Perspective

Resveratrol from Red Grapes - Pedestrian Polyphenol or Miraculous Anticancer Agent?

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Key words

Introduction
Phytochemicals with beneficial effects on health regularly make newspaper headlines. Media awareness reflects the considerable interest of the general public in naturally occurring remedies rooted in the assumptions that the plant kingdom harbours remedies against all sorts of ills, and that many of these remedies have still to be discovered. The realisation that fruits and vegetables can prevent diseases such as cancer has engendered an intense search for the constituents which may be responsible for these properties. Resveratrol (3,5,4’-trihydroxy-trans-stilbene) is an example of a dietary constituent which has been shown over the last decade to possess a fascinating spectrum of pharmacological properties. Resveratrol was first isolated from the roots of white hellebore (Veratrum grandiflorum O. Loes) in 1940 and later
from the roots of *Polygonum cuspidatum*, a plant used in Chinese and Japanese medicine. In 1992 resveratrol was suggested to explain some of the cardioprotective effects of red wine [1]. Since then many reports have shown that resveratrol can prevent, or decelerate, the progression of illnesses such as cancer, cardiovascular disease and ischaemic injury, as well as enhance stress resistance and extend the lifespan of various organisms from yeast to vertebrates. In 1997 Jang *et al.* [2] published a paper, in which the ability of resveratrol to inhibit diverse cellular events associated with initiation, promotion and progression of cancer was described. This paper fired the imagination of the cancer chemoprevention research community. During the 11 years since its publication, literally hundreds of reports have appeared in the literature in which cellular and biochemical mechanisms of resveratrol have been described, and their potential role in the putative cancer chemopreventive activity of this agent has been discussed. As a triphenol resveratrol is exceedingly prone to undergo metabolic conjugation, so that its bioavailability is extremely low. Its intriguing efficacy in spite of low bioavailability constitutes a conundrum [3]. This perspective aims at exploring whether recent results of investigations of resveratrol, especially those in humans, may help resolve the conundrum. It focuses in particular on mechanisms and pharmacokinetics of resveratrol and describes resveratrol analogues designed to optimise its pharmacology. There have been several incisive and comprehensive reviews of the health effects [e.g. 4] and pharmacokinetics [e.g. 5] of resveratrol, yet information of the pharmacology of resveratrol in humans remains scarce.
How can resveratrol prevent cancer?

Prominent among the mechanisms, which may mediate the cancer chemopreventive efficacy of resveratrol, are inhibition of cyclooxygenase enzymes, inhibition of angiogenesis, modulation of drug metabolising enzymes, antioxidation and alterations in cell cycle and apoptotic machinery. Jang et al originally proposed that resveratrol inhibits the enzymatic activity of both cyclooxygenase enzymes COX-1 and -2 [2]. Epidemiological evidence suggests that long-term inhibition of cyclooxygenase significantly reduces the risk of developing many cancers, and deletion of the gene that encodes COX-2 is protective in a mouse model of colorectal cancer [6](18 Oshima Cell). Resveratrol inhibited COX-1 in a somewhat selective fashion and reduced COX-2 at the mRNA level [7-10](21-24ev Baur). Resveratrol, delivered systemically, inhibited tumour-induced neovascularisation required to support solid tumour growth, illustrating its anti-angiogenic properties [11, 12] (31,32). Resveratrol can modulate the expression and activity of multiple drug-metabolising enzymes. It inhibited various cytochrome P450s [13-16] (35-38) and induced expression of phase II drug detoxifying enzymes [17] (44). These activities suggest that resveratrol may reduce the exposure of cells to carcinogens by decreasing carcinogen formation and/or increasing their detoxification.

Reactive oxygen species (ROS) have been shown to be involved with the initiation and progression of cancer via damaging DNA and other biomolecules. Resveratrol has antioxidant capacity, as reflected by its ability to increase plasma antioxidant activity and to decrease lipid peroxidation [18-20](62-64).

Resveratrol possesses antiproliferative and pro-apoptotic effects in tumour cell lines [65](50). These effects may be mechanistically linked to its abilities to downregulate cell cycle proteins [66-68](51-53) and increase apoptosis [69-71](54-56) in tumour
models *in vivo*. Resveratrol sensitized tumour cells to apoptosis induced by TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) [72](59), and this property may explain its pro-apoptotic effects *in vivo*.

At the end of 2006 two reports suggested that resveratrol is a caloric restriction mimetic in lower organisms and mice, associated with its ability to activate sirtuin proteins and to extend lifespan [73, 74]. Sirtuins are NAD-dependent deacetylases involved in gene silencing processes germane to aging, blockade of apoptosis and promotion of cell survival. So paradoxically, resveratrol can counteract radical oxygen species (ROS) production, induce apoptosis and inhibit cell growth, but it can also increase mitochondrial metabolism and, in turn, ROS production, thus enhancing cell survival. Scanning internet websites devoted to life enhancement shows that a surprisingly large number of healthy humans now ingest resveratrol at high doses with the objective to prolong their lives, without bearing in mind that the safety of high-dose resveratrol ingested for a long term is unknown.

**Pharmacokinetics of resveratrol**

In rodents resveratrol possesses a short initial half life, 8-14 min [75, 76] as it is metabolised extensively and rapidly. Results from clinical trials are essentially compatible with the rodent studies. Table 1 describes the widely varying situations in which resveratrol has been studied in humans, administered either as pure compound [77, 78] or as constituent of wine or grape fruit juice or grape extract [79-84](Zern, Meng, Vitaglione, Goldberg, Zamaroa, Lekakis). Walle et al [77] showed that the bulk of an iv dose of 25 mg per human resveratrol was converted to sulphate conjugates within 30 min. Serum peak levels of res were <22 nM. Five metabolites were identified in the urine, resveratrol mono sulphate, two isomeric
monoglucuronides, dihydroresveratrol monosulphate and dihydroresveratrol monoglucuronide. Total sulphate conjugates accounted for ~37% of the metabolites in the urine and total glucuronide conjugates ~19%, and there were only trace amounts of free res. Recently a study of the pharmacokinetics of single oral resveratrol administered at high doses (0.5, 1, 2.5 or 5g) was conducted in healthy human volunteers (10 per dose level) [78]. The doses were safe and resveratrol and six metabolites were recovered from plasma and urine. Peak plasma level of resveratrol at the highest dose (5g) was 2.4 μM, which occurred 1.5 h post-dose. Peak levels of two monoglucuronides and resveratrol-3-sulfate were 3-8 fold higher. The area under the plasma concentration curve values for resveratrol-3-sulfate and resveratrol monoglucuronides were up to 23 times greater than those of resveratrol. Urinary excretion of resveratrol and its metabolites was rapid, with 77% of all urinary agent-derived species excreted within 4 h after the lowest dose. Conjugate formation typically reduces cell permeability and aids drug excretion, therefore the reported in vivo efficacy of resveratrol in spite of its low bioavailability has led to speculation that its metabolites could mediate at least in part the efficacy exerted by the parent molecule. Concentrations of resveratrol in red wine vary widely, but a reasonable estimate is about 5 mgL\(^{-1}\) (22 μM) [85](180). Assuming a consistent daily intake of two glasses of wine (~375 ml) a person weighing 70 kg would receive a dose of ~27 μg per kg body weight each day. The plasma concentrations after such a low dose are clearly vanishingly small, in the 1-10 nM range [86](178), certainly orders of magnitudes below those found to elicit most pharmacological effects reported in vitro. Therefore it seems prudent to explore the pharmacology of resveratrol from two separate standpoints, firstly considering it as diet constituent, and secondly as agent in its own right which just happens to be diet-derived. Russo [87]
surmised that the diet constituent resveratrol, ie resveratrol at low doses, may be useful as a caloric restriction mimic, whilst at high doses, it may be a promising chemotherapeutic agent, but in neither case useful as cancer chemopreventive agent. This interpretation is based on the findings that low doses generate concentrations capable of mimicking caloric restriction, but insufficient to engage anticarcinogenesis, whilst high doses, which are pharmaceutically unfeasible in the chemoprevention paradigm, engender levels exerting antiproliferative and pro-apoptotic effects. Nevertheless in the light of the polymechanistic nature of the effect of resveratrol its seems premature to write off its potential chemopreventive activity. The maximum tolerated dose of resveratrol has not been properly established, but at 300 mg/kg it did not have detrimental effects in the rat [88](147).

**Resveratrol analogues**

Not surprisingly the diverse attractive pharmacological features of resveratrol in concert with its relatively simple chemical structure have inspired medicinal chemists to synthesize analogues with the aim to design novel agents with cancer chemopreventive/chemotherapeutic efficacy superior to that of parent resveratrol. Efforts have focussed on structure – activity relationships of substituted stilbene derivatives to optimise cytotoxic potency, ability to inhibit activation of NFkB, tyrosine kinase or cytochrome P450 or to exert antioxidation [89-93](12-15 JMEdChem. Heynekamp). For example a series of cis- and trans-stilbenes related to resveratrol with varying aryl, hydroxyl, methoxy and/or amine moieties have been synthesized and evaluated for ability to induce apoptosis in human leukaemia-derived HL60 cells [94] (Roberti). Among the analogues cis-3,4′,5-trimethoxy-3′-aminostilbene and cis-3,4′,5- trimethoxy-3′-hydroxystilbene (Fig. 1 ) were found to
possess potency superior to that of the lead compound resveratrol. Other workers have generated polyhydroxystilbenes, exemplified by 3,3’,4’,5-tetrahydroxystilbene (Fig. 1), to maximise the antioxidant activity of this type of molecule [95](Murias). A relatively subtle chemical change brought about by methylation of the stilbene hydroxy mioeties increased several pharmacological potencies of resveratrol. This increased potency may be, at least in part, due to the fact that methoxy moieties tend to be less vulnerable to attack by metabolising enzymes than hydroxy. Pertinent in this context is the finding that pterostilbene, bis-methylated resveratrol found in blueberries (Fig. 1), at 40 ppm in the diet potently suppressed aberrant crypt foci formation in the azoxymethane-induced colon carcinogenesis rat model and suppressed inducible nitric oxide synthase expression [96](Suh).

The recent realisation that resveratrol may extend lifespan, and the assumption that this property may ultimately be observed in humans, has engendered a flurry of medicinal chemistry projects in the pharmaceutical industry. These activities are primarily aimed at generating novel patentable molecules which activate sirtuins, possess life extending properties and mimic health effects of calorie restriction without requiring a change in eating habits, undoubtedly an attractive endeavour from a financial standpoint. It seems worthwhile to test these analogues, when they will be available, for cancer chemopreventive properties.

**Future work**

Are we any closer to resolve the conundrum posed by the undeniable efficacy of resveratrol in preclinical models in spite of its low systemic availability? Hardly. It still unclear whether metabolites can contribute to, or mediate, resveratrol efficacy. Experiments to explore this issue are afoot. The differential effects of dietary (ie low)
doses of resveratrol on the one hand and pharmacological (ie high) doses on the other has sparked investigations of dose-dependent differences in metabolite profiles obtained after consumption of either red wine or purified resveratrol. Results from studies of this type will undoubtedly help explain dose-response issues related to resveratrol pharmacology. Further chemical syntheses of resveratrol cogeners might well generate molecules with biological activities superior to those of resveratrol. It remains to be seen whether new synthetic caloric restriction mimetics modelled on resveratrol will prolong human life and whether such molecules exert cancer chemopreventive activity. Whilst studies in humans conducted hitherto support the tentative notion that resveratrol is a safe molecule, it will take a long time to establish whether chemically designed resveratrol analogues retain the safety of the parent molecule. Lack of knowledge of their safety will for a long time confound consideration of such novel molecules as potential cancer chemopreventive agents. The findings briefly highlighted here show that resveratrol is certainly not a pedestrian polyphenol. On the other hands, it is unlikely to turn out to be a miraculous anticancer remedy, even though it constitutes undoubtedly a molecule which will intrigue cancer pharmacologists for a long time to come.
Table 1. Clinical trials of resveratrol

<table>
<thead>
<tr>
<th>Study cohort</th>
<th>Intervention</th>
<th>Dose and route of resveratrol</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Healthy volunteers (44)</td>
<td>Lyophilized grape powder</td>
<td>58μg pd x 4 weeks po</td>
<td>[80]</td>
</tr>
<tr>
<td>Healthy volunteers (6)</td>
<td>Resveratrol in whisky/grape juice</td>
<td>0.32 – 70mg single dose po</td>
<td>[79]</td>
</tr>
<tr>
<td>Healthy volunteers (25)</td>
<td>Red wine</td>
<td>0.25, 0.48 or 1.9mg single dose po</td>
<td>[81]</td>
</tr>
<tr>
<td>Healthy volunteers (12)</td>
<td>Red wine, fruit or vegetable juice</td>
<td>25mg single dose po</td>
<td>[82]</td>
</tr>
<tr>
<td>Healthy volunteers (20)</td>
<td>Red wine</td>
<td>0.4 – 2.6mg pd x 4 weeks po</td>
<td>[83]</td>
</tr>
<tr>
<td>Coronary heart disease patients (30)</td>
<td>Grape extract</td>
<td>0.9mg single dose po</td>
<td>[84]</td>
</tr>
<tr>
<td>Healthy volunteers (6)</td>
<td>Pure resveratrol</td>
<td>25mg single dose iv/po</td>
<td>[77]</td>
</tr>
<tr>
<td>Healthy volunteers (40)</td>
<td>Pure resveratrol</td>
<td>0.5, 1, 2.5 or 5g single dose po</td>
<td>[78]</td>
</tr>
</tbody>
</table>
References


Cushman M, Nagarathnam D, Gopal D, He, HM, Lin CM, Hamel E. Synthesis and evaluation of analogues of (Z)-1-(4-methoxyphenyl)-2-(3,4,5-


Figure legend
Fig. 1. Structures of resveratrol (I) and resveratrol analogues discussed in the text, cis-3,4′,5′-trimethoxy-3′-aminostilbene (II), cis-3,4′,5′-trimethoxy-3′-hydroxystilbene (III), 3,3′,4′,5′-tetrahydroxystilbene (IV) and pterostilbene (V).