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Review

Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications

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Resveratrol is a phytoalexin polyphenolic compound found in various plants, including grapes, berries, and peanuts. Multiple lines of compelling evidence indicate its beneficial effects on neurological, hepatic, and cardiovascular systems. Also one of the most striking biological activities of resveratrol soundly investigated during the late years has been its cancer-chemopreventive potential. In fact, recently it has been demonstrated that this stilbene blocks the multistep process of carcinogenesis at various stages: tumor initiation, promotion, and progression. One of the possible mechanisms for its biological activities involves downregulation of the inflammatory response through inhibition of synthesis and release of pro-inflammatory mediators, modification of eicosanoid synthesis, inhibition of activated immune cells, or inhibiting such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) *via* its inhibitory effects on nuclear factor κB (NF-κB) or the activator protein-1 (AP-1). More recent data provide interesting insights into the effect of this compound on the lifespan of yeast and flies, implicating the potential of resveratrol as an anti-aging agent in treating age-related human diseases. It is worthy to note that the phenolic compound possesses a low bioavailability and rapid clearance from the plasma. As the positive effects of resveratrol on inflammatory response regulation may comprise relevant clinical implications, the purpose of this article is to review its strong anti-inflammatory activity and the plausible mechanisms of these effects. Also, this review is intended to provide the reader an up-date of the bioavailability and pharmacokinetics of resveratrol and its impact on lifespan.

Keywords: Anti-ageing / Anti-inflammatory / Resveratrol / Review

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Abbreviations: **AP-1**, activator protein-1; **cAMP**, cyclic adenosine monophosphate; **COPD**, chronic obstructive pulmonary disease; **COX**, cyclooxygenase; **ERK**, extracellular signal-regulated kinase; **fMLP**, formyl methionyl leucyl phenylalanine; **GM-CSF**, granulocyte macrophage-colony stimulating factor; **HMG**, half-mustard gas; **HX/XO**, hypoxanthine and xanthine oxidase; **I/R**, ischemia/reperfusion; **ICAM-1**, intracellular adhesion molecule-1; **IL**, interleukin; **iNOS**, inducible NO synthase; **JNK**, c-Jun NH_2-terminal kinase; **LPS**, lipopolysaccharide; **LT**, leukotriene; **MAPK**, mitogen-activated protein kinase; **NF-κB**, nuclear factor κ B; **NO**, nitric oxide; **PAF**, platelet-activating factor; **PG**, prostaglandin; **PKC**, protein kinase C; **PMA**, phorbol ester-induced activation; **PMN**, polymorphonuclear leukocytes; **PPAR**, peroxisome proliferator-activated receptor; **ROM**, reactive oxygen metabolites; **SIR**, sirtuin; **TNBS**, trinitrobenzenesulfonic acid; **TNF-α**, tumor necrosis factor α; **VCAM-1**, vascular cell adhesion molecule-l; **VEGF**, vascular endothelial growth factor.

1 Introduction

Resveratrol (*trans*-3,5,4′-trihydroxystilbene), is a nonflavonoid polyphenolic compound found in a large amount of

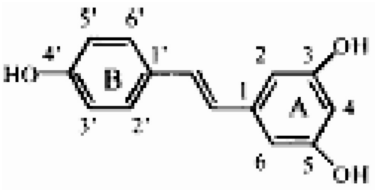

Figure 1. Chemical structure of resveratrol.

plant species (at least 72), a number of which are components of the human diet, including mulberries, peanuts, grapes, and red wines. Resveratrol exists as *cis*- and *trans*-isomeric forms, with *trans* to *cis* isomerization facilitated by UV exposure. Its stilbene structure is related to the synthetic estrogen diethylstilbestrol. Two phenol rings are linked by a styrene double bond to generate 3,4′,5-trihydroxystilbene (Fig. 1). Although the *trans*-isomer of resveratrol displays in the *in vitro*, *ex vivo*, and/or *in vivo* experiments a number of pharmacological effects, much less is known about the pharmacological activity of the *cis*-isomer, possibly as a result of the fact that *cis*-resveratrol, unlike the *trans*-isomer, is not commercially available [1].

The stilbene has been the focus of a number of studies investigating its beneficial effects on neurological, hepatic, and cardiovascular systems [2, 3]. A primary impetus for research on resveratrol has come from the paradoxical observation that a low incidence of cardiovascular diseases may coexist with intake of a high-fat diet and moderate consumption of 150 – 300 mL/day of red wines, a phenomenon known as the French paradox [4, 5]. This fact has resulted in the widespread use of resveratrol in dietary supplements with doses in the 10 – 20 mg range [6]. The possible mechanisms for the cardioprotective activity involve lipid metabolism and platelet function also the inflammatory response, including downregulation of pro-inflammatory mediators, alteration of eicosanoid synthesis, or inhibition of activated immune cells, primarily represented by neutrophils and monocytes/macrophages [7, 8]. Recently, Szewczuk and Penning [9] studied whether resveratrol was the sole agent responsible for the cardioprotective effects associated with red wine consumption. In this study, the authors showed that other red wine constituents, namely catechins and epicatechins, not only were as potent as resveratrol but were typically 15 times more concentrated than resveratrol in red wine [10, 11].

Besides endogenous defenses, the consumption of dietary antioxidants play an important role in protecting against pathological events of oxidative diseases, such as cardiovascular diseases, cancer, inflammation, and brain dysfunction [12]. Evidence has accumulated that resveratrol is both a free radical scavenger and a potent antioxidant [13] because of its ability to promote the activities of a variety of antioxidative enzymes. The ability of the polyphenolic compounds to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups and the potential for electron delocalization across the chemical structure [12].

One of the most striking biological activities of resveratrol soundly investigated during the lat years has been its cancer-chemopreventive potential. These properties were first appreciated when Jang *et al*. [14] demonstrated the inhibition of tumor formation induced by the aryl hydrocarbon 7,12-dimethylbenzanthracene after treatment with the phytoalexin. Since then, there has been a flurry of papers reporting the effects of resveratrol on critical events than regulate cellular proliferation and growth [3, 15]. In fact, recently Aziz *et al.* [16] have demonstrated that resveratrol possesses active cancer-chemopreventive activity against all the three major stages of carcinogenesis, *i. e.*, initiation, promotion, and progression. Essentially, the possible mechanisms for this observed cancer-chemopreventive activities include, for example: induction of apoptosis, inhibition of key steps in the signal transduction pathways, promotion of cellular differentiation, scavenging, or inhibition of oxygen-derived radicals and anti-inflammatory activity *via* downregulation of pro-inflammatory cytokines [3].

As the positive effects of resveratrol on inflammatory response regulation may comprise relevant clinical implications, the purpose of this article is to review its strong anti-inflammatory activity and the mechanisms of these effects. Demonstration of its mechanisms of action also implies the elucidation of the steps of its pharmacokinetics in cells and tissues. It is worthy to note that the phenolic compound possesses a low bioavailability and rapid clearance from the plasma [13]. On the other hand, it is well-established that reducing food intake (caloric restriction) extends lifespan in a wide range of species [17], from yeast to mammals [18]. Recent data provide interesting insight into the effect of resveratrol on the lifespan of yeast and flies, implicating its potential as an anti-aging agent in treating age-related human diseases. As a consequence of these above-mentioned evidences, there is a link in media reports to studies suggesting that longevity can be increased by a regular consumption of red wine [19, 20]. Accordingly, this review is intended to provide the reader an up-date of the bioavailability and pharmacokinetics of resveratrol and its impact on lifespan.

2 Synthesis, occurrence, and content of resveratrol in wine and plants

Resveratrol is a phytoalexin which synthesis in the plants can be induced by microbial infections, ultraviolet radiation (UV), and exposure to ozone [7]. Resveratrol is synthesized in the leaf epidermis and the skin (pericarp) of grape berries, but not in the flesh [21]. The phytoalexin is also

synthesized in lignified plant tissues, such as stalks and kernels of the berries [22]. In the grape species, this polyphenol reaches concentrations of 50 – 400 μg/g fresh weight in the leaves. Fresh grape skin contains about 50 – 100 μg of resveratrol per gram [23]. Consequently, the amount of this compound varies considerably in different types of grape juice and wine depending on the grape variety, environmental factors in the vineyard, juice extraction, and wine processing techniques. In grapes and wine, resveratrol occurs both as free resveratrol and piceid, the 3β-glucoside of resveratrol [24, 25]. In red wine, the concentration of resveratrol is in the range of 1.5 – 3 mg/L [25, 26]. Appreciable amounts of this polyphenol are also found in white and rosé wines: in a Spanish study, concentrations were 0.011 – 0.547 mg/L and 0.07 – 1.06 mg/L, respectively [25], while the content of resveratrol in Slovenian wines was up to 0.6 mg/L in white wine and 0.9 – 8.7 mg/L in red wine [24]. Other authors also reported higher values for red wines, up to 5 μg/mL, using high-performance liquid chromatography and highly sensitive fluorimetric detection [26 – 29]. The higher content of the stilbene in red wine is a consequence of the long contact time between the berry skin and must. Must for red wine is fermented with the berry skins for several days, whereas must for white wine is immediately separated from berry residues after mashing [30]. In grape juices, the content of resveratrol also highly differs with ranges varying from 3 to 15 μg/L [31] and 690 to 14 500 μg/L [32]. While the level of free resveratrol is rather low in grape juice, *cis*- and *trans*-piceid are the major resveratrol derivatives [32]. Recent studies show that total resveratrol and piceid levels in wines could vary from 0 to 25 000 μg/L [33]. The peanut appears the only other plant species used for human nutrition that contains resveratrol in significant amounts [34, 35].

Recently, Wang and colleagues [36] reported a liquid chromatography-mass spectrometry method (LC-MS) to analyze total resveratrol, including free resveratrol and resveratrol from piceid, in fruit products, and wine. Samples were extracted using methanol, enzymatically hydrolyzed, and analyzed using reversed-phase HPLC with positive ion atmospheric pressure chemical ionization (APCI) mass spectrometric detection. The results of this study showed that resveratrol was detected in grape, cranberry, and wine samples. Concentrations ranged from 1.56 to 1042 nmol/g in Concord grape products, and from 8.63 to 24.84 μmol/L in Italian red wine. The concentrations of resveratrol were similar in cranberry and grape juice at 1.07 and 1.56 nmol/g, respectively. Thus, the stilbene was detected in cranberry juice at a concentration similar to grape juice, which shows that cranberry may serve as an alternative dietary source for the antioxidant resveratrol. These authors also showed that grape and cranberry juices contained primarily *trans*-resveratrol, whereas processed juice products contained higher proportions of *cis*-resveratrol.

3 Pharmacokinetics and bioavailability of resveratrol

To date, the information relating to the pharmacokinetic steps and bioavailability of resveratrol is inconclusive. Studies in mice, rats, and dogs suggest consistently that resveratrol is satisfactorily absorbed and distributed in the blood stream which can be detected in significant concentrations in the blood and a number of organs [37]. It is rapidly metabolized by extensive first-pass glucuronidation and sulfated both in the liver and intestinal epithelial cells [37 – 42].

In a recent study, Meng and colleagues [43] found that more than 90% of total resveratrol, given as pure aglycone or as constituent of grape juice, circulating in the plasma of rats was in the conjugated form. Similarly, Marier *et al*. [44] analyzed the pharmacokinetics of resveratrol in its aglycone and glucuronide forms, following intravenous (15 mg/kg i.v.) and oral (50 mg/kg p.o.) administration in a solution of β-cyclodextrin to intact rats. Concentrations of aglycone and glucuronide in plasma and urine samples were determined using an electrospray ionization-liquid chromatography/tandem mass spectrometry method. After i.v. administration, plasma concentrations of the aglycone declined with a rapid elimination half-life ($T_{1/2}$, 0.13 h), followed by sudden increases in plasma concentrations 4 – 8 h after drug administration. The authors also showed that the aglycone was bioavailable at 38% when administered p.o. because of extensive first-pass glucuronidation, and that enterohepatic recirculation contributed to the overall systemic exposures of aglycone and glucuronide forms of resveratrol in rats. The fraction of aglycone and glucuronide excreted in urine appeared to be minimal compared with the biliary elimination pathways.

As mentioned above, glucuronidation clearly predominates the metabolism of resveratrol using *in vivo* studies in the rat [45, 46] or the perfused small intestine [38, 45], with a small contribution by sulfation. However, it is not clear which pathway(s) predominate in man. In man, using *in vitro* enzyme preparations [47 – 49], both glucuronidation and sulfation by liver microsomes may occur.

Kaldas and colleagues [6] studied whether resveratrol could be absorbed in man and enter the systemic circulation by measuring transport and metabolism of resveratrol (5 – 40 μM) by the human intestinal epithelial cell line Caco-2, cultured as monolayers on Transwells. The authors concluded that the transcellular transport of resveratrol by the human *in vitro* absorption model Caco-2 was high and independent of direction, suggesting facile intestinal absorption *in vivo* in man. However, limited linearity in the transport rate with time and extensive metabolism suggested that extensive presystemic metabolism of resveratrol may occur, leading

to low oral bioavailability. Metabolic studies demonstrated that sulfate conjugation was the major pathway for resveratrol in the Caco-2 cells and probably also in man *in vivo*. Highly polarized export of the sulfate conjugate from the Caco-2 cells occurred, which was concentration-dependent.

Recently, Meng *et al*. [43] have reported that, after oral administration of pure resveratrol to humans in a dose comparable to 2–3 glasses of wine (0.03 mg resveratrol/kg), resveratrol levels were readily detectable in biological fluids, such as plasma and urine, by HPLC coupled with electrochemical or mass spectrometric detections. The recovery of the phytoalexin in the circulating plasma suggested a rapid absorption of the stilbene in the gastrointestinal tract. At the given dose (0.03 mg/kg), more than half of the ingested resveratrol was recovered in the urine in 24 h, whereas at a higher dose of 1 mg/kg only a quarter of the administered dose could be recovered during the same period. In this experiment, with 200 and 400 mL grape juice, containing 0.16 mg of resveratrol/100 mL, mostly as the glycoside form, the level of resveratrol in the urine and plasma was below the level of detection, while when 600 and 1200 mL grape juice (containing 1 and 2 mg of total resveratrol, respectively) were administered, resveratrol was detectable in urine samples. However, the cumulative excretion of the polyphenol after drinking 1200 mL grape juice was only about 5% of the dose administered, being one-tenth that obtained with oral administration of pure resveratrol. This result suggests the lower bioavailability of resveratrol glycosides in grape juice in comparison to its pure aglycone.

With regard to how much resveratrol can be recovered from human organism after resveratrol consumption, assuming moderate wine ingestion (250 mL in a 70-kg person), the intake of resveratrol with wine in humans is ~18 μg/kg/day, being the resveratrol content of wine ~5 mg/L [50]. Soleas and colleagues [51] found that only 10–15% of resveratrol administered in white wine to humans was absorbed. Recently, Goldberg *et al.* [41] performed a study in healthy volunteers who received resveratrol at a dose of 360 μg/kg either dissolved in grape juice, vegetable juice, or white wine, *i.e.*, at a dose which was 20 times that associated with "normal" wine intake. These investigators found plasma peak levels of 20 nM authentic resveratrol and 2 μM total resveratrol (*i.e.*, genuine resveratrol plus resveratrol generated by hydrolysis of its conjugated) 30 min after ingestion, irrespective of dietary matrix, using a very sensitive gas chromatography-mass spectrometry method. Similarly, the latter plasma levels were confirmed by Walle *et al*. [52] who found peak plasma levels of resveratrol and metabolites about 2 μM and only trace amounts of unchanged resveratrol (<5 ng/mL) in plasma.

The concentrations of resveratrol shown to have biological activity *in vitro* range, approximately from 5–50 μM, although higher levels seem to be needed for some effects [53]. The percentage available for an *in vivo* effect is still not clear, as only limited data on the kinetics of resveratrol have been published. Results from preclinical studies in rats, using exclusively high-perfomance liquid chromatography methods, suggested consistent attainment of plasma peak levels 5–10 min post-oral administration of resveratrol and a rapid plasma elimination half-life of 12–15 min [50]. However, these studies differ as to the actual peak level values since doses of 2, 20, and 50 mg/kg resveratrol [44, 46, 54], each given p.o., generated peak values of 2, 1.2, and 6.6 μM, respectively. In the study performed by Marier and colleagues [44] the peak level of resveratrol glucuronide was as high as 105 μM, and, as aforementioned, the authors present convincing evidence for extensive enterohepatic circulation.

Little is known about the transport and the distribution of resveratrol through the body. Resveratrol must be bound to proteins and/or conjugated to remain at a high concentration in serum as a consequence of its low water solubility [55]. Furthermore, the efficiency of a therapeutic substance is related to its capacity (selectivity and affinity) to bind protein transporters [56]. In order to estimate the relationships between the amounts of resveratrol taken up by food or drink intake, and the several possible benefits illustrated from *in vitro/in vivo* experiments and from epidemiological studies, it is essential to demonstrate step by step the route of resveratrol from plasma to the cell-active site [57]. In plasma, resveratrol was shown to interact with lipoproteins [58–60]. *In vitro* assays showed that, on a protein basis, the concentrations of the stilbene added to plasma increased with the order of their lipid content, i.*e.*, high-density lipoprotein (HDL) < low-density lipoprotein (LDL) < very-low-density lipoprotein (VLDL), and that resveratrol is more associated with lipoproteins than with lipoprotein-free proteins [59]. Albumin appeared to be one of the plasmatic carriers transporting resveratrol in blood circulation in order to deliver the compound at the cell surface before cell membrane uptake and finally allowing its intracellular biological effect [57]. Although the intracellular proteic targets of resveratrol remain to be identified, Jannin *et al.* [57] proposed a schematic representation of the possible routes of resveratrol transport from plasma to intracellular targets. This scheme concerns only the transport of the unconjugated form of resveratrol. As concerns to resveratrol distribution, the phytoalexin is retrieved mainly in liver and kidney, but also in other tissues as colon, lung, heart, and brain [42, 61], although it is not clear whether the drug reaches the proposed sites of action *in vivo* after oral ingestion, especially in humans.

Although there is still poor information about the tissue-afinity of resveratrol, it is known that liver plays a key role in the bioavailability of dietary polyphenols. Bertelli *et al*.

[61] showed an accumulation of resveratrol in the liver of rats after oral administration. Vitrac and colleagues [42] confirmed this data studying the distribution of [^{14}C]*trans*-resveratrol in mouse tissues after oral administration and showed that the highest accumulation of radioactivity was found in liver. In a recent study [6], the authors showed the finding of an almost 40-fold accumulation of resveratrol in the Caco-2 cells in comparison with the incubation buffer, which is remarkable and emphasizes that the enterocyte could be a major biological target site for this dietary preventive compound. Recently, Lançon and colleagues [62] described that the uptake of resveratrol aglycone by hepatic cells involves two processes: a passive one and a carrier-mediated one. These authors took advantage of the fluorescent properties of resveratrol to observe its cell uptake and its cellular localization by fluorescence microscopy. Using radiolabelled resveratrol, they studied the time-, dose-, and temperature-dependencies of resveratrol influx to discriminate passive diffusion process from carrier-facilitated mechanism. Due to this last process, resveratrol, while tightly bound to blood proteins, could be largely delivered to body tissues.

As mentioned above, the efficacy of orally administrated resveratrol depends on its absorption, metabolism, and tissue distribution [40]. However, although several authors have investigated resveratrol pharmacokinetics [37, 38, 45, 63], none of these investigations have enabled a conclusion to be made about the metabolic profile of resveratrol. Indeed, recent studies have focused on resveratrol metabolism, but with contradictory results. Thus, according to a recent investigation performed by Yu and colleagues [40], no phase I metabolites of resveratrol resultant from oxidation, reduction, or hydrolysis were detected, whatever the tested systems (human liver microsomes, rat and human hepatocytes, *in vivo* studies). In contrast, in another study [64], two major metabolites of resveratrol (M1, M2) and one minor metabolite (M3) were found following *in vitro* incubations of the polyphenol with microsomal preparations of human cytochrome P450 1B1 (CYP1B1) from human lymphoblast or *Escherichia coli*-transfected cells. M2 was identified as piceatannol (3,5,3′,4′-tetrahydroxy-*trans*-stilbene) but M1 and M3 have not been conclusively identified due to the unavailability of authentic standards. M1 was suggested by these authors to be the 3,4,5,4′-tetrahydroxystilbene and M3 the 3,4,5,3′,4′-pentahydroxystilbene. More recently, Piver *et al*. [65] confirmed the formation of phase-I metabolites of resveratrol and the involvement of CYP1A2 in its metabolism. The characterization of these metabolites was performed by their chromatographic behaviors in HPLC, their UV and fluorescence spectra, or GC-MS analysis. One of them, metabolite M2, was identified as piceatannol while other one (M1) has not yet been conclusively identified due to the unavailability of an authentic standard. However, the proposed identity of M1 is the same as that proposed by Potter *et al*. [64] for the metabolite M1. These two metabolites were catalyzed by recombinant human CYP1A1, CYP1A2, and CYP1B1. Therefore, *trans*-resveratrol could play the role of a pro-drug, which would be converted into the active metabolite by CYP1A2 in the liver, CYP1A1 at the extra-hepatic level and CYP1B1 in tumors. Moreover, Wang and colleagues [66] described the characterization, chemical synthesis, and biological effects of the human metabolites of resveratrol. Using a combination of chromatographic, spectroscopic, and enzymatic analyses, the phase I clinical trial showed that resveratrol was rapidly converted into two metabolites after its oral administration in humans: resveratrol-3-*O*-glucuronide and resveratrol-4′-*O*-glucuronide [66].

Recently, Walle *et al*. [52] examined the absorption, bioavailability, and metabolism of [^{14}C]resveratrol after oral and i.v. doses in six human volunteers. Only trace amounts of unchanged resveratrol (<5 ng/mL) could be detected in plasma. Most of the oral dose was recovered in urine, and liquid chromatography/mass spectrometry analysis identified three metabolic pathways, *i.e.*, sulfate and glucuronic acid conjugation of the phenolic groups and, interestingly, hydrogenation of the aliphatic double bond, the latter likely produced by the intestinal microflora.

Therefore, the investigations have demonstrated the presence of resveratrol metabolites in the body. Thus, further studies are necessary to explore the biological effects of these metabolites, since if resveratrol metabolites were to possess efficacy, they could conceivably contribute to, or account for, the efficacy of resveratrol *in vivo* [50]. In that case, a lot of the extensive published data on the properties of resveratrol in cells *in vitro* would be rendered rather irrelevant with respect to explaining activity in animals and eventually in humans *in vivo*.

On the other hand, Zhou *et al*. [67] reported that some herbal/dietary constituents were shown to form reactive intermediates capable of irreversibly inhibiting various cytochromes (CYPs). The resultant metabolites lead to CYP inactivation by chemical modifications. Frequently, one of the underlying mechanisms of altered drug concentrations by concomitant herbal medicines is the induction or inhibition of hepatic and intestinal CYPs. According to recent findings, resveratrol inhibits some CYPs, namely aromatic hydrocarbon-induced CYP1A1 expression and activity [68, 69] as well as CYP1A2 and CYP1B1, which have been described as being crucial in tumor progression [70 – 73].

Resveratrol was also shown to be an irreversible (mechanism-based) inhibitor for CYP3A4 and a noncompetitive reversible inhibitor for CYP2E1 (which is involved in procarcinogen activation), in microsomes from rat liver, human liver, or cells containing cDNA-expressed CYPs [72 – 76]. CYP3A4 are expressed at high levels in the villus tip of

enterocytes, the primary site of absorption for orally administered drugs, and in the liver. Thus, the modulation of intestinal CYP3A represents an important mechanism for the enhanced or reduced bioavailability of coadministered drugs. In particular, the mechanism-based inhibition of CYP3A4 by herbal constituents may have important pharmacokinetic implications. In the case of resveratrol, aromatic hydroxylation and epoxidation of this compound mediated by CYP3A are possible because this stilbene is an electron-rich molecule with two aromatic benzene rings linked by an ethylene bridge. This results in a reactive *p*-benzoquinone methide metabolite that is capable of binding covalently to CYP3A4 and inactivating it [67]. Chan and Delucchi [75] showed that the incubation of resveratrol with Sf9 insect microsomes containing baculovirus-derived human CYP3A4 and NADPH-cytochrome P450 reductase showed that resveratrol inactivated CYP3A4 in a time- and NADPH-dependent manner. However, it is not one of the main red wine constituents that are responsible for CYP3A4 inactivation by red wine. Nevertheless, inactivation of CYP3A4 by resveratrol may cause clinically relevant drug interactions with CYP3A4 substrates. At this respect, grapefruit juice has been found to significantly increase oral bioavailability of most dihydropyridines, cyclosporine, midazolam, and verapamil, among other drugs, through the inhibition of CYP3A4 activity with no change of CYP3A4 mRNA and P-glycoprotein as the primary mechanism [77, 78]. However, the bioavailability of many other drugs like digoxin [79], diltiazem [80], and amlodipin in human volunteers [81], and indinavir in human immunodeficiency virus (HIV)-positive patients [67] were not altered by grapefruit juice, which indicates the difficulty in predicting herb-drug interactions. Nevertheless, more recent studies have shown that the mechanism of grapefruit juice interaction is more complex, because several moieties in the juice cause substrate-dependent competitive and/or mechanism-based inhibition of CYP3A4 and also inhibit various transporters, including P glycoprotein and organic anion transporting polypeptides [82, 83].

4 Effects of resveratrol on lifespan

It is well-established that reducing food intake (caloric restriction) extends lifespan in a wide range of species [17], from yeast to mammals [18]. In yeast, the sirtuin (SIR)2 gene mediates the life-extending effects of calorie restriction by nutrient withdrawal [84], although the overproduction of SIR2 enzyme can prolong the life of yeast grown under normal nutrient conditions [85]. The SIR2 enzyme belongs to a large family of evolutionarily conserved molecules termed SIRs, which is a family of nicotinamide adenine dinucleotide $(NAD)^+$-dependent protein deacetylases [86, 87]. These enzymes regulate a wide range of cellular activities, playing important roles in gene silencing, DNA repair, rDNA recombination, and ageing in model organisms [88–91], which affect lifespan in lower organisms, such as yeast and worms [92]. On the other hand, although how SIRs participate in mammalian ageing is not yet known, recent investigations indicated that in mammalian cells SIRs appear to act as regulators of programmed cell death and cell maturation (apoptosis and differentiation, respectively) [92].

In a recent report performed by Howitz and colleagues [93], using several small molecule libraries (which included analogues of ε-acetyl lysine, NAD^+, NAD^+ precursors, nucleotides, and purinergic ligands), these researchers found that resveratrol could extend yeast's lifespan, implicating the potential of resveratrol as an anti-aging agent in treating age-related human diseases through the stimulation of SIR2 activity. Interestingly, resveratrol seemed to be the most potent SIR2 activator of all the plant polyphenols tested by these investigators. The authors showed that, in yeast, the phytoalexin mimicked calorie restriction by stimulating SIR2, increasing DNA stability, and extending the lifespan by approximately 70%. As a consequence of those abovementioned evidences reporting an increased lifespan through the activation of SIRs after treatment of yeast with resveratrol, there appears to be a link in media reports to studies suggesting that longevity can increase by a regular consumption of red wine [19, 20]. Life extension in yeast is a long way from life extension in higher organisms. Thus, it is not a simple matter to extrapolate the results of yeast studies to human health, especially when the studies on yeast used much higher concentrations of resveratrol than are available from wine drinking [94], and its poor bioavailability after metabolization [41, 51, 54, 61]. In addition, the link between regular wine consumption and longevity is still not proven.

Recently, Wood *et al*. [95] showed that resveratrol was able to activate SIRs from the metazoans *Caenorhabditis elegans* and *Drosophila melanogaster*, and extend the lifespan of these animals without reducing fecundity. These data indicate that resveratrol slowed metazoan ageing by mechanisms that may be related to caloric restriction. Wood *et al.* [95] also suggested that the fact that resveratrol was capable to increase longevity without an apparent cost of reproduction, was counter to prevalent concepts of senescence evolution. More recently, Bauer *et al*. [96] reported the development of an assay in *Drosophila melanogaster* using the expression of molecular biomarkers that accelerates the ability to evaluate potential lifespan-altering interventions. Coupling the expression of an age-dependent molecular biomarker to a lethal toxin reduces the time needed to perform lifespan studies by 80%. The assay recapitulated the effect of the three best-known environmental

lifespan-extending interventions in the fly: ambient temperature, reproductive status, and calorie reduction. These scientists used this assay as a screen to identify drugs that extend lifespan in flies and proposed that it can be used to screen pharmacological as well as genetic interventions more rapidly for positive effects on lifespan. Thus, resveratrol was identified as being beneficial in their assay and shown to extend lifespan under normal laboratory conditions.

SIR1 is another member of the same family that is a human deacetylase that promotes cell survival by negatively regulating the p53 tumor suppressor. One known target of SIR1 is lysine 382 of p53 (K382). Deacetylation of this residue by SIR1 decreases the activity and half-life of p53, and increases cell survival under a variety of DNA damaging conditions [97–99]. At this respect, Howitz *et al*. [93] performed fluorescent deacetylation assays on human SIR1, using a synthetic peptide substrate that encompassed lysine 382 (K382) of p53. These investigators wanted to test whether resveratrol could stimulate this human SIR *in vivo*, and observed that the effects produced by resveratrol were concentration-dependent. Thus, whereas the stilbene stimulated SIR activity at relatively low doses (0.5 μM), higher doses (>50 μM) had the opposite effect, at least in certain assays. This may explain the dichotomy in the literature regarding the effects of resveratrol on cell viability [100–102].

Picard *et al*. [103] showed that SIR1 modulated adipogenesis through the activation of a critical component of calorie restriction in mammals (fat mobilization in white adipocytes), using mouse 3T3-L1 fibroblasts as an *in vitro* model. Adipogenesis in these cells is promoted by the nuclear receptor peroxisome proliferator-activated receptor (PPAR)-γ [104]. PPARs are nuclear receptors that are ligand-activated transcription factors that regulate different biological processes [105, 106]. To date, three isoforms, encoded by separated genes, have been identified: PPARα [NR1C1], PPARβ (NUC-1 or PPARδ) [NR1C2], and PPARγ [NR1C3]. All three PPARs are homologous in structure and three-dimensional conformation, with only few different modifications. PPARs are not only implicated in several physiological processes, but also in the pathophysiology of obesity, diabetes, immune response, ageing, atherosclerosis, and inflammatory response [107]. Although a precise biological role for PPAR-β remains unclear, PPAR-α and PPAR-γ play a key role in the metabolism of lipids and glucose [108, 109]. Repression of PPAR-γ by SIR1 was also evident in 3T3-L1 adipocytes, where overexpression of SIR1 attenuated adipogenesis, and RNA interference of SIR1 enhanced it. In differentiated fat cells, upregulation of SIR1 triggered lipolysis and loss of fat. The authors fully differentiated 3T3-L1 cells and subsequently (12 days after induction) applied the SIR1 activator resveratrol [93] over a wide range of concentrations. After staining the cells for fat content, a strong reduction in fat was observed at 50 and 100 μM resveratrol. The loss of fat was due to activation of SIR1, because there was no drug-mediated fat reduction in cells in which SIR1 levels were knocked down. These results provided a possible molecular pathway connecting calorie restriction to life extension in mammals, since a reduction in fat is sufficient to extend murine lifespan [110].

In addition to its immediate implications for aging and life extension, Hall [19] indicated that the new investigations bolster the notion that there is an evolutionarily conserved mechanism to stall the aging process during times of stress, such as when food is scarce. It also raises the possibility that the SIR-activating compound (STAC) reflects an interaction between plant and animal species [19]. Plants synthesize STACs, such as resveratrol, in response to stress and nutrient limitation [13], possibly to activate their own SIR pathways [93]. These molecules may activate animal SIRs because they serve as plant defense mechanisms against consumers or because they are ancestrally orthologous to endogenous activators within metazoans [95]. Alternatively, animals may use plant stress molecules as a cue to prepare for a decline in their environment or food supply [93]. Understanding the adaptive significance, endogenous function and evolutionary origin of SIR activators will lead to further insights into the underlying mechanisms of longevity regulation, and could aid in the development of interventions that provide the health benefits of caloric restriction [95].

5 Anti-inflammatory activity of resveratrol

5.1 Effects of resveratrol on activation of different cell types: mast cells, neutrophils, and monocytes/macrophages

In response to tissue injury, a multifactorial network of chemical signals initiate and maintain a host response designed to "heal" the afflicted tissue. This involves the activation and directed migration of leukocytes (neutrophils, monocytes and eosinophils) from the venous system to sites of damage. Tissue mast cells also have a significant role [111]. The inflammatory process has an initial response, acute inflammation, which is characterized by a brusque start showing manifestations, such as vascular and exudative phenomenons. When the reaction is uncontrolled, the subsequent changes are subject to the type and intensity of aggressor agent and the tissue affected. The acute inflammation has a duration of minutes or a few days, but when it does not resolve, the acute stage becomes converted into a chronic inflammation which has a major duration and also histologycally is characterized by the presence of lympho-

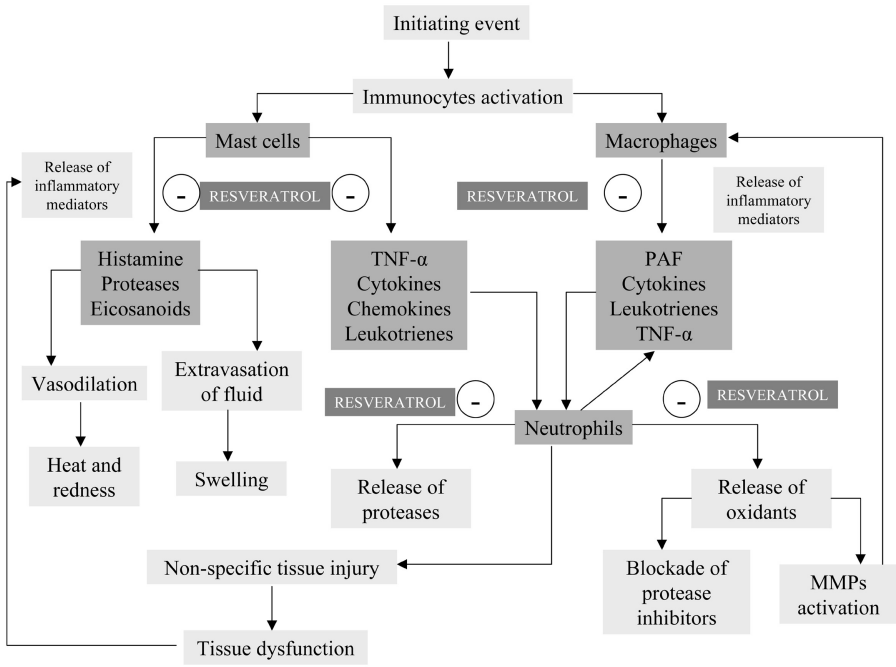

Figure 2. Amplification of inflammation and nonspecific injury by inflammatory mediators in inflammatory diseases. Regardless of the initiating event, immunocytes such as mast cells and macrophages respond by releasing a variety of inflammatory mediators and cytokines. These factors recruit neutrophils to the involved region through their chemotactic properties, and activate the neutrophils to release reactive oxygen metabolites and other oxidants and proteolytic enzymes. Moreover, the infiltrated neutrophils will release chemotactic inflammatory mediators, thereby amplifying the inflammatory response. Resveratrol elicits inhibitory effect at all physiopathological phases of the inflammatory response.

cytes and macrophages as well as blood vessel proliferation [112].

During the first stage of inflammation, morphological alterations occur: an intense vasodilation followed by an augment of blood flow and vascular permeability increase of the affected region. The result is the exudate and oedema production. The cellular migration is favored by the chemotactic action of exogenous as well as endogenous substances that specifically bind to membrane receptors. These chemotactic agents can be substances released from microorganisms, such as formyl methionyl leucyl phenylalanine (fMLP) peptide, or by mastocytes/macrophages which are able to induce the release of vasoactive mediators to damaged zone including the platelet-activating factor (PAF), leukotriene (LT)-B_4, complement fragment arachidonic acid metabolites, nitric oxide (NO) and cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, and reactive oxygen metabolites (ROMs) [113–115].

The pro-inflammatory TNF-α controls inflammatory cell populations as well as mediates many other aspects of the inflammatory process, such as endothelial permeability, possibly through an activation of tyrosine kinases [116]. In a recent study by Fulgenzi *et al*. [117], modification of TNF-α-induced vascular permeability in the presence of resveratrol was evaluated in a mouse liver perfusion model (1 μM). They showed the *in vivo* efficacy of resveratrol in reducing TNF-α-mediated vascular leakage. Table 1 (see Addendum) illustrates the anti-inflammatory efficacy and mechanims of action of resveratrol in preclinical animals models *in vivo*, but the list is certainly not exhaustive. Similarly, IL-8 is important in the development of inflammation because it participates in the recruitment of inflammatory leukocytes [118]. In a study by Donnelly *et al*. [119] resveratrol (1–100 μM) exhibited anti-inflammatory activity through the inhibition of IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF) release from A549 cells. Table 2 (see Addendum) includes some of the mechanistic properties of resveratrol *in vitro*.

Mast cells are another type of cells that take part in acute inflammation owing to their release of stored and newly synthesized inflammatory mediators, such as histamine, cytokines, and proteases complexed to highly sulfated proteoglycans, as well as lipid mediators [120] (Fig. 2). Thus, their activation plays an important role in the pathogenic process of inflammation and allergy. Activation of mast cells results in three types of biological response: secretion of the performed contents of their granules, synthesis and secretion of lipid mediators, and synthesis and secretion of

cytokines [121]. Baolin *et al*. [121] showed that resveratrol inhibited the release of TNF-α and histamine mediated by immunoglobulin E (IgE) in bone marrow-derived mouse mast cells. Also resveratrol was able to block the production and release of LTs and prostaglandin (PG) D_2.

Polymorphonuclear leukocytes (PMNs), basically neutrophils, play an essential role in the first stage of inflammation [122] because these are the first recruited effectors of the acute inflammatory response [123]. The results of some investigations suggest that resveratrol elicits inhibitory effect at all physiological phases of the inflammatory response (Fig. 2): from the initial recruitment of PMN to their activation and the subsequent release of inflammatory mediators [3, 124]. Once exposed to chemoattractants within the vasculature, the neutrophils become activated and capable of adhering tightly to the endothelium, the first committed step in the development of inflammation. Adherence of neutrophils to the vascular endothelium can be stimulated by LTB_4, TNF-α, PAF, and the complement fragment C5a, among others, which are capable of upregulating expression of adhesion molecules on the neutrophil, resulting in adherence to vascular endothelium. There are three classes of adhesive molecules present on leukocytes and endothelium: integrins (α- and β-integrins), selectins (L-, P-, and E-selectin), and members of the immunoglobulin superfamily of cell surface proteins (vascular cell adhesion molecule-l, VCAM-1, and intracellular adhesion molecule-1, ICAM-1) [124]. Integrins and selectins on leukocytes mediate the adhesion of circulating cells to endothelium, whereas selectins and members of the immunoglobulin superfamily on the endothelium mediate their "stickiness" for leukocytes [125 a + b].

The pro-inflammatory TNF-α plays a crucial activating role among the cytokines that modulate endothelial functions. TNF-α regulates the expression of some adhesion molecules and cytokines on endothelial cells. Ferrero *et al*. [126] performed an *in vitro* study to assess the expression of ICAM-1 and VCAM-1 by endothelial cells, following activation of cultured endothelial cells by TNF-α or lipopolysaccharide (LPS). Data from these authors showed that resveratrol inhibited ICAM-1 and VCAM-1 expression and neutrophil adhesion by TNF-α-stimulated human umbilical vein endothelial cells. Moreover, they demonstrated that resveratrol induced a significant inhibition in the adhesion of U937 monocytoid cells to LPS-stimulated endothelial cells. Analogously, resveratrol significantly inhibited the adhesion of neutrophils to TNF-α stimulated EA.hy.926 cells. In these experiments, the efficacy in modulating adhesion molecule expression was demonstrated for resveratrol concentrations of 100 nmol/L and 1 μmol/L [126]. Such concentrations are similar to those reached by resveratrol in rat plasma following oral ingestion of the drug [37].

More recently, Bertelli *et al*. [127] studied the effect of resveratrol treatment *in vitro* on the expression of VCAM-1 by TNF-α-stimulated human umbilical vein endothelial cells. The stilbene, used at the concentrations present in human plasma following moderate wine consumption (~μM range), was demonstrated to be an inhibitor of the adhesion molecule expression and significantly prevented the cytokine-induced vascular leakage.

On the other hand, considerable evidence implicates thrombin in downregulation of endothelial ectonucleotidase activity resulting in high levels of adenosine diphosphate (ADP) and adenosine triphosphate (ATP) which lead to platelet, leukocyte, and endothelial activation [128]. Resveratrol interfered with the pro-inflammatory signaling of thrombin resulting in the inhibition of adenosine nucleotide secretion from activated platelets and decreased neutrophil function *via* inhibition of protease-activated receptor (PAR) and P2-receptor signaling through interference with mitogen-activated protein kinase (MAPK) and c-Jun NH_2-terminal kinase (JNK). Thus, by inhibiting platelet activation through a diminished response to thrombin and by decreasing the liberation of inflammatory adenosine derivatives, the polyphenol appeared to affect oxygen free radical release and neutrophil-platelet interaction [129]. Moreover, the polyphenol protected endothelial adenosine nucleotide metabolism when downregulated by thrombin [130], preserving the suppression of endothelial CD39/ATPDase activity, which is critical in the inhibition of platelet and neutrophil activation by keeping adenosine nucleotide levels low [131]. These findings provide interesting insights into the effects of this compound on the cardiovascular system [129].

Some inflammatory mediators are also capable of priming or stimulating leukocytes to release ROM that include superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), or hypochlorous acid (HOCl), and other reactive species such as *N*-chloramines (RNHCl), or of stimulating the proliferation and/or differentiation of lymphocytes and other immunocytes [132]. Neutrophils as well as mastocytes/macrophages are able to endocytose tissue residues and foreign substances *via* the formation of a phagocytic vesicle, and also to release lysosomal enzymes and eicosanoids which all contribute to damage the tissue [122, 123].

The effects of resveratrol on PMN-induced pro-inflammatory signals has been investigated, for example, in a study by Rotondo and colleagues [133] upon PMN stimulated by exposure to fMLP, complement fragment C5a, the calcium ionophore A23187, or a monoclonal antibody to the β2-integrin MAC-1. Resveratrol exerted a strong inhibitory effect on ROM production, release of elastase and β-glucuronidase from neutrophil granules, and the expression of the β2-integrin MAC-1 on PMN surface [133]. More recently,

Cavallaro *et al*. [124] have demonstrated that resveratrol in whole blood also inhibited $O_2^{\bullet-}$ production of stimulated human neutrophils; it strongly decreased HOCl generation through exerting inhibitory effects on myeloperoxidase (MPO) and reduced NO production and chemotaxis.

Further evidence for the inhibition of neutrophil induced-ROM generation by resveratrol was reported by McClintock and colleagues [134] in acute lung injury in rat induced by the chemical warfare agent analog, 2-chloroethyl ethyl sulfide, known as 'half-mustard gas' (HMG) instilled *via* intrapulmonary injection. The induced damage may be related partially to complement mediated pathways and the generation by neutrophils of ROM. Administration of resveratrol provided significant protection when given up to 90 min after exposure of the lungs to HMG. Resveratrol has also been reported to inhibit the formation of phosphatidic acid and dygliceride in chemotactic fMLP peptide- or phorbol 12-myristate 13-acetate (PMA)-stimulated human neutrophils, suggesting that inhibition of phospholipase D activity may contribute to its anti-inflammatory effects [135].

After PMN, monocytes, which differentiate into macrophages in tissue, are next to migrate to the site of tissue injury, guided by chemotactic factors. Macrophages are also considered as the major players in the body's response to immunogenic challenges. A biological response modifier secreted from these cells could contribute to development of disease states such allergy and inflammation [136] because, once activated, macrophages are the main source of growth factors and cytokines, which profoundly affect endothelial, epithelial, and mesenchymal cells in the local microenvironment [137, 138]. A classical model of macrophage stimulation is bacterial endotoxic LPS. Under normal physiological settings this stimulation leads to a moderate increase in inducible NO synthase (iNOS) activity, resulting in NO production that has bactericidal effects. However, abnormally high concentrations of NO and its derivative peroxynitrite ($ONOO^-$) give rise to inflammation. For example, in LPS-activated RAW 264.7 macrophages, preincubation of cells with resveratrol reduced inflammation by downregulation of iNOS mRNA and protein [139, 140]. Similar data were found by Matsuda *et al*. [141] in mouse peritoneal exudate macrophages activated by LPS; in this model, resveratrol also inhibited NO production. Similarly, in another study using rat macrophages stimulated with thioglycollate, resveratrol at concentrations of 10 – 100 μM significantly and dose-dependently inhibited production of reactive nitrogen intermediates [142]. Another experiment performed in peritoneal macrophages of mice was reported by Zhong *et al*. [143] who studied the effects of resveratrol on IL-6 activity in the supernatant of these cells culture. The investigators found that the polyphenol dose-dependently inhibited IL-6 release by cultured macrophages induced by A23187 and fMLP, as well as calcium ion influx into the cells with the stimulation of fMLP. These results suggested that blocking of calcium ion influx into cells by the phytoalexin is one of the possible mechanisms of the IL-6 biosynthesis inhibitory action of resveratrol.

The effects of resveratrol on macrophage-induced pro-inflammatory signals were also investigated on chronic obstructive pulmonary disease (COPD) in which pulmonary inflammation is associated with predominant alveolar macrophage involvement. Alveolar macrophages were isolated from bronchoalveolar lavage (BAL) fluid from cigarette smokers and from patients with COPD and were stimulated with either IL-1β or cigarette smoke media (CSM) to release IL-8 and GM-CSF. Resveratrol reduced IL-1β stimulated IL-8 and GM-CSF release in both smokers and COPD patients to below basal levels. They concluded that resveratrol could be effective in the pharmacotherapy for macrophage pathophysiology in COPD [144].

5.2 Effects of resveratrol on enzyme systems involved in the synthesis of pro-inflammatory mediators

Two enzyme systems involved in the synthesis of pro-inflammatory mediators are the cyclooxygenase (COX) and the lipoxygenase pathways (Fig. 3). Both of them generate inflammatory substances, such as prostanoids and LTs, respectively. For example, thromboxane is an eicosanoid synthesized *via* COX that may contribute to tissue injury through modulation of inflammatory response because it has the capacity to stimulate LTB_4 release and the adherence of leukocytes to the vascular endothelium. Thromboxane is a very potent vasoconstrictor and agonist for platelet aggregation and also the major arachidonic acid metabolite produced by platelets [145]. Synthesis of LTs occurs mainly in immunocytes, epithelial cells, and endothelial cells. Although different types of LTs are known, they can be conveniently subdivided into two main subclasses: LTB_4 and peptido-LTs. The latter consists of both fatty acid and amino acid moieties. As mentioned above, LTB_4 is a very potent chemoattractant for neutrophils and can promote leukocyte recruitment from the vasculature through the upregulation of β_2 integrins (CD11/CD18) expression on those cells. The release of ROM from neutrophils can also be stimulated by the induction of LTB_4, while peptido-LTs (LTC_4, LTD_4, and LTE_4) are derived mainly from mast cells and although these substances increase the permeability of the vascular endothelium and promote the rolling of the leukocytes by increasing the expression of P-selectin on vascular endothelium cells, they exhibit little if any chemotactic activity [132]. Thus, literature has shown that blockade of synthesis of these sub-

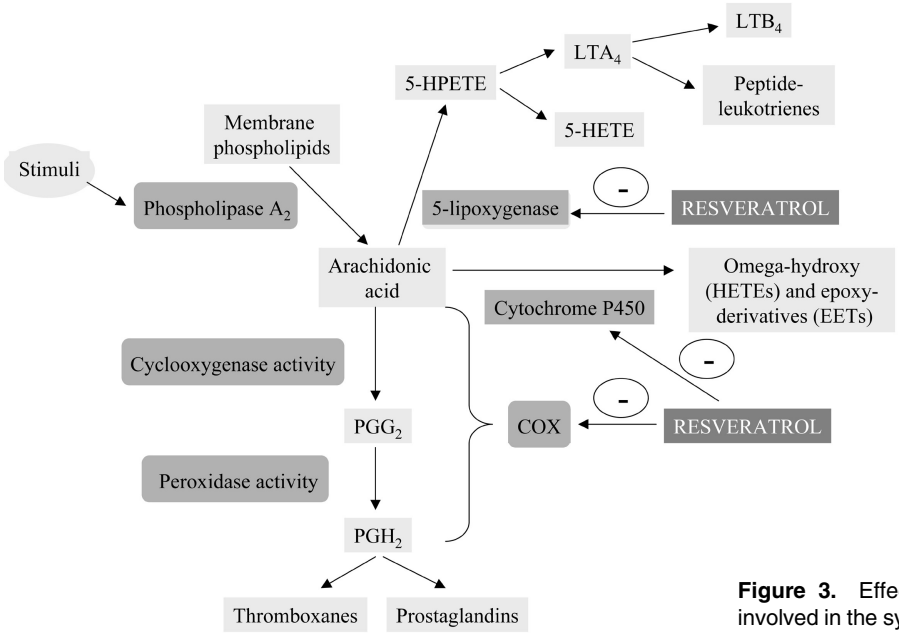

Figure 3. Effects of resveratrol on enzyme system involved in the synthesis of pro-inflammatory mediators.

stances, prostanoids and LTs, leads to a reduction of inflammation. In a recent report by Shigematsu *et al.* [146] using intravital microscopic approaches to quantify leukocyte/endothelial cell interactions and venular protein leakage in rat mesenteries exposed to either 20 min ischemia and 60 min reperfusion (I/R), resveratrol attenuated the pro-inflammatory effects of PAF, LTB_4-induced changes preventing leukocyte recruitment and endothelial barrier disruption induced by a number of superoxide-dependent pro-inflammatory stimuli, including I/R, hypoxanthine, and xanthine oxidase (HX/XO), or PAF. Likewise, Kimura and colleagues [147] found that resveratrol inhibited the production of the peptide-leukotriene LTC_4 (Fig. 3), while Rotondo *et al*. [133] showed the inactivation of the LTB_4 production by the phytoalexin from PMN stimulated with the calcium ionophore A23187. Furthermore, the researchers established that the inhibitory activity exerted by resveratrol on the 5-lipoxygenase product 5-HETE (5-hydroxy-6,8,11,14-eicosatetraenoic acid) and the COX products HHT (12-hydroxy-5,8,10-heptadecatrienoic acid) and thromboxane B_2 was directly responsible for the anti-platelet aggregation induced by the polyphenol [3].

On the other hand, PGs, considered not as mediators of inflammation but as modulators, are synthesized *via* COX (Fig. 3), in increased amounts during inflammation. PGs may downregulate inflammatory responses, for example, by modulating the activity of pro-inflammatory cytokines, neutrophil adherence, and extravasation and suppression of mast cell activation [148, 149].

Although the existence of three isoforms of COX [150] has been proposed, there is not any concrete scientific evidence for an actual third independent COX gene in the literature to date [151]. For the present, two isoforms of this enzyme have been identified in humans: COX-1 and COX-2 [152]. There is evidence that prostanoids derived from COX-1 exert immunomodulatory, cytoprotective, and proangiogenic effects [153] whereas those produced *via* COX-2 are involved both in the inflammatory and regeneration processes. COX-2 expression is affected by various stimuli, such as mitogens, oncogenes, tumor promoters and growth factors [154], and is clearly related to the development of various types of cancer [155, 156].

The anti-inflammatory properties of resveratrol were demonstrated in a rat model of carrageenan-induced paw edema, an effect attributed to inhibition of PG synthesis *via* inhibition of COX-1 [14]. Likewise, the same authors also demonstrated that the polyphenol was able to inhibit selectively the COX activity of COX-1 as well as the hydroperoxidase activity of this isoenzyme, and to a lesser extent COX-2 hydroperoxidase activity, but not COX activity of COX-2. However, a recent study performed by Szewczuk and colleagues [157] showed that resveratrol was able to discriminate between the two COX isoforms, since the phytoalexin was a peroxidase-mediated inactivator of COX-1 but not COX-2, suggesting that resveratrol led to the elimination of PGs synthesis *via* COX-1 whereas PGs synthesis by COX-2 was unaltered. In contrast, Subbaramaiah *et al*. [158] indicated that resveratrol suppressed the synthesis of PGE_2 by inhibiting COX-2 enzyme activity and moreover inhibited COX-2 gene transcription without altering the amount of COX-1. Subsequent studies found that this phytoalexin induced a reduction of PGs and expression of

COX-2 through the reduction of arachidonic acid release and induction of COX-2 by an antioxidant action, as resveratrol was able to diminish $O_2^{\bullet-}$ and H_2O_2 produced by murine peritoneal macrophages stimulated with LPS or phorbol esters [159]. Therefore, the anti-inflammatory activity of resveratrol may be explained by the inhibition of both COX-1 and COX-2 as well as its antioxidant effect.

In a recent study, our research group investigated the beneficial effects of resveratrol on the colon injury caused by intracolonic instillation of trinitrobenzenesulfonic acid (TNBS) in rats. We determined the production of PGE_2 and PGD_2 in colon mucosa and the expression of COX-1 and -2 immunohistochemically. Resveratrol significantly reduced the degree of colonic injury and neutrophil infiltration, and also produced a significant fall in the PGD_2 concentration. Compared with inflamed colon, no changes in staining for COX-1 were observed in colon of resveratrol and TNBS-treated rats. In contrast, COX-2 expression was decreased [160].

It has been reported that both COX isoforms are important sources of PGs that appear to contribute to gastric ulcer healing [161, 162]. Also, it is well-known that angiogenesis plays a central role in wound healing. Among many known growth factors, vascular endothelial growth factor (VEGF) is believed to be the most prevalent, efficacious, and long-term signal that is known to stimulate angiogenesis in wounds [163]. The wound site is rich in oxidants, such as H_2O_2, mostly contributed by neutrophils and macrophages. Topical application of resveratrol accelerated wound contraction and closure. In addition, resveratrol treatment was associated with a more well-defined hyperproliferative epithelial region, higher cell density, enhanced deposition of connective tissue, and improved histological architecture [164]. On the contrary, in a model of chronic gastric ulcer induced by serosal application of acetic acid, daily treatment with resveratrol during 14 days suppressed the PGE_2 generation in both nonulcerated and ulcerated gastric mucosa, prolonged ulcer healing which was accompanied by the fall in the gastric blood flow at the ulcer margin and a significant increase in plasma IL-1β and TNF-α levels [165].

PGs can also stimulate tumor cell growth by increasing cell proliferation, promoting tumor angiogenesis, and suppressing immune surveillance and apoptosis [166 – 170]. Thus, a strong correlation has been found between inflammation and cancer, suggesting that cancer is an inflammatory disease [171, 172]. In this line, the oxidation of arachidonate by COX generates other oxidative species raising the overall oxidative state of the cell. One consequence of this is that COX-2 will co-oxidize compounds such as benzo[*a*]-pyrene [173] to highly carcinogenic derivatives. Direct oxidative damage to DNA is a well-recognized mutagenic event [174] and such damage is increased following induction of COX-2 [175].

Several lines of evidence suggest that selective COX-2 inhibitors may provide an opportunity for both cancer prevention and therapy [176]. In order to find more selective COX-2 inhibitors, Murias *et al*. [177] synthesized and evaluated a series of methoxylated and hydroxylated resveratrol derivatives for their ability to inhibit both COX isoenzymes using *in vitro* inhibition assays for COX-1 and COX-2 by measuring PGE_2 production. Then, they compared the results with that of resveratrol and the selective COX-2 inhibitor celecoxib. The authors indicated that hydroxylated but not methoxylated resveratrol analogues showed a high rate of inhibition, being 3,3′,4′,5-tetra-*trans*-hydroxystilbene and 3,3′4,4′,5,5′-hexahydroxy-*trans*-stilbene the most potent resveratrol compounds with potency comparable or better than the clinically established celecoxib. In addition, to address the question whether there exists a quantitative relationship between chemical structure and biological activity, the effect of structural parameters on COX-2 inhibition was evaluated by quantitative structure-activity relationship (QSAR) analysis. These authors found a high correlation with the topological surface area (TPSA). Thus, hydroxylated resveratrol analogues showed significantly lower IC_{50} values against COX-2 than celecoxib that should result in lower doses necessary to achieve the same efficacy in clinical studies. However, the methoxylated derivatives were poor inhibitors of COX-2 activity and did not exhibit COX-2 specificity [177].

On the other hand, the cytochrome P4501A1 is able to induce the metabolic activation of pro-carcinoges, such as the polycyclic aromatic hydrocarbons, benzo[*a*]pyrene, and dimethylbenz[*a*]anthracene [53]. Resveratrol affects tumor initiation preventing the initial DNA damage by two different pathways [15]: (i) acting as an antimutagen through the induction of phase II enzymes [4, 178], such as quinone reductase, capable of metabolically detoxifying carcinogens by inhibiting the COX and cytochrome P4501A1 enzymes (Fig. 3) known to be able to convert substances into mutagens, as mentioned above; and (ii) acting as an antioxidant through inhibition of DNA damage by free radicals [14]. Thus, the antioxidant properties of resveratrol could also be the responsible for the cancer preventive activity of the stilbene. For example, resveratrol reduces oxidative damage induced by H_2O_2 in calf thymus DNA [179] and in several cancer cell lines [180, 181]. Moreover, the administration of resveratrol also reduced *in vivo* the levels of 8-oxo-7,8-dihydroxy-2′-deoxyguanosine, a known marker of oxidative DNA damage [182]. Resveratrol does all that at physiological concentration [14, 183, 184]. The inhibition of COX and hydroperoxidase functions, primarily of COX-1, and the downmodulation of COX levels in some cell types, constitutes a basis for the activity of resver-

atrol directed against the promotion of tumors [15]. Moreover, COX-2 isoenzyme is overexpressed in practically every premalignant and malignant condition involving the head, neck, esophagus, stomach, colon, liver, pancreas, breast, lung, bladder, and skin, as a consequence of deregulation of transcriptional and post-transcriptional control [185]. Therefore, the direct inhibition of COX-2 activity exerted by resveratrol provides a mechanistic basis for the chemopreventive properties of this polyphenol beyond its anti-inflammatory effects [158]. Moreover, resveratrol inhibited both COX-2 enzyme activity and PMA-induced activation of COX-2, which appeared to occur through a suppression of PMA-dependent activation of protein kinase C (PKC) and activator protein-1 (AP-1)-mediated gene expression [158]. These results were supported by data indicating that resveratrol inhibited COX-2 promoter-dependent transcriptional activity and PKC activation [186]. These data taken together with that resveratrol was able to inhibit nuclear factor kappa B (NF-κB), and AP-1 activation supply an additional mechanism for inactivation of COX-2 by the polyphenol, because COX-2 transcription can be stimulated by both transcription factors [187].

The effect that resveratrol exerts on cell cycle progression depends on the experimental system, being highly variable [188 – 191]. The stilbene inhibited in a dose-dependent manner cell proliferation in different human cancer cell lines, and the effect seemed rather specific for malignant cells [192]. Inhibition of polyamine biosynthesis through inhibition of ornithine decarboxylase activity [189] or through inhibition of ribonucleotide reductase [193] may be the possible mechanisms of action by which resveratrol exerts its anti-proliferative effect. Resveratrol also showed to decrease the proliferation of breast cancer cell lines [180, 194 – 196]. Nevertheless, increased proliferation by the polyphenol was also reported [197, 198]. Although the effects on cell growth are possibly mediated by an interaction with the estrogen receptor (ER), this seems not to be the only mechanism, as inhibition of proliferation has been observed in both ER+ and ER-cells [180, 194, 196].

Conflicting results have been obtained regarding the mechanisms associated with induction of the differentiation and apoptosis by resveratrol in tumor cells [14, 183, 191, 199]. The ability of resveratrol to induce the expression of CD95L, p53, and p21 may contribute to its growth-inhibitory effects [192, 200]. Thus, the phytoalexin could inhibit programmed cell death through induction of p53 activity [201 – 204] but a p53-independent pathway has also been proposed [199]. Cyclins (D1, A, B1), p21 expression, the apoptosis agonist bax, and the anti-apoptotic bcl-2 have also been reported to be involved [191, 199, 205, 206]. Therefore, resveratrol can inhibit all three stages of carcinogenesis: tumor initiation, promotion, and progression.

5.3 Effects of resveratrol on transcription factors

Inflammation involves a complex web of intracellular and intercellular cytokine signals, as well as other components of signalling networks that include several kinases, such as the family of proline-directed serine/threonine kinases named MAPKs, PKC, phosphoinositide-3-kinase, *etc*. MAPKs are activated by translocation to the nucleus, where they phosphorylate a variety of target transcription factors, including NF-κB and AP-1 [207, 208]. Many important cellular functions are under control of MAPKs and there are three well-characterized subfamilies of MAPKs in multicellular organisms: ERKs, JNKs, and the p38 enzymes [209].

There has been a great interest in investigating the effects of resveratrol on transcription factors that regulate the expression of inflammatory mediators. These include the Rel family of transcription factors, in particular nuclear factor NF-κB, AP-1, C/EBP, and fos/jun. Resveratrol interferes with the activation of these critical transcription factors at a step common to many stimuli and signal transduction pathways [3]. In fact, existing observations from both *in vitro* and *in vivo* studies have demonstrated differential effects of resveratrol on upstream kinases, such as MAPKs, which regulate activation of NF-κB and AP-1 [210].

NF-κB is a transcription factor for genes involved in cell survival, cell adhesion, inflammation, differentiation, and growth, which is activated by a variety of stimuli, such as carcinogenesis, inflammatory agents, such as TNF-α and H_2O_2, and tumor promoters [185]. Although NF-κB activation is regulated by MAPKs through multiple mechanisms, accumulating evidence indicates that NF-κB activation is modulated by MAPK/ERK kinase kinase-1, a kinase upstream of JNKs [211] which induces site-specific phosphorylation of an inhibitory protein called IkappaB (IκB). Activation of NF-κB is also modulated by p38 MAPK [212]. NF-κB is inactivated in resting cells by binding to one of the IκBs. Phosphorylation of IκB leads to liberation of NF-κB and its translocation to the nucleus [213]. Then, this factor is able to induce the expression of genes associated with the inflammatory process and the development of cancer, such as altered cell growth, immune and inflammatory responses [214]. Extensive research during the last few years has shown that most inflammatory agents mediate their effects through the activation of NF-κB and that most anti-inflammatory agents suppress NF-κB activation. Similarly, most carcinogen and tumor promoters activate NF-κB, whereas chemopreventive agents suppress it, thus suggesting a strong linkage with cancer [215, 216]. Thus, while activation of NF-κB promotes cell survival and proliferation, its downregulation sensitizes the cell to apoptosis. In a recent report performed by Manna and colleagues [217], the authors investigated the effect of resveratrol on NF-κB activation induced by various inflammatory agents.

Since concentrations used in their studies (a 5 μM solution of resveratrol) are comparable with that used in animal studies, the author suggested that resveratrol concentration used in their *in vitro* studies was achievable *in vivo* by consumption of grapes or wine. The results of this study showed that resveratrol blocked TNF-α-induced activation of NF-κB in a dose- and time-dependent manner. Resveratrol also suppressed TNF-α-induced phosphorylation and nuclear translocation of the p65 subunit of NF-kB, and NF-κB-dependent reporter gene transcription. Suppression of TNF-α-induced NF-κB activation by resveratrol was not only restricted to myeloid cells (U-937), but was also observed in lymphoid (Jurkat) and epithelial (HeLa and H4) cells. Resveratrol also blocked NF-κB activation induced by PMA, LPS, H_2O_2, okadaic acid, and ceramide. The suppression of NF-κB coincided with suppression of AP-1. Resveratrol also inhibited the TNF-α-induced activation of MAPK kinase and c-Jun *N*-terminal kinase, and abrogated TNF-α-induced cytotoxicity and caspase activation. Both ROM generation and lipid peroxidation induced by TNF-α were suppressed by resveratrol. Therefore, these investigators suggested that resveratrol's anticarcinogenic, anti-inflammatory, and growth-modulatory effects might thus be partially ascribed to the inhibition of activation of NF-κB and AP-1 and the associated kinases [217, 218]. Furthermore, Holmes-McNary and Baldwin [219] indicated that the inhibition of NF-κB activation and NF-κB-dependent gene expression were associated with the inhibition of the IκB kinase activation.

AP-1 is another transcription factor that regulates the expression of several genes that are involved in cell differentiation and proliferation. Its activation is implicated in tumor promotion as well as malignant transformation [220]. Thus, AP-1 activation can upregulate genes, such as IL-8, among others. In this line, Shen *et al.* [221] observed the modulation of IL-8 production in human monocytic cells by resveratrol and explored its mechanism at the gene transcription level. Resveratrol inhibited PMA-induced IL-8 production in U937 cells at protein and mRNA levels. The suppression of IL-8 gene transcription by resveratrol was, at least partly, due to inhibition of AP-1 activation. Furthermore, in a recent study performed by Donnelly *et al*. [119], the mechanism of resveratrol action was investigated using luciferase reporter genes stably transfected into A549 cells. Resveratrol inhibited NF-κB-, AP-1-, and cyclic adenosine monophosphate (cAMP) response element binding protein-dependent transcription to a greater extent than the glucocorticosteroid dexamethasone. They concluded that resveratrol has novel nonsteroidal anti-inflammatory activity that may have applications for the treatment of inflammatory diseases.

Therefore, the inhibition of the NF-κB or the AP-1 activation process by resveratrol may cause a significant suppression of cell proliferation, sensitizing cells for apoptosis as well as reducing the expression of inflammatory genes [185]. Moreover, in response to external stimuli, such as TNF-α, LPS, PMA, *etc.*, the expression of inflammatory genes, such as iNOS and PTGS2 (the gene of the COX-2 protein) which have been implicated in tumor promotion, are transiently upregulated through the activation of NF-κB and/or the AP-1 [222]. Thus, it is possible that resveratrol inhibits enzymes, such as iNOS and COX-2, *via* its inhibitory effects on NF-κB or the AP-1 [3].

On the other hand, Woo *et al.* [223] showed that resveratrol was able to inhibit several essential steps of PMA-induced matrix metalloproteinase-9 (MMP-9) expression. Metalloproteinases are particular proteases that contribute to invasion and metastasis, as well as tumor angiogenesis, and their expression is regulated by cytokines and signal transduction pathways [224, 225]. It is known that the human MMP-9 promoter contains *cis*-acting regulatory elements and transcription factors, including AP-1 and NF-κB, which participate in the regulation of the MMP-9 gene [226]. The role of MAPKs in the regulation of MMP-9 expression in malignant cells has been studied. At least two (ERK and JNK) of three so-called mitogenic pathways known so far in mammalian cells induced upregulation of MMP-9. Recently, it has been shown that the inhibition of p38MAPK leads to reduced phorbol ester-induced MMP-9 expression and invasion by tumor cells. The study performed by Woo *et al*. [223] demonstrated that the inhibition of PMA-induced MMP-9 by resveratrol was not only mediated by the regulation of transcription factors, such as AP-1 and NF-κB, but also through the inhibition of JNK activation and PKC-δ activation, which suggests the efficacy of resveratrol as it selectively targets JNK without affecting the ERKs. Therefore, anticarcinogenic, anti-inflammatory, and growth-modulatory effects of resveratrol can partially be explained through its ability to supress NF-κB and AP-1 activation as well as the associated upstream kinases [210].

Recently, investigators have shown that resveratrol was able to bind to the ligand-activated transcription factors receptors PPAR-α and γ suggesting that this phytoalexin may exert some of its anti-inflammatory capacity *via* this receptor. At this respect, Inoue and colleagues [227] indicated that resveratrol was a selective activator of PPAR-α and PPAR-γ using two experiments: in the first one, they employed a cell-based transfection assay in monkey kidney CV-1 cells using the ligand binding domain of nuclear receptors fused to the DNA-binding domain of the yeast transcription factor GAL4. The CV-1 cells were transfected for 6 h, washed, and incubated with resveratrol for 40 h. Resveratrol activated the nuclear receptors PPAR-α and PPAR-γ in a dose-dependent manner. In contrast, the stilbene did not activate other nuclear receptors including glucocorticoid receptor and PPAR-β even at 100 μM. In the second experiment, Inoue *et al*. [227] examined whether

the activation of PPAR-α and PPAR-γ by resveratrol could be induced in mouse primary cortical cultures and bovine brain microvessel vascular endothelial cells (BBMECs). To test this, they transfected a reporter vector of peroxisome proliferator-responsive element (PPRE) with the expression vector for PPAR-α or PPAR-γ. Treatment with 10 μM resveratrol for 24 h activated PPAR-α and PPAR-γ in both cell types. Comparable activation of PPAR-α and PPAR-γ by resveratrol was also found in human umbilical venous endothelial cells (HUVECs) and bovine arterial endothelial cells (BAECs) by the same authors. Inoue *et al*. [227] also demonstrated the protective effect exerted by resveratrol on the murine brain against ischemia through the binding to PPAR-α, suggesting that the anti-inflammatory activity of this phytoalexin could be mediated *via* this receptor.

6 Conclusions

Resveratrol has been the focus of a number of studies investigating its beneficial effects on neurological, hepatic, and cardiovascular systems. Also one of the most striking biological activities of resveratrol soundly investigated during the late years has been its cancer-chemopreventive and chemotherapeutic potential. In fact, recently it has been demonstrated that this stilbene blocks the multistep process of carcinogenesis at various stages: tumor initiation, promotion, and progression. The body of evidence presented here speaks volumes for the clinical potential of the stilbene. One of the possible mechanisms for its biological activities involves downregulation of the inflammatory response through inhibition of synthesis and release of pro-inflammatory mediators, modification of eicosanoid synthesis, inhibition of activated immune cells, or inhibiting, such as iNOS and COX-2, *via* its inhibitory effects on NF-κB or the AP-1. It can be concluded that resveratrol has novel nonsteroidal anti-inflammatory activity that may have applications for the treatment of inflammatory diseases. Finally, resveratrol has been shown to extend the lifespan of several species, implicating the potential of resveratrol as an anti-aging agent in treating age-related human diseases.

7 References

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8 Addendum

Table 1. Dose, anti-inflammatory efficacy, and mechanisms of resveratrol in preclinical animal models *in vivo*

Ref.	Model[a)]	Daily dose	Route	Mechanism	Efficacy[b)]
[117]	Mouse liver perfusion model	1 μM	i.p.	Reduction of TNF-α-induced liver vascular permeability	+
[134]	Rat model of HMG-induced lung injury	50 mg/kg	i.p.	Antioxidative effects	+
[146]	Rat model of I/R	0.7 mg/kg	i.v.	Attenuation of I/R-, hypoxanthine/xanthine oxidase-, and PAF-stimulated pro-inflammatory effects	+
[160]	Rat model of TNBS-induced colitis	5 and 10 mg/kg	i.g.	Reduction of PGD_2 and IL-1β levels Inhibition of MPO activity	+
[164]	Dermal wound model in BalbC mice	2.5 mg GSPE[c)]	Topical	Acceleration of wound contraction and closure	+
[165]	Acetic acid-induced chronic gastric ulcers in rats	10 mg/kg	i.g.	Inhibition of PGD_2 generation Prolongation of ulcer healing and reduction of gastric blood flow Increase in plasma IL-1β and TNF-α levels	–
[182]	$KBrO_3$-treated rats	16 mg/kg	i.p.	Inhibition of oxo^8dG[d)]-induced DNA damage	+
[206]	F344 rats	200 μg/kg	Drinking water	Modification of bax and p21 expression	+
[227]	Mouse experimental stroke model	20 mg/kg	p.o.	Reduction of infarct size through PPARα activation	+

a) HMG, half-mustard gas; I/R, ischemia/reperfusion; TNBS, trinitrobenzenesulfonic acid; $KBrO_3$, potassium bromate
b) +: efficacious; –: inefficacious
c) GSPE: grape seed proanthycyanidin extract containing 5000 ppm resveratrol
d) oxo^8dG: 8-oxo-7,8-dihydroxy-2′-deoxyguanosine

Table 2. Mechanisms and efficacious concentrations of resveratrol in cells *in vitro* related to anti-inflammatory activity

Ref.	Experimental system	Mechanism	Efficacious concentrations
[119]	A549 cells	Inhibition of IL-1β-mediated release of IL-8 and GM-CSF	100 μM
[121]	Bone marrow-derived mouse mast cells (BMMC)	Inhibition of IgE-mediated release of LT and PGD_2	10 and 100 μM
		Inhibition of A23187-mediated release of LT and histamine	10 and 100 μM
		Inhibition of IgE-mediated release of histamine and TNF-α	100 μM
[124]	Human blood or isolated cells	Inhibition of zymosan- or fMLP-stimulated human neutrophil production	10^{-6}–10^{-2} mg/mL
	Isolated neutrophils	Inhibition of zymosan-stimulated HOCl production	10^{-6}–10^{-2} mg/mL
		Inhibition of fMLP-induced chemotaxis	10^{-6}–10^{-4} mg/mL
		Inhibition of spontaneous neutrophil motility	10^{-6}–10^{4} mg/mL
		Inhibition of fMLP-stimulated nitrite production	10^{-2} mg/mL
	RAW 246.7 cells	Inhibition of fMLP-stimulated nitrite production	10^{-2} mg/mL
[126]	Human saphenous vein endothelial cells (HSVEC)	Inhibition of LPS-stimulated expression of VCAM-1	100 nM and 1 μM
		Inhibition of LPS-stimulated adhesion of monocytoid U937 cells	1 μM
	Human umbilical vein endothelial cells (HUVEC)	Inhibition of TNF-α-stimulated expression of ICAM-1	100 nM and 1 μM
	Ea.hy 926 endothelial cells	Inhibition of TNF-α-stimulated neutrophil adhesion	100 nM and 1 μM
[127]	HUVEC	Inhibition of TNF-α-stimulated expression of VCAM-1	~μM range
[129]	HUVEC	Inhibition of thrombin-stimulated adenosin nucleotide release	1 pM to 100 μM
		Decrease of thrombin-stimulated neutrophil function	1 pM to 100 μM

Table 2. Continued

Ref.	Experimental system	Mechanism	Efficacious concentrations
[133]	Cellular suspension of PMN	Inhibition of fMLP-stimulated ferric cytochrome *c* reduction	44 μM
		Inhibition of fMLP-, C5a- and A23187-stimulated elastase and β-glucuronidase release	22–440 μM
		Inhibition of fMLP-stimulated MAC-1 expression	220–440 μM
		Inhibition of A23187-stimulated LTB4 production	220 μM
[135]	Human neutrophils	Inhibition of fMLP- and PMA-stimulated phosphatidic acid and diglyceride	IC_{50} (fMLP) = 42.4 μM IC_{50} (PMA) = 60.9 μM
[139]	RAW 264.7 macrophages	Inhibition of LPS-activated iNOS protein induction	3–30 μM
[140]	RAW 264.7 macrophages	Downregulation of LPS-activated iNOS mRNA and protein	0.1 mM
[141]	Mouse peritoneal exudate macrophages	Inhibition of LPS-activated NO induction	100 μM
[142]	Rat peritoneal macrophages	Inhibition of thioglycollate-stimulated reactive nitrogen intermediates production	10–100 μM
		Inhibition of PMA-stimulated ROM extracellular and intracellular production	1–10 μM
		Inhibition of *Kluyveromyces lactis* cell phagocitosis	10 μM
[143]	Mouse peritoneal macrophages	Inhibition of A23187- and fMLP-stimulated IL-6 release	$5 \cdot 10^{-6}$ to $4 \cdot 10^{-5}$ M
		Inhibition of fMLP-stimulated calcium ion influx into cells	10^{-8} to 10^{-5} M
[144]	Alveolar macrophages from broncho-alveolar lavage from cigarette smokers and from patients with chronic obstructive pulmonary disease (COPD)	Reduction of IL-1β-stimulated IL-8 and GM-CSF release	100 μM
[147]	Human polymorphonuclear leukocytes (PMN-L)	Inhibition of lypoxygenase products 5-HETE, 5,12-diHETE and COX product HHT	10^{-6}–10^{-3} M
		Inhibition of 15-HETE and PGE_2	10^{-4}–10^{-3} M
		Inhibition of A23187-stimulated lysozyme	10^{-4}–10^{-3} M
		Inhibition of A23187-stimulated β-glucuronidase release	10^{-3} and $5 \cdot 10^{-7}$ M to 10^{-6} M
		Inhibition of LTC_4	10^{-6}–10^{-4} M
[14]	Microsomes derived from sheep seminal vesicles as a crude source of COX-1 or recombinant human COX-2	Inhibition of COX activity of COX-1	ED_{50} = 15 μM
		Inhibition of hydroperoxidase activity of COX-1	ED_{50} = 3.7 μM
		Inhibition of hydroperoxidase activity of COX-1	ED_{50} = 85 μM
	Human promyelocytic leukemia (HL-60) cells	Inhibition of PMA-induced free radical formation	ED_{50} = 27 μM
	Salmonella typhimurium strain TM677	Inhibition of 7,12-dymethylbenz[*a*]anthracene (DMBA)-stimulated mutagenic response	ED_{50} = 4 μM
	Cultured mouse hepatoma (Hepa 1c1c7) cells	Induction of quinone reductase activity	21 μM
	Cultured HL-60 cells	Induction of expression of nitroblue tetrazolium reduction activity	ED_{50} = 11 μM
		Induction of acid sterase activity	ED_{50} = 19 μM
		Inhibition of [^{3}H]thymidine incorporation	ED_{50} = 18 μM
	Mouse mammary gland culture	Inhibition of the development of DMBA-induced preneoplastic lesions	ED_{50} = 3.1 μM
[157]	COX-1 purified homogeneity from ram seminal vesicles and human COX-2 purified from baculovirus infected *Sf*-21 cells	Inhibition of hydroperoxidase activity of COX-1	100 μM
[158]	Human mammary epithelial cell line 184B5/HER	Inhibition of synthesis of PGE_2 by suppression of COX-2 activity	10–30 μM
		Inhibition of PMA-induced COX-2 activity	2.5–30 μM
		Inhibition of PMA-mediated PGE_2 production	2.5–20 μM
		Inhibition of PMA-mediated COX-2 mRNA induction	2.5–20 μM
		Inhibition of PMA-mediated COX-2 transcription induction	15 μM
		Inhibition of PMA-mediated COX-2 promoter activity induction	5–15 μM
		Inhibition of PMA-mediated activation of PKC	15 μM

Table 2. Continued

Ref.	Experimental system	Mechanism	Efficacious concentrations
		Inhibition of PKC-α- and ERK-1-mediated induction of COX-2 promoter activity	15 μM
		Inhibition of AP-1-mediated induction of COX-2 promoter activity	15 μM
[159]	Murine resident peritoneal macrophages from CD-1 mice (Harlam)	Inhibition of LPS- and PMA-stimulated ROM effect	30 μM
		Decrease of LPS-stimulated [^{3}H]AA release	30 μM
		Decrease of PMA-stimulated [^{3}H]AA release	3–30 μM
		Decrease of ROS-stimulated [^{3}H]AA release	30 μM
		Inhibition of LPS-, PMA-, and ROM-stimulated COX-2 induction	30 μM
		Inhibition of LPS- and PMA-mediated PEG_2 induction	3–30 μM
[164]	Immortalized human keratinocytes line HaCaT	Increase of H_2O_2-induced VEGF protein and mRNA	2.5–10 μg/mL GSPE[a]
		Induction of VEGF transcription by GSPE	10 μg/mL GSPE
[177]	Cell-free immunoassay system. Purified ovine enzyme as source of COX-1 and human recombinant enzyme as source of COX-2	Inhibition of COX-1	IC_{50} = 0.535 μM
		Weak inhibition of COX-2	IC_{50} = 0.996 μM
[178]	Primary cultures of human mammary epithelial cells (17-CA, 18-CA and 46-CA)	Inhibition of *O*-acetyltransferase and sulfotransferase activation	50 μM
		Estimulation of PhIP[b]-DNA adducts in kinase reaction	50 μM
		Inhibition of PhIP-associated DNA adduct formation by *O*-acetyltransferase and sulfotransferase catalysis	50 μM
	Breast cancer cell lines ER[c]-positive MCF7 and ZR-75-1	Suppression of *O*-acetyltransferase and sulfotransferase activation	50 μM
		Reduction of PhIP-DNA adducts	50 μM
[179]	Calf thymus DNA	Inhibition of free radical-stimulated DNA damage	0.05–10 μM
[180]	Three different human breast cancer cell lines: ER[c]-positive MCF7, ER-positive T47D, and ER-negative MDA-MB-231	Inhibition of H_2O_2-stimulated T47D and MCF7 cell lines (anti-oxidant activity)	10^{-8} M
		Inhibition of PMA-induced T47D	10^{-8} M
		Decrease cell proliferation	10^{-12}–10^{-6} M
[181]	Multiple cell lines	Reduction of cells in the G2/M phase of the cell cycle	% IC_{50} ranged from 1.6 to 11.4
		Inhibition of cell growth	IC_{50} ranged from 22 to 109 μM
	Normal rat fibroblast (Rat-1)	Prevention of ROM production and DNA damage induced by oxidative stress	30 and 90 μM
[15] (review)	Multiple cell lines	Inhibition of cell growth	~5–10 μM
[158]	Mammary epithelial cells	Decrease in COX-2 expression	~5 μM
[186]	Porcine PKCα expressed in Sf9 insect cells by infection with a high titer recombinant baculovirus	Incorporation of resveratrol into phospholipid membranes	64–200 μM
		Inhibition of activated PKCα	IC_{50} = 30 μM
[188]	Promyelocytic cell line HL-60	Complete and reversible cell cycle arrest at the S-phase checkpoint	30 μM
[189]	Caco-2 human colon cancer cells	Inhibition of cell growth at the S/G2 phase transition of the cell cycle (antiproliferative effect)	25 μM
		Decrease of ornithine decarboxylase activity	25 μM
[190]	Human histiocytic lymphoma U937 cells	Complete and reversible cell cycle arrest at the S-phase checkpoint	30–60 μM

a) GSPE, grape seed proanthycyanidin extract containing 5000 ppm resveratrol
b) PhIP, 2-amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine
c) ER, estrogen receptor

Table 2. Continued

Ref.	Experimental system	Mechanism	Efficacious concentrations
[191]	Several human cancer cell lines	Inhibition of growth	100 μM
		Induction of apoptosis	300 μM
		Induction of S-phase cell cycle arrest	300 μM
		Decrease in the expression levels of cyclins D1, A and B1	300 μM
		Decrease in β-catenin expression and induction of COX-2 expression	300 μM
		Decrease of cyclin D1mRNA levels	300 μM
		Induction of cyclins D1 degradation	100 μM
[192]	HL-60 human leukemia cell line, T47D human breast carcinoma cells and normal human peripheral blood lymphocytes (PBLs)	Induction of caspase-mediated apoptosis in HL-60 cells	32 μM
		Enhance of CD95L and induction of CD95 signaling-dependent apoptosis in HL-60 and T47D cells	32 μM
		No affection of cell survival in normal human PBLs	32 μM
[193]	Several cell lines	Full destruction of tyrosil radical	10 μM
		Inhibition of ribonucleotidase reductase activity	100 pmol/mg-min
		Inhibition of DNA-synthesis	IC_{50} = 8–10 μM
[194]	Immortal and neoplastic human breast epithelial cells (MCF-7, ER-negative MCF-10F and MDA-MB-231)	Inhibition of cell growth	22–175 μM
[195]	Human breast cancer cell line MCF-7	Inhibition of cell growth	10^{-5} M
[196]	Human breast cancer cell line MDA-MB-231	Inhibition of cell growth	$5 \cdot 10^{-5}$ M
[197]	Three human breast cancer cell line (MCF-7, MDA-MB-231 and T47D cells)	Induction of T47D proliferation	10 μM
[198]	Human breast cancer cell line MCF-7	Stimulation of cell proliferation	10 nM to 10 μM
[199]	Colon tumor cells	Induction of p53-independent apoptosis	100 μM
[200]	Pulmonary artery endothelial cells (BPAE cells)	Inhibition of cell progression through S- and G2-phases of the cell cycle	50–100 μM
		Increase of tumor suppressor gene protein p53 expression	50–100 μM
		Elevation of cyclin-dependent kinase inhibitor p21 level	50–100 μM
[203]	Mouse epidermal cells	Activation of p53	20 μM
[204]	Androgen-sensitive prostate cancer cells (LNCaP)	Activation of p53	10^{-5} M
[205]	Leukemia cells	Induction of apoptosis	32–100 μM
[217]	Myeloid (U937), lymphoid (Jurkat), and epithelial (HeLa and H-4) cells	Inhibition of PMA-, LPS-, H_2O_2-, okadaic acid-, ceramide- and TNF-induced NF-κB activation	5 μM
		Inhibition of TNF-dependent phosphorylation and nuclear translocation of p65 subunit of NF-κB	5 μM
		Repression of TNF-induced NF-κB-dependent reporter gene expression	5 μM
		Inhibition of TNF-induced c-Jun kinase and MEK activation	5 μM
		Inhibition of TNF-induced AP-1 activation	5 μM
		Inhibition of TNF-induced cytotoxicity and caspase activation	5 μM
		Inhibition of TNF-induced reactive oxygen intermediates (ROIs) generation and lipid peroxidation	5 μM
[218]	Human pancreatic carcinoma cell line Mia PACA-2 and rat pancreatic carcinoma BSp73AS cells	Enhance of apoptosis	100 μM
		Inhibition of NF-κB activation	100 μM
		Activation ofcaspase-3	100 μM
[219]	Human monocyte (THP-1) and macrophage (U937) cells	Inhibition of TNF- and LPS-induced NF-κB activation	30 μM
		Inhibition of TNF-induced IKK activity	30 μM
[221]	Human monocytic cells U937	Inhibition of PMA-induced IL-8 protein production and mRNA accumulation	0.1–10 μM
[119]	A549 cells	Inhibition of NF-κB-, AP-1-, and cAMP-response element-dependent transcription	10^{-4} M

Table 2. Continued

Ref.	Experimental system	Mechanism	Efficacious concentrations
[223]	Human cervical cancer cell line (Caski cells)	Inhibition of PMA-stimulated MMP-9 release	50–100 μM
		Decrease of PMA-stimulated MMP-9 mRNA	75 μM
		Inhibition of PMA-induced NF-κB DNA-binding activity	75 μM
		Inhibition of PMA-mediated PKC-δ translocation	50 μM
[227]	Cell-based transfection assay in monkey kidney CV-1 cells	Activation of nuclear receptors PPAR-α and PPAR-γ	100 μM
	Several cell lines	Activation of nuclear receptors PPAR-α and PPAR-γ	10 μM

