

Resveratrol: A Natural Compound with Pharmacological Potential in Neurodegenerative Diseases

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Resveratrol is a phytoalexin structurally related to stilbenes, which is synthesized in considerable amounts in the skin of grapes, raspberries, mulberries, pistachios and peanuts, and by at least 72 medicinal and edible plant species in response to stress conditions. It was isolated in 1940 and did not maintain much interest for around five decades until its role in treatment of cardiovascular diseases was suggested. To date, resveratrol has been identified as an agent that may be useful to treat cancer, pain, inflammation, tissue injury, and other diseases. However, currently the attention is being focused in analyzing its properties against neurodegenerative diseases and as antiaging compound. It has been reported that resveratrol shows effects in *in vitro* models of epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and nerve injury. However, evidences *in vivo* as well as in human beings are still lacking. Thus, further investigations on the pharmacological effects of resveratrol *in vivo* are necessary before any conclusions on its effects on neurodegenerative diseases can be obtained.

Introduction

Flavonoids and stilbenes are biosynthesized through the shikimate pathway. They are important because they contribute to flavor and color of many fruits and plants, as well as normal growth, development, and defense [1]. Stilbenes are molecules of two phenolic rings connected by an ethene molecule, and can be classified according to the presence of different substituents on the aromatic rings and to the degree of ring saturation. Resveratrol is the main stilbene generated in considerable amounts in the skin of grapes, raspberries, mulberries, pistachios and peanuts, and by at least 72 medicinal and edible plants species such as *Polygonum cuspidatum*, *Veratrum grandiflorum*, *Arachis hypogaea*, *Rhizoma polygoni cuspidate*, *Yucca Shidigera*, *Cassia quinquangulata*, *Rheum rhamponiticum* among others [2,3]. It can be found as glucoside [4] forming oligomers [5], tetramers [6], trimers [7], and oxyresveratrol, which is also known as piceatannol [8].

Resveratrol (3,4',5-trihydroxystilbene) is a phytoalexin polyphenolic structurally related to stilbenes. It is found in two isomers, cis- (Z) and trans- (E) resveratrol. Ini-

tially, cis-resveratrol was considered biologically inactive; however, several reports have shown that both isoforms possess biological activity, although trans-resveratrol seems to be more potent than cis-resveratrol in most of the comparative studies indicating certain stereo-specific activity of the molecule [9]. Physically, it is a white powder with slight yellow cast, which is soluble in ethanol and dimethyl sulfoxide and very slightly soluble in warm water.

Resveratrol represents 5–10% of the biomass of grape skins and it is contained in concentrations ranging from 0.05 to 25 mg/L in most wines and grape juices [10] whereas its concentration in peanuts, pistachios, blueberries, and bilberries reach up to 5.1 $\mu\text{g/g}$. In addition, plants as *R. rhamponiticum* contain around 3.9 mg/g of this compound [3]. The fact that resveratrol is contained in a wide variety of dietary resources gives a reason to think that regular consumption of resveratrol in the diet may be useful for treatment of multiple illnesses in which it has shown pharmacological activity. The present review attempts to integrate the established pharmacological properties of resveratrol on central nervous system function.

Other actions such as those related with its cardiovascular [11] and cancer [12,13] effects have been previously reviewed.

History

The ancient cultures already knew beneficial properties of red wine. In the medicinal book of Hindus titled Ayurveda is described a fermented red grape juice used as a cardiogenic compound [14]. Consequently, the Holy Bible described to Timothy, a Christian believer who was allowed to drink a little wine because of his stomach and frequent illnesses. In 1940, resveratrol was isolated and identified from *V. grandiflorum* [15] and later on from the roots of *P. cuspidatum*, an oriental plant used in traditional medicine to treat fungal infections, dermatitis and hyperlipidemic diseases [16]. At the beginning, resveratrol was described as a phytoalexin synthesized in the leaf epidermis and grape skin from *Vitis vinifera* in response to infection or injury [17].

This compound did not maintain much interest for about 50 years until 1992, when Siemann and Creasy suggested that the resveratrol was the active ingredient in wines causing reduction of serum lipids. After this paper, many authors focused on its cardiovascular properties and resveratrol was postulated as a candidate to explain the low incidence of heart diseases in France (French paradox), a country with high-fat diet and moderate red wine consumption [18,19].

The attention came back to resveratrol after 1997, when it was shown that it could have chemopreventive activity [20]. Since then, the number of articles published each year on resveratrol as well as its properties has been increasing in an exponential manner. To date, several reports have clearly established that resveratrol is an anti-inflammatory, analgesic, antioxidant, and anti-ischemic compound [3,21,22]. Currently, the new interest on resveratrol is in identifying its properties and mechanisms in neurodegenerative diseases and aging [23].

Pharmacology

Epilepsy

The term epilepsy refers to a neuronal disorder characterized by the periodic and unpredictable occurrence of seizures whereas the term seizure refers to a transient alteration of behavior due to the disordered, synchronous, and rhythmic firing of populations of brain neurons [24]. Resveratrol has shown anticonvulsant activity in the pentylentetrazole, ferric chloride, and kainic acid models. In the model of pentylentetrazole, a chemconvulsant useful in identifying compounds that are ef-

fective against absence seizures in human beings, resveratrol reduced the incidence of generalized convulsions by an adenosinergic mechanism that did not involve A₂ adenosine receptor [25]. On the other hand, the ferric chloride model simulates seizures produced after a post-traumatic event where free radical formation seems to play a critical role in the neuronal disorder genesis. In this model, resveratrol delays the onset of epileptiform electroencephalographic changes and significantly reduces malondialdehyde levels, a marker of oxidative stress [26]. In addition, it attenuates the incidence of convulsions in the kainic acid model when this is administered as pre- and post-treatment or in chronic administration protecting from the damage in the olfactory cortex and hippocampus [27,28]. The main mechanism of action of classical antiseizure drugs, as carbamazepine, phenytoin, or valproate, is to promote the inactivated state of voltage-activated Na⁺ channels. In this regard, resveratrol has shown to act through this mechanism in dorsal root ganglion cells [29].

Alzheimer's Disease

The pathological hallmarks of Alzheimer's disease are due to the loss of hippocampal and cortical neurons that clinically manifest a gradual impairment of cognitive abilities accompanied often of aphasia, disorientation, and disinhibition. The alterations are produced by the formation of neurofibrillary tangles and senile plaques of protein β -amyloid which are the product of larger amyloid precursor proteins [30]. Epidemiological studies have shown that moderate red wine consumption can attenuate clinical dementia produced by Alzheimer's disease in humans [31]. Accordingly, red wine diminishes deterioration of Alzheimer's disease-related spatial memory in mice [32] and it increases the cellular viability in cultures [33]. Furthermore, resveratrol has shown a memory improvement in different behavioral tests [34,35].

Resveratrol treatment markedly inhibits polymerization of the β -amyloid peptide [36] by a mechanism that does not involve β -amyloid production because resveratrol has no effect on activity of β - and γ -secretases but stimulates indirectly the proteosomal degradation of β -amyloid peptides [37]. Han *et al.* [38] have reported that the protective effects of resveratrol on β -amyloid protein-induced toxicity in cultured rat hippocampal cells are specifically related to activation of protein kinase C. In addition, Miloso *et al.* [39] showed that resveratrol activates ERK1/2 kinases in neuroblastoma cells. Besides these described pathways, it has been postulated that resveratrol produces its neuroprotective activity against Alzheimer's disease through enhancing the intracellular free-radical scavenger glutathione [40], decreasing malondialdehyde

Table 1 Pharmacokinetic parameters of resveratrol and its main metabolites

Dose	Treatment	Specie	Cmax	T max	t _{1/2}	AUC	CL _T	References
Oral resveratrol								
Free resveratrol								
25 mg	Single dose	Humans	<0.02 μM	1.0 h	–	–	–	96
25 mg	Single dose	Humans	~0.03 μM	0.5 h	–	–	–	92
500 mg	Single dose	Humans	0.3 μM	0.8 h	2.9 h	1.0 $\mu\text{M}\cdot\text{h}$	2.2 L/h	97
5000 mg	Single dose	Humans	2.4 μM	1.5 h	8.5 h	5.9 $\mu\text{M}\cdot\text{h}$	4.9 L/h	97
0.1 mg/kg	Single dose	Rats	0.07 μM	0.5 h	–	–	–	98
0.1 mg/kg	15 days	Rats	0.03 μM	–	–	–	–	98
2 mg/kg	Single dose	Rats	2.4 μM	0.16 h	–	–	–	100
5 mg/kg	Single dose	Rats	0.1 μM	1.5 h	–	–	–	128
20 mg/kg	Single dose	Rabbits	1.1 μM	0.05 h	0.24 h	–	–	99
20 mg/kg	Single dose	Rats	1.2 μM	0.1 h	–	–	–	99
20 mg/kg	Single dose	Mice	2.6 μM	0.5 h	–	–	–	99
2.6 mg/kg	10 days	Mice	0.08 μM	–	–	–	–	99
50 mg/kg	Single dose	Rats	6.6 μM	0.3 h	1.5 h	7.1 $\mu\text{M}\cdot\text{h}$	32.4 L/h/kg	101
4000 mg	Single dose	Mice	30.2 μM	0.5 h	–	–	–	102
Resveratrol glucuronide								
500 mg	Single dose	Humans	1.8 μM	2.0 h	2.9 h	8.6 $\mu\text{M}\cdot\text{h}$	282.7 L/h	97
5000 mg	Single dose	Humans	5.7 μM	2.0 h	7.9 h	44.2 $\mu\text{M}\cdot\text{h}$	590.6 L/h	97
50 mg/kg	Single dose	Rats	105.2 μM	0.4 h	1.6 h	324.7 $\mu\text{M}\cdot\text{h}$	0.7 L/h/kg	101
4000 mg	Single dose	Mice	534 μM	1.0 h	–	–	–	102
Resveratrol sulphate								
500 mg	Single dose	Humans	5.1 μM	1.5 h	3.2 h	18.0 $\mu\text{M}\cdot\text{h}$	131.2 L/h	97
5000 mg	Single dose	Humans	19.1 μM	2.1 h	7.7 h	137.6 $\mu\text{M}\cdot\text{h}$	207.8 L/h	97
4000 mg	Single dose	Mice	386 μM	1.0 h	–	–	–	102
Intravenous resveratrol								
Free resveratrol								
15 mg/kg	Single dose	Rats	–	–	1.3 h	5.6 $\mu\text{M}\cdot\text{h}$	11.7 L/h/kg	101
Resveratrol glucuronide								
15 mg/kg	Single dose	Rats	–	–	1.5 h	38.7 $\mu\text{M}\cdot\text{h}$	1.7 L/h/kg	101

of resveratrol in several studies is not clear because the red wine used in that studies contains very low amounts of resveratrol (0.88 μM), which is 10-fold lower than the minimal effective concentration shown to produce its *in vitro* effects (10–40 μM , Table 1; 32,37).

Parkinson's Disease

Parkinson's disease is a neurodegenerative disease characterized by a selective loss of dopaminergic neurons in the substantia nigra pars compacta that provide dopaminergic innervations to the striatum and its neuronal toxicity is related with the aggregates formation called Lowry bodies. Clinical syndrome includes resting tremor, muscle rigidity, bradykinesia, impairment of postural balance, and difficulty in walking [30].

Numerous theories have been described to explain the cell death of nigrostriatal dopaminergic neurons in Parkinson's disease. Dopamine theory establishes that this catecholamine can be oxidized spontaneously or by some enzymes as monoamine oxidase B or

cyclooxygenase-2 to generate free radicals [46,47], then dopamine oxidation products can suffer polymerization to form a neurotoxin called neuromelanin [48]. In this regard, low doses of resveratrol (5 μM) markedly attenuate dopamine-induced cell death in neuroblastoma cells by activation of the antiapoptotic factor Bcl-2 and inhibition of caspase-3 [49]. Another theory suggests that the nitric oxide is involved in the generation of superoxide radicals and lipid peroxidation, which leads to the release of arachidonic acid. Chalimoniuk et al. [50] demonstrated that resveratrol (0.1 mM) prevents nitric oxide- and cyclic GMP-dependent inhibitory effect on arachidonic acid incorporation into phosphatidylinositol possibly by nitric oxide synthase inhibition. Although this mechanism may be used to explain the neuroprotective effect of resveratrol in Alzheimer's and others neurodegenerative diseases, as resveratrol concentrations greater than 6 μM were used, it is likely that this mechanism has no clinical relevance. In addition, it has been postulated that the induction of heme-oxygenase by antioxidants may protect against neuronal disorders [51].

Huntington's Disease

Huntington's disease is a fatal neurodegenerative disorder caused by the expansion of a cytosine adenine guanine (CAG) triplet-repeat in the huntingtin gene, which is localized on chromosome 4p16.3 and that leads to an extended polyglutamine domain called PolyQ. The repeat length in normal subjects varies from 9 to 34 repeats but in patients with the illness the number of repeats is over 40. This neurodegenerative disorder is characterized by prominent loss of neurons in the striatum of the brain although other areas can be affected in a minor grade. Atrophy of these structures produces a gradual onset of ataxia, personality changes, and dementia in midlife [30]. At molecular level, mutant huntingtin polypeptides acquire an unusual conformation facilitating their aggregation into inclusion bodies, which impair vesicular and mitochondrial traffic and cause mitochondrial dysfunction leading to an increase in free radicals production and cell death [52].

Kumar et al. [53,54] showed that resveratrol (5–10 mg/kg) reverses motor and cognitive impairment generated in rats by administration of 3-nitropropionic acid or colchicine through its antioxidant activity. In another study, Solans et al. [52] developed a Huntington's disease-like model in yeast cells where they found that the interactions of huntingtin polypeptides aggregate to the mitochondrial and actin networks cause alterations in the function of mitochondrial respiratory chain complex II and III activity leading to mitochondria-dependent cell death. In this model, resveratrol (10–50 μM) significantly reduced the amount of free radicals produced in the mutant strain but it did not diminish the size of polyQ aggregates, mitochondrial fragmentation, or the rate of growth of the strains control and mutant suggesting that the resveratrol plays a role delaying but not inhibiting disease development. Others have demonstrated that resveratrol (10–100 μM) decreases cell death of neurons derived from 111Q knock-in mice by sirtuin activation (*Sir2*) and not simply for its antioxidant properties [55]. Likewise, it rescues *Caenorhabditis elegans* worms carrying 128Q in a dependent-manner of *sir-2.1* and *daf-16* being the latter a member of FOXO family. However, reduction of free radicals leading to a delay of Huntington's disease as well as sirtuin activation should be taken with caution as these mechanisms were observed after high resveratrol concentrations (10–100 μM).

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis is a neuromuscular disorder generated in motor neurons of the spinal, bulbar, and cortical regions. It is a progressive degenerative disease

leading to muscle weakness and eventually paralysis with a fatality within 2–3 years of onset [30]. Only 2% of all cases are due to a mutation in the enzyme superoxide dismutase 1 [56]; however, the most cases are sporadic and their mechanisms are unknown.

It has been observed that resveratrol (50–500 nM) reduces mutant superoxide dismutase-induced neurotoxicity in transfected neurons of mice by sirtuin-1 protein activation [35]. Moreover, resveratrol and riluzole stimulate activity of large-conductance Ca^{2+} -activated K^{+} channels in vascular endothelial and neuroendocrine cells, respectively. As riluzole is a drug used for the treatment of amyotrophic lateral sclerosis [57], data suggest that the resveratrol could be a promise of treatment in this disease.

Neuronal Injury

It is well established that the neurons are highly susceptible to oxidative injury due to its high consumption of oxygen. Several preclinical studies have suggested that resveratrol may be useful in protecting brain and spinal cord against traumatic neuronal injury following contusion or ischemia-reperfusion injury because resveratrol modulates different oxidative stress neuronal markers *in vivo* at the injury site. In brain, resveratrol decreases lactate dehydrogenase, xanthine oxidase, metalloproteinase 9, heme-oxygenase, and malondialdehyde levels, and it increases reduced glutathione levels induced by several types of injury such as cerebral artery occlusion, cerebral ischemia-reperfusion, focal cerebral ischemia, and streptozotocin-induced diabetes [58–60]. Tsai et al. [60] found that, after ischemia-reperfusion injury, resveratrol produces a differential expression of nitric oxide synthases. In this study, inducible nitric oxide synthase was decreased, whereas endothelial nitric oxide synthase was increased and there was no change in neuronal nitric oxide synthase expression giving a possible explanation of controversial effects that produce resveratrol on nitric oxide. Moreover, resveratrol inhibits peroxisome proliferator-activated receptors alpha (PPARs; 61] and decreases $\text{NF}\kappa\text{B}$ p65 expression [62] involved in inflammation after ischemia-reperfusion injury. In cultures, resveratrol inhibits postsynaptic glutamate receptors, voltage-activated K^{+} channels [63], and it increases heme-oxygenase activity [64]. All these effects decrease anxiety, improve motor and cognitive skills, and attenuate edema and cellular death as well as glial cell activation [65,66]. Moreover, there are suggestions that neuroprotective effects of resveratrol on memory improvement are mainly due to its antioxidant properties [53,54].

The effects in spinal cord are similar to those described in brain [67,68]. In addition, ionic microenvironment

changes after spinal cord injury have been suggested as potent therapeutic targets. In support of this idea, resveratrol improved Na^+ , K^+ -ATPase activity and augmented Ca^{2+} -ATPase, Mg^{2+} -ATPase and Ca^{2+} , Mg^{2+} -ATPase activities protecting the spinal cord of ionic and energetic imbalance produced by ischemic injury or contusion [69]. Studies in cultures show that resveratrol not only has a protective role against reactive oxygen species but also modulates important glial functions, such as glutamate uptake activity, glutathione content and S100B secretion improving functional recovery, diminishing DNA fragmentation and apoptosis [70,71].

Although *in vivo* experiments have shown that the resveratrol has beneficial effects on spinal cord injury in rodents [65,67,69,60], some of the putative mechanisms of resveratrol have been obtained using *in vitro* preparations and concentrations greater (10–250 μM) than those reached in humans (6 μM) [61,63,71]. Thus, caution is mandatory when interpreting these mechanisms.

Pain

Neuropathic pain is a chronic pain state that affects up to 10% of people in United States and Europe. It is characterized by abnormal sensory processing resulting from lesions to nervous system where the conventional analgesics often have limited efficacy and undesirable side-effects [72].

Resveratrol reduces tactile allodynia produced by neuropathic pain in some animal models as the L5/L6 spinal nerve ligation [73] and diabetic neuropathy [74–76]. Bermúdez-Ocaña et al. [73] observed that the antiallodynic activity of spinal resveratrol can be attributed to the activation of the nitric oxide-cyclic GMP-protein kinase G-large-conductance Ca^{2+} -activated K^+ channel pathway, whereas the antiallodynic effect observed in diabetic neuropathy was attributed to the reduction of oxidative stress produced in that pathology. The proposed mechanisms in this model include inhibition of reactive oxygen species (ONOO^-), inhibition of the synthesis and release of pro-inflammatory mediators (tumor necrosis factor- α), and activation of endogenous antioxidant enzymes (catalase) that leads to a reduction in lipid peroxidation and DNA fragmentation. These effects also improve nerve blood flow and motor nerve conduction velocity deficits of diabetic rats attenuating the hyperalgesia and allodynia associated to oxidative stress [54,74–76].

Recently, a study carried out by Kim et al. [29] showed that the resveratrol inhibits both tetrodotoxin-sensitive and -resistant Na^+ channels in rat dorsal root ganglion neurons causing a hyperpolarizing shift of the steady-state inactivation and reducing the recovery from inactivation of both Na^+ currents. All these activities point

toward the potential of resveratrol in the treatment of neuropathic pain.

Lifespan

Calorie restriction is a dietary regimen in which an organism is provided with 30–50% fewer calories than it would naturally consume *ad libitum* [77]. It has been demonstrated that the calorie restriction increases lifespan in a wide diversity of organisms, such as yeast, fruit flies, nematodes, crustaceans, spiders, rodents, primates, and humans [78,79]. Emerging data point out that sirtuins are responsible for extending lifespan in all these animals, they are NAD^+ -dependent deacetylases and mono-ADP-ribosyl transferases that receive their names of silent information regulator (Sir2) protein discovered in *Saccharomyces cerevisiae*, Sir2. Sirtuins are conserved proteins evolutionarily that have been associated to silencing of genes at the mating-type loci and telomeres, as well as cell cycle progression and chromosome stability to promote survival and stress resistance under adverse conditions.

In 2003, Howitz et al. [80] screened over 20,000 molecules to identify those that enhance SIRT1 activity *in vitro*, turning out resveratrol to be (11–200 μM) the most potent SIRT1-activating compound. Researchers found that resveratrol (5–10 μM) mimics calorie restriction by stimulating Sir2, extending yeast lifespan by 70%. In support of this data, resveratrol had no effect on lifespan under glucose restriction or in Sir2 null mutant yeast. A second study [81] showed that resveratrol (100 μM) could extend lifespan of *Caenorhabditis elegans* and *Drosophila melanogaster* up to 14 and 29%, respectively, but failed to extend lifespan in Sir2 null mutant animals or in animals with a restricted diet suggesting that the resveratrol extends lifespan through a mechanism related to calorie restriction. Likewise, resveratrol modestly increased egg production in fruit flies at the beginning of the adult life. Valenzano et al. [82] have reported similar results in *Nothobranchius furzeri*, a short-lived seasonal fish. Supplementation with resveratrol resulted in a maximum extended lifespan of 59%. In addition, resveratrol delayed locomotor and cognitive aging and the offspring of resveratrol-treated fishes developed into normal adults. Recently, Baur et al. [3] administered resveratrol to mice on a high-caloric diet finding an increment of lifespan of 31% with improved motor skills. These mice had lower levels of markers that predict diabetes, decreased liver, pancreas and heart damage, and increased hepatic mitochondrial number suggesting that at the tested doses (12.5–50 μM) resveratrol not only extends lifespan but also improves general health. So far, it is difficult to assess the relevance of these mechanisms, as some of them are produced with concentrations greater than those

reached in human beings. Thus, future studies should consider the concentration issue in order to get relevant conclusions.

Others

Besides its above-mentioned pharmacological properties in several diseases, resveratrol protects against high-caloric diet-induced-obesity [83,84] and it increases athletic performance [84]. In addition, several studies have found that resveratrol inhibits the replication and shows activity against several viruses such as herpes simplex [85], human cytomegalovirus [86], influenza virus A [87], varicella-zoster [88], and human immunodeficiency virus-1 [89]. Moreover, resveratrol has protective effects on noise-induced hearing loss [90] and it diminishes glucose in normal and diabetic rats [91].

Pharmacokinetics

Absorption

Resveratrol is well absorbed after oral administration, but it has a low bioavailability (around 2%) because it is metabolized rapidly in intestine and liver [92]. Two studies carried out in human intestinal epithelial cells show that resveratrol is absorbed via multidrug resistance-associated proteins 2 and 3 with extensive sulphate conjugation [93,94]. However, resveratrol sulphatation can be inhibited by different natural flavonoids or by some drugs [95], which have demonstrated to inhibit sulfo-transferases, suggesting that the consumption of these polyphenols with resveratrol could increase the bioavailability of the free compound. However, another study disputed these results, as they were not able to detect free compound in humans [96]. Doses of 0.5 and 5 g showed blood peaks of 0.3 and 2.4 μM at 0.83 and 1.5 h, respectively [97].

In addition, the daily administration of 2-mL of red wine containing 6.5 mg/L of total resveratrol for 15 days in rats showed an accumulation of this compound in heart, liver, and kidney but the concentrations were lower than those required for its pharmacological activity [98].

In animals, bioavailability of free resveratrol seems higher than humans, so the administration from 2 to 50 mg/kg in rats reaches blood concentrations from 1.2 to 6.6 μM in around 10 min [99–101]. A second peak is observed over the 4- to 8-h time period when enterohepatic recirculation occurs [97,101].

Interestingly, peak levels of resveratrol conjugates are up to 17 times higher than parent molecule [97,101,102] suggesting that some pharmacological activities could be due to resveratrol metabolites. In this regard, a study car-

ried out in mice showed that the resveratrol-glucuronide and resveratrol-sulfate reached a maximum concentration of 534 and 386 μM , respectively. In contrast, free resveratrol reached only 30.2 μM in mice after a dose of 4 g/kg [102]. However, as conjugation is a process to inactivate xenobiotics rendering them water-soluble to facilitate their elimination, it is unlikely that these conjugates may have the potency of resveratrol.

Distribution

After a single oral dose of 240 mg/kg, the higher peak concentrations and the greater area under tissue concentration of resveratrol were found in small intestine, heart, liver, and lung [103]. At 18 h, free resveratrol is the main form retained in liver, heart, lung and brain [104]. The binding of the compound to plasma proteins seems to occur particularly on albumin [105].

The plasma half-life of free resveratrol is variable in humans and it is ranging from 2 to 14 h [96,97], whereas in rats the half-life has a value average of 1.4 h [101]. The rest of the pharmacokinetic parameters are summarized in Table 1.

Metabolism

It has been demonstrated in mice, rats, and humans that the resveratrol undergoes extensive first-pass glucuronidation and enterohepatic recirculation, which contribute to the rapid plasmatic declination observed over the first 2 h. In addition, the uptake of resveratrol in hepatic cells occurs through a carrier-mediated transport besides passive diffusion [93,101].

Isoforms of human liver cytochrome P450 enzymes involved in the hepatic biotransformation of resveratrol include CYP1A1, CYP1A2, and CYP1B1 [106]. In turn, resveratrol can inhibit activity or expression of CYP1A1, CYP1A2, CYP1B1, CYP2A4, CYP2A5, CYP2B9, CYP3A4, CYP3A5, and CYP2E1 [107–111]. Recently, a study performed in HepG2 cells demonstrated that resveratrol autoinduces three phase II metabolizing enzymes, two isoforms of UDP-glucuronosyltransferases (UGT1A1 and UGT2B7) and a sulfotransferase (ST1E1; 112). These effects on drug metabolism suggest that the resveratrol acts to increase body detoxification, which may contribute to delaying the onset of neurodegenerative diseases.

Several metabolites have been identified in human and rats. At least, 11 of them have been well characterized [Fig. 2].

Excretion

Approximately 50–80% of the compound is excreted in urine and feces and only 0.04% of free resveratrol is

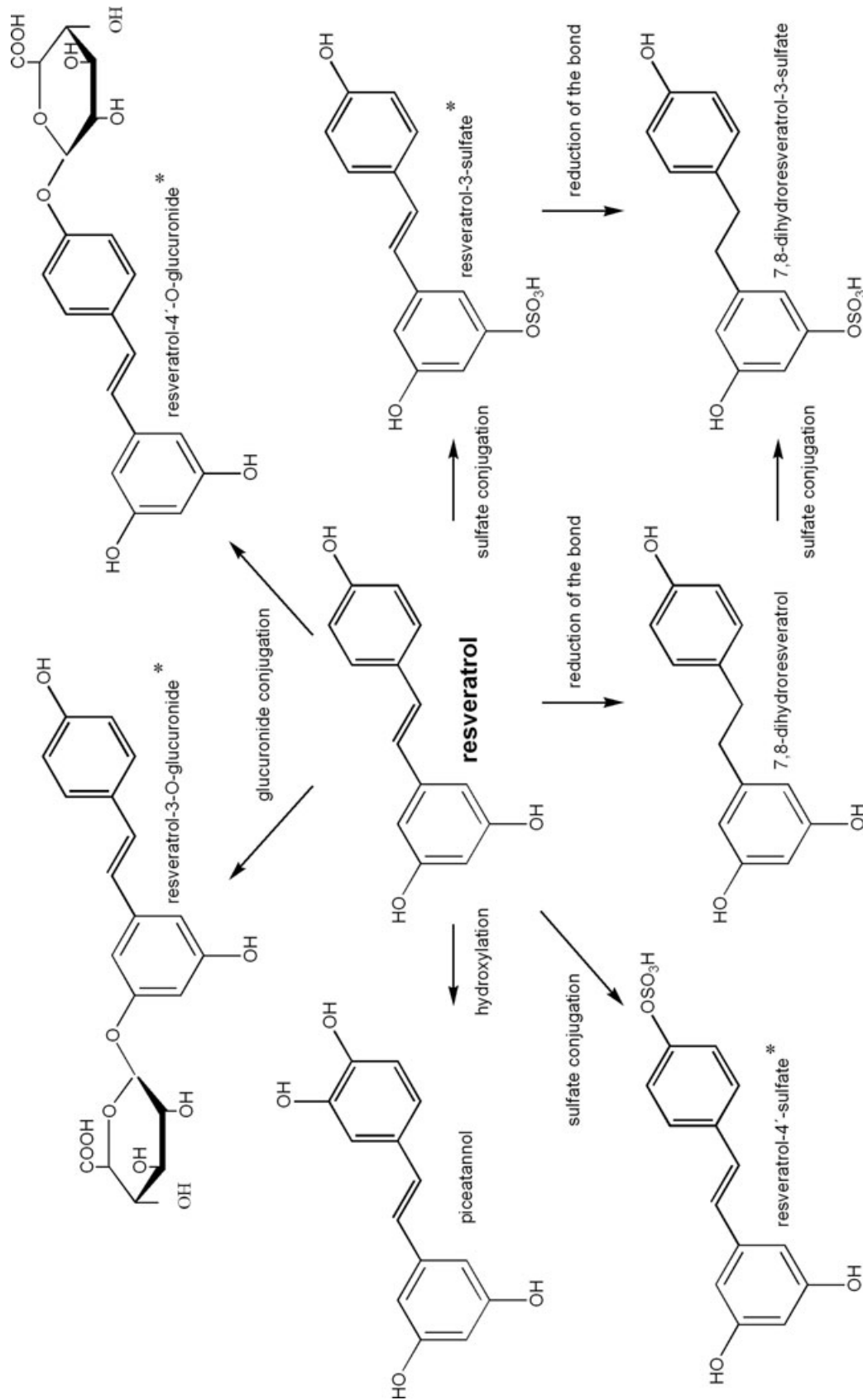


Figure 2 Proposed metabolic pathway of resveratrol. Metabolites were identified in rat or human urine and in human liver microsomes. The asterisk indicates the metabolites identified as cis- and trans-isomers but were excluded for image clarity. Cis-isomers are glucuronidated and sulfated faster than trans-isomers. Data obtained by Piver *et al.* [106], Wang *et al.* [126], and Urpi-Sarda *et al.* [127].

excreted in the urine within 24 h post-administration [96,97].

Toxicology

While the therapeutic potential of resveratrol has been increasing year by year and its health benefits seem promising, there are only few studies about resveratrol toxicity. A study performed by Juan et al. [113] showed that the repeated oral administration of resveratrol (20 mg/kg/day) did not produce any hematological or biochemical change nor signs of toxicity in the organs. However, rats that received higher doses of resveratrol (3 g/kg/day for 4 weeks) showed a significant increase in the clinical signs of toxicity. These signs included reduced body weight and food consumption, as well as biochemical and hematological changes. In addition, histopathology revealed nephropathy and hyperplasia in the bladder [114]. In mice, resveratrol (2 and 4 g/kg/day) induced mortality associated with impaction and accumulation of the compound in the gastrointestinal tract. In this study, authors found increased liver weight and serum cholesterol and mild anemia at the highest dose, as well as hydronephrosis and epithelial hyperplasia in the urinary bladder [102].

In humans beings, the consumption of single doses of resveratrol (0.5, 1, 2.5, or 5 g) did not cause serious adverse effects [97]. However, more randomized clinical trials with protocols of chronic administration are necessary to establish a valid conclusion about the safety of resveratrol in human beings.

To investigate the estrogenicity of trans-resveratrol during the development and its possible teratogenic effects, several studies have been performed in animals. In a study, adult mice received 3 mg/L of resveratrol in drinking water. After the offspring, animals were exposed to resveratrol during gestation, lactation, prepubertal period and pubertal period, up to adulthood. Mice parents did not show any alteration but the offspring presented a decreased kidney weight compared to parents. In addition, males and females in the first generation presented a decreased weight in seminal vesicles and ovaries, respectively [115]. In support of this study, Turner et al. [116] reported that resveratrol had no toxic effects in weanling rats of 3-week old. In contrast, some authors have observed changes in adult and weanling rats treated with resveratrol, which include alterations in sociosexual behavior and in organs related to reproduction [117–120]. In summary, resveratrol produces minor effects in adult animals and their offspring although more exhaustive studies are required before resveratrol can be recommended for pregnant mothers and children.

Finally, resveratrol is a polyphenol, which can be oxidized by polyphenol oxidases to quinonic compounds that, in turn, are capable to react with amino acids or proteins and to promote cancer initiation [121]. For instance, CYP3A converts resveratrol into p-benzoquinone, a quinonic compound, which binds covalently to CYP3A4 and inactivates it [122].

Clinical Studies

The Food and Drug Administration authorized trans-resveratrol as an investigational new compound on January 30, 2001. To date, it is only sold as a nutritional supplement and there are few studies on its bioavailability and pharmacological effects in humans. Goldberg et al. [123] investigated 24 healthy subjects who consumed red or white wine and two different grape juices during 4 weeks. They concluded that the favorable effects of wines observed in plasmatic lipids and lipoprotein concentrations are probably due to their alcohol content because these effects were not reproduced by grape juices. Accordingly, two studies showed an efficient absorption but very low bioavailability of unchanged resveratrol in plasma [92,96] in 12 and 6 volunteers, respectively, demonstrating that orally administered resveratrol is insufficient to explain any possible biological activity in humans. The main problem of low availability of free resveratrol seems to be its extremely rapid conjugation by the intestine and liver. Considering this, a phase I study, carried out in forty volunteers who received high doses of resveratrol (until 5 g) found that maximal concentrations of free resveratrol at the highest dose was around 2.4 μM [97]. This value was below the resveratrol concentrations required in *in vitro* assays to produce diverse pharmacological effects such as inhibition of NF- κB and AP-1 or activation and inhibition of cytochrome P450 enzymes [124] but it could explain inhibition of adhesion molecules and phosphorylation of ERK_{1/2} [39,125].

So far, there are no clinical trials about resveratrol's effects on epilepsy, pain, depression, Huntington's disease, Parkinson's disease, and aging. Contrariwise, Orgogozo et al. [31] conducted a prospective study in 3,777 volunteers aged 65 and over to assess the effects of red wine, and indirectly resveratrol, on incidence of dementia and Alzheimer's disease. This study showed that people drinking three to four glasses of red wine per day had an 80% decreased incidence of dementia and Alzheimer's disease three years later, compared to those who drank less or did not drink at all. Larger follow-up as well as possible side effects associated to red wine consumption are not reported in this study. Although this is a well-conducted study, it does not support the claimed beneficial effect of resveratrol on Alzheimer's disease

in humans because such effects could be due to other flavonoids present in red wine. Taken together, clinical data suggest that the resveratrol's effects, if any, still need to be demonstrated.

Concluding Remarks

In the recent years, there has been an extraordinary interest in resveratrol activities. Based on preliminary data, resveratrol could provide cellular resistance against insults that involve oxidative stress-induced injury. However, more systematic preclinical work as well as well-conducted clinical trials are necessary before it can be prescribed as a potential prophylactic compound in either acute or chronic conditions such as stroke, amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, neuronal injury, neuropathic pain, and a variety of age-related disorders.

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Conflict of Interest

The authors have no conflict of interest.

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