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Effect of long-term piceatannol treatment on eNOS levels in cultured endothelial cells

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ABSTRACT

Piceatannol (3, 3′, 4, 5′-tetrahydroxy-*trans*-stilbene) is a naturally occurring phytochemical found in passion fruit (*Passiflora edulis*) seeds. Previously, we demonstrated that piceatannol has acute vasorelaxant effects in rat thoracic aorta. It was suggested that endothelial NO synthase (eNOS) might be involved in piceatannol-induced acute vasorelaxation. Here, we investigated the expression of eNOS in EA.hy926 human umbilical vein cells after long-term treatment with piceatannol, and compared this effect with that of resveratrol, an analog of piceatannol. Long-term treatment with piceatannol up-regulated eNOS mRNA expression and increased eNOS protein expression in a dose-dependent manner. Moreover, piceatannol increased the levels of phosphorylated eNOS. Treatment with resveratrol also increased eNOS expression, but to a lesser degree than piceatannol. These findings indicate that piceatannol may improve vascular function by up-regulating eNOS expression.

1. Introduction

Endothelial cells maintain vascular tone and structure by regulating the balance between vasorelaxation and vasoconstriction [1]. Some vascular risk factors, such as oxidative stress and inflammation, induce vascular endothelial dysfunction [2]. Intake of poorly balanced food is also a vascular risk factor; for example, overconsumption of high-fat foods can lead to severe blood vessel disease such as atherosclerosis and hypertension [3]. In contrast, intake of polyphenols derived from foods and beverages such as fruits, vegetables, red wine, cacao, and tea, can prevent blood vessel disease [4]. Polyphenols show various anti-oxidant properties, including radical-scavenging ability [5], xanthine oxidase- and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-inhibiting activity [5,6], as well as the ability to activate endogenous anti-oxidant enzymes [5] and protect blood vessels against oxidative stress.

Nitric oxide (NO), a major vasorelaxant factor, regulates blood flow by relaxing blood vessels [7]. NO is produced by the enzyme endothelial nitric oxide synthase (eNOS) *via* the conversion of L-arginine to L-citrulline [8]. It is reported that NO bioactivity is reduced by aging [9,10], endogenous eNOS inhibitors [11], and reactive oxygen species (ROS) [12]. Decreased eNOS activity and NO levels cause impaired endothelium-dependent vasorelaxation, and lead to endothelial dysfunction [13,14]. However, dietary polyphenols have the potential to improve vascular function by promoting eNOS activity and enhancing NO bioavailability [15–18].

Piceatannol (3, 3′, 4, 5′-tetrahydroxy-*trans*-stilbene) is a polyphenolic stilbene phytochemical. We previously reported that piceatannol is present in large amounts in passion fruit (*Passiflora edulis*) seeds [19]. Piceatannol is also found in grapes, wine, *Polygonum cuspidatum*, *Rheum rhaponticum*, and *Vitis amurensis* [20]. Piceatannol displays a wide spectrum of biological activities such as anti-oxidant activity [21], anti-mutagenic activity [22], anti-parasitic activity [23], anti-bacterial activity [24], anti-inflammatory activity [25], anti-tumor activity [26], melanogenesis-inhibiting, and promotion of collagen synthesis [19]. Piceatannol also induces endothelium-dependent acute vasorelaxation in rat thoracic aorta [27]. Resveratrol, an analog of piceatannol, also exerts acute vasorelaxant effects on thoracic aorta [28,29]. Furthermore, it has been demonstrated that long-term treatment with resveratrol up-regulates eNOS mRNA expression, as well as eNOS protein expression, in cultured endothelial cells [17,18].

In this study, we investigated the effect of long-term piceatannol treatment on eNOS levels in the human umbilical vein cell line EA.hy926. Moreover, we also compared piceatannol to resveratrol with regard to eNOS expression and phosphorylated eNOS (p-eNOS) content.

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2. Materials and methods

2.1. Materials

Piceatannol and resveratrol were obtained from Tokyo Chemical Industry, Co., Ltd. (Tokyo, Japan). All other reagents were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Cell culture and treatment

Human EA.hy926 endothelial cells (American Type Culture Collection, Rockville, MD, USA) were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in low-glucose Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (HyClone, Logan, UT, USA), 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mM glutamine. Cells were seeded and grown in 6-well plates, and confluent cells were further incubated for 2 to 48 h in cell culture media containing different concentrations of piceatannol or resveratrol. Cell culture media containing piceatannol or resveratrol were replaced with new media every 24 h. Control cells were incubated with the same medium containing solvent (dimethyl sulfoxide [DMSO]).

2.3. Determination of eNOS mRNA expression

Total RNA was extracted from cells with the QIAshredder and the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration of RNA was determined by spectrophotometry at 260 nm. RNA samples (2 μg) were reverse transcribed into complementary DNA using the High Capacity complementary DNA Reverse transcription Kit (Applied Biosystems, Forster City, CA, USA) according to the manufacturer's recommendations. Real-time PCR reactions were performed in triplicate on a Light Cycler 480 Real-time PCR system II (Roche Diagnostics, Mannheim, Germany) using the TaqMan Gene Expression Master Mix (Applied Biosystems) and Universal ProbeLibrary Probes (Roche Diagnostics). The assay was performed with probe No. 5 for eNOS and probe No. 60 for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), respectively. PCR primers used were forward (5′-gaccctcaccgctacaacat-3′) and reverse (5′-ccgggtatccaggtccat-3′) for eNOS and forward (5′-agccacatcgctcagacac-3′) and reverse (5′-gcccaatacgaccaaatcc-3′) for GAPDH, respectively. Amplification conditions were 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. eNOS mRNA expression was normalized to GAPDH mRNA expression levels, after which relative eNOS mRNA expression was determined in comparison with the levels in the control cells.

2.4. Western blotting analysis of eNOS and p-eNOS expression

EA.hy926 cells were lysed in 20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 10 mM sodium pyrophosphate, 20 mM NaF, 10 nM okadaic acid, 2 mM orthovanadate, 1% Triton X-100, and Complete Protease Inhibitor Cocktail (Roche, Indianapolis, IN, USA). The lysates were sonicated and centrifuged at 15000 rpm for 20 min at 4 °C. The total protein content of the supernatant was analyzed by DC Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). A total of 1.5 μg of protein was separated by 7.5% SDS–PAGE and transferred to PVDF membranes. The membranes were blocked using 3% BSA in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 1 h and incubated with mouse anti-eNOS (1:1000, BD Transduction Laboratories, San Diego, CA, USA), rabbit anti-p-eNOS (phospho-Ser^{1177}) (1:1000, Cell Signaling, Beverly, MA, USA), or mouse anti-β-tubulin (1:20000, Sigma, St. Louis, MO, USA) overnight at 4 °C. Membranes were washed with TBST and incubated with horseradish peroxidase (HRP)-conjugated anti-mouse IgG (1:10000 for eNOS detection and 1:20000 for β-tubulin detection, Cell Signaling) or anti-rabbit IgG (1:10000 for p-eNOS detection, Cell Signaling) for 1 h at room temperature. All antibodies were diluted with 3% BSA in TBST. Visualization of immunoreactive bands was performed using the ECL Plus western blotting detection system (GE Healthcare, Little Chalfont, UK). The intensity of each band was determined by ImageJ software (National Institutes of Health, Bethesda, MD, USA), and normalized to the intensity of the β-tubulin band.

2.5. Statistical analysis

Data are presented as mean and standard deviation (S.D.). Statistical analyses were performed with a Tukey's HSD for multiple comparisons using SPSS software, version 13.0J for windows (SPSS Inc.). A value of $p < 0.05$ was considered significant.

3. Results

3.1. Comparison of the effects of piceatannol and resveratrol on eNOS mRNA expression in endothelial cells

To evaluate the effect of piceatannol on eNOS mRNA expression, we performed real-time PCR. Treatment with 50 μM piceatannol for 6 or 9 h significantly up-regulated eNOS mRNA expression 1.9- and 2.4-fold, respectively ($p < 0.01$) (Fig. 1). On the other hand, resveratrol treatment tended to increase eNOS mRNA expression, but the effect was not statistically significant. In addition, treatment with piceatannol (50 μM for 6 or 9 h) significantly up-regulated eNOS mRNA levels compared to resveratrol treatment ($p < 0.05$).

3.2. Effect of piceatannol and resveratrol on eNOS protein expression in endothelial cells

To investigate the effects of piceatannol and resveratrol on eNOS regulation, we examined the expression of eNOS protein in EA.hy926 cells by using western blot analysis. After 24 h stimulation with piceatannol, eNOS protein expression increased in a dose-dependent manner (Fig. 2). Piceatannol-induced eNOS protein expression significantly increased by 1.5-fold at 50 μM ($p < 0.01$). Resveratrol increased eNOS protein expression, and 50 μM treatment significantly increased eNOS protein expression by 1.4-fold ($p < 0.05$).

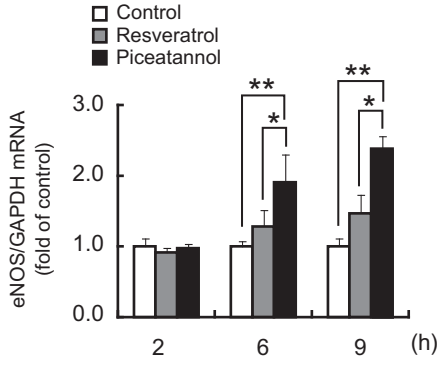

Fig. 1. Treatment with piceatannol induced eNOS mRNA expression in EA.hy926 cells.EA.hy926 cells were treated with 0.1% DMSO (control), 50 μM resveratrol, or 50 μM piceatannol for 2–9 h. Induction of eNOS mRNA expression was examined by quantitative PCR. The ratio of eNOS to GAPDH was determined and expressed as fold of control. Data are shown as the mean + S.D. ($n = 4$). $^{**}p < 0.01$, $^{*}p < 0.05$ (Tukey's HSD test).

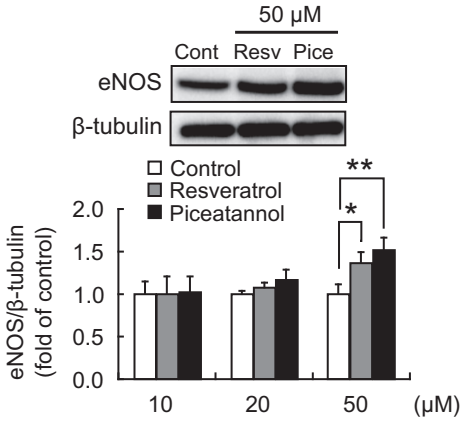

Fig. 2. Treatment with piceatannol dose-dependently increased eNOS protein expression. EA.hy926 cells were treated with 0.1% DMSO (control), 10–50 μM resveratrol, or 10–50 μM piceatannol for 24 h. Induction of eNOS protein was examined by western blot analysis. The ratio of the eNOS band to the β-tubulin band was determined and expressed as fold of control. Data are shown as mean + S.D. ($n = 4$). The upper panel depicts a representative blot, treated with 0.1% DMSO (control), 50 μM resveratrol, or 50 μM piceatannol for 24 h.$^{**}p < 0.01$,$^{*}p < 0.05$ (Tukey's HSD test). Cont: control; Resv: resveratrol; Pice: piceatannol.

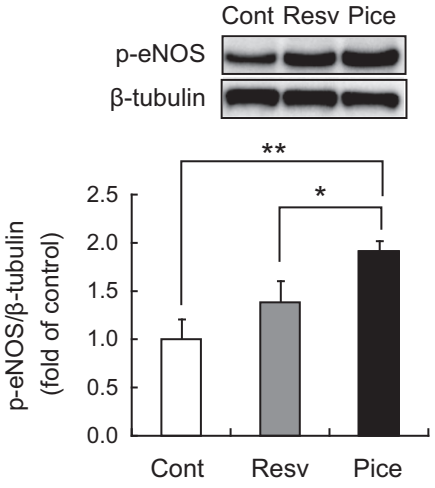

Fig. 4. Treatment with piceatannol increased p-eNOS protein.EA.hy926 cells were treated with 0.1% DMSO (control), 20 μM resveratrol, or 20 μM piceatannol for 48 h. Induction of p-eNOS protein expression was then examined by western blot analysis. The ratio of the p-eNOS band to the β-tubulin band was determined and expressed as fold of control. Data are shown as mean + S.D. ($n = 4$). The upper panel depicts a representative blot, treated with control, 20 μM resveratrol, or 20 μM piceatannol for 48 h. $^{**}p < 0.01$, $^{*}p < 0.05$ (Tukey's HSD test). Cont: control; Resv: resveratrol; Pice: piceatannol.

To investigate the effects of extending piceatannol and resveratrol treatment time on eNOS protein expression, the treatment time was extended from 24 h to 48 h and eNOS protein expression was measured. A preliminary experiment showed that cell viability was inhibited by stimulation with 50 μM resveratrol for 48 h (data not shown), so the cells were treated with 20 μM piceatannol or resveratrol. Treatment with 20 μM piceatannol for 48 h significantly enhanced eNOS protein expression by 1.3-fold ($p < 0.01$) (Fig. 3). Treatment with resveratrol for the extended period of time also appeared to enhance eNOS protein expression, but the increase was not statistically significant. Treatment of cells with 20 μM piceatannol for 48 h resulted in a significant increase in eNOS protein expression compared to that observed after treatment with 20 μM resveratrol ($p < 0.01$), suggesting that piceatannol is more effective than resveratrol in promoting eNOS protein expression.

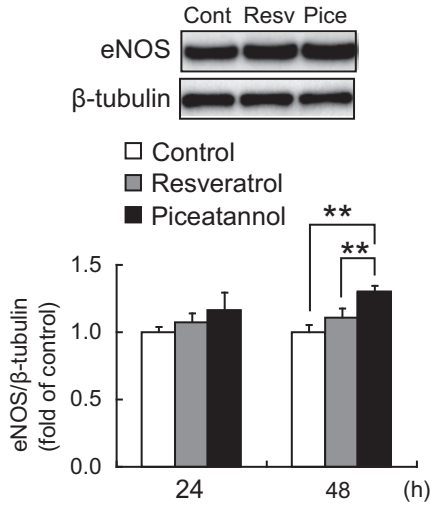

Fig. 3. Treatment with piceatannol increased eNOS protein expression as a function of time. EA.hy926 cells were treated with 0.1% DMSO (control), 20 μM resveratrol, or 20 μM piceatannol. After 24 or 48 h, eNOS protein induction was examined by western blot analysis. The ratio of the eNOS band to the β-tubulin band was determined and then expressed as fold of control. Data are shown as mean + S.D. ($n = 4$). The upper panel depicts a representative blot, treated with control, 20 μM resveratrol, or 20 μM piceatannol for 48 h. $^{**}p < 0.01$ (Tukey's HSD test). Cont: control; Resv: resveratrol; Pice: piceatannol.

3.3. Effect of piceatannol and resveratrol on p-eNOS content in endothelial cells

Phosphorylation of eNOS-Ser^{1177} is important for eNOS activation [30]. Therefore, the phosphorylation state of the eNOS protein was determined. After a 48 h stimulation with piceatannol, p-eNOS content significantly increased by 1.3-fold (Fig. 4). Stimulation with resveratrol also appeared to increase p-eNOS content, but a significant increase was observed only in cells treated with 20 μM piceatannol.

4. Discussion

In this study, we investigated the effect of piceatannol on eNOS expression in endothelial cells. We found that treatment with piceatannol increased eNOS mRNA expression, eNOS protein expression, as well as the levels of p-eNOS.

Previously, we demonstrated that piceatannol induces acute vasorelaxation in rat thoracic aorta [27]. This acute vasorelaxation was significantly inhibited by treatment with an NO synthase inhibitor, and the vasorelaxant effect was also not observed in endothelium-denuded aortas, suggesting that piceatannol induces vasorelaxation *via* endothelium-derived NO. In the present study, long-term treatment with piceatannol increased eNOS production in endothelial cells. These results suggest that piceatannol has multimodal vasorelaxant effects on endothelial cells, including both acute and long-term effects. It is thus predicted that both single and continuous consumption of piceatannol may have a vasorelaxant effect, and therefore piceatannol would provide protection against vascular dysfunction.

NO, an important factor for vasorelaxation, is produced by eNOS in endothelial cells and contributes to the control of blood flow [7]. Endothelial function declines with age, and a major reason for this age-related decline is a decrease in eNOS protein levels [31]. Oxidized LDL, a factor in the development of atheromatosis, is known

to diminish eNOS activity, and asymmetric dimethylarginine (ADMA), a natural inhibitor of eNOS, has also been reported to inhibit eNOS activity [13]. Conversely, many studies have reported that some polyphenols, especially resveratrol, increase eNOS activity in endothelial cells [15,17,18]. Long-term treatment with piceatannol increased eNOS levels in our study, suggesting that increased eNOS activity induced by piceatannol will increase NO bioactivity in endothelial cells, resulting in improvement of endothelial dysfunction.

Phosphorylation of eNOS has been associated with eNOS activation, and NO production increases when eNOS Ser^{1177} is phosphorylated [30]. eNOS Ser^{1177} is phosphorylated by stimulation with various factors, such as shearing stress [32], estrogen [33], vascular endothelial growth factor (VEGF) [32], insulin, and some polyphenols [34]. In our study, long-term stimulation with piceatannol increased p-eNOS levels, which will ultimately lead to increase NO production. The ratio of p-eNOS content to total eNOS content appeared to be increased by treatment with piceatannol (data not shown); however, additional studies are needed to further elucidate the effects of piceatannol on eNOS activation.

Treatment with 50 μM piceatannol for 24 h significantly increased eNOS protein levels. However, when the duration was extended to 48 h, even 20 μM piceatannol was sufficient to significantly increase eNOS protein levels. These results indicate that even low concentrations of piceatannol can increase eNOS expression when the duration of piceatannol exposure is extended. Although the pharmacokinetics of piceatannol is yet to be elucidated, the initial serum piceatannol level after intravenous administration at 10 mg/kg was reported to be 8.5 μg/mL (35 μM) [35]. Thus, we suspect that the concentration of the treatment used in this study is within a reasonable range.

Here, we showed that long-term treatment with piceatannol significantly increases eNOS mRNA and protein levels, and also observed that the effect of piceatannol on eNOS production is higher than that of resveratrol. Although the mechanism of polyphenol-induced eNOS up-regulation is not fully understood, it is thought that transcriptional regulators are associated with the increase in eNOS levels. SIRT1, a histone deacetylase, is thought to contribute to increased eNOS expression in endothelial cells. For example, overexpression of SIRT1 was shown to increase eNOS protein levels in mouse aorta [36]. In endothelial cells, up-regulation of eNOS mRNA by treatment with resveratrol is prevented by knockdown of SIRT1 [37]. Piceatannol and resveratrol activate the deacetylase activity of SIRT1, and the effect of piceatannol is reported to be higher than that of resveratrol [38]. Strong up-regulation of eNOS mRNA by piceatannol is considered to be partly due to the activation of SIRT1. It has also been reported that activation of estrogen receptors is associated with the up-regulation of eNOS expression by resveratrol. Both piceatannol and resveratrol act as estrogen receptor agonists [39,40], and estrogen receptor activation is associated with the up-regulation of eNOS protein levels [41]. Even in the present study, piceatannol activation of estrogen receptors may be associated with the up-regulation of eNOS expression.

Anti-oxidative activity is one factor underlying the effect of polyphenol-mediated protection against vascular dysfunction. Piceatannol has strong anti-oxidative activity, and the radical-scavenging activity of piceatannol is thought to be higher than that of resveratrol [20]. Superoxide immediately reacts with NO to generate peroxynitrite, which leads to eNOS uncoupling and enzyme dysfunction [4]. It has been reported that some polyphenols prevent superoxide from breaking down NO, and reduce atherosclerosis *via* inhibition of LDL oxidation [4]. In addition to the up-regulation of eNOS by piceatannol, the strong anti-oxidative activity of piceatannol is expected to contribute to the improvement of vascular function.

In summary, we demonstrated that long-term treatment with piceatannol increases eNOS and p-eNOS levels in endothelial cells. The data suggest that piceatannol is more effective than resveratrol in inducing eNOS mRNA expression, eNOS protein expression, and p-eNOS content. The increased eNOS protein and p-eNOS content induced by piceatannol will enhance NO bioactivity in endothelial cells, resulting in the improvement of vascular function.

References

[1] T. Hirase, K. Node, Endothelial dysfunction as a cellular mechanism for vascular failure, Am. J. Physiol. Heart Circ. Physiol. 302 (2012) H499–505.
[2] Y. Hirata, D. Nagata, E. Suzuki, H. Nishimatsu, J. Suzuki, R. Nagai, Diagnosis and treatment of endothelial dysfunction in cardiovascular disease, Int. Heart J. 51 (2010) 1–6.
[3] N.M. Kaplan, The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension, Arch. Intern. Med. 149 (1989) 1514–1520.
[4] J.C. Stoclet, T. Chataigneau, M. Ndiaye, M.H. Oak, J. El Bedoui, M. Chataigneau, V.B. Schini-Kerth, Vascular protection by dietary polyphenols, Eur. J. Pharmacol. 500 (2004) 299–313.
[5] R.J. Nijveldt, E. van Nood, D.E. van Hoorn, P.G. Boelens, K. van Norren, P.A. van Leeuwen, Flavonoids: a review of probable mechanisms of action and potential applications, Am. J. Clin. Nutr. 74 (2001) 418–425.
[6] F. Orallo, E. Alvarez, M. Camina, J.M. Leiro, E. Gomez, P. Fernandez, The possible implication of trans-Resveratrol in the cardioprotective effects of long-term moderate wine consumption, Mol. Pharmacol. 61 (2002) 294–302.
[7] N. Toda, H. Toda, Coronary hemodynamic regulation by nitric oxide in experimental animals: recent advances, Eur. J. Pharmacol. 667 (2011) 41–49.
[8] D.A. Geller, T.R. Billiar, Molecular biology of nitric oxide synthases, Cancer Metastasis Rev. 17 (1998) 7–23.
[9] J. Hoffmann, J. Haendeler, A. Aicher, L. Rossig, M. Vasa, A.M. Zeiher, S. Dimmeler, Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli: important role of nitric oxide, Circ. Res. 89 (2001) 709–715.
[10] H. Matsushita, E. Chang, A.J. Glassford, J.P. Cooke, C.P. Chiu, P.S. Tsao, ENOS activity is reduced in senescent human endothelial cells: Preservation by hTERT immortalization, Circ. Res. 89 (2001) 793–798.
[11] R.J. MacAllister, H. Parry, M. Kimoto, T. Ogawa, R.J. Russell, H. Hodson, G.S. Whitley, P. Vallance, Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase, Br. J. Pharmacol. 119 (1996) 1533–1540.
[12] U. Forstermann, T. Munzel, Endothelial nitric oxide synthase in vascular disease: from marvel to menace, Circulation 113 (2006) 1708–1714.
[13] R.O. Cannon 3rd, Role of nitric oxide in cardiovascular disease: focus on the endothelium, Clin. Chem. 44 (1998) 1809–1819.
[14] R.R. Giraldez, A. Panda, Y. Xia, S.P. Sanders, J.L. Zweier, Decreased nitric-oxide synthase activity causes impaired endothelium-dependent relaxation in the postischemic heart, J. Biol. Chem. 272 (1997) 21420–21426.
[15] S.K. Nicholson, G.A. Tucker, J.M. Brameld, Physiological concentrations of dietary polyphenols regulate vascular endothelial cell expression of genes important in cardiovascular health, Br. J. Nutr. 103 (2010) 1398–1403.
[16] Y. Steffen, T. Schewe, H. Sies, (−)-Epicatechin elevates nitric oxide in endothelial cells via inhibition of NADPH oxidase, Biochem. Biophys. Res. Commun. 359 (2007) 828–833.
[17] T. Wallerath, G. Deckert, T. Ternes, H. Anderson, H. Li, K. Witte, U. Forstermann, Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase, Circulation 106 (2002) 1652–1658.
[18] T. Wallerath, H. Li, U. Godtel-Ambrust, P.M. Schwarz, U. Forstermann, A blend of polyphenolic compounds explains the stimulatory effect of red wine on human endothelial NO synthase, Nitric Oxide 12 (2005) 97–104.
[19] Y. Matsui, K. Sugiyama, M. Kamei, T. Takahashi, T. Suzuki, Y. Katagata, T. Ito, Extract of passion fruit (*Passiflora edulis*) seed containing high amounts of piceatannol inhibits melanogenesis and promotes collagen synthesis, J. Agric. Food Chem. 58 (2010) 11112–11118.
[20] H. Piotrowska, M. Kucinska, M. Murias, Biological activity of piceatannol: leaving the shadow of resveratrol, Mutat. Res. 750 (2012) 60–82.
[21] S.K. Lee, Z.H. Mbwambo, H. Chung, L. Luyengi, E.J. Gamez, R.G. Mehta, A.D. Kinghorn, J.M. Pezzuto, Evaluation of the antioxidant potential of natural products, Comb. Chem. High Throughput Screen 1 (1998) 35–46.
[22] M. Larrosa, F.A. Tomas-Barberan, J.C. Espin, Grape polyphenol resveratrol and the related molecule 4-hydroxystilbene induce growth inhibition, apoptosis, S-phase arrest, and upregulation of cyclins A, E, and B1 in human SK-Mel-28 melanoma cells, J. Agric. Food Chem. 51 (2003) 4576–4584.
[23] L. Kedzierski, J.M. Curtis, M. Kaminska, J. Jodynis-Liebert, M. Murias, In vitro antileishmanial activity of resveratrol and its hydroxylated analogues against leishmania major promastigotes and amastigotes, Parasitol. Res. 102 (2007) 91–97.
[24] J.J. Docherty, H.A. McEwen, T.J. Sweet, E. Bailey, T.D. Booth, Resveratrol inhibition of Propionibacterium acnes, J. Antimicrob. Chemother. 59 (2007) 1182–1184.
[25] C. Gerhauser, K. Klimo, E. Heiss, I. Neumann, A. Gamal-Eldeen, J. Knauft, G.Y. Liu, S. Sitthimonchai, N. Frank, Mechanism-based in vitro screening of

potential cancer chemopreventive agents, Mutat. Res. 523–524 (2003) 163–172.

[26] R.M. Niles, C.P. Cook, G.G. Meadows, Y.M. Fu, J.L. McLaughlin, G.O. Rankin, Resveratrol is rapidly metabolized in athymic (nu/nu) mice and does not inhibit human melanoma xenograft tumor growth, J. Nutr. 136 (2006) 2542–2546.

[27] S. Sano, K. Sugiyama, T. Ito, Y. Katano, A. Ishihata, Identification of the strong vasorelaxing substance scirpusin B, a dimer of piceatannol, from passion fruit (Passiflora edulis) seeds, J. Agric. Food Chem. 59 (2011) 6209–6213.

[28] H.F. Li, Z.F. Tian, X.Q. Qiu, J.X. Wu, P. Zhang, Z.J. Jia, A study of mechanisms involved in vasodilatation induced by resveratrol in isolated porcine coronary artery, Physiol. Res. 55 (2006) 365–372.

[29] O. Rakici, U. Kiziltepe, B. Coskun, S. Aslamaci, F. Akar, Effects of resveratrol on vascular tone and endothelial function of human saphenous vein and internal mammary artery, Int. J. Cardiol. 105 (2005) 209–215.

[30] D. Fulton, J.P. Gratton, T.J. McCabe, J. Fontana, Y. Fujio, K. Walsh, T.F. Franke, A. Papapetropoulos, W.C. Sessa, Regulation of endothelium-derived nitric oxide production by the protein kinase Akt, Nature 399 (1999) 597–601.

[31] C.R. Woodman, E.M. Price, M.H. Laughlin, Aging induces muscle-specific impairment of endothelium-dependent dilation in skeletal muscle feed arteries, J. Appl. Physiol. 93 (2002) 1685–1690.

[32] S. Dimmeler, I. Fleming, B. Fisslthaler, C. Hermann, R. Busse, A.M. Zeiher, Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation, Nature 399 (1999) 601–605.

[33] R.L. Lantin-Hermoso, C.R. Rosenfeld, I.S. Yuhanna, Z. German, Z. Chen, P.W. Shaul, Estrogen acutely stimulates nitric oxide synthase activity in fetal pulmonary artery endothelium, Am. J. Physiol. 273 (1997) L119–L126.

[34] F. Kim, B. Gallis, M.A. Corson, TNF-alpha inhibits flow and insulin signaling leading to NO production in aortic endothelial cells, Am. J. Physiol. Cell Physiol. 280 (2001) C1057–C1065.

[35] K. Roupe, X.W. Teng, X. Fu, G.G. Meadows, N.M. Davies, Determination of piceatannol in rat serum and liver microsomes: pharmacokinetics and phase I and II biotransformation, Biomed. Chromatogr. 18 (2004) 486–491.

[36] Q.J. Zhang, Z. Wang, H.Z. Chen, S. Zhou, W. Zheng, G. Liu, Y.S. Wei, H. Cai, D.P. Liu, C.C. Liang, Endothelium-specific overexpression of class III deacetylase SIRT1 decreases atherosclerosis in apolipoprotein E-deficient mice, Cardiovasc. Res. 80 (2008) 191–199.

[37] A. Csiszar, N. Labinskyy, J.T. Pinto, P. Ballabh, H. Zhang, G. Losonczy, K. Pearson, R. de Cabo, P. Pacher, C. Zhang, Z. Ungvari, Resveratrol induces mitochondrial biogenesis in endothelial cells, Am. J. Physiol. Heart Circ. Physiol. 297 (2009) H13–20.

[38] T. Kahyo, S. Ichikawa, T. Hatanaka, M.K. Yamada, M. Setou, A novel chalcone polyphenol inhibits the deacetylase activity of SIRT1 and cell growth in HEK293T cells, J. Pharmacol. Sci. 108 (2008) 364–371.

[39] M. Maggiolini, A.G. Recchia, D. Bonofiglio, S. Catalano, A. Vivacqua, A. Carpino, V. Rago, R. Rossi, S. Ando, The red wine phenolics piceatannol and myricetin act as agonists for estrogen receptor alpha in human breast cancer cells, J. Mol. Endocrinol. 35 (2005) 269–281.

[40] J. Wober, F. Moller, T. Richter, C. Unger, C. Weigt, A. Jandausch, O. Zierau, R. Rettenberger, M. Kaszkin-Bettag, G. Vollmer, Activation of estrogen receptor-beta by a special extract of Rheum rhaponticum (ERr 731), its aglycones and structurally related compounds, J. Steroid Biochem. Mol. Biol. 107 (2007) 191–201.

[41] B.D. Gehm, J.M. McAndrews, P.Y. Chien, J.L. Jameson, Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor, Proc. Natl. Acad. Sci. USA 94 (1997) 14138–14143.