

δ-viniferin was prepared from resveratrol by biotransformation with horseradish peroxidase.[28] Reaction mixture composed of resveratrol (500 mg) in 125 mL of acetone, 950 mL of 0.1 M sodium acetate buffer (pH 5), peroxidase (3 units/mL) in 100 mL of 20 mM sodium acetate buffer (pH 5), and 75 mL of 0.3% hydrogen peroxide, was incubated at 27°C for 30 minutes, and then 1250 mL of 5% trichloroacetic acid was added to inactivate enzyme activity. Reaction product, δ-viniferin, in the mixture was extracted with 500 mL of ethyl acetate, concentrated, and applied to a column (φ5.6 × 20 cm) of Wakogel N60 (Wako Pure Chemicals) pre-equilibrated with a solvent system of chloroform-ethyl acetate (6:4, vol./vol.). Elution was carried out with the same solvent system and desired fractions were combined. The resultant δ-viniferin fraction was further purified by preparative HPLC equipped with InertSustain C18 (14 mm I.D. × 250 mm, GL Science). Mobil phase and flow rate were 55% methanol and 7.41 mL/min, respectively. The structure of purified δ-viniferin was confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy (in DMSO-d_6 containing 0.03% tetramethylsilane).[11, 14]

2.3 | Cell culture

VECs were derived from porcine aortas obtained from Tsuchiura, Japan. VECs were cultured in DMEM containing 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified 5% CO_2 incubator and analyzed between passages 8 and 10. The cells were serum-starved by incubation for 16 hours in DMEM containing 1% FBS before starting the experiments.

2.4 | Wound repair assay

VECs (3×10^5 cells/well) were seeded in 3.5-cm dishes and pretreated with resveratrol, ε-viniferin, or δ-viniferin at various concentrations. Then, the VEC monolayer was wounded by scratching using a sterile 200-μL pipette tip to make an open area with a consistent diameter. The wounds were photographed with a microscope attached to a digital camera at 0 and 24 hours after scratching. Cells that had migrated into the wound area were counted.

2.5 | NOS activity assay

Intracellular NO was detected using the fluorescence reagent DAF-2DA. VECs (3×10^3 cells/dish) were plated in a 96-well plate, starved for 16 hours, washed three times with HBSS and incubated with 10 μM DAF-2 DA for 45 minutes. Following this incubation period, the cells were washed three times with HBSS and incubated with 100 μM L-arginine and resveratrol or ε-viniferin for 24 hours. Finally, the fluorescence of the cells was measured using a spectrofluorophotometer (Powerscan HT; Dainippon Pharmaceutical, Osaka, Japan) with excitation and emission wavelengths of 488 and 530 nm, respectively.

2.6 | Western blotting

For western blotting, the cells were resuspended in lysis buffer comprised of 50% glycerol, 100 mM NaF, 10 mM sodium pyrophosphate, 1% Triton X-100, 1 mM Na_3VO_4, 1 mM PMSF, 10 μg/mL antipain, 10 μg/mL leupeptin, and 10 μg/mL aprotinin. Afterwards, the sample lysates were centrifuged at 14 000 rpm at 4°C for 15 minutes, and the resultant supernatant was used for subsequent experiments. For western blotting, the protein concentrations of the cell supernatants were assayed by a Pierce BCA protein assay kit, and proteins were separated by 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked in 5% BSA at room temperature for 1 hour and then incubated overnight with anti-SIRT1, anti-HO-1, anti-phos-eNOS, anti-catalase, and anti-α-tubulin antibodies (diluted 1:1000 in 5% BSA) at 4°C. The membranes were then incubated with an HRP-conjugated secondary antibody for 2 hours at room temperature. The bands were detected by a chemiluminescence kit (Nacalai Tesque), and band intensity was quantified by an LAS-4000 mini imaging system (Fujifilm, Tokyo, Japan).

2.7 | Cell viability assay

VECs (1×10^5 cells/well) were seeded in 24-well plates, starved for 16 hours, and incubated with or without 10 μM resveratrol, 10 μM ε-viniferin, and 5 μM δ-viniferin for 24 hours. After washing with PBS, the cells were further incubated with 800 μM H_2O_2 for 24 hours. The cells were then washed three times with PBS and harvested in DMEM. Collected VECs were stained with 1 × trypsin-EDTA and 3% trypan blue inhibitor mixed 1:1 with a 5% trypan blue staining solution

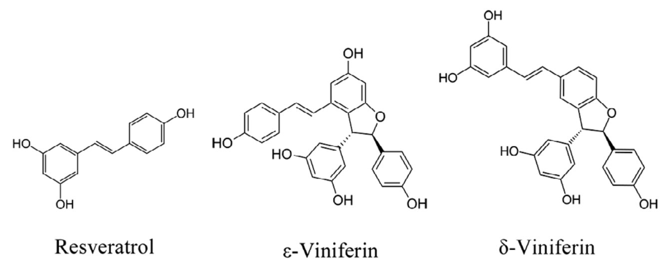

FIGURE 1 Chemical structures of resveratrol, ε-viniferin, and δ-viniferin

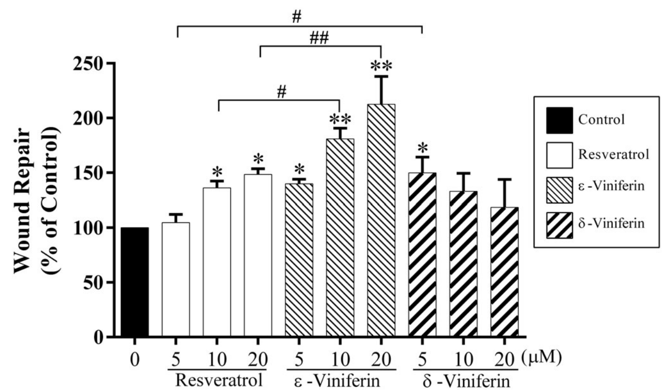

FIGURE 2 Effect of resveratrol, ε-viniferin, and δ-viniferin on wound repair in VECs. Serum-starved VECs were wounded with a 200-μL pipette tip and then incubated with 5, 10, and 20 μM resveratrol, ε-viniferin, and δ-viniferin, respectively, for 24 hours. Wound repair was assessed by counting cells that had proliferated and migrated in the cell-free area. The data are presented as the mean ± SD of three independent experiments. **P* < .05 and ***P* < .01 compared to the control group. #*P* < .05 and ##*P* < .01 between two groups

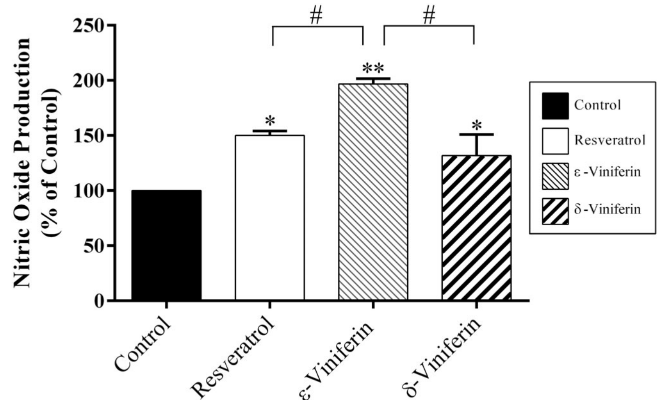

FIGURE 3 Effect of resveratrol, ε-viniferin, and δ-viniferin on NOS activity by VECs. Serum-starved VECs were pretreated with 10 μM DAF2-DA for 45 minutes and then treated with or without 10 μM resveratrol, 10 μM ε-viniferin, or 5 μM δ-viniferin for 24 hours. Thereafter, the fluorescence of the cells was measured. The data are presented as the mean ± SD of three independent experiments. **P* < .05 and ***P* < .01 compared to the control group. #*P* < .05 between two groups

(Nacalai Tesque). Cell viability was determined with a Countess II FL cell counter.

2.8 | Statistical analysis

Statistical analysis was performed using GraphPad Prism 6. All data are expressed as the mean ± SD (S.D.) of at least three different experiments. The differences between groups were analyzed by using one-way ANOVA followed by Tukey's test for multiple comparisons. Values with $P < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Resveratrol, ε-viniferin, and δ-viniferin stimulate wound repair in VECs

We compared the effect of resveratrol, ε-viniferin, and δ-viniferin (Figure 1) on wound repair of VECs at different concentrations because VEC wound repair prevents arteriosclerosis in the early stages of development. After incubation for 24 hours, 10 and 20 μM resveratrol, 5-20 μM ε-viniferin, and 5 μM δ-viniferin significantly stimulated wound repair (Figure 2 and S2). Unlike resveratrol, ε-viniferin and δ-viniferin were significantly effective at a low concentration (5 μM). The reason why δ-viniferin loses function in higher concentration is supposed to be resistance or induced toxicity of the compound. Moreover, 10 and 20 μM ε-viniferin elicited a significant greater increase in wound repair ability than resveratrol at the same concentrations. These results indicate that all three compounds can protect VECs via wound repair although they have different potency and efficacy with each other.

3.2 | Resveratrol, ε-viniferin, and δ-viniferin promote wound repair by producing NOS activity in VECs

Because wound repair is dependent on NO. NO induced cell proliferation to promoted wound repair in VECs.[8, 29] we investigated NOS activity by resveratrol, ε-viniferin, and δ-viniferin in VECs. We have previous tested two red wine compounds (resveratrol and ε-viniferin) on NO production at concentrations of 10, 20, and 30 μM, and we find that the effect is in a dose-dependent manner.[8] Here, we test three compounds on NOS activity at single dose. All three compounds significantly stimulated NO production (Figure 3). Resveratrol at 10 μM and δ-viniferin 5 μM increased the level of NO to approximately 150% of that of the control. ε-Viniferin at 10 μM increased the NO level to approximately 200% of that of the control, which is significantly stronger than the effect of the other compounds. These data are consistent with the data on the wound repair in Figure 2.

To confirm the involvement of NO in the wound repair induced by these compounds, we cultured VECs with or without L-NAME, an

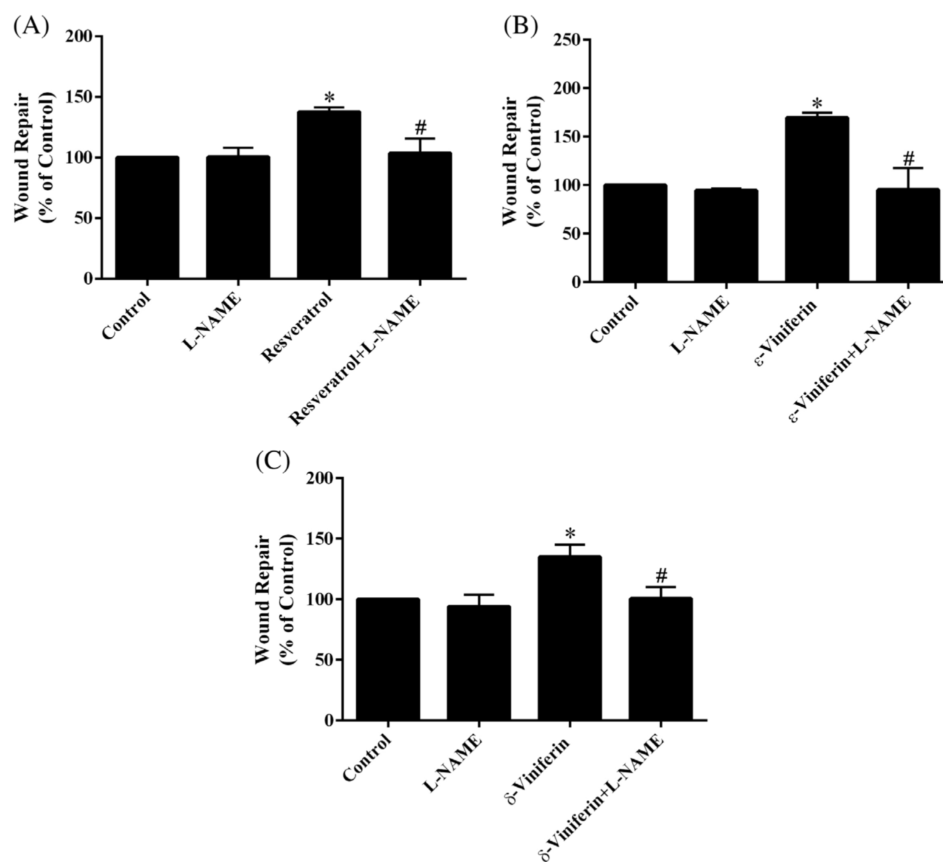

FIGURE 4 Suppressive effect of eNOS inhibitor L-NAME on wound repair induced by resveratrol, ε-viniferin, or δ-viniferin in VECs. Serum-starved VECs were pretreated with 1 mM L-NAME for 1 hour, wounded with a 200-μL pipette tip, and then treated with, A, 10 μM resveratrol; B, 10 μM ε-viniferin; or C, 5 μM δ-viniferin for 24 hours. The data are presented as the mean ± SD of three independent experiments. *$P < .05$ compared to the control group. #$P < .05$ compared to the resveratrol-, ε-viniferin-, or δ-viniferin-treated groups

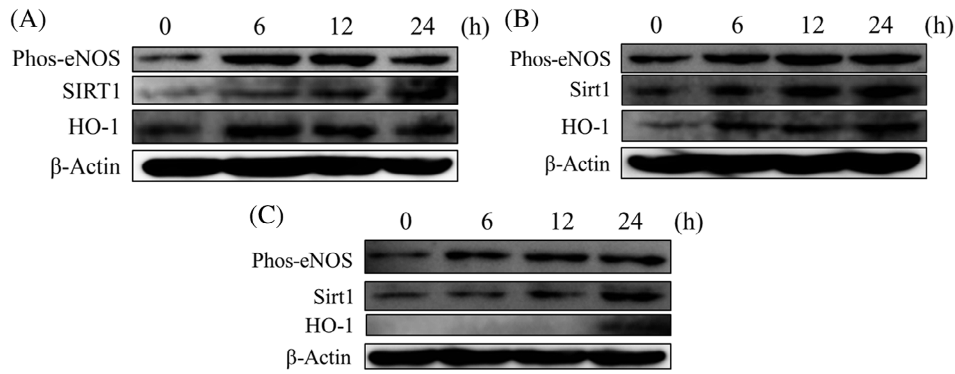

FIGURE 5 Effect of resveratrol, ε-viniferin, and δ-viniferin on phosphorylation of eNOS and expression of SIRT1 and HO-1 in VECs. Serum-starved VECs were treated with, A, 10 μM resveratrol; B, 10 μM ε-viniferin; or C, 5 μM δ-viniferin for 6, 12, and 24 hours, respectively. Thereafter, the cells were lysed and analyzed by western blotting using anti-phos-eNOS, anti-SIRT1, ant-HO-1, and β-Actin antibodies. The data are representative of three independent experiments

FIGURE 6 Suppressive effect of SIRT1 and HO-1 inhibitors EX527 and ZnPPIX, respectively, on resveratrol-, ε-viniferin-, and δ-viniferin-stimulated wound repair in VECs. Serum-starved VECs were pretreated with 10 μM EX527 for 1 hour, wounded with a 200-μL pipette tip, and then treated with, A, 10 μM resveratrol; B, 10 μM ε-viniferin; or C, 5 μM δ-viniferin. Similarly, serum-starved VECs were pretreated with 1 μM ZnPPIX for 1 hour, wounded with a 200-μL pipette tip, and then treated with; D 10 μM resveratrol; E, 10 μM ε-viniferin; or (F) 5 μM δ-viniferin. The data are presented as the mean ± SD of three independent experiments. *$P < .05$ compared to the control group. #$P < .05$ compared to the resveratrol-, ε-viniferin-, or δ-viniferin-treated groups

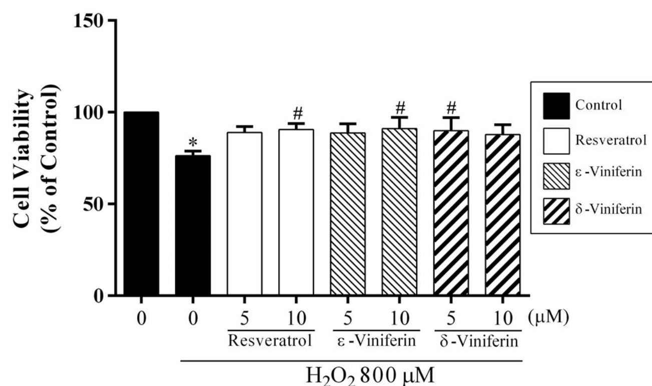

FIGURE 7 Suppressive effect of resveratrol, ε-viniferin, and δ-viniferin on oxidative stress-induced reduction of cell viability in VECs. Serum-starved VECs were incubated with 5 or 10 μM resveratrol, ε-viniferin, or δ-viniferin for 24 hours, washed with PBS, and then incubated with 800 μM H_2O_2 for 24 hours. Cell viability was assayed with a trypan blue dye exclusion test as described in Section 2. The data are presented as the mean ± SD of three independent experiments. **P* < .05 compared to the control group. #*P* < .05 compared to resveratrol-, ε-viniferin-, or δ-viniferin-treated groups

NOS inhibitor (Figure 4 and S3). The inhibition of NO production nearly completely blocked the wound repair induced by each compound, which supports our notion that these three compounds enhance wound repair primarily by producing NO, thereby preventing arteriosclerosis.

3.3 | Resveratrol, ε-viniferin, and δ-viniferin induce eNOS phosphorylation and expression of SIRT1 and HO-1 in VECs

In VECs, NOS activity is regulated by eNOS, which is activated by phosphorylation site Ser 1177 and inhibited by Thr 495,[24, 29, 30] we investigated eNOS Ser 1777 in this study. As shown in Figure 5, each compound phosphorylated eNOS. Furthermore, the expression of SIRT1 and HO-1 was also increased by these compounds. It is known that SIRT1 and HO-1 are involved in promoting NO production and wound repair. Taken together, these results suggest that the three compounds increase NO production via the phosphorylation of eNOS and that SIRT1 and HO-1 may contribute to this NO production.

3.4 | SIRT1 and HO-1 inhibitors EX527 and ZnPPIX, respectively, suppress resveratrol-, ε-viniferin-, and δ-viniferin-stimulated wound repair in VECs

Because SIRT1 and HO-1 are known regulators of NO generation and wound repair, we investigated the effect of the SIRT1 inhibitor EX527 and the HO-1 inhibitor ZnPPIX on wound repair promoted by the three compounds. Each inhibitor nearly completely blocked the wound repair induced by these compounds (Figure 6, Figure S4, and Figure S5). These data indicate that both SIRT1 and HO-1 are critically implicated in wound repair stimulated by resveratrol, δ-viniferin, and ε-viniferin, respectively, in VECs.

3.5 | Resveratrol, ε-viniferin, and δ-viniferin protect VECs from oxidative stress

Antioxidants play an important role in protecting cardiovascular function; therefore, we examined whether the three compounds protect VECs from oxidative stress. VECs were incubated with each compound for 24 hours, respectively, and washed with PBS to remove the added compounds. Thereafter, H_2O_2 was added to the cells. As shown in Figure 7, H_2O_2 treatment without the compounds for 24 hours reduced the cell viability. However, pretreatment of the cells with 10 μM resveratrol, 10 μM ε-viniferin, and 5 μM δ-viniferin, respectively, significantly reversed the decreased cell viability. These results strongly demonstrate that the three compounds make VECs resistant to oxidative stress.

3.6 | Resveratrol, ε-viniferin, and δ-viniferin raise catalase expression in VECs

Catalase contributes to cellular defense against H_2O_2-induced oxidative stress. We investigated the effects of 5 μM δ-viniferin and 10 μM ε-viniferin and resveratrol on catalase expression in VECs. Figure 8 shows that catalase protein level was significantly increased by all three compounds in a time-dependent manner. It should be noted that δ-viniferin-dependent induction response of catalase expression was a little slower compared to that of the other two compounds. This data is in agreement with the data on the HO-1 expression in Figure 5.

4 | DISCUSSION

We investigated the effect of resveratrol and its dimers, ε-viniferin and δ-viniferin, on NO production and wound repair in VECs. All three compounds increased wound repair of VECs by producing NO and increasing the expression of SIRT1 and HO-1. Moreover, the three compounds raised the expression of catalase, leading to the protection of cell viability from oxidative stress.

Dysfunction of ECs is the major cause of atherosclerosis. NO production induced cell proliferation and promoted wound repair, furthermore protect of ECs functions and prevent atherosclerosis.[8] NO production dependently by eNOS activity, it regulated by two important phosphorylation multiple sites, activation site Ser1177 and the inhibitory site Thr495.[30] Zhao et al[19] demonstrated that high glucose generated ROS, leading to the reduced cell viability, and δ-viniferin efficiently suppressed this reduced cell viability in HUVECs. However, Zhao et al[19] did not show the wound repair of the cells by δ-viniferin. The present study indicated that δ-viniferin showed wound repair as

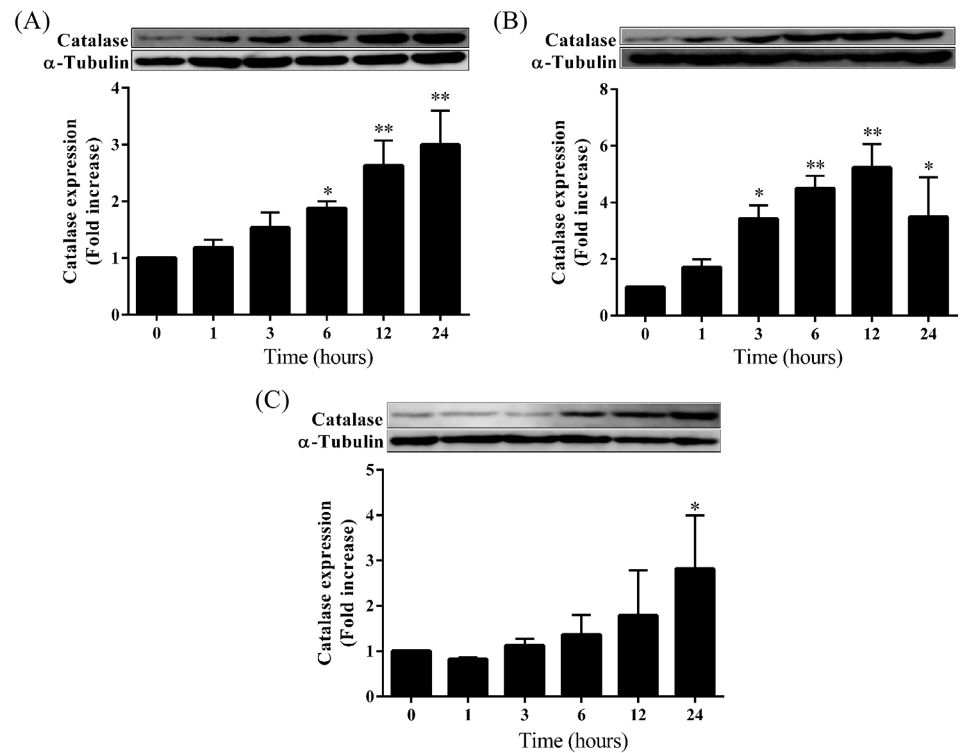

FIGURE 8 Stimulatory effect of resveratrol, ε-viniferin, and δ-viniferin on catalase expression in VECs. VECs were incubated with, A, 10 μM resveratrol; B, 10 μM ε-viniferin; and C, 5 μM δ-viniferin for 1, 3, 6, 12, or 24 hours, respectively, lysed, and then subjected to western-blot analyses using anti-catalase antibody. Catalase expression was normalized to the amount of α-tubulin. The data are presented as the mean ± S.D. of three independent experiments. *$P < .05$ and **$P < .01$ compared to the control group

well as suppression of oxidative stress-induced cell death. Moreover, not only δ-viniferin, but also resveratrol and ε-viniferin exhibited the same functions, suggesting that resveratrol and its dimers have the common beneficial effects on VECs.

We found that HO-1 and SIRT1 are critically involved in the wound repair induced by the three compounds. Mattagajasingh et al[31] demonstrated that SIRT1 deacetylated eNOS, stimulating eNOS activity and increasing NO in VECs. Actually, inhibition of SIRT1 in the endothelium of arteries inhibited endothelium-dependent vasodilation with decreased NO levels. Similarly, Araujo et al[32] described that HO-1 also plays an important role in the protection against atherosclerosis by acting as an antioxidant, antiinflammatory, antiapoptotic, antiproliferative, and immunomodulatory factor. They also indicated that HO-1 catalyzes heme degradation, leading to the generation of biliverdin, free iron, and carbon monoxide (CO), and CO induces the generation of NO in VECs. These reports suggest that both SIRT1 and HO-1 are important for the production of NO in VECs. In the report by Sodhi et al,[33] fructose-induced decrease in SIRT1 protein levels was reversed by the HO-1 inducer Co protoporphyrin (CoPP), which was suppressed by Sn-mesoporphyrin (SnMP), an inhibitor of HO-1 activity in hepatocytes. Fructose-dependent increases in triglycerides and FAS were suppressed by CoPP, which was reversed by SIRT1 siRNA in hepatocytes. Moreover, administration of CoPP to mice fed a high fructose diet increased SIRT1 expression and ameliorated lipid accumulation and fibrosis in liver along with decreasing vascular dysfunction. These data indicate that HO-1 activity is an upstream of SIRT1 in hepatocytes, hepatic tissue, and an artery. HO-1 may also be an upstream of SIRT1 in the presses of wound repair induced by resveratrol, ε-viniferin, and δ-viniferin in our study.

Three limitations of this study, first we did not test combination of these three active compounds and calculate the combination index. Whether the combination of these three compounds have synergetic effect on VECs and wound healing needs to be investigated in future. The second is whether the three compounds affect antioxidant enzyme SOD in VECs remains unclear. Although one study indicated red wine compound ε-viniferin induced expression of MnSOD, the effect three compounds on antioxidant enzyme SOD in VECs remain further investigated.[34] The third is although we find that three compounds affect the functions of VECs by a similar molecular mechanism with different potency and efficacy. The detailed difference among three compounds remains need to be further studied.

In conclusion, resveratrol and its dimers, ε-viniferin, and δ-viniferin, in red wine may be effective in preventing atherosclerosis by a similar molecular mechanism with different potency and efficacy.

CONFLICT OF INTEREST

All the authors declare no conflicts of interest.

REFERENCES

1. Cai H. Hydrogen peroxide regulation of endothelial function: Origins, mechanisms, and consequences. Cardiovasc Res. 2005;68(1):26–36.
2. Yang X, Li Y, Li Y, Ren X, Zhang X, Hu D, et al. Oxidative stress-mediated atherosclerosis: Mechanisms and therapies. Front Physiol. 2017;8:600.
3. Waddington E, Puddey IB, Croft KD. Red wine polyphenolic compounds inhibit atherosclerosis in apolipoprotein E-deficient mice independently of effects on lipid peroxidation. Am J Clin Nutr. 2004;79 (1):54–61.
4. Ferrières J. The French paradox: Lessons for other countries. Heart (Br Cardiac Soc). 2004;90(1):107–111.

5. Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: Is the effect due to beer, wine, or spirits. BMJ. 1996;312(7033):731–736.
6. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature. 2006;444(7117):337–342.
7. Klinge CM, Wickramasinghe NS, Ivanova MM, Dougherty SM. Resveratrol stimulates nitric oxide production by increasing estrogen receptor alpha-Src-caveolin-1 interaction and phosphorylation in human umbilical vein endothelial cells. FASEB J. 2008;22(7): 2185–2197.
8. Zghonda N, Yoshida S, Ezaki S, Otake Y, Murakami C, Mliki A, et al. Epsilon-Viniferin is more effective than its monomer resveratrol in improving the functions of vascular endothelial cells and the heart. Biosci Biotechnol Biochem. 2012;76(5):954–960.
9. Li H, Xia N, Forstermann U. Cardiovascular effects and molecular targets of resveratrol. Nitric Oxide. 2012;26(2):102–110.
10. Privat C, Telo JP, Bernardes-Genisson V, Vieira A, Souchard JP, Nepveu F. Antioxidant properties of trans-epsilon-viniferin as compared to stilbene derivatives in aqueous and nonaqueous media. J Agric Food Chem. 2002;50(5):1213–1217.
11. Pezet R, Perret C, Jean-Denis JB, Tabacchi R, Gindro K, Viret O. Delta-viniferin, a resveratrol dehydrodimer: One of the major stilbenes synthesized by stressed grapevine leaves. J Agric Food Chem. 2003;51(18):5488–5492.
12. Lambert C, Richard T, Renouf E, Bisson J, Waffo-Teguo P, Bordenave L, et al. Comparative analyses of stilbenoids in canes of major *Vitis vinifera* L. cultivars. J Agric Food Chem. 2013;61(47): 11392–11399.
13. Vitrac X, Bornet A, Vanderlinde R, Valls J, Richard T, Delaunay JC, et al. Determination of stilbenes (delta-viniferin, trans-astringin, trans-piceid, cis- and trans-resveratrol, epsilon-viniferin) in Brazilian wines. J Agric Food Chem. 2005;53(14):5664–5669.
14. Wilkens A, Paulsen J, Wray V, Winterhalter P. Structures of two novel trimeric stilbenes obtained by horseradish peroxidase catalyzed biotransformation of trans-resveratrol and (–)-epsilon-viniferin. J Agric Food Chem. 2010;58(11):6754–6761.
15. Piver B, Berthou F, Dreano Y, Lucas D. Differential inhibition of human cytochrome P450 enzymes by epsilon-viniferin, the dimer of resveratrol: Comparison with resveratrol and polyphenols from alcoholized beverages. Life Sci. 2003;73(9):1199–1213.
16. Quiney C, Dauzonne D, Kern C, Fourneron JD, Izard JC, Mohammad RM, et al. Flavones and polyphenols inhibit the NO pathway during apoptosis of leukemia B-cells. Leuk Res. 2004;28(8): 851–861.
17. Oshima Y, Namao K, Kamijou A, Matsuoka S, Nakano M, Terao K, et al. Powerful hepatoprotective and hepatotoxic plant oligostilbenes, isolated from the oriental medicinal plant Vitis coignetiae (Vitaceae). Experientia. 1995;51(1):63–66.
18. Yanez M, Fraiz N, Cano E, Orallo F. (–)-trans-epsilon-viniferin, a polyphenol present in wines, is an inhibitor of noradrenaline and 5-hydroxytryptamine uptake and of monoamine oxidase activity. Eur J Pharmacol. 2006;542(1–3):54–60.
19. Zhao H, Ma T, Fan B, Yang L, Han C, Luo J, et al. Protective effect of trans-delta-viniferin against high glucose-induced oxidative stress in human umbilical vein endothelial cells through the SIRT1 pathway. Free Radic Res. 2016;50(1):68–83.
20. Morris BJ. Seven sirtuins for seven deadly diseases of aging. Free Radic Biol Med. 2013;56:133–171.
21. Winnik S, Auwerx J, Sinclair DA, Matter CM. Protective effects of sirtuins in cardiovascular diseases: From bench to bedside. Eur Heart J. 2015;36(48):3404–3412.
22. Zhang QJ, Wang Z, Chen HZ, Zhou S, Zheng W, Liu G, et al. Endothelium-specific overexpression of class III deacetylase SIRT1 decreases atherosclerosis in apolipoprotein E-deficient mice. Cardiovasc Res. 2008;80(2):191–199.
23. Stein S, Lohmann C, Schafer N, Hofmann J, Rohrer L, Besler C, et al. SIRT1 decreases Lox-1-mediated foam cell formation in atherogenesis. Eur Heart J. 2010;31(18):2301–2309.
24. Stein S, Schafer N, Breitenstein A, Besler C, Winnik S, Lohmann C, et al. SIRT1 reduces endothelial activation without affecting vascular function in ApoE$^{-/-}$ mice. Aging (Albany, NY). 2010;2(6):353–360.
25. Cheng Y, Takeuchi H, Sonobe Y, Jin S, Wang Y, Horiuchi H, et al. Sirtuin 1 attenuates oxidative stress via upregulation of superoxide dismutase 2 and catalase in astrocytes. J Neuroimmunol. 2014;269 (1–2):38–43.
26. Ota H, Eto M, Kano MR, Kahyo T, Setou M, Ogawa S, et al. Induction of endothelial nitric oxide synthase, SIRT1, and catalase by statins inhibits endothelial senescence through the Akt pathway. Arterioscler Thromb Vasc Biol. 2010;30(11):2205–2211.
27. Zghonda N, Yoshida S, Araki M, Kusunoki M, Mliki A, Ghorbel A, et al. Greater effectiveness of epsilon-viniferin in red wine than its monomer resveratrol for inhibiting vascular smooth muscle cell proliferation and migration. Biosci Biotechnol Biochem. 2011;75(7): 1259–1267.
28. Takaya Y, Terashima K, Ito J, He YH, Tateoka M, Yamaguchi N, et al. Biomimic transformation of resveratrol. Tetrahedron. 2005;61(43): 10285–10290.
29. Zrelli H, Wu C, Zghonda N, Shimizu H, Miyazaki H. Combined treatment of Hydroxytyrosol with carbon monoxide-releasing Molecule-2 prevents TNFα-induced vascular endothelial cell dysfunction through NO production with subsequent NFκB inactivation. Biomed Res Int. 2013;2013:912431.
30. Mount PF, Kemp BE, Power DA. Regulation of endothelial and myocardial NO synthesis by multi-site eNOS phosphorylation. J Mol Cell Cardiol. 2007;42(2):271–279.
31. Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, Jung SB, et al. SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci U S A. 2007;104(37):14855–14860.
32. Araujo JA, Zhang M, Yin F. Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. Front Pharmacol. 2012;3:119.
33. Sodhi K, Puri N, Favero G, Stevens S, Meadows C, Abraham NG, et al. Fructose mediated non-alcoholic fatty liver is attenuated by HO-1-SIRT1 module in murine hepatocytes and mice fed a high fructose diet. PLoS One. 2015;10(6):e0128648.
34. Fu J, Jin J, Cichewicz RH, Hageman SA, Ellis TK, Xiang L, et al. Trans-(–)-epsilon-Viniferin increases mitochondrial sirtuin 3 (SIRT3), activates AMP-activated protein kinase (AMPK), and protects cells in models of Huntington disease. J Biol Chem. 2012;287(29): 24460–24472.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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