Cardiovascular Protective Effects of Resveratrol

Silvia Bradamante, Livia Barenghi, and Alessandro Villa

CNR — ISTM, Istituto di Scienze e Tecnologie Molecolari, Milan, Italy

Keywords: Adenosine — Antioxidants — Cardiovascular protection — LDL oxidation — Nitric oxide — Platelet aggregation — Preconditioning — Resveratrol.

ABSTRACT

Resveratrol (3,4’,5-trihydroxy-trans-stilbene), a phytoalexin found in grape skins, peanuts, and red wine, has been reported to have a wide range of biological and pharmacological properties. It has been speculated that at low doses (such as consumed in the common diet) resveratrol may have cardioprotective activity. In this article we describe recent in vitro and in vivo studies in animal models. The results of these studies suggest that resveratrol modulates vascular cell function, inhibits LDL oxidation, suppresses platelet aggregation and reduces myocardial damage during ischemia-reperfusion. Although the reported biological data indicate that resveratrol is a highly promising cardiovascular protective agent, more studies are needed to establish its bioavailability and in vivo cardioprotective effects, particularly in humans.

INTRODUCTION

Resveratrol (RSV) is a naturally occurring polyphenol. It is found in grapes, and grape products, such as wine, as well as other botanical sources, such as peanuts. In grapes, resveratrol is present only in the skin, and as both, free RSV and piceid (3-O-mono-D-glucoside of RSV). The chemical structures of RSV (3,4’,5-trihydroxy-stilbene, MW = 228.2) and of piceid are shown in Fig. 1. RSV exists in cis- and trans-isomeric forms; the trans to cis isomerization is induced by UV exposure. In wines the trans-form is by far the most abundant (35). The trans-RSV structure is similar to that of the synthetic estrogen, diethylstilbestrol. The biological antioxidant, estrogenic and anticancer activities of RSV (Table 1) have been reported and discussed in recent reviews (26,29,34,43,76,99).
majority of publications quoted in this review article deal with the pharmacological activity of trans-RSV. Indeed, it has been indicated that the trans-RSV has greater anticancer and cardioprotective activities than the cis isomer.

CHEMICAL DETECTION METHODS

The pharmacological activity of RSV has stimulated the development of analytical methods for measuring it in various matrices, including plant extracts, wine, serum and tissue. Most of these methods are based on HPLC and UV absorbance (82,103) with a detection limit of 5 μg/L. The difference in the maximum UV absorption of the two RSV isomers (trans-: 307 nm and cis-: 288 nm) allows their separation.

Fluorometric (77) and electrochemical detection (2,63) methods (sensitivities of the two methods to stilbene are 0.01 and 1 μg/L, respectively) have also been used, as well as capillary electrophoresis (35). Gas chromatography-mass spectrometry (GC-MS) analysis is highly sensitive and specific (30,88), but it requires prior derivatization of RSV in order to increase its thermal stability and volatility. The high temperature used with this approach might cause inaccurate quantitation due to partial isomerization or degradation of

<table>
<thead>
<tr>
<th>TABLE 1. Major biological activities of resveratrol (26)</th>
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<tbody>
<tr>
<td>Inhibition of lipid peroxidation;</td>
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<tr>
<td>Chelation of copper;</td>
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<td>Free radical scavenging;</td>
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<td>Alteration of eicosanoid synthesis;</td>
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<td>Inhibition of platelet aggregation;</td>
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<td>Vasorelaxing activity;</td>
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<td>Modulation of lipid metabolism;</td>
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<td>Anticancer activity;</td>
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<td>Estrogenic activity;</td>
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RESVERATROL

BIOLOGICAL ACTIVITY

Antioxidant Activity in Vitro

Natural antioxidants, such as vitamins and polyphenols, play an important role in the prevention of diseases associated with free radicals. RSV reduces the rate of cytochrome c oxidation by hydroxyl radicals, produced as a result of the UV irradiation of hydrogen peroxide (H₂O₂), with an IC₅₀ = 33 μM (92). RSV is also very effective in scavenging hydroxyl radicals (86) in an ethanolic solution of β-carotene (Fig. 2).

RSV has a strong inhibitory effect on the superoxide radical (O₂•⁻) and H₂O₂ produced by macrophages stimulated by lipopolysaccharides (LPS) or phorbol esters (PMA). It also significantly decreases the [³H]arachidonic acid release induced by LPS, PMA, or by means of exposure to O₂•⁻ or H₂O₂ (62). The antioxidant activity of RSV has been assessed by its capacity to prevent Fe²⁺-induced lipid peroxidation in microsomes and by low-density lipoprotein (LDL) oxidation by Cu²⁺ (24), as well as by inhibition of iron- or UV irradiation-catalyzed lipid peroxidation (IC₅₀ = 4.8 and 3.9 μM, respectively) in rat liver microsomes (64). A detailed study (55) has confirmed that RSV is an effective scavenger of hydroxyl, superoxide, and metal-induced radicals. RSV has also been found to have antioxidant effects in cells producing reactive oxygen species (ROS), a protective effect from lipid peroxidation in cell membranes as well as from DNA damage caused by ROS. The calculated RSV reaction rate with •OH (9.45 × 10⁻¹⁰ 1/M/sec) is significant. RSV is, however, less potent than other well-established antioxidants, such as ascorbate (1.2 × 10⁻¹⁰ 1/M/sec), GSH (1.5 × 10⁻¹⁰ 1/M/sec) and cysteine (1.3 × 10⁻¹⁰ 1/M/sec).

The antioxidant activity of RSV has been recently evaluated in Saccharomyces cerevisiae cell cultures (86), and compared with those of butyl hydroxy toluene (BHT), propyl gallate (PG) and vitamins C and E. At concentrations of up to 0.1 mM, RSV efficiently scavenged 1,1-diphenyl-2-picrylhydrazyl (DPPH•, 0.25 mM) free radicals. Similar results

FIG. 2. Inhibition of β-carotene oxidation by the antioxidants: Butylated hydroxytoluene (BHT), propyl gallate (PG), RSV, and vitamins C and E (0.025 μM) in the presence of H₂O₂ and Fe²⁺. Equal signs indicate no statistically significant differences using Tukey’s test (p ≤ 0.05) in scavenging hydroxyl radicals. Adapted from ref. 92.
were obtained by Wang et al. (95), who demonstrated that at high concentrations RSV forms a dimer (of unknown toxicity) that lacks the capacity to scavenge DPPH.

Bioavailability, Pharmacokinetics, and Metabolism

Numerous studies have shown that peak concentration of trans-RSV in rat blood or serum is reached very rapidly, at 10 to 15 min after oral administration (46,89). Its mean residential time in an organism has been calculated to be 33.83 min (Table 2). These data confirm the preliminary findings by Bertelli et al. (7), who demonstrated significant cardiac bioavailability of trans-RSV and its strong affinity for liver and kidney.

Studies on the distribution of [14C]- and [3H]trans-RSV in tissues confirm that the drug is bioavailable after oral administration. Vitrac et al. (93) have clearly shown that RSV is absorbed, metabolized and distributed to various tissues in a mammalian whole-body (mouse). Radioactivity derived from [14C]trans-RSV was found in various organs, such as liver and kidney, with peak concentrations in the duodenum and to a lesser extent in brain, heart, lung and testis. Trans-RSV was identified on the basis of HPLC co-chromatography of organ extracts with the authentic standard. According to the same report trans-RSV is eliminated primarily by renal excretion. On the basis of the amount of radioactivity excreted in feces and urine over 24 h, Soleas et al. (89) reported that in rats at least 50% of orally administered [3H]trans-RSV is absorbed by the digestive tract.

In the perfused rat small intestine (a model of intestinal absorption), the bioavailability of luminally administered RSV was 20.5%. At a vascular site, 16.8% was conjugated to give RSV glucuronide together with RSV sulphate (3.4%) and free RSV (0.3%) (2). Using the same model, Kuhnle et al. (51) found that almost all of the absorbed RSV is conjugated as glucuronide, the reaction being mediated by UDP-glucuronosyltransferase 1A1 (4). A detailed investigation has determined the pharmacokinetics of trans-RSV in its aglycone (RESAGL) and glucuronide (RESGLU) forms following i.v. or p.o. administration to rats (59). The same study also found sudden increases in RSV plasma concentrations at 4 to 8 h after its administration. The short $t_{1/2}$ is, therefore, the apparent elimination half-life before enterohepatic recirculation, whereas the terminal elimination half-life with enterohepatic recirculation is 1.5 to 2 h.

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<tr>
<th>Parameter</th>
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<th>Compartmental</th>
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<td>514.21</td>
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<tr>
<td>$t_{max}$ (min)</td>
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<td>7.35</td>
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<tr>
<td>$t_{1/2}$ (min)</td>
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<td>13.23</td>
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<tr>
<td>Cl/F</td>
<td>0.116</td>
<td>0.139</td>
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<tr>
<td>MRT (min)</td>
<td>33.83</td>
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Note. $C_{max}$, peak plasma concentration; $t_{max}$, time to peak plasma concentration; $t_{1/2}$, half-life; Cl/F, clearance/F; MRT, mean residential time.
RVS is a plant-derived polyphenol. Its presence in common dietary sources suggests low side effects, although its lethal doses, as a single agent, have never been determined. RSV produced no toxic effects in rats by oral administration at 20 mg/kg/day over a period of 28 days (47). Incubation of isolated rat hepatocytes with RSV decreased cell viability (LC$_{50}$ (2 h) = 470 ± 39 nM) (68). Cell death has been attributed to mitochondrial toxicity (27).

**Cardiovascular Protection**

Epidemiological studies (18,50) suggested that dietary factors, including moderate red wine consumption, may reduce the risk of cardiovascular diseases, at least partly because of the presence of polyphenols. Evidence for cardioprotection by alcoholic beverages and their polyphenols has been extensively reported. Polyphenols have a multitude of biological activities. Their antioxidant, free radical scavenging properties, and their ability to interact with the pathway leading to the generation of NO from vascular endothelium are relevant to cardioprotection. Although there are no extensive clinical studies on the cardioprotective effects of polyphenols, a few reports on the effects of polyphenols in volunteers have recently been published (22,32).

Attention to RSV was drawn in 1992, when Seimann and Creasy (83) reported the presence of trans-RSV in red wine. They pointed out that RSV is an active ingredient of the oriental folk medicine kojo-kon, which is used for many therapeutic indications including heart diseases. It has been suggested that RSV is responsible for the cardiovascular benefits associated with moderate wine consumption (15) and that the presence of RSV in red wine may explain the so-called “French paradox,” a very low mortality rate due to coronary heart disease (CHD) in France despite a high-fat diet and smoking habits (18).

In the absence of clinical studies, the cardioprotective effect of RSV in humans has yet to be demonstrated. However, in vitro studies and experiments on animal models suggest that it may provide protection against CHD by a number of mechanisms:

1) inhibition of (LDL) oxidation,
2) inhibition of platelet aggregation, synthesis of proatherogenic eicosanoids and expression of procoagulant tissue factor,
3) inhibition of cell proliferation,
4) increased vasorelaxation and upregulation of NO synthase.

**Inhibition of LDL oxidation**

A number of studies have shown that oxidative stress is a major causative factor for atherosclerosis, and that lipoprotein (mainly LDL) oxidation appears to be a crucial event in atherogenesis (49). Oxidized LDL (ox-LDL) is present in atherosclerotic lesions, and may contribute to a series of deleterious proatherogenic reactions (101). RSV is an effective scavenger of hydroxyl, superoxide and metal-induced radicals, as well as an antioxidant in ROS-producing cells (55). It is worth noting that, because of its lipophilic properties, trans-RSV binds isolated lipoproteins (HDL < LDL < VLDL) in a fixed ratio with the lipoprotein phospholipid content (6). Given the above properties, RSV (2 – 6 µM) inhibits both the lipid oxidation and protein modification caused by two relevant physio-
logical oxidants: ferrylmyoglobin and peroxynitrite (12) (Fig. 3). The RSV-induced protection of lipids and apoprotein from oxidative damage preserves the ability of LDL to enter CHO-K1 cells by means of the native LDL receptor pathways, whereas ox-LDL are taken up by unregulated scavenger receptors that induce the formation of foam cells, and cause dysfunction in vascular smooth muscle and endothelial cells (EC) (1). The high degree of RSV’s effectiveness in the prevention of LDL oxidation was first demonstrated by Frankel et al. (25). It has been confirmed by Sanchez Moreno et al. (81), who used the new oxidizability index, CLT50 (the concentration of antioxidant that increases the lag time by 50% in comparison with controls regardless of the antioxidant status of LDL). In general, polyphenols have been found to be more potent antioxidants than ascorbic acid or tocopherol with CLT50 for RSV, caffeic acid and catectin in the range of 0.38 – 0.43 \( \mu \text{M} \) vs. 2.1 \( \mu \text{M} \) for ascorbic acid and 17.15 \( \mu \text{M} \) for tocopherol.

It has also been reported that RSV is an efficient lipoxygenase inhibitor (58), and thus may prevent the subendothelial oxidative modification of LDL (49). The inhibitory effects of RSV have been evaluated in K562 cells. The IC50 of RSV for inhibition of 5-lipoxygenase was 2.5 ± 0.3; of 15-lipoxygenase, 25 ± 3.0; of cyclooxygenase, 20 ± 2.0; and of peroxidase, 15 ± 2.0 \( \mu \text{M} \).

Despite its reported antioxidant activity, RSV, like other polyphenols, is metabolized by peroxidase enzymes to form a prooxidant phenoxy radical (27), which, in the presence of \( \text{H}_2\text{O}_2 \), acts as a catalyst of LDL oxidation (78). The relevance of the pro- or antioxidant action of RSV on oxidation of different lipoproteins in vivo is still unknown.

Although the treatment of human liver cells in vitro for 24 h with 5 \( \mu \text{M} \) RSV caused a 50% reduction in secreted LDL (comparable with that obtained using 10 \( \mu \text{M} \) atorvastatin)
RSV only slightly inhibited \( K_i = 35 - 69 \mu M \) purified human squalene monooxygenase, a rate-limiting enzyme in cholesterol biosynthesis (52). In line with these results, it has been reported that RSV affects liver lipid metabolism \textit{in vitro} by reducing the hepatic production of apoprotein B and the secretion of cholesterol esters and triglycerides in HepG2 human hepatocarcinoma cells (31). By \textit{in vivo} administration RSV did not affect, however, lipoprotein profile or normolipidemia (26,87,92,98,99). In contrast with the above mentioned \textit{in vivo} studies (92,98), it has been demonstrated that RSV reduces:

1) proteinuria and hyperlipidemia secondary to nephritis in rats (5 mg/day/100 g body weight for 14 days) by improving liver lipid synthesis and liver fatty acid oxidation (70);

2) hyperlipidemia in hepatoma-bearing rats (10 – 50 ppm for 20 days) by partially increasing the fecal excretion of sterols and bile acid (65) (Fig. 4).

In these two cases, the mechanism by which RSV reduces serum lipid levels (hypotriglyceridemic > hypocholesterolemic action) remains unclear, but is presumed to be related to its antioxidant and/or antiinflammatory properties. The discrepancies between older and more recent studies can be ascribed to the different hyperlipidemic models used.

In relation to its antiinflammatory and growth inhibiting properties, it has been reported that RSV interferes with NF-kB, a transcription factor that controls the expression of a number of cytokines, growth factors, adhesion molecules, and the JNK and ERK signaling pathways (76). Shigematsu et al. have recently demonstrated in a rat model of ischemia/reperfusion that RSV (0.7 mg/kg i.v. during stabilization period) exerts a powerful antiinflammatory effect and inhibits leukocyte adhesion and microvascular barrier dysfunction by detoxification of superoxide (85).
Inhibition of platelet aggregation, synthesis of proatherogenic eicosanoids, and expression of procoagulant tissue factor

It has been demonstrated that RSV interferes with two prominent features of the atherogenesis: apoptosis and thrombosis. Apoptosis contributes to plaque instability, rupture and thrombus formation (61). Although it has not been proven in a vascular system, RSV (50 μM) blocks apoptosis induced by oxidized VLDL and LDL in PC12 cells (21), and its antiapoptotic activity has been shown to depend on the direct inhibition of the main arachidonate metabolizing enzymes (58).

The thrombotic disorders are the main causes of myocardial infarction and stroke (17). Since in vitro experiments demonstrated inhibition of platelet function and of endothelial tissue factors by RSV (74), it is conceivable that RSV may decrease blood coagulation and thrombus formation. The recently reviewed (26,87) in vitro inhibition of platelet aggregation in response to various agonists (thrombin, ADP and collagen) has been linked to the inhibition of eicosanoid synthesis (76) and to platelet calcium channels (20). However, it is more likely that RSV interferes with some of the other factors involved in hemostasis and thrombosis, rather than platelet aggregation. In fact, the IC\textsubscript{50} of RSV for inhibition of platelet aggregation (>100 μM, depending on the agonist used) is rather high (26,87). ADP-induced platelet aggregation was inhibited by RSV and its glucosylated derivative, (E)-resveratrol 3-O-β-D-glucopyranoside, with IC\textsubscript{50} of 129.9 ± 64 and 84.90 ± 6.50 μM, respectively (72).

Many other proatherogenic functions can be inhibited by RSV at lower concentrations:

1) the main arachidonate-metabolizing enzymes involved in eicosanoid production (IC\textsubscript{50} = 2 – 25 μM) (58,76);
2) procoagulant tissue factor expression (IC\textsubscript{50} = 10 – 20 μM) (74), and
3) monocyte adhesion to EC (IC\textsubscript{50} = 10 – 25 μM) (75).

It should be noted that the in vivo cardiovascular protective effect of dietary RSV, as a consequence of normal wine consumption, cannot be attributed to the inhibition of platelet aggregation. Ethanol is thought to be the main anti-aggregant component of wine (26,87) and the antiplatelet activity of RSV should be weakened or masked in circulation (48). Indeed, in circulation RSV is largely bound to serum proteins (53) and erythrocytes (8).

Inhibition of cell proliferation

The proliferation of vascular smooth muscle cells (VSMC) plays an important role in the pathogenesis of proliferative cardiovascular disorders: atherosclerosis and post-angioplasty restenosis. Numerous studies indicate that RSV has an antiproliferative effect. At concentrations as low as 0.01 to 1.0 μM (attainable in plasma at therapeutic doses) it inhibits proliferation of VSMC induced by advanced glycation end-products (AGEs), as well as the synthesis of DNA and collagen (66). More recently RSV (25 μM) has been found to affect differently quiescent (no effect) and proliferating VSMC (induction of apoptosis) by acting on intracellular p53 and p21\textsuperscript{WAF1/CIP1} levels (67). RSV inhibits angiotensin II-induced VSMC hypertrophy by interfering with the PI3/Akt and p70\textsuperscript{s6k} and ERK\textsuperscript{1/2} signaling pathways (37). This effect suggests that RSV may be capable of selectively eliminating abnormally proliferating VSMC. These results open up the possibility of using RSV (at higher concentration, >25 μM) clinically to “delete” unwanted proliferating VSMC in certain pathological conditions, such as post-angioplasty restenosis (37,67).
antiproliferative effect has also been demonstrated in EC exposed to VEGF to stimulate peroxide release (57). In addition to the in vitro evidence, RSV was effective in vivo as well. By repeated administration, at 2 to 4 mg/kg/day for five weeks, RSV inhibited intimal hyperplasia (vascular thickening) of an injured artery in an experimental rabbit model (104).

**Vascular relaxation and upregulation of NO synthase**

It is thought that among polyphenols RSV is the most effective relaxant of vascular smooth muscle. RSV has been reported to inhibit norepinephrine- (NE) and phenylephrine-induced contraction of rat aorta (14). In guinea pigs, Naderali et al. (69) found that at 5–70 μM RSV induces concentration-dependent relaxation of both mesenteric and uterine arteries preconstricted with NE (10 μM) or KCl (125 mM). His data indicate that RSV has a greater effect on resistance than conductance arteries. RSV-induced vascular relaxation may be endothelium-dependent (attenuated by L-NAME) or endothelium-independent: vasodilating prostanoids were apparently not involved. Jager et al. (45) described the vasorelaxant effects of trans-RSV (EC50 = 4.5 μM) on porcine coronary arteries, while Li et al. (56) reported a stimulant effect of RSV on the Ca2+-activated K+ current in vascular EC (EC50 = 20 μM). These findings stimulated Orallo et al. (71) to reexamine endothelium-dependence of the vasodilator effect of RSV. The characteristic endothelium-dependent vasorelaxant effect of trans-RSV in rat aorta seems to be caused by the inhibition of vascular NADH/NADPH oxidase, and a subsequent decrease in basal cell superoxide radical generation and, therefore, NO biotransformation.

Among the endogenous mediators of cardiovascular disorders, the 21 amino acid peptide endothelin-1 (ET-1) is a primary antecedent in coronary heart disease. Results from pre-clinical studies in humans, as well as animal studies, have shown that plasma ET-1 levels are consistently elevated in many spasm-related cardiovascular diseases, and that ET receptor blockers can substantially alleviate the complications of these diseases (97). RSV has been found to function as an ET-1 antagonist (100). It inhibits MAPK activity and nuclear translocation in coronary artery smooth muscle by reversing ET-1 stimulatory effects (23). There is evidence that oxidative stress (produced by stimulation of VSMC by H2O2) increases ET-1 generation and autocrine ET-1 activity in VSMC, a mechanism that might contribute to endothelial dysfunction in atherosclerosis. This effect is inhibited by RSV (100 μM) (80). In hypercholesteremic rabbits RSV (3 mg/kg/day for 12 weeks) decreased plasma ET-1 levels and significantly increased NO levels (105). These results show that RSV (and red wine) improves endothelial function, which may be one of the mechanisms by which it exerts alcohol-independent cardioprotective effects.

Another therapeutically relevant effect of RSV on the cardiovascular system is its ability to interact with the pathway leading to the generation of NO by the vascular endothelium. NO has vasorelaxant and anti-aggregatory properties. In a longer term, it induces the expression of genes that protect the cardiovascular system (13) and limits the flux of atherogenic plasma proteins into the arterial wall. At 50 to 100 μM RSV has been found to cause a 3-fold increase in eNOS expression in cultured pulmonary artery EC (41), whereas at 10 μM it had no effect. Subsequently, it has been demonstrated (94) that RSV in a concentration-dependent manner upregulates (up to 2.8 fold) eNOS mRNA expression in HUVECs incubated for 24 to 72 h. eNOS protein expression and eNOS-derived NO production also increased. Leikert et al. (54) obtained analogous results using RSV and
red wine polyphenol extract (RPWE), and suggested that an increase in active eNOS levels with subsequent endothelial NO release may antagonize the development of endothelial dysfunction, supporting long-term cardiovascular protective effects of RSV or polyphenols.

Cardioprotective Profile in Isolated Rat Hearts

Most of the studies considered so far concentrated on the positive effects of RSV in the prevention of atherosclerosis and coronary heart disease. We will now consider its possible use as a therapeutic drug in the treatment of acute conditions, such as ischemia-reperfusion (I-R) injury.

It has been found that RSV (10 μM administered at 15 min before 30 min global ischemia-2h reperfusion) has a cardioprotective action in isolated perfused working rat hearts (79). The RSV-treated group showed a significant reduction in malonaldehyde (MDA) formation and infarct size in comparison with the control group. The authors attributed this effect to RSV’s peroxyl radical scavenging activity. In a model of transient regional ischemia in anesthetized rats, infusion of RSV effectively prevented I-R-induced arrhythmias and cardiac cell damage. Because of very low concentrations of RSV the authors doubted that a direct antioxidant effect was responsible and suggested that the observed effect may have been due to a significant upregulation of NO production by RSV (42).

We (10) found that in Langendorff-perfused hearts RSV infusion (10 μM) for 10 min caused a 40% decrease in the baseline phosphorylation potential \((P < 0.05\) vs. pre-infusion value) without affecting contractility. During the infusion, the level of effluent adenosine was increased by 68% and paralleled a 50% increase in coronary flow (Table 3). We suggested that the metabolic pattern after RSV infusion is similar to that produced by ischemic preconditioning, thus indicating that an increase in adenosine availability is involved in the cardioprotective activity of RSV. We tested this hypothesis using a low-flow ischemia protocol that we believe is clinically more relevant (11). The results showed that RSV stimulates adenosine release and significantly increases coronary flow. This effect

<table>
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<tr>
<th>Time (min)</th>
<th>PCr (μmol/gww)</th>
<th>P&lt;sub&gt;c&lt;/sub&gt; (μmol/gww)</th>
<th>PP (1/mM)</th>
<th>Ado (nmol/min/gww)</th>
<th>CF (mL/min)</th>
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<tbody>
<tr>
<td>0</td>
<td>4.99 ± 0.33</td>
<td>1.57 ± 0.12</td>
<td>107.6 ± 16.0</td>
<td>0.18 ± 0.02</td>
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<tr>
<td>10</td>
<td>4.35 ± 0.22</td>
<td>2.18 ± 0.25</td>
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Note. PCr, phosphocreatine; P<sub>c</sub>, inorganic phosphates; PP, phosphorylation potential; Ado, adenosine; CF, coronary flow; gww, gram wet weight. Data from Bradamante et al. (11); mean values ± S.E.M.

In control hearts, adenosine release was 0.20 ± 0.02 at \(t = 0\) min and 0.22 ± 0.02 at \(t = 10\) min.
was almost completely abolished by the adenosine receptor antagonist 8-\textit{p}-sulfophenyltheophylline (SPT) (Fig. 5).

It has been shown that activation of adenosine receptors is a pharmacological preconditioning (36,90,102) that can elicit late-phase protection (12 to 24 h after the stimulus). In addition, Bolli (9) has pointed to NO as a trigger and mediator of late preconditioning. We tested (11) the effects of the prolonged administration of RSV (15 days, 25 mg/L) in rats by observing their Langendorff-perfused hearts at 24 h after drug treatment. The hearts subjected to a low-flow ischemia protocol (1h with a reduction in natural coronary flow to 0.6 mL/min, followed by 30 min of reperfusion) showed vasodilation and improved functional recovery at reperfusion. Neither RSV nor SPT were present in the buffer during reperfusion. At \( t = 0 \) min, the Res group showed significantly increased CF in comparison with \( t = -10 \) min (\$, \( P < 0.05 \)), and * \( P < 0.05 \) vs. IC and ResSPT. During reperfusion, there was a significant increase in CF in the Res group: \( t = 45 \) min \# \( P < 0.01 \) vs. IC and ResSPT; \( t = 60 \) min * \( P < 0.05 \) vs. IC and ResSPT. Adapted from ref. 11.

RSV (10 \textmu M) was also tested as a pharmacological preconditioning agent in isolated perfused working rat hearts subjected to I-R injury (30 min global ischemia-2h reperfusion). The RSV-treated hearts showed greater postischemic functional recovery, with reduced myocardial infarction (Fig. 8) and cardiomyocyte apoptosis (Fig. 9). The cardioprotective effects were abrogated by L-NAME or aminoguanine (AG). RSV induced inducible NO synthase (iNOS) mRNA, which was completely blocked by AG (39). The fact that RSV was unable to precondition iNOS knockout mouse hearts (whereas it successfully preconditioned wild-type hearts), confirms the essential role of iNOS in RSV-induced cardiac preconditioning (44).
COMMENTS

RSV is attractive as a potential cardioprotective agent since the long-term use of polyphenols in the diet suggests its safety. However, its safety cannot be considered established since a proper toxicological evaluation of RSV alone, as a single agent, has not been performed.

While many in vitro studies and limited experiments on animal models clearly demonstrate that RSV is a potent antioxidant and is capable of interfering with many important pathways (i.e. lipid and eicosanoid synthesis and NO production), its therapeutic efficacy in humans has not been established.

The beneficial effects of RSV may be attributed to its antioxidant, platelet aggregation inhibitory, vasorelaxant activities or its ability to inhibit proliferation of VSMC or EC. An interplay of different mechanisms cannot be excluded, but has to be demonstrated. It is worth noting, however, that the majority of the experiments have been performed in vitro.
in non-human models using high concentrations of RSV. In addition, most of the studies focused on short-term responses. Up to now, only the antioxidant and the vasodilatory effects have been proven not only *in vitro*, but also in animal models, while the ability of RSV to modify the lipoprotein profile is still controversial. More experiments should be performed using physiologically relevant RSV concentrations and a wider selection of models.

We shall not discuss the possible benefits associated with dietary polyphenol intake in CHD, as this has been extensively discussed in recent reviews (16,19,60); we simply suggest caution in drawing optimistic assumptions of cardiovascular benefits from *in vitro* data. Although it is widely known that red wine has cardioprotective effects, the real existence of the “French paradox” has been questioned by recent and more precise epidemi-

**FIG. 7.** Changes in rate-pressure product (A) and coronary flow expressed (B) as percentages of baseline values (for 100% values see Fig. 3) in Langendorff-perfused hearts of rats after chronic RSV consumption. *P < 0.05 of R vs. C, R-LNAME and R-SPT. For abbreviations and protocol, see legend to Fig. 6. Adapted from ref. 11.
logical data concerning effects of wine on human health (5,18). Since RSV is one of the more active polyphenols, we should be also concerned whether RSV in red wine can be responsible for the cardioprotective effects of wine in humans. If we assume that the RSV content of red wine is \(~5\) mg/L and “normal” wine consumption of a 70 kg person in Mediterranean countries is 0.5 L/day, the intake of RSV with wine will be 2.5 \(\mu\)g/day or 36 \(\mu\)g/kg/day. In a recent study of healthy volunteers (33), RSV was administered at a dose of 360 \(\mu\)g/kg (10 times the dose possibly consumed in wine). The authors demonstrated that absorption of RSV is broadly equivalent in aqueous and alcoholic matrices, and found peak plasma levels of 2 \(\mu\)M of total RSV (conjugated and free) at 30 min after ingestion (only 20 nM was free RSV). They concluded that the peak concentrations of 10 – 40 nM free RSV is inadequate to account for observed biological activity. On this
basis the potential health benefits of red wine suggested by many research groups (48,94) have been recently questioned as unrealistic and greatly overstated (84,91). Gescher and Steward (29) considered the evidence from several studies indicating the possibility that high bioavailability and plasma levels of RSV were due to inclusion of the glucuronidated and sulfate derivatives, and indicated the need for further studies in animals and humans. While these proposed investigations are not expected (34) to greatly improve our knowledge of RSV properties, some recent results on compounds activating life-extending genes (SIRT genes) (38,40) or inhibiting AGEs (66) suggest new lines of research into the therapeutic use of polyphenols, including RSV. Indeed, it has been demonstrated that RSV increases the replicative lifespan of *Saccharomyces cerevisiae* by 70% and is the most potent among tested polyphenols.
REFERENCES


89. Takano H, Bolli R, Black RG et al. A(1) or A(3) adenosine receptors induce late preconditioning against infarction in conscious rabbits by different mechanisms. *Circ Res* 2001;88:520–528.


