Alcoholism: Clinical and Experimental Research

The biological responses to resveratrol and other polyphenols from alcoholic beverages

Lindsay Brown, Paul A. Kroon, Dipak K. Das, Samarjit Das, Arpad Tosaki, Vincent Chan, Manfred V. Singer and Peter Feick

School of Biomedical Sciences, The University of Queensland, Brisbane, Australia (LB, VC); Institute of Food Research, Norwich, UK (PAK); Cardiovascular Research Center, University of Connecticut School of Medicine, Farmington, USA, (DKD, SD); Department of Pharmacology, University of Debrecen, Debrecen, Hungary (AT); Department of Medicine II, (Gastroenterology, Hepatology and Infectious Diseases), University Hospital of Heidelberg at Mannheim, Germany (MVS, PF)

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Correspondence:
Manfred V. Singer, M.D. (Hon. Doc. mult.)
Professor of Medicine and Chairman
Department of Medicine II
(Gastroenterology, Hepatology and Infectious Diseases)
University Hospital of Heidelberg at Mannheim
Theodor - Kutzer - Ufer 1-3
D-68135 Mannheim
Germany
Tel.: ++49 - 621 - 383 - 3284
Fax: ++49 - 621 - 383 - 3805
E-mail: manfred.v.singer@med.ma.uni-heidelberg.de

ABSTRACT

Although excessive consumption of ethanol in alcoholic beverages causes multi-organ damage, moderate consumption, particularly of red wine, is protective against all-cause mortality. These protective effects could be due to one or many components of the complex mixture of bioactive compounds present in red wine including flavonols, monomeric and polymeric flavan-3-ols, highly coloured anthocyanins as well as phenolic acids and the stilbene polyphenol, resveratrol. The therapeutic potential of resveratrol, firstly in cancer chemoprevention and then later for cardioprotection, has stimulated many studies on the possible mechanisms of action. Further indications for resveratrol have been developed, including the prevention of age-related disorders such as neurodegenerative diseases, inflammation, diabetes and cardiovascular disease. These improvements are remarkably similar yet there is an important dichotomy: low doses improve cell survival as in cardio- and neuro-protection yet high doses increase cell death as in cancer treatment. Fewer studies have examined the responses to other components of red wine, but the results have, in general, been similar to resveratrol. If the non-alcoholic constituents of red wine are to become therapeutic agents, their ability to get to the sites of action needs to be understood. This mini-review summarizes recent studies on the possible mechanisms of action, potential therapeutic uses and bioavailability of the non-alcoholic constituents of alcoholic beverages, in particular resveratrol and other polyphenols.

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INTRODUCTION

Alcohol-related disorders account for an enormous part of the global mortality and morbidity (Rehm et al., 2003). Chronic alcohol abuse may lead to organ damage and carcinoma, especially to the liver and pancreas, but also damage to the brain (Harper, 2007) and immune system (Cook, 1998) as two of many examples. On the other hand, epidemiological studies indicate that light to moderate consumption of alcoholic beverages reduces all-cause mortality (De Lorimier, 2000). In particular, red wine consumption is inversely associated with mortality due to cardiovascular diseases (Gronbaek, 2002), and the protective effects are observed for consumption levels up to 300 ml wine per day (Rotondo et al., 2001). The reductions in risk from low to moderate consumption of alcoholic beverages are greater for red wine compared to white wine, beer and most spirits (Gronbaek, 2002). The potential association between red wine consumption and risk of cardiovascular disease mortality has been highlighted by the 'French Paradox' which refers to the finding that people in France suffer from a relatively low incidence of coronary heart disease, despite their diet being rich in saturated fats (Renaud & de Lorgeril, 1992). This concept has been intensely dissected with Ferrières (2004) concluding "that the promotion of primary prevention, based on an optimal diet rich in fruit and vegetables, regular physical exercise, and life without smoking, is worthwhile."

This phenomenon was first noted by the Irish physician Samuel Black (1819) who attributed the observation to the French habit of drinking red wine with meals, while the first scientific study was published by Cabot (1904), and the first comparison of coronary heart disease mortality between wine-drinking countries and those where beer or spirits were consumed was made 75 years later (St. Leger et al., 1979). The effect has since been ascribed to a number of red wine components including ethanol, which at low doses has beneficial effects on cardiovascular disease risk, and various polyphenols derived from the grape skins, seeds and stems or during maturation in oak barrels (Booyse et al., 2007).

Red wine contains a complex mixture of bioactive compounds that are predominantly phenolic in nature (Soleas et al, 1997). These include flavonols such as myricetin, kaempferol and the predominant quercetin, the flavan-3-ol monomers catechin and epicatechin, the oligomeric and polymeric flavan-3-ols or proanthocyanidins, various

highly coloured anthocyanins, various phenolic acids (gallic acid, caftaric acid, caffeic acid, p-coumaric acid) and the stilbene resveratrol (figure 1). These compounds occur in red wine but are virtually absent from white wine because the skins, seeds and stems are present during the fermentation of red wine but not white wine. Red wine is one of the richest sources of polyphenols in human diets. Highly tannic red wines can contain up to 3 g of total polyphenols per litre, and moderate red wine drinkers will consume polyphenols at levels well above the population average (Waterhouse, 2002). Urinary resveratrol measurements have been proposed as a robust marker of wine intake (Zamora-Ros et al, 2009). Resveratrol is synthesized in the skins of grapes as a response to fungal infection. Once present, the compound acts as a phytoalexin, preventing the proliferation of pathogens. Rich natural sources of resveratrol include *Polygonum cuspidatum* (Japanese knot-weed, 0.524 mg/g), red wines (0.1 – 14.3 mg/l), red grape skins, berries such as blueberries, as well as peanuts and other nuts (Baur & Sinclair, 2006).

There is a substantial literature on the biological activities of phenolic compounds that are present in red wine. Much of the early interest centred on quercetin, a potent anti-oxidant that is present as a mixture of glycosides and the aglycone in red wine (Burns et al., 2000). Quercetin has been shown, *in vitro* and in animal models, to possess a range of biological activities relevant to protecting against cardiovascular diseases (Perez-Vizcaino et al., 2006) and against cancer (Birt et al., 2001; Moon et al., 2006a).

There has been considerable interest in resveratrol and other polyphenols in red wine in relation to human health. This mini-review summarizes the current knowledge about the various beneficial effects of resveratrol and other polyphenols from wine in the prevention of ageing-related disorders such as cancer, neurodegenerative disorders, inflammation and cardiovascular disease; further, the possibility that resveratrol may be useful in the treatment of these diseases will be mentioned. A critical issue for resveratrol and other polyphenols as potential therapeutic agents is whether adequate concentrations can be achieved at the sites of action; thus, the bioavailability and efficacy of these compounds in humans are outlined at the end of this article.

Resveratrol: the evidence for potential benefits to human health

There is substantial evidence that resveratrol prevents or delays the onset of chronic diseases such as diabetes, inflammation, Alzheimer's disease and cardiovascular disease that increase in prevalence with age as well as inducing neuroprotection and inhibiting proliferation of human cancer cell lines (Aggarwal *et al*, 2004; Baur & Sinclair, 2006; Das & Maulik, 2006; Das & Das, 2007b; Opie & Lecour, 2007; Vidavalur *et al*, 2006; Harikumar & Aggarwal, 2008; Raval *et al*, 2008; Saiko *et al*, 2008). The breadth of the therapeutic potential of resveratrol is shown by the extension of the lifespan and improved motor function in mice fed a high-calorie diet (Baur *et al*, 2006). This impressive study indicates new approaches for treating obesity-related diseases and the diseases of ageing. The prevention or treatment of cardiovascular disease has been a major research theme in resveratrol studies. The potential benefits cover the range of cardiovascular problems, including myocardial infarction, arrhythmias, hypertension, hypertrophy, inflammation leading to fibrosis, atherosclerosis and thrombosis.

Resveratrol extends the lifespan of species such as yeast, worms and flies by activation of the silent information regulator 2 (SIR2), one of the sirtuins (Baur & Sinclair, 2006). The sirtuins as class III histone deacetylases regulate key pathways by removing acetyl groups from the ε-amino group of lysine to silence transcription. Multiple metabolic pathways are regulated by SIRT1, the mammalian analogue of SIR2, as this enzyme is involved in lifespan regulation, the cellular response to stress, glucose homeostasis, insulin secretion and lipid mobilization (Jiang, 2008). SIRT1 is a key regulator of vascular endothelial homeostasis controlling angiogenesis, vascular tone and endothelial dysfunction (Potente & Dimmler, 2008). The cardiac responses to low (2.5 fold) or moderate (7.5 fold) overexpression of SIRT1 in transgenic mouse hearts included attenuation of age-dependent increases in heart weight, apoptosis, fibrosis and cardiac dysfunction while high (12.5 fold) overexpression increased apoptosis, heart weight and decreased cardiac function (Alcendor *et al*, 2007).

While the pharmacological actions of resveratrol have been linked to antioxidant actions, the possible link between sirtuin activation and redox regulation by

resveratrol is not yet clear. Hypoxia-induced apoptosis in embryonic rat heart-derived H9c2 cells was greatly decreased following treatment with resveratrol by activation of SIRT1 increasing the ability of FOXO1 (Forkhead box factors regulated by insulin/Akt) to induce cell cycle arrest (Chen et al, 2009). The ability of the FOXO transcription factor FOXO3 to induce cell cycle arrest and resistance to oxidative stress was increased by SIRT1 (Brunet et al, 2004). Moderate expression of SIRT1 protected the heart from oxidative stress and increased expression of antioxidant enzymes but high expression increased oxidative stress (Alcendor *et al*, 2007). Cilostazol, a selective inhibitor of phosphodiesterase III, dose-dependently increased SIRT1 expression to protect human endothelial cells after ischemic vascular damage through increased production of nitric oxide (Ota et al, 2008). These studies suggest that sirtuins are important in the resistance to oxidative stress induced by resveratrol, but the details need to be determined.

The breadth of the therapeutic potential for resveratrol hides a contradiction: how can the same compound improve the survival of cells as a cardioprotective or neuroprotective agent, yet kill cancer cells? Although the limited literature makes comparison of doses difficult, the general trend is that higher doses are needed to kill cancer cells than to improve cell survival. Possible reasons include dose-dependent effects, either at the level of the molecular mechanisms as described above for SIRT1, or by activation of different pathways at different resveratrol doses. In the heart, resveratrol at lower doses (5 mg/kg) activated survival signals by up-regulating the antiapoptotic and redox proteins Akt and Bcl-2 while higher doses of 25 mg/kg potentiated a death signal by down-regulating redox proteins and up-regulating pro-apoptotic proteins (Dudley et al, 2008). Although resveratrol is usually considered as an antioxidant, primarily by increasing NO bioavailability, resveratrol can also exhibit pro-oxidant properties in the presence of transition metal ions such as copper, leading to oxidative breakage of cellular DNA (Alarcón de la Lastra & Villegas, 2007). These authors suggest that this pro-oxidant action is the common mechanism for anticancer and chemopreventive properties of plant polyphenols.

Resveratrol and chemoprevention

The initial reports of anti-cancer responses to resveratrol led to the increased interest in this compound from 1992 (Baur & Sinclair, 2006). Resveratrol inhibited or retarded

the growth of a wide range of human cancer cells in culture as well as implanted tumours usually in mice (Aggarwal et al, 2004; Athar et al, 2007). The compound inhibited experimental tumourigenesis in a wide range of animal models by targeting many components of intracellular signaling pathways including pro-inflammatory mediators, regulators of cell survival and apoptosis, and tumour angiogenic and metastatic switches by modulating a distinct set of upstream kinases, transcription factors and their regulators (Kundu & Surh, 2008; Pirola & Fröjdö, 2008). However, the role of sirtuin activation by resveratrol has not been defined. Khan et al (2008) have presented the key findings from studies on the effects of dietary antioxidants including tea polyphenols, curcumin, genistein, resveratrol, lycopene, pomegranate and lupeol against cell lines from human skin, prostate, breast, lung and liver cancers. Many of the same compounds, including resveratrol, curcumin and epigallocatechin gallate, modulated the effects of deregulated cell cycle checkpoints, and this could contribute to the prevention of cancer (Meeran & Katiyar, 2008). However, it should be emphasised that these results are measurements of resveratrol responses on human cancer cells in culture, or taken as conclusions from epidemiological studies, rather than the results from clinical trials with cancer patients. No clinical trials with resveratrol in human cancers have been reported although 5 trials on human cancers are underway or finished (http://www.clinicaltrials.gov/ct2/results?term=resveratrol).

Resveratrol and inflammation

Inflammation is a pervasive cause of disease, implicated in diseases as diverse as metabolic syndrome (Dandona et al, 2005), rheumatoid arthritis and neurodegenerative disorders (Stolp & Dziegielewska, 2008). The cardioprotective and cancer preventive actions of resveratrol may be attributable to anti-inflammatory effects such as the inhibition of synthesis and release of pro-inflammatory mediators, modifications of eicosanoid synthesis, inhibition of some activated immune cells, or inhibiting enzymes such as cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2) through to the inhibitory effect of resveratrol on transcription factors such as nuclear factor kappaB (NFkappaB) or activator protein-1 (AP-1) (Das & Das, 2007a; Udenigwe et al, 2008). Numerous studies have confirmed that resveratrol suppresses TNF-α activation (Das et. al., 2006b). Some preclinical as well as clinical studies have shown significant suppression of IL-1, IL-6 and IL-8 with resveratrol (Aggarwal

et. al., 2004, Marier et. al., 2005, Donnelly et al. 2004). In an acute *in vivo* study, resveratrol inhibited the generation of adhesion molecules (Das et al., 2006b).

Resveratrol and diabetes

In rat models of diabetes, resveratrol reduced blood glucose and triglyceride concentrations following streptozotocin treatment (Su et al, 2006) and prevented the increase in blood pressure and cardiac hypertrophy while restoring the mesenteric and cardiac eNOS activity and decreasing oxidative stress in fructose-fed rats (Miatello *et al*, 2005). In obese Zucker rats, a genetic model of type-2 diabetes, resveratrol increased GLUT-4 expression and reduced endothelin expression and cardiac apoptosis in ischaemic-reperfused hearts (Lekli *et al*, 2008). Resveratrol increased insulin sensitivity by lowering the blood glucose level in high calorie diet mice with reduced insulin-like growth factor-1 (IGF-I) and increased AMP-activated protein kinase (AMPK) playing a major role (Baur et al, 2006). Resveratrol attenuated diabetic nephropathy in rats (Sharma et. al., 2006a), reduced thermal hyperalgesia and cold allodynia in streptozotocin-induced diabetic rats (Sharma et. al., 2006b) and attenuated diabetic neuropathic pain through changes in NO and TNF-α (Sharma et. al., 2007).

Resveratrol and gastrointestinal diseases

Helicobacter pylori causes peptic ulcer and gastrointestinal cancers. Cancer prevention requires control of the infection and the subsequent inflammatory processes. Plant products including resveratrol inhibited *H. pylori* colonization, decreased gastric inflammation by inhibiting cytokine and chemokine release, and repressed precancerous changes by inhibiting nuclear factor-kappa B DNA binding, inducing profuse levels of apoptosis and inhibiting mutagenesis (Lee et al, 2008).

Resveratrol and neuroprotection

The first evidence that resveratrol crosses the blood-brain barrier was published by Virgili & Contestabile (2000). In this study in young adult rats, chronic administration of resveratrol protected against the damage caused by the systemic injection of the excitotoxin kainic acid in the olfactory cortex and the hippocampus. Resveratrol effectively protected the brain from traumatic brain injury (Ates et al., 2006) and also inhibited the excitatory synaptic transmission in rat hippocampus (Gao et al., 2006).

Resveratrol preconditioning and protection of brain cells *in vitro* from ischemic injury was mediated by SIRT1 (Raval et al, 2006). A similar study showed the neuroprotective effect of resveratrol by inhibiting the voltage-activated potassium currents in hippocampal neurons (Gao and Hu, 2005). In a study of spinal cord ischemia model, resveratrol-induced neuroprotection was mediated by decreased oxidative stress and increased NO release (Kiziltepe et al., 2004). Zamin et al. (2006) suggested that PI3-kinase/Akt pathway but not mitogen-activated protein kinase (MAPK) is involved in the neuroprotection.

Resveratrol and myocardial injury

Preconditioning describes the development of resistance to damage caused by prolonged ischemia and reperfusion to the heart (Bolli, 2007). Coordinated up-regulation of iNOS–VEGF–KDR–eNOS by resveratrol has been suggested by Das et al (2005a) as the preconditioning mechanism in rat hearts. Resveratrol likely activates both adenosine A_1 and A_3 receptors which phosphorylate PI-3 kinase, which then phosphorylates protein kinase B (Akt) and thus preconditions the heart by producing NO as well as by the activation of antioxidant Bcl-2 (Das et al, 2005a, 2005b). Activation of adenosine A_3 receptors could also precondition the heart by a survival signal through the cAMP response element-binding protein (CREB) phosphorylation via PI-3 Kinase–Akt and via MEK (Mitogen-activated extracellular signal-regulated protein kinase)–CREB pathways (Das et al., 2005c). Recent studies have also demonstrated that NO can induce the expression of heme oxygenase-1. Das et al. (2006a) showed that tin protoporphyrin (SnPP), a heme oxygenase-1 inhibitor, abolished the increased cardiac function parameters, reduced myocardial infarct size and decreased cardiomyocyte apoptosis that characterise the cardioprotection with resveratrol. The heme oxygenase-1 mediated mechanisms were related to the p38MAP kinase and Akt survival signaling, but independent of NFκB activation. Being a polyphenol, resveratrol protects the heart by its anti-oxidative properties through various redox signalling mechanisms. The ability of resveratrol to modulate redox signalling has been extensively reviewed (Das & Maulik, 2006; Vidavalur et al, 2006) but the role of sirtuin activation in the heart is yet to be determined. White wine lacking polyphenols but containing antioxidant compounds such as caffeic acid and tyrosol mediated cardioprotection against

ischemia-reperfusion injury in rat hearts by a similar survival pathway involving Akt/FOXO3a/NFκB (Thirunavukkarasu et al, 2008).

Resveratrol and hypertension

The concept that reactive oxygen species contribute to the cause and the development of complications of hypertension is well supported by many clinical and experimental studies (Li & Shah, 2004). However, systolic blood pressure was not lowered by resveratrol in young stroke-prone spontaneously hypertensive rats (SHRSP) although oxidative damage to DNA and glyoxidative damage *in vivo* were decreased (Mizutani et al, 2001). Consistent with these findings, administration of quercetin in the diet of young SHR did not delay or lessen the onset or severity of cardiovascular complications, including hypertension (Carlstrom et al, 2007). In contrast, rats with abdominal aortic constriction showed anti-hypertensive and anti-hypertrophic responses to quercetin administered in the food, but without changes in vascular and myocardial function (Jalili et al, 2006).

Resveratrol and angiogenesis

The formation of new blood vessels is a potential mechanism to reduce ischemic damage to the heart. Ischemia induces VEGF release to encourage the development of collateral coronary circulation. Reactive oxygen species may have an important role in activating this process of myocardial angiogenesis (Maulik, 2006). Resveratrol enhanced angiogenesis both *in vivo* and *in vitro* by induction of VEGF which was regulated by thioredoxin-1 and heme oxygenase-1 (Kaga et al, 2005). This could be an important mechanism mediating cardioprotection in the chronic ischemic myocardium.

Resveratrol and the endothelium

Endothelial dysfunction is a characteristic finding in coronary heart disease. Resveratrol appears to protect endothelial cells at multiple targets. In a rat model of injured aorta, a lower dose of resveratrol (10 mg/kg) increased the proliferative, migrative and adhesive activities of endothelial progenitor cells, enhanced eNOS expression and accelerated the repair of the injured artery; however, a higher dose (50 mg/kg) had minimal effects (Gu et al, 2006). Chemokines in endothelial cells such as fractalkine are critical in the initiation, maintenance and resolution of

inflammation. Resveratrol inhibited monocyte adhesion to human endothelial cells induced by TNF-α and then suppressed arterial endothelial fractalkine expression in organs such as the heart (Moon et al, 2006b). The expression of the atherogenic cytokine, monocyte chemoattractant protein-1, was suppressed by about 45% at a resveratrol concentration of 50 μmol/l; this suppression was not altered by heme oxygenase-1 inhibition or knockdown indicating a distinct signalling pathway (Cullen et al, 2007). At submicromolar resveratrol concentrations, TNF-α-induced NFκB activation, inflammatory gene expression and monocyte adhesiveness to human coronary arterial endothelial cells were inhibited; these anti-inflammatory actions on endothelial cells could lead to cardioprotection (Csiszar et al, 2006).

As the cells lining blood vessels, endothelial cells are sensitive to increased levels of reactive oxygen species in the blood, or produced in the vasculature by NADPH oxidase. The stimulation of this enzyme by oxidised LDL in vascular endothelial cells was inhibited by resveratrol by reducing the membrane association of two of the proteins in the active enzyme complex, gp91 (phox) and Rac1 (Chow et al, 2007). Resveratrol pre-treatment prevented the increase in caspase 3/7 activity produced by oxidised LDL and TNF-α in endothelial cells; this effect was attenuated by inhibition of the antioxidant systems, glutathione peroxidase and heme oxygenase-1 (Ungvari et al, 2007). The oxidant species, peroxynitrite, induced apoptotic changes in aortic endothelial cells in culture; resveratrol concentrations from 1-25 μmol/l caused a concentration-dependent decrease in these apoptotic changes (Brito et al, 2006). Leukocyte activation, transmigration and target cell adhesion following ischemia-reperfusion are important in the development of graft rejection following transplantation. Following tissue allotransplantation, rats treated with resveratrol showed a moderate survival prolongation and reduction of lymphocytic infiltration and necrosis (Hsieh et al, 2007).

Flavanoids such as quercetin and wine polyphenols prevented endothelial dysfunction and reduced blood pressure, oxidative stress and end-organ damage in hypertensive animals, while flavanoid-rich foods improved endothelial function in cardiovascular patients (Perez-Vizcaino et al, 2006). An acute improvement in flow-mediated vasodilatation was measured after administration of a red grape extract

containing epicatechin, catechin, resveratrol, rutin and other polyphenols to patients with coronary heart disease (Lekakis et al, 2005).

Resveratrol and collagen deposition

Resveratrol inhibited cardiac fibroblast proliferation and differentiation to the hypersecretory myofibroblast phenotype in rat cardiac fibroblasts in culture; these are two critical steps in cardiac collagen deposition (Olson et al, 2005). Fibrosis is the final result of an inflammatory challenge and thus inflammation plays a key role in organ damage in chronic cardiovascular disease. Resveratrol interacts at multiple sites within the inflammatory pathways to decrease inflammation (Das & Das, 2007a).

Multiple actions of dietary polyphenols have been proposed for reducing inflammation as well as in the processing of lipids in the body to improve the blood lipid profile to eventually reduce the risk of coronary heart disease (Zern & Fernandez, 2005). The processes of initiation, progression and rupture of atherosclerotic plaques offer many targets to delay or prevent cardiovascular disease. There is much experimental evidence that the phenolic compounds in wine alter these processes as part of their cardioprotective actions (Szmitko & Verma, 2005).

Resveratrol and blood coagulation

Tissue factor (TF) is the primary initiator of the blood coagulation cascade. Thrombus formation at the site of complicated atherosclerotic lesions following exposure to TF is a major cause of death in industrialized countries. In human umbilical vein endothelial cells and mononuclear cells, resveratrol and quercetin reduced the expression of TF mRNA by bacterial lipopolysaccharide, interleukin-1β or TNF-α (Di Santo et al, 2003). Several resveratrol derivatives were 2-10-fold more effective in inhibiting TF production in human peripheral blood mononuclear cells than the parent compound (Kaur et al, 2007).

The prevention of excessive or inappropriate aggregation of platelets decreases thrombus formation, reduces the subsequent blockage of small blood vessels and thereby reduces the risk of stroke and myocardial infarction. Many studies, reviewed

by Baur & Sinclair (2006), have shown that resveratrol prevents platelet aggregation *in vitro*, probably by selective inactivation of cyclooxygenase-1.

One of the potential mechanisms to explain this wide range of cardioprotective and vascular protective actions is the removal of highly reactive free radicals such as superoxide following increased expression of heme oxygenase-1 (Huang et al, 2005; Juan et al, 2005). Low concentrations of resveratrol (1-10 μmol/l) likely to be physiologically relevant increased heme oxygenase-1 expression, at least partly through actions on the NFκB pathway in human aortic smooth muscle cells in culture (Huang et al, 2005; Juan et al, 2005). However, the increased expression of heme oxygenase-1 has been attributed to regulation by MAP kinase and Akt survival signalling in the rat heart, not by activation of the NFκB pathway (Das et al, 2006a).

Bioavailability and bioefficacy of resveratrol and other polyphenols in humans

At high doses, orders of magnitude higher than would be achievable through red wine consumption by humans, orally ingested resveratrol is also able to extend lifespan in a number of organisms ranging from mono-cellular yeasts to animals such as mice. These findings are remarkable, with lifespan extension of greater than 50% reported for certain species of yeast, nematode, insect and fish (Howitz et al., 2003; Wood et al., 2004; Valenzano et al., 2006). It has also been demonstrated that high doses of resveratrol can prevent the detrimental effects of a high fat diet in a mouse model of obesity (Baur et al., 2006), another truly remarkable observation with wide-ranging implications for mankind considering the obesity epidemic that is sweeping the western world. But, it must be recognised that all these observations have been made with doses of resveratrol that are well above those achievable in humans though normal diet. Red wine is almost the only source of resveratrol in human diets, and the richest red wines contain about 7 mg resveratrol per litre. To achieve the equivalent dose of resveratrol that was fed to the fish in order to achieve a >50% lifespan extension (Valenzano et al., 2006) and to mice in order to prevent the detrimental effects of a high fat diet (Baur et al., 2006), one would need to consume around 60 litres per day of high resveratrol red wine, which is clearly not feasible. Therefore, these potential effects of resveratrol remain achievable through pharmaceutical means only and not through dietary means. It is interesting to note

that other dietary components have been shown to be effective in extending lifespan, including blueberry polyphenols (Wilson et al., 2006).

Flavan-3-ols, or the catechins and their oligo- and poly-meric derivatives the proanthocyanidins, have also attracted considerable interest due to their ability to improve endothelial function, vascular tone and platelet reactivity *in vivo*. In particular, ingestion of cocoa flavan-3-ols has been shown, consistently, to improve vascular function (Schroeter et al., 2006; Heiss et al., 2007) and platelet reactivity (Rein et al., 2000). These observations have been consistent across a number of studies and the required dose of epicatechin has been estimated at 1-2 mg per kg body weight (Schroeter et al., 2006). The concentration of (-)-epicatechin in red wine is in the range of 20-200 µM but (+)-catechin is present at 200-500 µM (Burns et al., 2000).

Absorption from the gut

The ability of a dietary component to influence vascular function and prevent cardiovascular disease is dependent on how much of the oral dose is absorbed from the gut, the influence of metabolism during the first pass (small intestine and liver) and the subsequent exposure that is a function of the excretion rate which is reflected in the plasma half life. It is possible that some wine compounds may accumulate in certain tissues and hence increase the exposure at these specific sites. Although there is some evidence that certain polyphenols accumulate to higher concentrations in certain body tissues compared to others, there is considerably more evidence (from animal experiments) showing that dietary polyphenols do not become concentrated in specific tissues, besides those of the gut. Nevertheless, there is some evidence that resveratrol accumulates in a time- and dose-dependent manner in the liver (Vitaglione et al., 2008) and brains (El-Mohsen et al., 2006) of rats intragastrically administered resveratrol over a period of days, while another report indicates preferential fixation of ^{14}C-resveratrol in the organs of absorption and elimination (stomach, intestine, liver, kidney) (Vitrac et al., 2003).

Polyphenols are only partially absorbed and only a fraction is excreted via the urine in humans. The plasma concentrations achieved and the urinary yield as a function of oral dose depends on a number of factors, but by far the most important is the structure of the polyphenol. Almost without exception, phenolics in plants are

glycosylated, and this is true of most plant-derived foods and beverages also. Glycosylation can have an enormous influence on the efficiency of absorption and the site of absorption – monoglucoside derivatives are preferentially absorbed from the small intestine and are better absorbed than other glycosides such as rhamnoglucosides, that are absorbed later (probably from the colon) and to a lesser extent. The enhanced absorption of monoglucosides from the small intestine is due to human small intestinal lactase enzyme that has the capacity to hydrolyse flavonoid glucosides and which is required for absorption and metabolism of these compounds (Németh et al., 2003). Some polyphenols are quite well absorbed and a significant proportion is excreted in the urine; examples include gallic acid, the isoflavones genistein and daidzein, and the (citrus) flavanones hesperetin and naringenin (Manach et al., 2005). However, for others such as anthocyanins, much less than 1% of the oral dose is recovered in urine and plasma concentrations peak in the low nanomolar range (Manach et al., 2005). To date, the highest concentrations of polyphenols observed in plasma following oral ingestion by humans have been around 1-10 µM. These concentrations are only observed for relatively large doses of polyphenols from supplements or polyphenol-rich foods and beverages that contain the more bioavailable flavonoids, such as monomeric flavan-3-ols, isoflavones and flavanones.

Metabolism during the first pass

Flavonoids and other polyphenols are extensively metabolised during absorption from the gut, and the typical products are phase-2 conjugates (i.e. glucuronidated, sulfated and methylated derivatives of the parent polyphenol (Kroon et al., 2004)). Indeed, for many polyphenols, the conjugation process is so efficient that the aglycone is not detectable in plasma and urine (Donovan et al., 2006). The first pass includes the small intestine and the liver, and both are capable of metabolising polyphenols (Manach et al., 2004). The kidney also has significant capacity to modify polyphenols but there is little evidence to support a major role for this organ, probably because it is precluded from contributing by the efficiency of the small intestine and liver in these activities. The major plasma and urinary forms resulting from ingestion of different polyphenols have been summarised by Kroon et al. (2004). Many red wine polyphenols (flavonols, catechin / epicatechin, resveratrol, phenolic acids) are efficiently conjugated and are present in plasma predominantly as phase-2

conjugated metabolites. For example, following oral ingestion, 100% of quercetin and virtually all resveratrol is present as glucuronidated and sulfated conjugates of the aglycone or a methylated derivative (Day et al., 2001; Vitaglione et al., 2005) (see Figure 2).

The flavanol monomers, (+)-catechin and (-)-epicatechin, are also conjugated during absorption. The major products are glucuronidated and sulfated conjugates of the parent aglycone or methylated derivatives (Kroon et al., 2004). However, anthocyanins (the brightly coloured components responsible for the red colour of wine) are often not conjugated during absorption, and in this respect, they are rather unique. Although the mechanisms of absorption for anthocyanins are not well understood, the fact that they appear in plasma un-metabolised and at extremely low concentrations, and that the urinary yield typically is extremely low (Manach et al., 2005), may indicate that these compounds escape intestinal metabolism by passing between the enterocytes rather than through them.

What are effects of metabolism on biological activity?

Due to the lack of commercially-available standards, and the technical difficulties associated with their synthesis, there are only a limited number of reports concerned with the biological activities of authentic polyphenol phase-2 conjugates. Readers are referred to Kroon et al. (2004) and Williamson et al. (2005) that discuss much of the available data. Many of the studies concerned with the effects of conjugation on biological activity have focussed on quercetin, the conjugates of which have been synthesised chemically (Needs & Kroon, 2006). For example, conjugation has been shown to detrimentally affect the ability of quercetin to inhibit xanthine oxidase and lipoxygenase enzyme activities *in vitro*, although the loss of potency was heavily dependent on the position of conjugation; the 3'- and 4'-glucuronides were only slightly less effective inhibitors of xanthine oxidase (Day et al., 2000). Quercetin glucuronides have also been shown to inhibit the N-acetylation of the arylamine carcinogen 2-aminofluorene by human acute myeloid leukaemia (HL-60) cells (Kuo et al., 2002), inhibit lung cancer cell growth via cell cycle arrest and induction of apoptosis (Yang et al., 2006), prevent angiotensin-II-induced vascular cell hypertrophy in cultured rat aortic smooth muscle cells via inhibition of JNK and AP-1 signalling pathways (Yoshizumi et al., 2002) and down-regulate transcription of

human cyclooxygenase-2 (COX-2) (O'Leary et al., 2004; de Pascual-Teresa et al., 2004). In contrast, Donnini et al. (2006) provide evidence that whereas quercetin and quercetin-3-glucuronide inhibited vascular endothelial growth factor (VEGF)-induced changes in cell functions and angiogenesis, quercetin-3'-sulfate promoted cell proliferation and angiogenesis. Therefore, phase-2 conjugation is an important factor to consider when assessing the potential biological activities of polyphenols, as the effects can be both profound and unpredictable. To date, there are no reports documenting the relative biological activity of resveratrol phase-2 conjugates, even though these represent the predominant forms *in vivo* following resveratrol ingestion.

In summary, further research is required to understand the role of resveratrol and other polyphenols in providing the additional benefits of consumption of low to moderate amounts of red wine, compared to white wine, beer or spirits. However, the various risks associated with drinking alcoholic beverages has so far prevented a fully controlled clinical trial to determine the effects of moderate red wine consumption on disease risk.

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FIGURE LEGENDS

Figure 1: Chemical structures of resveratrol, epicatechin, gallic acid and quercetin.

Figure 2. Intestinal metabolism and transport of resveratrol.

Resveratrol aglycone is taken up by the enterocytes, probably by passive diffusion, and is efficiently conjugated with glucuronic acid and sulfate through the action of UDP-glucuronosyl transferase and aryl-sulfatase enzymes. Methylation has not been observed, presumably due to the lack of a catechol function in the resveratrol molecule, which is a requirement for catechol-O-methyltransferase (COMT). The conjugates are transported out of the enterocytes by as yet unconfirmed processes, but it is thought that efflux transporters of the multi-drug resistance proteins (MRP) family are involved (several are indicated). Resveratrol sulfate is preferentially transported to the apical (luminal) side of polarised enterocytes, while resveratrol glucuronides are preferentially effluxed to the basolateral (serosal) side (Kaldas et al., 2003). Hence, the predominant form of resveratrol in human blood following oral dosing is resveratrol glucuronide (95-99% of total plasma resveratrol).

Solid arrows, probable active transport process; Dashed arrows, diffusion; Res, resverastrol; GlcA, glucuronic acid; $SO4^-$, sulphate; MRP, multi-drug-resistance protein; BCRP, breast cancer resistance protein; PgP, P-glycoprotein.

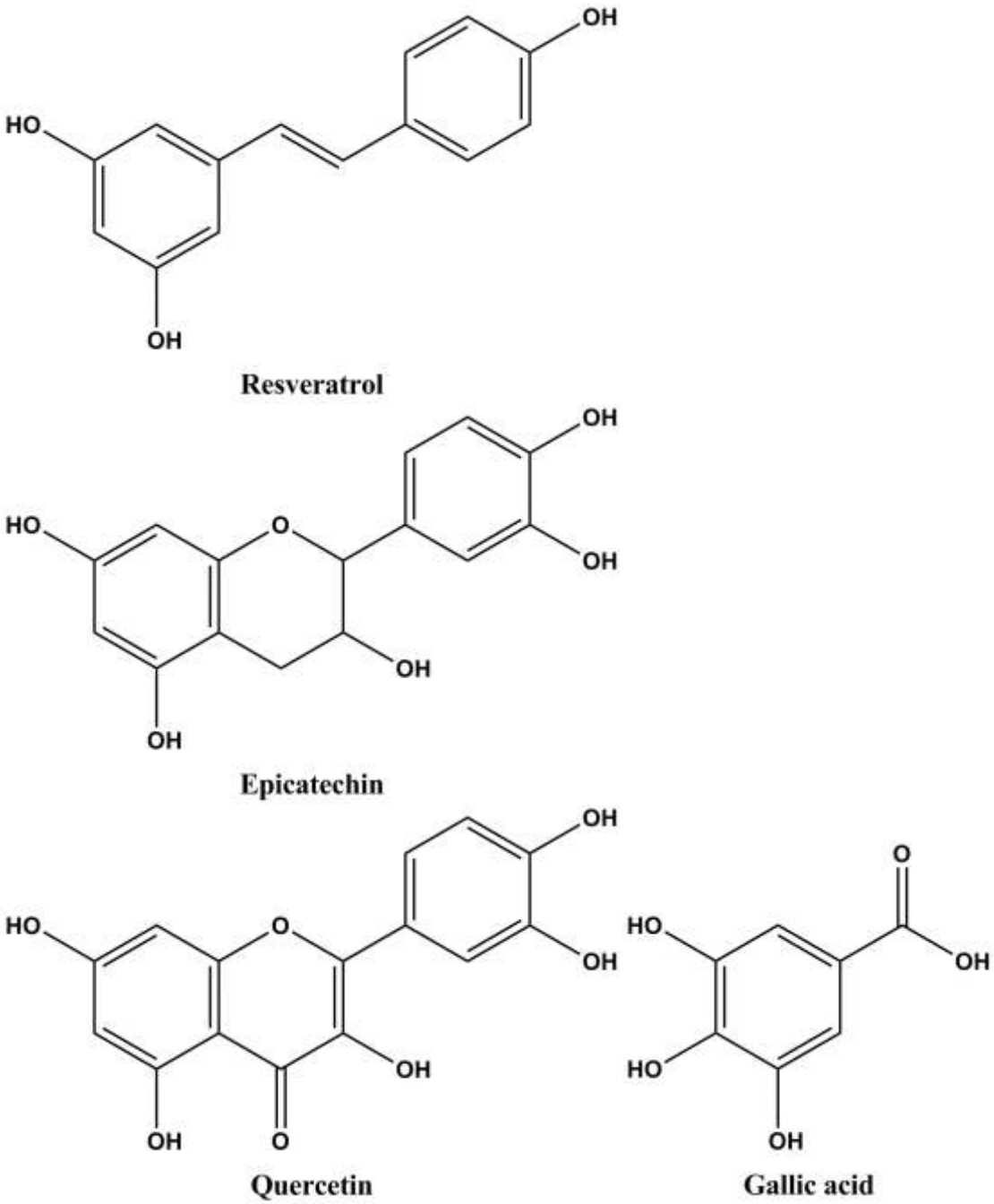
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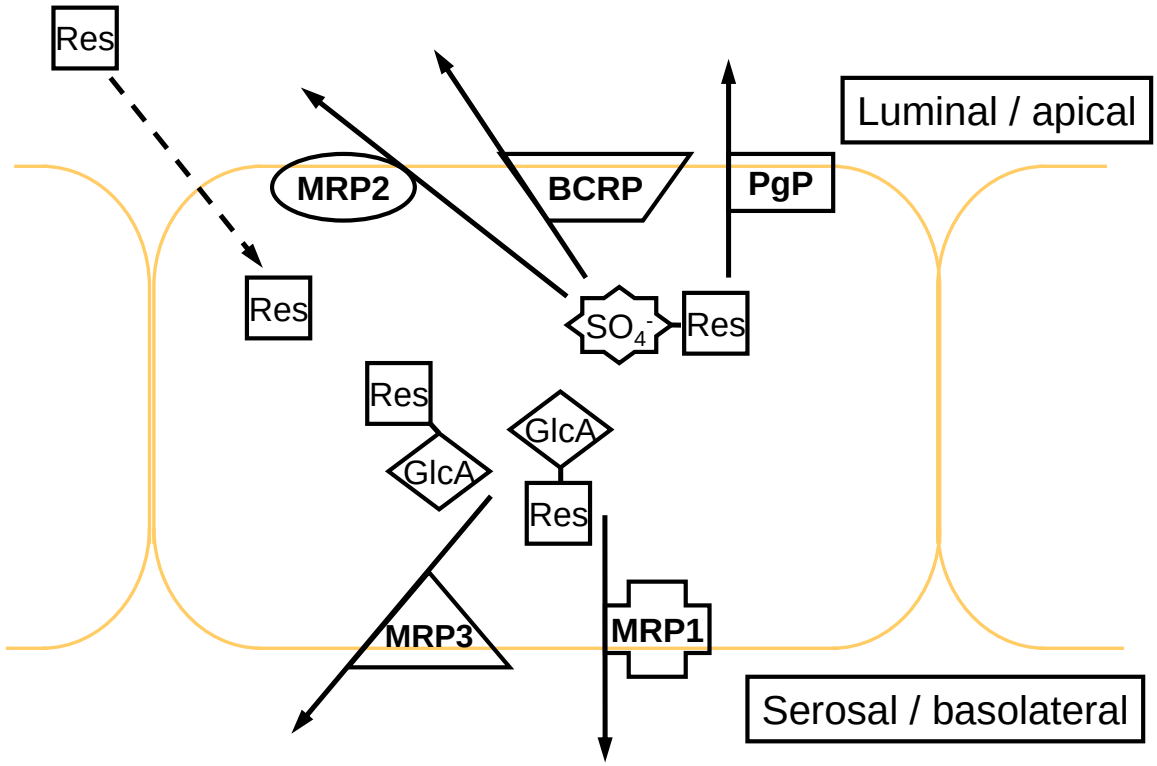
Figure 1

Figure 2


Res
Luminal / apical
MRP2
BCRP
PgP
Res
SO_4^-
Res
Res
GlcA
GlcA
Res
MRP3
MRP1
Serosal / basolateral