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Antioxidant, prooxidant and cytotoxic activity of hydroxylated resveratrol analogues: structure–activity relationship

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Abstract

Resveratrol (*trans*-3,4′,5-trihydroxystilbene), a naturally occurring hydroxystilbene, is considered an essential antioxidative constituent of red wine possessing chemopreventive properties. However, resveratrol and even more its metabolite piceatannol were reported to have also cytostatic activities. In order to find out whether this is related to antioxidative properties of those compounds, we synthesized five other polyhydroxylated resveratrol analogues and studied structure–activity relationships between pro-/antioxidant properties and cytotoxicity. Radical scavenging experiments with $O_2^{\bullet -}$ (5,5-dimethyl-1-pyrroline-*N*-oxide/electron spin resonance (DMPO/ESR)) and 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) (photometry) revealed that 3,3′,4′,5-tetrahydroxystilbene (IC_{50}: 2.69 μM; k_9: 443000 M^{-1} s^{-1}), 3,4,4′,5-tetrahydroxystilbene (IC_{50}: 41.5 μM; k_9: 882000 M^{-1} s^{-1}) and 3,3′,4,4′,5,5′-hexahydroxystilbene (IC_{50}: 5.02 μM), exerted a more than 6600-fold higher antiradical activity than resveratrol and its two other analogues. Furthermore, in HL-60 leukemic cells hydroxystilbenes with *ortho*-hydroxyl groups exhibited a more than three-fold higher cytostatic activity compared to hydroxystilbenes with other substitution patterns. Oxidation of *ortho*-hydroxystilbenes in a microsomal model system resulted in the existence of *ortho*-semiquinones, which were observed by ESR spectroscopy. Further experiments revealed that these intermediates undergo redox-cycling thereby consuming additional oxygen and forming cytotoxic oxygen radicals. In contrast to compounds with other substitution patterns hydroxystilbenes with one or two resorcinol groups (compounds **1** and **3**) did not show an additional oxygen consumption or semiquinone formation. These findings suggest that the increased cytotoxicity of *ortho*-hydroxystilbenes is related to the presence of *ortho*-semiquinones formed during metabolism or autoxidation.

Keywords: Resveratrol; Hydroxystilbenes; Antioxidants; Prooxidants; Cytotoxicity; HL-60 cells

1. Introduction

Cancer is one of the main reasons of death in both men and women, claiming over 6 million people each year worldwide. Chemoprevention in combination with anticancer treatment is therefore important to reduce morbidity and mortality [1].

One promising natural chemopreventive product is resveratrol (3,4′,5-trihydroxy-*trans*-stilbene), a phytoalexin found in grapes, which is present in concentrations of up to 10 μM in red wines and to a much lesser extent in white wines [2,3]. The anticancer activity of resveratrol was first revealed by its ability to reduce incidences of carcinogen-induced development of cancers in experimental animals [4,5]. It has since been demonstrated that it possesses chemopreventive and cytostatic properties via the inhibition of tumor initiation, promotion and progression [4]. It causes cell arrest in the S and G2 phases of the

Abbreviations: COX, cyclooxygenase; DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; DPPH$^\bullet$, 2,2-diphenyl-1-picrylhydrazyl; DTPA, diethylenetriaminepentaacetic acid; ESR, electron spin resonance; LPO, lipid peroxidation; $O_2^{\bullet -}$, superoxide radical; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen species; HO–Stilb–OH, hydroxystilbene; HO–Stilb–O^-, deprotonated hydroxystilbene; [HO–Stilb–O–H–$O_2^{\bullet -}$]$^{\neq}$, adduct of hydroxystilbene with $O_2^{\bullet -}$; HO–Stilb–O$^\bullet$, phenoxyl radical; O=Stilb=O, two-electron oxidation product of hydroxystilbenes; $^-$O–Stilb–O$^\bullet$, semiquinone anion; SOD, superoxide dismutase

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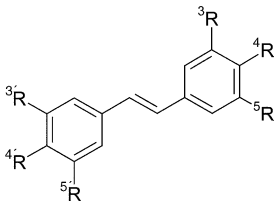
cell cycle [6] and is capable of inducing differentiation and apoptosis in a multitude of tumor cell lines, such as human leukemia, breast cancer and esophageal cells via CD95-dependent or independent mechanisms or through activation of caspase 3 or cleavage of PARP [7–9]. It has also been demonstrated that resveratrol inhibits the ribonucleotide reductase catalyzing the rate limiting step of de novo DNA synthesis [10]. While resveratrol exerts a non-selective cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibition [11], we could further show that hydroxylated resveratrol analogues are selective COX-2 inhibitors with a selectivity index (IC_{50} for COX-1/IC_{50} for COX-2) in part higher than celecoxib, a selective COX-2 inhibitor already established on the market [12]. These data are clinically important, as several lines of evidence suggest that selective COX-2 inhibitors may be beneficial both for cancer prevention and therapy.

Besides anticancer activities resveratrol also exhibits pronounced antioxidant properties by its ability to inhibit hydrogen peroxide- or lipid hydroperoxide-dependent lipid peroxidation of cellular membrane lipids [13,14]. Moreover, resveratrol reduces metal ion-dependent and independent oxidation of low-density lipoproteins [15,16], a process that is responsible for promoting atherogenesis. It also effectively protects isolated rat hearts from ischemia reperfusion injuries [17,18] reducing myocardial infarct size compared to control rat hearts [19]. Due to these experimental findings resveratrol in red wine was made responsible for the French paradox, the fact that the incidence of heart infarction in Southern France is 40% lower than in the rest of Europe despite the population's high-fat diet [3].

In contrast to the detailed knowledge of resveratrol activities in biological systems much less is known about the effects of higher hydroxylated stilbenes. Cai et al. compared the inhibiting activities of resveratrol and seven other hydroxylated *trans*-stilbenes with respect to an azo compound-induced peroxidation of linolic acid in vitro and to induce apoptosis in cultured HL-60 and Jurkat human leukemia cells [20]. They found that both antioxidant and apoptotic activities of the analogues containing 3,4-dihydroxyl groups namely 3,4-*trans*-dihydroxystilbene, 3,4,4′-*trans*-trishydroxystilbene and 3,4,5-*trans*-trihydroxystilbene were significantly higher than those of resveratrol and the other analogues. These data were supported by other investigators who also found free radical scavenging activity that was several times better, along with a higher growth-inhibitory activity of 3,3′,4′,5-tetrahydroxystilbene (piceatannol, astringinin) and 3,4,4′,5-tetrahydroxystilbene compared to resveratrol in tumor cells [21]. Resveratrol and its hydroxylated derivatives may be oxidized in an enzymatic or non-enzymatic manner via the one-electron pathway to a phenoxyl radical ($ArO^{\bullet}$) and subsequently yield quinone or quinone–methide type prooxidant or alkylating products. Several studies showed that the quinone products from oxidation of catecholic estrogen [22] and dopamine [23] are indeed responsible for the observed apoptotic effects of these drugs on cells.

Table 1
Structures of resveratrol (**1**) and its analogues **2–6**



Compound	^{3}R	^{4}R	^{5}R	$^{3'}R$	$^{4'}R$	$^{5'}R$
1	–OH	–H	–OH	–H	–OH	–H
2	–OH	–OH	–OH	–H	–OH	–H
3	–OH	–H	–OH	–OH	–H	–OH
4	–OH	–H	–OH	–OH	–OH	–H
5	–OH	–OH	–OH	–OH	–H	–OH
6	–OH	–OH	–OH	–OH	–OH	–OH

To clarify the possible link between antioxidant-derived radicals and cytotoxicity in cancer cells, we investigated radical production, prooxidation and radical scavenging activity of resveratrol and five synthesized hydroxylated analogues (Table 1) on the cytotoxicity of HL-60 human promyelocytic leukemia cells. Furthermore, the mechanism and structure–activity relationship of the double role as radical scavengers and prooxidants in biological systems will be discussed.

2. Materials and methods

2.1. Chemicals

5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) and DMSO were purchased from Sigma–Aldrich, Munich, Germany, potassium superoxide (KO_2) was obtained from Fluka, Switzerland and dibenzo-18-crown-6 (crown ether) was purchased from Merck, Germany. All other chemicals, obtained from commercial suppliers, were used as received and were of analytical grade purity.

2.2. Synthesis of hydroxylated resveratrol analogues

Hydroxylated resveratrol analogues **2–6** were synthesized using standard chemical methodologies with purity within ±0.4% of the theoretical values as previously described [12]. Resveratrol (**1**) was obtained from Sigma (Munich, Germany) with a purity of approximately 99%. Structures of synthesized compounds are presented in Table 1.

2.3. Competition of a spin trap compound and resveratrol derivatives for superoxide radicals

In order to assess the $O_2^{\bullet -}$ scavenging activity (a major aspect of the antioxidative activity) of resveratrol deriva-