Resveratrol: From Basic Studies to Bedside

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Abstract

Plants produce a remarkable amount of low molecular mass natural products endowed with a large array of pivotal biological activities. Among these molecules, resveratrol (3,5,4'-trihydroxystilbene) has been identified as an important modulator of cell phenotype with a complex and pleiotropic mode of action. Extensive literature regarding its activity, mainly employing cellular models, suggests that this polyphenol controls cell proliferation, induces differentiation, and activates apoptosis and autophagy. The compound also modulates angiogenesis and inflammation. Similarly, studies on implanted cancers and chemical-induced tumors confirm the potential chemotherapeutical interest of the compound. Likewise, several reports clearly demonstrated, in animal models, that the compound might positively affect the development and evolution of chronic diseases including type 2 diabetes, obesity, coronary heart disease, metabolic syndrome, and neurogenerative pathologies. Finally, a number of investigations stated that the toxicity of the molecule is scarce. Despite these promising observations, few clinical trials have yet been performed to evaluate the effectiveness of the molecule both in prevention and treatment of human chronic disease. Preliminary findings therefore suggest the need for more extensive clinical investigations.

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Abbreviations

AKT	cellular homolog of the transforming v-Akt protein
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
BSA	Body surface area
CD95	Cluster of differentiation 95
cdc2 kinase	Cell division control protein 2 kinase
Egr-1	Early growth response protein 1
FOXO	Forkhead box class O
HIF	Hypoxia-inducible factor
IGF-1	Insulin-like growth factor 1
MAP kinase	Mitogen-activated protein kinase
NAD	Nicotinamide adenine dinucleotide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
p21^{Cip1}	21 kDa protein cyclin-dependent kinase inhibitor protein 1
PDE	Phosphodiesterase
PGC-1α	Peroxisome proliferator–activated receptor gamma coactivator 1-alpha
PI3K	Phosphatidylinositol 3-kinases
PKC	Protein kinase C
Sirt-1	Sirtuin 1
TRAIL	TNF-related apoptosis-inducing ligand

Contents

1   Introduction

Resveratrol (Fig. 1) was mentioned for the first time in 1939 by Takaoka, who isolated it from "*Veratrum album*" [89]. The name of the polyphenol presumably comes from its occurrence in the resin of a *Veratrum* species. In 1997, Pezzuto and
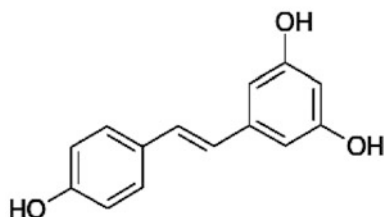
Resveratrol (3,5,4'-trihydroxy-
trans-stilbene)

Fig. 1 Chemical structures of trans-resveratrol. Resveratrol (3,5,4′-trihydroxystilbene) is a derivate of stilbene (stilbenoid) and exists as two diastereomers: cis- (Z) and trans- (E). The trans-form is the preferred steric form in nature and is relatively stable. It can undergo isomerization to the cis- form when exposed to ultraviolet irradiation

colleagues published a study reporting that extracts of the non-edible Peruvian legume "*Cassia quinquangulata Rich*" (Leguminosae) showed a potent cycloxygenase 2 inhibitory activity [45]. They also found that resveratrol was the active principle of the extract [45].

In Pezzuto's paper, as harbinger of things to come, a large number of resveratrol anticancer activities were reported, affecting all the steps of cancerogenesis, namely initiation, promotion, and progression. Thereafter, an exponential number of reports on resveratrol accumulated and, so far, more than 5000 studies have been published (Fig. 2). In 1998, the effect of resveratrol on the growth and
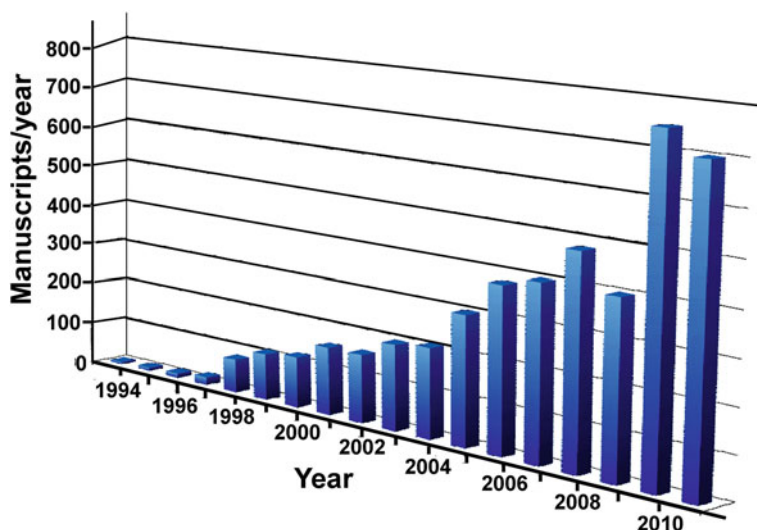

Fig. 2 Number of papers concerning resveratrol per year. The graph reports the number of published investigations during the period 1994–2011. The values were derived from Pub.Med.gov

differentiation of HL-60 cell line, a human promyelocytic cell line, was reported. In this study it was demonstrated, for the first time, that the molecule induces the myeloid commitment of the cells by hampering a specific cell cycle transition, that is, from G2 to M [29]. Importantly, biochemical analysis demonstrated definite changes in the cell division cycle engine, that is, the accumulation of cyclin A and the hyperphosphorylation of cdc2 kinase [29].

Although resveratrol is ubiquitous in nature, it is found in a limited number of edible substances, most notably in grapes. In turn, due to the peculiar processing methodology, resveratrol is found predominantly in red wines. Thus, resveratrol received intense and immediate attention. The notion that red wine prevents cancer and other diseases was very appealing and was strictly correlated to the so-called French Paradox. The correlation is now strongly questioned and, most intriguing, even whether or not a "French Paradox" exists is a matter of debate.

In brief, since 1997, resveratrol has been suggested to promote health in relation to various diseases or sufferings, covering a broad range of pathologies including cancer, heart disease, neurodegenerative pathologies, aging, inflammation, obesity, and diabetes. These diseases are clearly strictly interconnected and, for example, positive effects on obesity, inflammation, and aging might be important in the prevention of malignant transformation. Here, we will discuss the relationship between resveratrol and cancer, also taking into account the possibility of its use in therapy.

2   Resveratrol Effects on Established Cell Lines

The activities of resveratrol on cells have been extensively investigated and several hundred studies have been published on this topic. The majority of cellular models employed have been established from malignant tissues. Thus, these investigations suffer not only from the artificial in vitro growth conditions, but also from the strong intrinsic phenotypic variability due to the genetic and molecular alterations specific to the cancer of origin. Conversely, few analyses have been performed on cells derived from normal tissues.

The major phenotypic effects include the arrest of growth (at different phases of the cell cycle) [12, 13, 23, 29, 33, 52, 69]; the induction of differentiation [5, 14, 27, 29, 46, 47, 53, 104]; the activation of apoptosis, necrosis, and autophagy [1, 35, 55–57, 61, 63, 72, 73, 76, 92, 102]; anti-inflammatory activity [54, 107]; and interference with tumor angiogenesis [2, 16, 19, 85] among others. These activities appear particularly interesting in the field of human cancer treatment since they affect the major aspects of human malignant transformation [40]. In some studies, resveratrol has been associated with other compounds (frequently chemotherapeutics) that increase or hamper its effects. Several excellent reviews have critically appraised the ample literature on the ex vivo resveratrol activities, and this is not the aim of the present review [96 and references therein]. Interestingly, some studies report that the effects of the molecule are different at distinct

concentrations, inducing proliferation at low level and showing an anticancer function at higher concentration [18].

Some general conclusions might be drawn from the studies on cell lines. First, the polyphenol is endowed with a very large variety of promising biological activities that, in the main, are not related to resveratrol antioxidant capability. Second, the efficacious concentrations generally range between 10 and 50 μM. Third, the effects of the molecule frequently vary in relation to the concentration employed. Thus, resveratrol might be considered a hormetic compound in that the amount of molecule used is of critical importance for its activity [18]. This variability must be taken into consideration when translating in vitro experiments into clinical settings.

3   The Molecular Bases of Resveratrol Activity

The evaluation of the efficacious doses of an anticancer agent requires robust knowledge of its mechanisms of action. Over the past two decades, the molecular activities of resveratrol have been the subject of a vast number of investigations.

A multitude of data implicates resveratrol in an intricate web of pathways confirming the pleiotropic nature of the compound.

The molecule modulates various transduction pathways, including those correlated to MAP kinase [8, 12, 31, 105]; JNK [73, 79, 100], NF-κB [13, 14, 33, 42, 48, 55], AKT/PI3K [38, 39, 69, 98], PKC [7, 60, 75, 80, 86], CD95/TRAIL [26, 35, 56, 77, 78], and FOXO [23, 85].

Resveratrol controls apoptosis by altering the level of p53 [36, 90, 108]; caspases [3, 21, 64]; survivin [6, 41]; Bax, Bcl-2, and Bcl-xL [68, 70, 92]. The compound inhibits cyclooxygenase [87, 88, 110] and cytochrome P450 [22]; induces phase II drug metabolizing enzymes [24, 25, 51]; up-regulates antioxidant enzymes such as glutathione peroxidase [84], catalase [34], and quinone reductase [74]; and inhibits ornithine decarboxylase [95]. Intriguingly, resveratrol has been shown to regulate cathepsin D [94] and to inhibit HIF-α (hypoxia-inducible factor α) function [20, 101]. The effect on HIF-α factors (HIF-1α and HIF-2α) is particularly important since these proteins modulate the metabolism of glucose by enhancing its internalization and glycolitic metabolism [81].

During our studies on resveratrol, we demonstrated that, in K562 cells (an erythroleukemic cell line), the molecule up-regulated the cellular content of Egr-1 (early growth response) transcription factor by activating MAP kinase pathway [30]. In turn, Egr-1 increased the gene transcription of p21$^{\text{Cip1}}$, an inhibitor of cyclin-dependent kinases. p21$^{\text{Cip1}}$ accumulation was responsible for the resveratrol antiproliferative effect and, at least in part, for the induction of erythroid differentiation [30]. Resveratroinduced p21$^{\text{Cip1}}$ accumulation has also been observed by us and other research groups in different cell line models. This finding demonstrates, in general, that resveratrol affects specifically gene expression and the cell division cycle engine.

An additional mechanism of resveratrol action requires, however, particular attention and discussion, that is, its effect on sirtuin. Sirtuins are a family of enzymes that deacetylate proteins at the expense of NAD, thus possessing either protein deacetylase or mono-ribosyltransferase activity [37, 109]. Sirtuins have been implicated in the promotion of life extension in several species and in the modulation of gene transcription, apoptosis, and stress resistance, as well as energy expenditure control under low-calorie conditions [58, 103, 109].

In 2003, Howitz and colleagues reported that resveratrol is a powerful naturally occurring activator of yeast Sir2, the homolog of mammalian Sirt-1, and is also able to extend the life length of *Saccaromyces cerevisiae* [44]. Subsequently, the capability of resveratrol to elongate the duration of life was also confirmed in a worm (*Caenorhabditis elegans*) and in the fruit fly *Drosophila melanogaster* [9].

Subsequently, some of these results have been questioned, and now the anti-aging effect of resveratrol would appear to be unlikely [59, 67]. Similarly, whether the effect of resveratrol on Sirt-1 exists in vivo or it is only an in vitro activity has been the object of debate [11, 28]. On this aspect, however, no definite conclusion is available.

In the context of the effects of an altered nutrition in human physiology and pathology, it has been reported that resveratrol strongly ameliorates the performances of mice fed with a high-fat diet. The positive effect was correlated to Sirt1-dependent deacetylation and activation of PGC-1α, a master gene that activates oxidative metabolism by increasing the respiratory chain components and the mitochondria number and activity [50, 67]. Very recently, further studies on this topic have been reported. First, it has been demonstrated that the primary molecular effect of resveratrol is the inhibition of phosphodiesterase (PDE) that results in a cyclic AMP increase [65]. The up-regulation of the cyclic mononucleotide triggers a series of reactions resulting in the activation of AMP kinase (AMPK), a pivotal player in the control of caloric restriction. Then, AMPK regulates Sirt1 and PGC-1α (Fig. 3a). A different study reports that initial targets of resveratrol are Sirt1 or AMPK, alternatively, depending on the amount of resveratrol employed [71]. In both cases, the final result is the activation of PGC-1α (Fig. 3b).

Thus, although the two reports propose different primary resveratrol targets, final effectors are both AMPK and PGC-1α [65, 71]. Studies with knock-out mice might help in clarifying whether the differences in the observed mechanisms may depend or not on the resveratrol concentration employed. In both cases, the two investigations definitely demonstrate that resveratrol affects "in vivo" energy metabolism.

Up to the end of 2011, more than 50 studies analyzed the effect of resveratrol as an anticancer compound in animal models of different cancers, including skin cancer (non-melanoma skin cancer and melanoma); breast, gastric, colorectal, esophageal, prostate, and pancreatic cancers; hepatoma, neuroblastoma, fibrosarcoma, and leukemia (reviewed in [96]). In general, these preclinical studies suggest a positive activity of the molecule in lowering the progression of cancer, reducing its dimension, and decreasing the number of metastases.
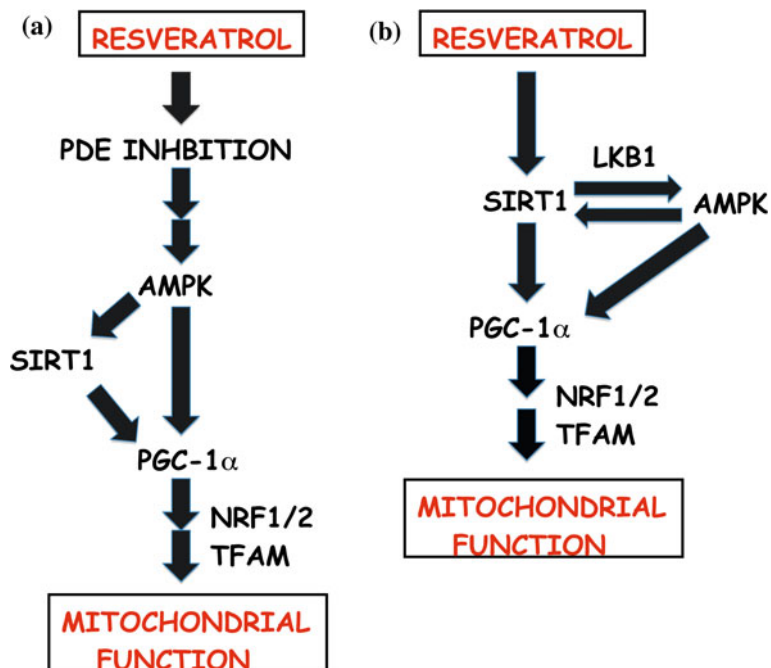
Fig. 3 Proposed molecular mechanisms of resveratrol affecting energy metabolism *Panel* a. The primary target of resveratrol is the phosphodiesterase activity that results in an increase in cyclic AMP. The up-regulation of cAMP stimulates the activity of AMPK. This kinase activates SIRT-1 and PGC-1α independently. Furthermore, SIRT-1 activates PGC-1α. Finally, PGC-1a along with additional transcription factors (NRF1/2 and TFAM) positively modulates the mitochondrial activity. *Panel* b. In this mechanism, the primary target of resveratrol is SIRT-1. The up-regulation of this enzyme results into the activation of both AMPK and PGC-1α. Moreover, AMPK also activates PGC-1α. Finally, PGC-1α and the two factors, NRF1/2 and TFAM, increase mitochondrial activity

These findings prompted studies to evaluate the possibility of translating the anticancer activities observed in preclinical studies into the use of resveratrol for cancer treatment. It is, however, necessary to emphasize that a large number of naturally occurring compounds show an anticancer activity in animal models but, when evaluated in clinical trials, the results obtained are very frequently unsatisfactory in terms of efficacy and toxicity.

4   Resveratrol Pharmacokinetics

It is indisputable that resveratrol modulates in vivo and in vitro a large array of intracellular molecular mechanisms as well as complex biological events and that the natural polyphenol has a positive effect on numerous experimental models of cancers. However, the doses of resveratrol required for reaching serum levels

comparable with those found efficacious in vitro have cast severe doubts on the potential usefulness of the molecule, particularly in dietary prevention strategies.

Resveratrol is usually well tolerated at least in the short-term or acute exposure experiments performed in humans. When eight healthy subjects were exposed for eight days to 2 grams of resveratrol twice/day, six of eight subjects had mild episodic diarrhea/loose stool. The symptoms typically appeared at the beginning of the treatment period, and one of the subjects developed a temporary rash and headache [49].

In a double-blinded, randomized, placebo-controlled study, up to 975 mg/day was given to healthy volunteers. Two adult subjects (male and female) of each group were treated with 25, 50, 100, or 150 mg, six times/day, for two days in total. Adverse effects were mild in severity and similar between all groups [4]. In a different study, 270 mg resveratrol was given to 19 volunteers for one week without causing any discomfort [99]. In a further report, healthy volunteers tolerated resveratrol well in a seven-day exposure study, but experimental details were not given, thus making the assessment of results challenging [32]. The same article describes a trial that included daily exposure to 2.5 g or 5 g resveratrol for 28 days. The authors reported that the adverse events were generally mild in nature and reversible, but the experimental details are scarce [32].

On the other hand, the prevention and/or treatment of malignancies (and other chronic diseases) might require therapies extended for several months/years and, thus, data on long-term resveratrol toxicity are of crucial importance. Unfortunately, this information is so far not available. Therefore, while resveratrol might be considered a food supplement and a relatively safe natural medication, further investigations are absolutely necessary to determine its long-term effects.

A central issue that needs to be clarified is resveratrol bioavailability compared with its therapeutic efficacy. This complex issue might be approached in different ways. As reported in a previous section, resveratrol in vitro effects (i.e., on cell systems) are mainly observed at concentrations ranging from 10 to 50 μM. However, these values do not consider that the polyphenol interacts with components of the culture medium (e.g., proteins, lipoproteins and others), and thus, the actual free resveratrol effective concentration might be significantly lower. In humans, when resveratrol was administered in a single dose of approximately 25 mg [4, 82, 83, 97], the plasma concentration of the free molecule ranged from 1 to 5 ng/ml (4-20 nM); administration of a higher dose (5 g) led to a value of serum free resveratrol of about 2.3 μM [4, 82, 83, 97]. The maximum peak plasma concentration was reached in the first 30–90 min after intake. Under these conditions, the corresponding concentration of the three main resveratrol metabolites (resveratrol-3-O-sulfate, resveratrol-3-O-glucuronide and resveratrol-4-O-glucuronide) exceeded that of the free compound by approximately 20-fold [4, 82, 83, 97]. Plasma half-lives of resveratrol and of its three major conjugates were similar (between 2.9 and 11.5 h). In urine, within 24-h postdose, excretion rates were highest during the initial 4-h collection period, while traces of resveratrol metabolites were detected in feces, consistent with an enterohepatic recirculation [4, 82, 83, 97]. Thus, bioavailability studies showed that, even after a high dose of

resveratrol administration, only a small amount of the free form is present in plasma and that treatments with high resveratrol amounts are required to reach serum levels corresponding to those necessary for the in vitro biological activities.

This methodological approach, however, is intrinsically poor, as it does not directly correlate the strategy of treatment and the serum dosage with the biological effects.

An interesting alternative methodology is to consider the resveratrol dosage employed in studies where clear in vivo effects were observed. In this respect, the study of Baur and colleagues might be useful [10]. The authors employed, in mouse treatment, a dosage of about 22.4 ± 0.4 mg kg^{-1} day^{-1} and observed significant phenotypic effects after approximately 110 weeks. The value corresponds to about 1568 mg for a man of 70 kg. As Baur and Sinclair [9] reported a concentration of 5 mg resveratrol per liter of some red wines, the above value would correspond to about 300 l of wine to be consumed every day.

This estimation, however, does not consider the so-called body surface area (BSA), a parameter that is necessary for a correct dose translation from mice to humans. Employing this parameter, 22.4 mg kg^{-1} (in mice) corresponds to 1.82 mg kg^{-1} (in humans), and, in turn, 1568 mg to about 128 mg. However, protective effects have been observed at a lower resveratrol dose, that is, 5.9 mg kg^{-1} day^{-1} in mice [50], equivalent to 33 mg for a man of 70 kg (using BSA correction). Since 33 mg resveratrol is contained in 6 l of wine, this still suggests that the importance of the dietary phytoalexin is questionable.

However, two more aspects should be taken into account which might allow us to suggest that resveratrol effects occur (at least in part) even in the presence of a normal diet (about 0.4 l of wine day^{-1}, around 1.5 mg day^{-1}).

First, the efficacy of dietary resveratrol might be higher than that showed by the compound taken in pill form as a purified preparation. Indeed, it has been suggested (but not demonstrated) that the presence in foods of other compounds (for example, other polyphenols) interfering with resveratrol removal might diminish the catabolism of the phytoalexin and increase its serum level [50]. Second, bioavailability data suggest that prolonged resveratrol treatment leads to an increase in serum resveratrol content as well as to its accumulation in specific cellular compartments (i.e., cellular membranes) or tissues, due to the molecule lipophilicity.

5   Clinical Studies on Resveratrol

Although few studies on the clinical effect of resveratrol treatments in humans are available, the existing data seem promising enough to warrant further investigation.

A very recent report, based on a cohort of one thousand people, showed a direct correlation between resveratrol dietary consumption and improvement of several cardiac risk parameters. The intake of resveratrol was evaluated by determining

levels of resveratrol itself and of its metabolites in urine [106]. A further investigation reported a trial where 75 subjects were divided into 3 groups and treated with placebo, grape extract, and grape extract enriched with a low amount of resveratrol. The authors claimed that after treatment for 1 year, a number of cardiac risk factors (C-reactive protein, tumor necrosis factor-α, plasminogen activator inhibitor type 1, interleukin-6/interleukin-10 ratio) were significantly decreased only in the group of subjects treated with resveratrol [93]. Finally, a pivotal study, published in Cell Metabolism, showed that in 11 obese patients, treatment with 150 mg/day of resveratrol for 30 days, strongly and positively influenced several parameters (i.e., increase in intramyocellular lipid levels and decrease in intrahepatic lipid content, circulating glucose, triglycerides, alanine-aminotransferase, and inflammation markers) and, biochemically, induced the up-regulation of muscle AMPK, SIRT-1, and PGC-1alpha activities, which are similar to the observations reported in the animal models [91]. The authors emphasized that the treatment was, however, performed employing resveratrol at a concentration 400-fold lower than that used in mice.

These three studies [91, 93, 106], suggest, but clearly do not definitely prove, that resveratrol affects metabolic parameters and risk factors which are also important for cancerogenesis.

Two other interesting studies were published in Cancer Research in 2010. These investigations evaluated the toxicity and metabolism of the polyphenol and its accumulation in both normal and malignant colon tissue [17, 66]. In these cases, the treatment was at high doses for a short period. The results of one study demonstrated that resveratrol shows very low toxicity and might reach concentrations negatively affecting IGF-1 level [17]. Lowering IGF-1 is considered to be one important parameter in anticancer activity. In the second investigation, patients affected by colon carcinomas were treated with resveratrol for 7 days before tumor removal. The results showed a clear, although limited, decrease in proliferation of malignant cells. Moreover, resveratrol accumulated in colon tissues ranging in concentration from 20 to 200 μM [66].

In addition to the studies reported above, several clinical trials of either dietary or supplemented resveratrol are currently at different stages of completion. Particularly, a search in www.clinicaltrials.gov by using the key term "resveratrol" revealed 53 studies at different stages (retrieved October 4, 2012). More specifically, 7 studies were active but not yet recruiting, 18 studies were active and recruiting, 21 studies were completed, 2 studies terminated, 4 studies with unknown status (i.e., information has not been updated recently), and 1 investigation was withdrawn. The majority of these trials investigate the effect of the molecule on type-2 diabetes, obesity, and cardiovascular diseases.

Eight studies directly evaluated the effect of resveratrol on cancer development. Six trials were completed and the results of four of these have been published [15, 43, 62, 66]. One trial is still recruiting the patients while the status of the last one is unknown.

The majority of studies (four) were devoted to investigating the effect of the polyphenol treatment on colon cancer while one analyzed the activity of the molecule on the development of cancers. The remaining three studies focused on resveratrol effects on gastrointestinal cancer, follicular lymphoma and multiple myeloma (using an association between resveratrol and bortezomib), but no conclusions are yet available from these investigations. In general, the major inference that can be deduced from the published studies [15, 43, 62, 66] is that the positive cancer chemopreventive properties showed by resveratrol warrant further investigation. The study designs for these trials (e.g., dosages/formulations of resveratrol, length of trial) vary greatly, with doses as high as 5 g in healthy adults.

A main limitation and criticism of the clinical resveratrol research already available is a lack of trials examining the long-term health effects of resveratrol. Importantly, it has been recently announced that the Danish Council for Strategic Research has granted $3.4 million for a four-year study to investigate resveratrol on the management of metabolic syndrome, osteoporosis, and chronic inflammation. The main purpose of this landmark study is to demonstratively prove that supplementary intake of resveratrol can neutralize the detrimental effects of excess body weight, specifically obesity. The effects to be measured include low-grade inflammation that is often associated with type 2 diabetes, non-alcoholic fatty liver disease, osteoporosis, and cancer.

In summary, further controlled clinical trials are clearly required to prove the preventive and therapeutic efficacy of either dietary or supplemented resveratrol.

6   Conclusion and Perspectives

The emerging data from human clinical trials on resveratrol suggest that the positive effects obtained in vitro and in animal models must be taken into serious consideration in view of a possible protective or therapeutic use of the molecule. As a matter of fact, a trivial comparison of the serum level reached by the molecule after treatment with the amount necessary in the cellular growth medium for obtaining phenotypic effects appears quite simplistic.

Indeed, recent findings indicate that even a very low dose of resveratrol treatment (8 mg/die) prolonged for one year significantly reduces a number of cardiac risk factors [93]. Probably, 8 mg resveratrol is still a value too high to be reached only by drinking wine (it corresponds to 1–3 liters, depending on the wine), but it is not extremely elevated considering that resveratrol occurs in various foods. On the other hand, the scarce toxicity of the molecule suggests that the use of the compound in the prevention or treatment of chronic diseases might warrant serious consideration.

On the other hand, it is not clear whether long-term resveratrol supplementation will preserve the benefits to ultimately impact the development of chronic disease, and the small number of clinical trials remains dwarfed compared to the thousands of basic science experiments.

Finally, to further evaluate resveratrol's potential for widespread use in human medicine, continued research exploring a gamut of responses in humans is obviously necessary. Future studies should aim to:

1. Investigate different dosages and/or formulations of resveratrol, in terms of both bioavailability and efficacy.
2. Evaluate the efficacy of resveratrol as a putative alternative for a given outcome/treatment. In some chronic diseases (type 2 diabetes, obesity, metabolic syndrome, cardiovascular diseases, and neuro-degenerative pathologies), resveratrol may be considered a serious alternative option in their prevention and treatment. In this regards, only the results of numerous and independent trials must be considered.
3. Study the effects of long-term resveratrol supplementation.
4. Determine the activity of resveratrol's metabolites.
5. Establish if resveratrol can have either additive or synergistic effects in combination with other therapies.
6. Determine whether genetic factors might explain differences in bioavailability and physiological responses to resveratrol between individuals.
7. Develop new strategies for resveratrol supplementation.

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