Review

Recent Advances in the Study on Resveratrol

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Appropriate long-term drinking of red wine is associated with a reduced risk for lifestyle-related diseases such as cardiovascular disease and cancer, making resveratrol, a constituent of grapes and various other plants, an attractive compound to be studied. Historically, resveratrol has been identified as a phytoalexin, antioxidant, cyclooxygenase (COX) inhibitor, peroxisome proliferator-activated receptor (PPAR) activator, endothelial nitric oxide synthase (eNOS) inducer, silent mating type information regulation 2 homolog 1 (SIRT1) activator, and more. Despite scepticism concerning the biological availability of resveratrol, a growing body of in vivo evidence indicates that resveratrol has protective effects in several stress and disease models. Here, we provide a review of the studies on resveratrol, especially with respect to COX, PPAR, and eNOS activities, and discuss its potential for promoting human health.

Key words resveratrol; cyclooxygenase; peroxisome proliferator-activated receptor; endothelial nitric oxide synthase; silent mating type information regulation 2 homolog 1

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene, Fig. 1) was first isolated from the roots of the white hellebore (Veratrum grandiflorum O. Loes) and was named in 1940 by Dr. Michio Takaoka, in his thesis.1) Dr. Takaoka was supervised by Dr. Riko Majima, a pioneer of Japanese organic chemistry for natural products, and had many disciples, including the famous protein chemist Dr. Shiro Akabori and the first Japanese female chemistry graduate, Dr. Chika Kuroda. Dr. Kuroda identified various natural chemicals such as shikonin from a medicinal herb (Lithospermum erythrorhizon)2) and carthamin from safflowers.3) The identification of these agents as well as the identification of resveratrol by Dr. Takaoka were the first steps toward the scientific efficacy of the Chinese “material medica,” a collection of traditional Asian medicines and therapeutic foods. Remarkably, the grape was described as “Good for muscle, bone, and longevity” in Shin-No-Hon-Zou-Kyo, the oldest book of materia medica, estimated to have been published in A.D. 22–250. In 1963, resveratrol was isolated from the roots of Polygonum cuspidatum, a plant used in the traditional medicine Ko-jo-kon and described as a “prescription for inflammation, carcinogenesis, and cardiovascular diseases” in Mei-I-Betsu-Roku, a book on materia medica published about 1500 years ago. Surprisingly, the actions described for grape and Ko-jo-kon are almost identical to those recently described for resveratrol.

Resveratrol was initially characterized as a phytoalexin,5) which is an antimicrobial substance synthesized by plants in response to infection. There were several pioneering reports on resveratrol, including a study of resveratrol as an inhibitor of arachidonate metabolism via interactions with 5-lipoxygenase and cyclooxygenase (COX) pathways in leukocytes.6) However, resveratrol attracted little interest until 1992, when it was postulated to explain some of the cardioprotective effects of red wine.7) Since that time, many studies have shown that resveratrol can prevent or slow the progression of a variety of conditions, including cancers, cardiovascular diseases, and ischemic injuries,8–12) as well as enhance stress resistance and extend lifespan. Attempts to demonstrate favorable effects in vitro have met with almost universal success and have led to the identification of multiple direct targets of resveratrol. This review discusses the effects of resveratrol on COX, peroxisome proliferator-activated receptor (PPAR), and endothelial nitric oxide synthase (eNOS). Additional reviews are needed to cover the entire literature on resveratrol with respect to COX and silent mating type information regulation 2 homolog 1 (SIRT1).13,14)

1. FROM COX SUPPRESSOR TO PPAR ACTIVATOR

COX, a key enzyme in prostaglandin (PG) synthesis, has two isoforms, COX-1 and -2. COX-1 is constitutively expressed in most cells, whereas COX-2 is induced by inflammatory stimuli such as endotoxin lipopolysaccharide (LPS), suggesting that COX-2 plays a critical role in inflammation.15,16) However, growing evidence indicates that COX-2 expression is regulated differently among cell types, and that COX-2 also plays key roles in tumorigenesis.17) Development,18–20) and circulatory homeostasis.21,22) Anti-inflammatory and cancer-preventative properties of resveratrol have been demonstrated in a rat model of carrageenan-induced paw edema and in a mouse skin cancer model using dimethyl benzanthracene (DMBA) and 12-O-tetradecanoyl phorbol 13-acetate (TPA), respectively. In both models, the effects of resveratrol were attributed to the inhibition of PG synthesis via the inhibition of COX-1.8) Szewczuk et al.23) showed that resveratrol could discriminate between COX-1 and COX-2, suggesting that resveratrol leads to the elimination of PG synthesis via COX-1. This is consistent with a report showing that COX-1 as well as COX-2 is involved in tumorigenesis.24) In contrast, our collaborators have indicated that 5 μM resveratrol suppressed PGE2 synthesis by inhibiting COX-2 activity; moreover, it inhibited COX-2 gene transcription without altering the amount

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of COX-1 in human breast carcinoma 184B5/HER cells.\textsuperscript{25} We found that 5μM resveratrol did not suppress COX-2 expression in human umbilical vein endothelial cells (HUVECs) or in bovine arterial endothelial cells (BAECs) (unpublished result), indicating that COX-2 expression is cell-type specific.

PPARs are members of a nuclear receptor family of ligand-dependent transcription factors.\textsuperscript{26} The PPAR subfamily comprises three isoforms, PPARα, β/δ, and γ, which play various roles in lipid and carbohydrate metabolism, cell proliferation and differentiation, and inflammation; they are considered molecular targets against lifestyle-related diseases.\textsuperscript{27,28} The PGD\textsubscript{2} metabolite 15-deoxy-Δ\textsubscript{12,14} PGJ\textsubscript{2} (15d-PGJ\textsubscript{2}) has been identified as a potent natural ligand of PPARγ.\textsuperscript{29,30} We reported that 15d-PGJ\textsubscript{2} suppressed the LPS-induced expression of COX-2 in macrophage-like U937 cells, but not in vascular endothelial cells, and that the expression of COX-2 was regulated by a negative feedback loop mediated through PPARγ, especially in macrophages.\textsuperscript{31} These findings indicate that PPARs participate in cell type-selective control of COX-2 expression, leading us to suspect that resveratrol may be an activator of PPARs. This concept was confirmed in vitro by cell-based reporter assays using HUVECs and BAECs, as 5μM resveratrol activated PPARα, β/δ, and γ in these cells.\textsuperscript{12,32} In a study using PPARα-knockout mice, resveratrol treatment (20mg/kg weight/day for 3 d) was shown to protect the brain against ischemic injury through a PPARα-dependent mechanism, indicating that resveratrol activates PPARα in vivo.\textsuperscript{29} Concerning tissue-selective expression of COX-2, we reported that physiological shear stress induced COX-2 expression for the production of prostacyclin\textsuperscript{33} as well as PGD\textsubscript{2}\textsuperscript{34} in vascular endothelial cells. Corticosteroids have been shown to induce COX-2 expression in cardiomyocytes,\textsuperscript{35,36} and the production of PGD\textsubscript{2} via COX-2 was involved in protection against ischemia/reperfusion injury in the heart.\textsuperscript{37} Clinical investigations have demonstrated an association between the risk for cardiovascular disease and COX-2 selective inhibitors.\textsuperscript{38,39} These results indicate that because resveratrol seems to act as a tissue-selective suppressor of COX-2, resveratrol may be a more effective inhibitor than COX-2 selective inhibitors (Fig. 2). We found that 5μM resveratrol also selectively activates PPARβ/δ,\textsuperscript{32} which directs the expression of the alternative phenotype in Kupffer cells and adipose tissue macrophages. These phenotypic changes act to dampen inflammation and maintain homeostasis, avoiding metabolic problems such as insulin resistance.\textsuperscript{40,41} Furthermore, prostacyclin analogues have been reported as ligands for PPARβ/δ.\textsuperscript{42}

Vaticanol C, a resveratrol tetramer, activates PPARα and PPARβ/δ \textit{in vitro} (5μM) and \textit{in vivo} (0.04% of diet for 8 weeks), but has no effect on SIRT1 activation.\textsuperscript{32} Polyphenolic compounds such as apigenin, chrysin,\textsuperscript{43,44} and humulon\textsuperscript{45} (Fig. 1) suppress COX-2 expression and also activate PPARα and/or γ.\textsuperscript{46,47} Similarly, essential oil components such as carvacrol and citral activate PPARs and suppress COX-2 expression.\textsuperscript{48—50} These findings suggest that various food-derived components, including resveratrol, have similar effects on PPARs and COX-2.

2. LINK TO eNOS

A growing body of evidence supports the theory that polyphenolic compounds have activities that maintain healthy cardiovasculature. Epidemiological studies have demonstrated that dietary polyphenol intake, especially red wine consumption, may improve endothelial function and reduce the risk...
for cardiovascular diseases.\textsuperscript{51—53} The inverse correlation between red wine consumption and incidence of coronary heart disease and atherosclerosis is now recognized as the French paradox.\textsuperscript{54,55} Resveratrol, a key compound implicated in the cardiovascular benefits associated with red wine consumption, exerts cardiovascular protection by diverse mechanisms in vivo.\textsuperscript{56—58} At pharmacological doses, resveratrol increases vascular nitric oxide (NO) levels and improves NO bioavailability in animal models.\textsuperscript{59—64} NO in the vasculature is constitutively synthesized by endothelial NO synthase (eNOS) and plays a crucial role in maintaining cardiovascular homeostasis.\textsuperscript{65,66} NO relaxes vascular smooth muscle cells, thereby upregulating blood flow, and it prevents thrombogenic and atherogenic processes by vasodilatory and anti-aggregatory effects. Accordingly, these effects of NO may contribute to the cardioprotective effect of resveratrol.

In vitro studies have been conducted to determine whether resveratrol acts directly on blood vessels or endothelial cells, facilitating NO production. Most reports focusing on short-term effects (within 1 h) have demonstrated that resveratrol induces increased NO bioavailability or production. Chen and Pace-Asciak reported that 30\textmu M resveratrol inhibited the contractile response to phenylephrine in isolated rat aorta.\textsuperscript{67} Similarly, resveratrol (70\textmu M) caused relaxation of isolated human saphenous vein and internal mammary artery rings,\textsuperscript{68} and polyphenols relaxed porcine arterial rings pre-contracted with KCl, with an IC\textsubscript{50} of 38.67\textmu M for resveratrol.\textsuperscript{69} In these studies, the inhibitory effect of resveratrol was reversed by removal of the endothelium or inhibition of NOS. These results suggest that NO can mediate the biological activities of resveratrol, although it is unclear whether NO production in endothelial cells is promoted by resveratrol. Orallo \textit{et al.} reported that resveratrol (1—30\textmu M) relaxed the contractile response of rat aortic rings to phenylephrine and KCl in a NO-dependent manner.\textsuperscript{70} However, resveratrol did not affect eNOS activity, but instead inhibited NADH/NADPH oxidase and the subsequent decrease in superoxide generation, leading to an improved NO bioavailability.

According to Wallerath \textit{et al.}, resveratrol rapidly increased NO production in cultured endothelial EA.hy926 cells, although comparatively high concentrations (at least 10\textmu M) of resveratrol were required for a significant increase in NO production.\textsuperscript{71} Wang \textit{et al.} reported that 100\textmu M resveratrol caused the phosphorylation of Akt, extracellular signal-regulated kinase (ERK)1/2, and eNOS within 15 min, resulting in increased NO production in bovine aortic endothelial cells.\textsuperscript{72} Klinge \textit{et al.} claimed that even at nanomolar concentrations, resveratrol served as a phytoestrogen in endothelial cells, increasing NO production through membrane estrogen receptors (ERs).\textsuperscript{73,74} Resveratrol induced the ER-mediated rapid activation of Src and mitogen-activated protein kinase ERK1/2, leading to eNOS activation in endothelial cells. However, in other studies, resveratrol-stimulated NO production was not prevented by pretreatment with an ER antagonist.\textsuperscript{69,71,75} Compared with estradiol, resveratrol binds to ER\textalpha{} with a much lower affinity, having an IC\textsubscript{50} of approximately 100\textmu M.\textsuperscript{76} It is not yet clear how ER mediates the effect of nanomolar resveratrol. In contrast, other researchers have not detected any resveratrol-induced vasodilation. Resveratrol, even at 100\textmu M, failed to relax isolated rat aortic rings in one study,\textsuperscript{77} and it had no effect on the contractile response or NO production in isolated porcine coronary artery in another study.\textsuperscript{78} We found that resveratrol had no effect at low concentrations (<20\textmu M), but high concentrations of resveratrol (>50\textmu M) increased NO production in endothelial F-2 cells.\textsuperscript{79} In fact, resveratrol at low concentrations inhibited the increase in NO production in response to vascular endothelial growth factor.

Overall, it remains unclear whether resveratrol rapidly increases NO production. A major complication in the studies using resveratrol at high concentrations is that cell viability and damage were not examined. It is well known that resveratrol has cytotoxic actions toward cancer cells, and resveratrol at high concentrations can reduce cell viability and induce disruption of the plasma membrane, resulting in Ca\textsuperscript{2+} influx in endothelial F-2 cells.\textsuperscript{75} Furthermore, an injurious effect of resveratrol was reported in HUVECs.\textsuperscript{79} These observations suggest that an increased cytosolic Ca\textsuperscript{2+} concentration subsequent to cell damage may result in Ca\textsuperscript{2+}-dependent eNOS activation in resveratrol-treated endothelial cells. In such a case, resveratrol at high concentrations may transiently increase NO production, but this effect would not be beneficial. Moreover, although high concentrations of resveratrol can have pharmacological effects, high concentrations are not produced by dietary intake; serum resveratrol concentrations are only 20—50\textmu M after oral administration of 25 mg of resveratrol in healthy volunteers.\textsuperscript{80,81}

In general, routine consumption of red wine is required for cardioprotection, suggesting that genes specifically expressed in the vasculature play important roles in the beneficial effects of resveratrol. Consequently, the long-term effects of resveratrol have been examined. Wallerath \textit{et al.} reported that the exposure of cultured endothelial cells to resveratrol for 24—72 h upregulated eNOS mRNA and protein expression levels, resulting in increased bioavailability of NO.\textsuperscript{79} However, their experiments were not conducted under dietary conditions, as the biological activity of resveratrol was observed between 10 and 100\textmu M. Räthel \textit{et al.}\textsuperscript{72} and Appledoorn \textit{et al.}\textsuperscript{83} confirmed that resveratrol at high concentrations significantly enhanced eNOS gene expression and enzyme activity, and NO production. In contrast, Nicholson \textit{et al.}\textsuperscript{84} reported that the exposure of HUVECs to nanomolar concentrations of resveratrol for 24 h increased the eNOS mRNA level, although neither eNOS protein nor NO production were determined. We also examined the long-term effect of nanomolar resveratrol on functional eNOS expression in HUVECs as a model of routine wine consumption.\textsuperscript{85} Resveratrol at 50\textmu M for 24 h did not alter eNOS protein levels or NO production, whereas daily treatment for 5 d significantly increased both eNOS protein and NO production without a loss of viability. Thus, eNOS induction may result from the cumulative effect of nanomolar concentrations of resveratrol. Our findings related to resveratrol account in part for the cardiovascular benefits of routine consumption of red wine. Further investigations are needed to resolve the effect of resveratrol on endothelial NO production.

3. LINK TO SIRT1 AND OTHER TARGETS

Resveratrol has been considered to be a caloric restriction (CR) mimetic in lower organisms, primarily on the basis of its activation of sirtuin proteins and its capacity to extend lifespan.\textsuperscript{86,87} In mammals, CR and resveratrol treatment afford
protein against a similar spectrum of diseases. However, there is controversy as to whether resveratrol is a direct activator of SIRT1, there are several other molecular targets of CR mimetics. Corton et al. reported that PPARα mediates some CR effects and proposed that a pharmacological approach toward mimicking many of the beneficial effects of CR may be possible. Tanno et al. reported that manganese superoxide dismutase (Mn-SOD) was induced by 100 μM resveratrol via nuclear translocation and activation of SIRT1 in mouse myoblast C2C12 cells, and that the oral administration of resveratrol (0.4% in diet for 35 weeks) to TO-2 hamsters increased cardiomyocyte Mn-SOD levels, suppressed fibrosis, preserved cardiac function, and significantly improved survival. These results are consistent with our finding that hepatic expression of SIRT1 and Mn-SOD genes was induced by 0.02% resveratrol in the diet for 4 weeks in wild-type mice, but not in PPARα knockout mice (manuscript in preparation). However, 50 μM resveratrol promoted fat mobilization in white adipocytes by repressing PPARγ in 3T3-L1 cells, which is inconsistent with our finding that 5 μM resveratrol activates PPARα, β/δ, and γ in BAECs.

Other candidate targets of resveratrol such as quinone reduce tase 2 (QR2), AMP-activated protein kinase (AMPK), and PPARγ coactivator (PGC-1α) have also been reported. Resveratrol inhibits purified QR2, with a dissociation constant of 35 nM; 10 μM resveratrol as well as apigenin stimulated AMPK in HepG2 hepatoma cells; and 50 μM resveratrol induced PGC-1α-responsive genes such as medium chain acyl-CoA dehydrogenase, cytochrome C, and estrogen receptor-related receptor α in C2C12 cells infected with an adenovirus expressing PGC-1α.

CONCLUSION

It is no exaggeration to say that the literature on resveratrol is contradictory and confusing. The wide range of concentrations and doses used to achieve the various effects reported for resveratrol in both in vitro cell culture and animal studies raises many questions about the concentrations achievable in vivo. Furthermore, resveratrol has a short half-life and is metabolized extensively in the body. Nevertheless, beneficial effects of resveratrol have been observed for several thousands of years in the form of “materia medica” such as grapes and Ko-jo-kon, establishing the concept of the long-term effects of low levels of pharmacologically active substances. In this regard, it will be important to elucidate the molecular mechanisms of the long-term effects of resveratrol. We assume that COX-2, PPAR, and eNOS are important clues to understanding the cellular basis for the long-term effects of resveratrol involving communication among several functionally different cells.

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REFERENCES

1) Takaoka M. Of the phenolic substances of white hellebore (Veratrum grandiflorum L. of the Family Liliaceae). J. Fac. Sci. Hokkaido Imperial University, 3, 1—16 (1940).
23) Szewczuk LM, Forti L, Stivala LA, Penning TM. Resveratrol is a
peroxidase-mediated inactivator of COX-1 but not COX-2: a mecha
nistic approach to the design of COX-1 selective agents. J. Biol.
24) Tiano HF, Loftin CD, Akunda J, Lee CA, Spalding J, Sessoms
A, Dunson DB, Rogan EG, Morham SG, Smart RC, Langenbach
R. Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters
epiperal differentiation and reduces mouse skin tumorigenesis.
25) Subbaramaiah K, Chung WJ, Michaluart P, Telang N, Tanabe T,
Inoue H, Jang M, Pezzuto JM, Dannenberg AJ. Resveratrol inhibits
cyclooxygenase-2 transcription and activity in phosphor ester-treated
human mammary epithelial cells. J. Biol. Chem., 273, 21875—21882
26) Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G,
Inoue H, Taba Y, Miwa Y, Yokota C, Miyagi M, Sasaguri T.
27) Bresalier RS, Sandler RS, Quan H, Bolognese JA, Ozenius B,
Horgan K, Lines C, Riddell R, Morton D, Lanas A, Konstant MA,
Baron JA; Adenomatous Polyp Prevention on Vioxx (APPROVe)
Trial Investigators. Cardiovascular events associated with rofecoxib
in a colorectal adenoma chemoprevention trial. N. Engl. J. Med.,
352, 1092—1102 (2005).
B, Lee CH. Adipocyte-derived Th2 cytokines and myeloid PPARδ
regulate macrophage polarization and insulin sensitivity. Cell
29) Hertz R, Berman I, Keppeler D, Bar-Tana J. Activation of gene tran-
scription by prostacyclin analogues is mediated by the peroxisome-
proliferator-activated receptor (PPAR). Eur. J. Biochem., 235,
30) Odegaard JI, Ricardo-Gonzalez RR, Red Eagle A, Vats D, Morel
CR, Goforth MH, Subramanian V, Mukundan L, Ferrante AW,
Chawla A. Alternative M2 activation of Kupffer cells by PPARδ
ameliorates obesity-induced insulin resistance. Cell Metab., 7,
31) Liang YC, Tsai SH, Lin-Shiau SY, Chen CF, Lin JK. Suppression of in-
ducible cyclooxygenase and inducible nitric oxide synthase by
apigenin and related flavonoids in mouse macrophages.
32) Hocking SW, Jeong YJ, Inoue H, Park JW, Kwon TK. Chrysin suppresses
lipopolysaccharide-induced cyclooxygenase-2 expression through the
inhibition of nuclear factor for IL-6 (NF-IL6) DNA-binding activity.
33) Yamamoto K, Wáng J, Yamamoto S, Tobe H. Suppression of cy-
clooxygenase-2 gene transcription by humulone of beer hop extract
studied with reference to glucocorticoid receptor. FEBS Lett.,
34) Liang YC, Tsai SH, Tsai DC, Lin-Shiau SY, Lin JK. Suppression of in-
ducible cyclooxygenase and nitric oxide synthase through activa-
tion of peroxisome proliferator-activated receptor-γ by flavonoids in
35) Yajima H, Ikeshima E, Shiraki M, Kanaya T, Fukushima D, Odai
H, Tsuyobo-Kasaoaka N, Ezaki O, Oikawa S, Kondo K. Isolumi-
honulules, bitter acids derived from hops, activate both per-
oxisome proliferator-activated receptor α and γ and reduce insulin
Carvacrol, a component of thyme oil, activates PPARα and γ and
37) Katsukawa M, Nakata R, Takizawa Y, Hori K, Takahashi S, Inoue
H. Citral, a component of lemongrass oil, activates PPARα and γ
and suppresses COX-2 expression. Biochim. Biophys. Acta, 1801,
1214—1220 (2010).
38) Yajima H, Ikeshima E, Shiraki M, Kanaya T, Fukushima D, Odai
H, Tsuyobo-Kasaoaka N, Ezaki O, Oikawa S, Kondo K. Isolumi-
honulules, bitter acids derived from hops, activate both per-
oxisome proliferator-activated receptor α and γ and reduce insulin
Carvacrol, a component of thyme oil, activates PPARα and γ and
40) Katsukawa M, Nakata R, Takizawa Y, Hori K, Takahashi S, Inoue
H. Citral, a component of lemongrass oil, activates PPARα and γ
and suppresses COX-2 expression. Biochim. Biophys. Acta, 1801,
1214—1220 (2010).
41) Katsukawa M, Nakata R, Koeji S, Hori K, Takahashi S, Inoue H.
Citral, a component of lemongrass oil, activates PPARα and γ
and suppresses COX-2 expression. Biochim. Biophys. Acta, 1801,
1214—1220 (2010).
61) Miate


