Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications

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Abstract
Resveratrol (3,4′,5′-trihydroxystilbene) is a phytoalexin found in various plants, including grapes, berries and peanuts. It is also present in wines, especially red wines. During the last years, it has been the focus of numerous in vitro and in vivo studies investigating its biological attributes, which include mainly antioxidant and anti-inflammatory activities, anti-platelet aggregation effect, anti-atherogenic property, oestrogen-like growth-promoting effect, growth-inhibiting activity, immunomodulation and chemoprevention. In fact, recently, it has been demonstrated that the stilbene blocks the multistep process of carcinogenesis at various stages: tumour initiation, promotion and progression. More recent results provide interesting insights into the effect of this compound on the life span of yeasts and flies, implicating the potential of resveratrol as an anti-aging agent in treating age-related human diseases.

Introduction
Resveratrol (3,4′,5′-trihydroxystilbene) is a phytoalexin found in a wide variety of dietary sources including grapes, plums and peanuts. It is also present in wines, especially red wines and to a much lesser extent in white wines. Its stilbene structure is related to the synthetic oestrogen diethylstilbestrol. Resveratrol exists as cis- and trans-isomers. Trans-resveratrol is the preferred steric form and is relatively stable if it is protected from high pH and light. The synthesis of trans-resveratrol in the plants can be induced by microbial infections, UV radiation and exposure to ozone [1–3].

A primary impetus for research on resveratrol was initiated from the paradoxical observation that a low incidence of cardiovascular diseases may co-exist with a high-fat diet intake and moderate consumption of red wine [4,5], a phenomenon known as the French paradox [1]. The possible mechanisms by which resveratrol exerts its cardio- and vascular-protection involve inhibition of platelet aggregation, arterial vasodilation mediated by NO (nitric oxide) release, favourable changes in lipid metabolism such as LDL (low-density lipoprotein)-cholesterol oxidation, antioxidant effects, stimulation of angiogenesis [6], induction of cardioprotective protein expression, and insulin sensitization. Indeed, it reduces the synthesis of certain lipids and eicosanoids that tend to promote inflammation and atherosclerosis; likewise, it suppresses certain cardiac arrhythmias [7]. Some of these effects may be due in part to resveratrol being a phyto-oestrogen, i.e. a plant compound that has biologically similar properties to those of oestrogens [8].

More recent results provide interesting insights into the effect of this compound on the lifespan of yeasts and flies, implicating its potential as an anti-aging agent in treating age-related human diseases [9,10]. Additionally, some investigators have indicated a potential neuroprotective activity for resveratrol based on its beneficial effects in several brain damage models. Similarly, several studies, including ours, have identified resveratrol as a beneficial agent in the control of inflammatory disorders such as arthritis and inflammatory bowel disease [11,12]. Potential mechanisms implicated include: inhibition of synthesis and release of pro-inflammatory mediators, modification of eicosanoid synthesis, inhibition of activate immune cells and inflammatory enzymes such as iNOS [inducible NOS (nitric oxide synthase)] and COX-2 (cyclo-oxygenase-2) through its inhibitory effects on NF-κB (nuclear factor κB) or the AP-1 (activator protein-1) signalling pathways [9].

One of the most striking biological activities of resveratrol intensely investigated during the last years has been its cancer-chemopreventive or anticancer properties. These properties were first appreciated when Jang et al. [13] demonstrated that resveratrol possesses cancer-chemopreventive and cytostatic properties via the three major stages of carcinogenesis, i.e. initiation, promotion and progression [14]. Since then, there has been a flurry of papers reporting the implication of resveratrol in cancer chemoprevention through a wide range of actions that are poorly understood. It appears to help detoxify carcinogens, to reduce the synthesis of various cancer-related
Inflammation 1157

compounds and to interfere with cell survival programmes; for instance, resveratrol has been shown to promote apoptosis in cancer cells by blocking anti-apoptotic proteins expression or by inhibiting signal transduction through the PI3K (phosphoinositide 3-kinase), MAPK (mitogen-activated protein kinase) or NF-κB pathways [3,10,15].

Most of the scientific evidence for resveratrol’s benefits is based on in vitro studies in which the diastereomers trans- or cis-resveratrol have been tested. However, from animal studies and human trials, we know that the predominant isomer that is orally ingested with foods is trans-resveratrol glucoside (piceid), which is biotransformed and rapidly eliminated. In addition, these derivatives might be less biologically active due to their esterified hydroxy groups. However, the chemopreventive activity of orally administered trans-resveratrol has almost been demonstrated in cancer-induced animal models [16]. Nonetheless, future studies are needed to know the effective dose required to achieve the health benefits evidenced in experimental models.

**Resveratrol as free radical scavenger and antioxidant**

Over the last few years, a number of studies have provided evidence of an important role of ROS (reactive oxygen species) in mediating the development of oxidative stress. Excessive ROS accumulation may induce the oxidative modification of cellular macromolecules (lipid, proteins and nucleic acids) with deleterious potential. In fact, DNA damage by ROS has been implicated in mutagenesis, oncogenesis and aging. Oxidative lesions in DNA include base modifications, sugar damage, strand breaks and abasic sites [17]. Since gene transcription can be regulated by oxidants, antioxidants and other determinants of the intracellular redox state, ROS can also produce protein damage, inducing other types of mutations.

One of the biological activities that have been ascribed to resveratrol involves its antioxidant potential. Resveratrol is both a free radical scavenger and a potent antioxidant because of its ability to promote the activities of a variety of antioxidant enzymes (Figure 1). The ability of the polyphenolic compounds to act as antioxidants depends on the redox properties of their phenolic hydroxy groups and the potential for electron delocalization across the chemical structure [18].

The common recognition of resveratrol as a natural antioxidant was clarified by Zini et al. [19], who suggested three different antioxidant mechanisms: (i) competition with coenzyme Q and, to decrease the oxidative chain complex, the site of ROS generation, (ii) scavenging O$_2^\bullet$ radicals formed in the mitochondria and (iii) inhibition of LP (lipid peroxidation) induced by Fenton reaction products. In fact, numerous studies have demonstrated the ability of resveratrol to scavenge both O$_2^\bullet$ and *OH radicals [20–22]. By contrast, in a study by Orallo et al. [23], using the enzymatic hypoxanthine oxidase-XO (xanthine oxidase) system, resveratrol neither affected the XO activity nor scavenged O$_2^\bullet$ radicals in rat macrophage extracts.

In order to protect tissues against the deleterious effects of ROS, all cells possess numerous defence mechanisms that include enzymes such as SOD (superoxide dismutase), catalase, glutathione reductase and glutathione peroxidase. Resveratrol can maintain the concentration of intracellular antioxidants found in biological systems. For instance, in a study by Losa [21], stilbene appeared to maintain the glutathione content in peripheral blood mononuclear cells isolated ex vivo from a healthy human from oxidative damage caused by 2-deoxy-D-ribose. In a previous study, in human blood platelets, resveratrol markedly decreased oxidation of thiol groups of proteins in these cells [24]. Similarly, resveratrol induced an increase in glutathione levels in a concentration-dependent manner in human lymphocytes activated with H$_2$O$_2$. In another study, resveratrol increased the amounts of several antioxidant enzymes, including glutathione peroxidase, glutathione S-transferase and glutathione reductase [25].

**Effects of resveratrol on RNS (reactive nitrogen species) generation**

It is now widely accepted that a moderate concentration of NO appears to play cardio- and neuro-protective effects, and, along these lines, several reports have shown the role of resveratrol in the regulation of NO production from vascular endothelium in the ischaemic heart, brain or kidney [26,27].

However, abnormally high concentrations of NO and its derivatives RNS have been associated with tumour growth and vascular invasion. In a previous study [28], the effects of...
resveratrol and oxyresveratrol on nitrosative and oxidative stress derived from microglial cells was investigated. Phytoalexin considerably diminished NO production upon the inducible isomeric of NOS (iNOS expression), and it also induced an inhibitory effect on the iNOS enzyme activity.

Bacterial endotoxic LPS (lipopolysaccharide) is one of the most important stimuli for iNOS induction, resulting in NO production that has bactericidal effects. For example, in LPS-activated RAW 264.7 macrophages, pre-incubation of cells with resveratrol reduced inflammation by down-regulation of the iNOS and mRNA [29,30]. The results obtained demonstrate that resveratrol is a potent inhibitor of the antipathogen responses of rat macrophages and thus suggest that this agent may have applications in the treatment of diseases involving macrophage hyper-responsiveness [31,32].

**Antioxidant activity of resveratrol and carcinogenesis**

Resveratrol prevents the initial DNA damage by two different pathways: (i) acting as an antimutagen through the induction of Phase II enzymes, such as quinine reductase, capable of metabolically detoxifying carcinogens by inhibiting COX and cytochrome P450, and (ii) acting as an antioxidant through inhibition of DNA damage by ROS [33]. It has been proposed that ROS derived from LP may function as tumour initiators [20]. Leonard et al. [20] have shown that resveratrol exhibits a protective effect against LP in cell membranes and DNA damage caused by ROS.

The antipromotional properties of resveratrol can be partly attributed to its ability to enhance gap-junctional intercellular communications in cells exposed to tumour promoters such as PMA [34]. The tumour-promoting activity mediated by PMA has also been associated with oxidative stress by increased production of O$_2^•−$ and H$_2$O$_2$, reduction of SOD activity and interference with glutathione metabolism. In a model of PMA application to mouse skin, resveratrol induced the restoration of H$_2$O$_2$ and glutathione levels, and also myeloperoxidase, glutathione reductase and SOD activities [35].

The development of skin cancer is related to accumulative exposure to solar UVB as well as the nuclear transcription factor NF-κB, which plays a critical role in skin biology. NF-κB is involved in the inflammatory and carcinogenic signalling cascades, and resveratrol was able to block the damage caused by UVB exposure via its antioxidant properties blocking UVB-mediated NF-κB activation. Finally, resveratrol could inhibit tumour progression, partly by an inhibition of DNA polymerase and deoxyribonucleotide synthesis through its ability to scavenge the essential tyrosine radical of the ribonucleotide reductase and partly by inducing cell cycle arrest [18] (Figure 2).

**Effects of resveratrol on intracellular redox state**

Recent results have provided interesting insight into the effect of resveratrol on intracellular redox state. These results seem to support both anti- and pro-oxidant activities of this compound, depending on the concentration of resveratrol and the cell type, leading to oxidative breakage of cellular DNA. Lately, it has been proposed that such pro-oxidant action could be an important action mechanism of its anticancer and apoptotic inducing properties. Furthermore, it has been shown that there is an interesting correlation among the antioxidant and pro-oxidant activities and cytotoxicity of dietary polyphenols [36].

Every antioxidant is in fact a redox (reduction–oxidation) agent and thus might become a pro-oxidant to accelerate LP and/or induce DNA damage under special conditions. Studies have revealed pro-oxidant effects of antioxidant vitamins and several classes of plant-derived polyphenols such as flavonoids [37], tannins [38] and curcumin [39].

Ahmad et al. [40] observed that exposure of human leukaemia cells to low concentrations of resveratrol (4–8 μM) inhibited caspase activation and DNA fragmentation induced by incubation with H$_2$O$_2$. At these concentrations, resveratrol elicited pro-oxidant properties as evidenced by an increase in intracellular O$_2^•−$ concentration. Likewise, in rat hepatocytes exposed to ferrylmyoglobin-induced oxidative stress, physiological concentrations (100 pM–100 nM) of resveratrol exerted pro-oxidant activities [22]. It has also been shown that resveratrol has a pro-oxidative effect on DNA damage during interaction with ADP-Fe$^{3+}$ in the presence of H$_2$O$_2$ in tumour cell line cultures [34].

Similarly, the pro-oxidant effects of resveratrol were shown on rat liver microsomal systems. Resveratrol inhibited LP; however, resveratrol increased *OH generation, indicating that *OH played a minor role in LP [41]. In addition, it is well known that haem (iron-protoporphyrin IX) is a pro-oxidant and its rapid degradation by haem oxygenase is believed

![Figure 2](image-url)
to be neuroprotective. Using primary neuronal cultures, resveratrol was able to significantly induce haem oxygenase 1. This study indicated that the increase of haem oxygenase activity by resveratrol is a unique pathway by which this compound can exert its neuroprotective actions. Further corroborating the pro-oxidant activity of resveratrol, there are data that demonstrate its inefficiency in protecting proteins (BSA) from oxidative damage induced by metal-catalysed reaction or alkylperoxyl radicals.

Fukuhara and Miyata first reported the pro-oxidant activity of resveratrol in a plasmid-based DNA cleavage assay in the presence of transition metal ions such as copper. DNA degradation by resveratrol in the presence of copper (10–100 µM) or alone (200 µM) (in the absence of added copper) has also been shown in a cellular system of peripheral lymphocytes isolated from human blood.

Copper is one of the most redox-active metal ions present in the nucleus, serum and tissues. Approximately 20% of copper is located in the nucleus and is closely associated with DNA bases, in particular, guanine. Furthermore, it has been shown that the concentration of copper is greatly increased in various malignancies. Copper ions from chromatin can be mobilized by metal-chelating agents, giving rise to internucleosomal DNA fragmentation, a property that is the hallmark of cells undergoing apoptosis.

The cytotoxic mechanism of resveratrol probably involves mobilization of endogenous copper ions, possibly chromatin-bound copper. First, resveratrol undergoes oxidation in the presence of Cu(II). The oxidative product of resveratrol is a dimer, which possibly might be formed by dimerization of resveratrol phenoxyl radical as a result of the reductive activation of molecular oxygen. Indeed, this initial electron transfer generates the reduction of Cu(II) to Cu(I). Interestingly, DNA strand scission occurred at neutral pH, indicating that resveratrol can induce DNA cleavage without the oxygenation of the benzene nuclei to the catechol moiety. However, the structural feature of the copper–peroxide complex as the reactive species responsible for the DNA cleavage is still unknown. Secondly, the Cu(II)–peroxide complex is capable of binding DNA and forms a DNA–resveratrol–Cu(II) ternary complex. The high binding affinity of a 4-hydroxy group at the 4-position with both Cu(II) and DNA makes it possible and therefore cleaves DNA efficiently (Figure 3).

Clinical implications
The body of evidence presented here speaks volumes about the clinical potential of resveratrol as an antioxidant and pro-oxidant. Insufficient activation of apoptosis because of defects in apoptosis programmes or because of the dominance of survival signals may result in cancer cell resistance. Despite aggressive therapies, resistance of many tumours to current treatment protocols still constitutes a major problem in cancer therapy. Poly-mechanistic phytochemicals such as resveratrol may offer the advantage over targeted therapeutics and may open new perspectives in cancer therapy. By blocking survival and anti-apoptotic mechanisms or causing DNA degradation, as a consequence of its pro-oxidant action, resveratrol can sensitize cancer cells, which may result in synergistic anti-tumour activities when resveratrol is combined with conventional chemotherapeutic agents or cytotoxic compounds. However, further insights into the signalling network and interaction points modulated by resveratrol may provide the basis for novel discovery programmes to exploit resveratrol for the prevention and treatment of human diseases.

References

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