Oxidative stress and protective effects of polyphenols: Comparative studies in human and rodent kidney. A review☆

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Abstract

Reactive oxygen species (ROS) play a key role in the pathophysiological processes of a wide range of renal diseases. Thus, antioxidants are expected to decrease the vulnerability of the kidney to oxidative challenges. Polyphenols, particularly abundant in red wine, could act as ROS scavengers, iron chelators and enzyme modulators. In addition, chronic exposure to moderate amounts of ethanol results in increased activity of the renal antioxidant enzymes, further supporting a renoprotective effect of red wine based on its antioxidant properties. An enhancement of plasma antioxidant capacity following red wine consumption has been reported both in man and rodents, thereby providing a contributory factor to its renoprotective effect because the kidney is a highly perfused organ. Although phenol concentration of red wine does not influence the activity of antioxidant enzymes of the kidney, the concentration of these compounds is negatively correlated with tissue lipid peroxidation, assessed by thiobarbituric acid reactive substances, and positively correlated with the antioxidant capacity of plasma. Moreover, amelioration of myoglobinuric renal damage was found in rats following chronic exposure to flavonol-rich red wine. Also, pretreatment with resveratrol, or other red wine polyphenols, decreased kidney damage caused by ischaemia–reperfusion. The aim of the present review is to examine the pathophysiological basis of the renoprotective effect of red wine in man and rodents, based on functional, biochemical and ultrastructural evidence.

Keywords: Antioxidants; Oxidative stress; Antioxidant enzymes; Red wine; Ethanol; Renoprotection; Polyphenols; Lipid peroxidation

Contents

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1. Introduction

Considerable experimental evidence has contributed to support a key role of reactive oxygen species (ROS) in the numerous mechanisms of seemingly unrelated nephropathies (Rodrigo and Rivera, 2002). While enzymatic and non-enzymatic systems preserve the antioxidant/oxidant status, these defense systems become overwhelmed during oxidative stress, a metabolic derangement due to an imbalance caused by excessive generation of ROS or a diminished antioxidant capacity. It has long been recognized that ROS are harmful for cells, mainly because they injure lipids, proteins, and nucleic acids, which leads to structural and functional impairments (Freeman and Crapo, 1982; Mantle and Preedy, 1999). Numerous interventions have been put forward to counteract the effects of ROS, by reinforcing the antioxidant defense systems. Dietary supplementation with the antioxidant vitamin E slowed the rate of progression of renal deterioration (Fryer, 1997), attenuated the nephrotoxicity caused by ferric nitrilotriacetate (Fe-NTA) (Iqbal and Athar, 1998) and ameliorated the glomerulosclerosis occurring in the nephrectomy remnant kidney model in the rat (Hahn et al., 1999). Also, gentamicin-induced nephrotoxicity was ameliorated with garlic, known to be rich in polyphenols (Pedraza-Chaverri et al., 2000). Recently, the possible advantage of a moderate wine consumption in patients with chronic renal failure was hypothesized (Caimi et al., 2004). Therefore, it is expected that the naturally occurring nutritional sources of antioxidants, such as fruits, vegetables, tea or wine, would also attenuate the renal damage caused by oxidative challenges. Polyphenolic compounds, abundant in these nutritional sources, could play a major role in enhancing the antioxidant system, since they behave as ROS scavengers, metal chelators and enzyme modulators (Pietta et al., 1998). In agreement with this view, it was demonstrated that resveratrol, a stilbene polyphenol found in grapes and red wine, suppresses the proteinuria, hypoalbuminemia and hyperlipidemia induced by anti-rat kidney antiserum (Nihei et al., 2001). Also, renoprotective effects have been reported for other polyphenols such as quercetin (Ishikawa and Kitamura, 2000) and alpha-G-rutin (Shimoi et al., 1997). Although these studies have been performed in rodents, it was suggested that this protection may be useful to prevent or treat myoglobinuric acute renal failure in humans (Stefanovic et al., 2000), two species with great similarity on the mechanism of renal injury in this setting. Although dietary supplements containing polyphenols have been used in humans, a safety assessment of the applied dose has been recommended due to the possibility of some adverse effect of this mode of consumption (Mennen et al., 2005). The aim of the present review is to examine the pathophysiological basis of the renoprotective effect of red wine polyphenols in humans and rodents, based on functional, biochemical and ultrastructural evidences.

2. Protective effects of wine polyphenols in man

Considerable effort has been devoted to the study of the prevention of coronary heart diseases by antioxidants (Giugliano, 2000; Cordova et al., 2005), but the antioxidant prevention against renal diseases has been poorly analyzed. The renoprotective effect of polyphenols is thought to be mainly due to their large array of biological actions, such as free radical-scavenging, metal chelation and enzyme modulation abilities (Pietta et al., 1998). The polyphenolic components of wine include substances such as anthocyanins, resveratrol, galic acid, catechin, myricetin, quercetin, etc. All these compounds are likely responsible for an enhancement of the antioxidant capacity of plasma in humans (Duthie et al., 1998; Durak et al., 1999), thereby modulating the systemic antioxidant defense system. However, the bioavailability of wine polyphenols remains to be fully established.

Anthocyanin pigments that are responsible for the color of red wine, were found in human plasma after wine consumption (German and Walzem, 2000). Absorbed quercetin is metabolized to conjugated derivatives retaining antioxidant properties in plasma (Manach et al., 1998). Although phenolic compounds may undergo chemical modifications once absorbed into the bloodstream, such as glycosylation, methylation, or glucuronidation, their availability and capability to exert biological activity still remain (Howard et al., 2002; Hollman and Katan, 1999). It should be mentioned that wine ethanol content could aid the enteric absorption of polyphenols (Duthie et al., 1998). It has been reported that red wine is a poor source of bioavailable polyphenols in men (De Vries et al., 2001), because wine flavonols are poorly absorbed relative to onions or tea flavonols (Hollman and Katan, 1997). Nevertheless, after oral administration of red wine, resveratrol shows significant bioavailability and strong affinity for liver and kidney (Bertelli et al., 1996a). Even though the amount of resveratrol found in these tissues was lower than that required for pharmacological activity, it is possible that prolonged administration of red wine in the diet could lead to an increased resveratrol concentration in different tissues, including kidney, and this would explain its beneficial effect (Bertelli et al., 1996b). Further investigations in chronic models to get a better understanding of the bioavailability of polyphenols in moderate chronic wine consumers are still lacking.

Other biological actions of polyphenols include the reduction of the susceptibility of low density lipoproteins (LDL) to oxidation both in vitro (Kerry and Abbey, 1997) and in vivo (Nigdikar et al., 1998), an effect likely due to the property of these compounds to scavenge free radicals (Dugas et al., 2000). Also, polyphenols may participate in the regulation of vascular tone (Adriantsitohaina, 1999) or in the inhibition of platelet aggregation (Keevil et al., 2000). Together with scavenge free radicals polyphenols may avoid their formation through the

Haber-Weiss/Fenton reactions, due to their chelating properties. Thus, quercetin chelates intracellular iron (Ferrali et al., 1997), thereby avoiding its catalyzing effect on the formation of ROS. Also, quercetin is able to inhibit the activity of transcription factors involved in the production of inflammatory lesions of the kidney (Rangan et al., 1999a,b), thus behaving as an anti-inflammatory agent (Kuhlmann et al., 1998). Resveratrol, another wine polyphenol, was shown to inhibit the expression of adhesion molecules of the endothelium (Ferrero et al., 1998) and the activity of cyclooxygenase-2 (Subbaramaiah et al., 1998). Other indirect evidence of the beneficial effects of red wine consumption was reported in a follow-up study revealing that the risk of stone formation decreased by 59% in moderate wine drinkers (Curhan et al., 1998).

3. Renal alterations associated with oxidative stress

Oxidative stress mediates a wide range of renal impairments, ranging from acute renal failure (Paller et al., 1998; Baliga et al., 1999; Shah, 2001), rhabdomyolysis (Vanholder et al., 2000), obstructive nephropathy (Klahr, 2001), hyperlipidemia (Wanner et al., 1997; Sakatsume et al., 2001) and glomerular damage (Kitamura and Ishikawa, 1999) to chronic renal failure and hemodialysis and associated inflammation (Handelman et al., 2001). Thus, increased levels of malondialdehyde and F2-isoprostanes, two products of lipid peroxidation, have been reported in various clinical settings associated with renal damage (Martín-Mateo et al., 1999), although most of these studies have been performed in rats or mice. The mechanisms of glomerular and tubulointerstitial alterations induced by oxidative stress and the effects of polyphenols to counteract the oxidative damage will be discussed below.

3.1. Glomerular alterations

Oxidative stress may alter the structure and function of the glomerulus because of the effect of ROS on mesangial and endothelial cells (Klahr, 1997). The glomerulus is considerably more sensitive to oxidative injuries than other nephron segments. Lipoprotein glomerulopathy has been characterized by a relatively rapid progression to renal impairment and the development of glomerulosclerosis (Sakatsume et al., 2001). Both native and oxidized forms of LDL (LDL-ox) may be involved in the glomerular damage mediated by oxidative stress. Oxidative stress participates in the renal damage induced by hyperlipoproteinemia (Scheuer et al., 2000), mainly associated with the glomerular accumulation of LDL (Lee and Kim, 1998). Subsequently, oxidation of LDL by mesangial cells could occur (Wheeler et al., 1994), thereby activating the apoptosis pathway of endothelial and mesangial cells, as shown by studies of these cells of humans in vitro, an effect prevented by antioxidants (Galle et al., 1999). Also, native LDL has shown a dose-dependent stimulation of proliferation of cultured mesangial cells (Nishida et al., 1999), a response attributed to an enhancement of expression of c-*jun* and c-*fos* genes, involved in the cellular proliferation and DNA synthesis in mesangial cells during LDL exposure (Gröne et al., 1996). Antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD), but not glutathione peroxidase (GSH-Px), may partially inhibit the effect of LDL on DNA synthesis (Greiber et al., 1996). Native LDL was found to induce the generation of ROS in rat glomerular cells (Wanner et al., 1997), although other studies found no effect of LDL in the production of superoxide anion by mesangial and endothelial cells in vitro (Galle et al., 1999). This controversy may be due to the different conditions of the experimental models that were used. Although the presence of an excessive amount of LDL is recognized as a factor of glomerular damage, its role in the production of oxidative stress has yet to be fully elucidated. This damage could be direct or indirect, because oxidation of LDL is induced by infiltrating leukocytes resulting in increased glomerular damage. In addition, native LDL can stimulate fibronectin secretion by mesangial cells. LDL-ox may stimulate the genic expression of fibronectin through the autocrine secretion of transforming growth factor-β (TGF-β) in cultured human glomerular epithelial cells (Ding et al., 1997). These data support a role of oxidative stress and dyslipoproteinemia in the pathogenesis of glomerulosclerosis associated with renal diseases. Studies in rats demonstrated that long-term wine exposure reduced LDL-cholesterol through its nonalcoholic components, thereby protecting the kidney against the deleterious effects of LDL and their oxidized derivatives on the glomerulus (Cascon et al., 2001); this effect could be reinforced by a preservation of polyunsaturated fatty acids of kidney phospholipids also attributed to polyphenols (Araya et al., 2001).

Oxidative stress could also be involved in other inflammatory lesions caused by a series of mediators, including cytokines and chemokines leading to leukocyte activation, production of ROS and increased glomerular damage (Takemura et al., 1994). Also, the molecules causing inflammation could be produced by the resident renal cells, such as glomerular mesangial and endothelial cells, proximal tubular epithelial cells, and interstitial fibroblasts (Rovin and Phan, 1998). The nuclear factor-kappaB (NF-κB) is one of the most important regulators of proinflammatory gene expression (Tak and Firestein, 2001), and it has been demonstrated that ROS can stimulate its activation in mesangial cells (Massy et al., 1999).

The antioxidants may play a key role against the glomerular inflammatory processes, through a diminution of the activity of inflammatory enzymes (Ozaki et al., 1999) and cytokine secretion, or by inhibiting the activity of NF-κB (Massy et al., 1999), as shown for the wine polyphenol quercetin (Ishikawa and Kitamura, 2000). Also, resveratrol, a stilbene polyphenol found in grapes and wine, is a potent antiglomerulonephritic factor capable of suppressing proteinuria, hypoalbuminemia, and hyperlipidemia induced by anti-rat kidney antiserum (Nihei et al., 2001). In addition, it has been documented that superoxide anion participates in tumor necrosis factor-α (TNF-α)-induced mesangial cell apoptosis (Moreno-Manzano et al., 2000). Polyphenols may counteract this mechanism through a cytoprotective action of the glomerular mesangial cells, exerted by a restriction on apoptosis (Kitamura and Ishikawa, 1999). The mechanisms whereby antioxidants exert

such effects are still unknown, but their renoprotective effects could be expected in renal pathologies, such as glomerulosclerosis. It should be noted that, in the case of wine, ethanol could also be involved in glomerular protection. In addition to the evidence that acute ethanol consumption reduces glomerular damage (Cecchin and De Marchi, 1996), its renoprotection against oxidative injury may also be postulated on the basis of data found in experimental models of chronic ethanol consumption (Scott et al., 2000; Orellana et al., 1998), but the physiological relevance of these findings has not been yet established.

3.2. Tubulointerstitial alterations

The renal tubular epithelia could be exposed to injurious chemical species when molecules appear in the urinary space because of the loss of glomerular permselectivity occurring in chronic renal diseases, or because of their increased plasma levels. Among these macromolecules are LDL-ox (Chen et al., 2000), transition metals (Shah, 2001; Barrouillet et al., 1999), hemoglobin and myoglobin (Zager and Burkhart, 1997) or potentially nephrotoxic drugs (Baliga et al., 1999). LDL-ox may induce a pro-oxidant environment (Agarwal et al., 1996). In turn, this oxidative stimulus may activate heme-oxygenase, an enzyme that catalyzes the degradation of the heme groups of hemoglobin and myoglobin (Zager and Burkhart, 1997), two hemopigments found in the urinary space in numerous glomerulopathies in which the glomerular barrier is impaired. Subsequently, iron liberation results in tubular production of hydroxyl radicals and lipid peroxidation. This cytotoxic effect is attenuated by the administration of iron chelators, or by hydroxyl radical scavengers (Shah and Walker, 1988). During myoglobinuria, tubular cells show an increased production of hydrogen peroxide (Zager and Burkhart, 1997) and a dramatic drop of reduced glutathione (GSH) (Abul-Ezz et al., 1991).

The accumulation of macrophages within the interstitial space of the renal cortex plays a pathogenic role in the development of tubular injury and interstitial fibrosis in progressive chronic renal diseases (Vielhauer et al., 2001). Proximal tubular epithelial cells are thought to mediate the interstitial macrophage infiltration because of their anatomic position and their ability to produce chemotactic cytokines, chemokines, and other inflammatory mediators. It was reported that ROS may induce gene expression of these mediators in the tubular epithelial cells, resulting in the recruitment of leukocytes. Thus, in renal tubular cells, the expression of chemokines, such as monocyte chemoattractant protein (MCP)-1, MCP-3, macrophage inflammatory protein1 (MIP-1), and T cell activation gene 3 (TCA3), precedes the production of infiltrates containing monocytes, macrophages and T lymphocytes, in experimental acute tubulointerstitial nephritis (Ou et al., 1999).

In contrast, the effect of NF-κB, an important modulator of inflammatory responses occurring in tubular epithelial cells, can be inhibited by various antioxidants. In this context, the quercetin-mediated inhibition of NF-κB was associated with the reduction of both pro-[interleukin-1β (IL-1β), TNF-α, MCP-1, and MCP-2] and anti-inflammatory (TGF-β) and IL-10 cytokine transcription in proximal tubular cells (Kuhlmann et al., 1998). In vitro quercetin prevented cisplatin-induced cellular injury and upregulation of chemokines in the renal cortex (Jones and Shoskes, 2000). Also, pretreatment with quercetin or curcumin resulted in preservation of histological integrity, with a decrease in tubular damage and interstitial inflammation. These wine polyphenols were associated with a strong attenuation of the expression of MCP-1 and regulated upon activation normal T-cell expressed and secreted (RANTES), two chemokines activated by ischemia–reperfusion (Shoskes, 1998). However, further studies are needed to determine whether the administration of quercetin could attenuate the chronic upregulation of NF-κB in tubulointerstitial diseases in vivo (Rangan et al., 1999a,b).

Ureteral obstruction is another renal alteration where generation of renal damage is closely related with the occurrence of oxidative stress. ROS may play an important role in the tubulointerstitial inflammation associated with obstructive nephropathy (Klahr, 2001). The mechanical disturbance due to a complete ureteral obstruction causes tubular injury resulting in a pro-inflammatory and tubulointertitial fibrosis (Ricardo and Diamond, 1998). The antioxidant enzymes CAT and SOD from tubular cells from the obstructed kidney show a downregulation, which causes increased susceptibility of the kidney to oxidative damage (Cvetkovic et al., 1998), a process exacerbated by sodium depletion (Kinter et al., 1999).

Alternatively, angiotensin II (Ang II) also plays a pivotal role in the progression of renal diseases, including obstructive nephropathy (Klahr, 1998). Ang II mediates the activation of membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and, subsequently, the generation of superoxide anion that, in turn, leads to hypertrophy of renal tubular cells (Hannken et al., 1998). In addition, upregulation of the expression of TGF-β and TNF-α between other cytokines by Ang II was also reported in obstructive nephropathy. TGF-β is a major cytokine involved in the process of expansion and fibrosis of the tubulointertitial space, but its expression is inhibited by polyphenols (Shi et al., 2004). TNF-α, secreted by renal tubular cells, has a role in the recruitment of inflammatory cells to the renal interstitium (Klahr, 2001).

Calcium oxalate urolithiasis constitutes a frequent example of obstructive nephropathy. Together with the pathogenic role of oxalate in the formation of stones, it was reported early that it has the ability to generate free radicals, causing lipid peroxidation. Studies in animal models are in agreement with these data, as shown by the association of hyperoxaluria-induced lipid peroxidation (Thamilselvan et al., 1997) accompanied by a diminution of GSH levels (Muthukumar and Selvam, 1998).

The possibility that the effects of antioxidants ameliorate tubulointertitial damage has been studied in rats and mice using two experimental models. The nephrotoxicity caused by ferric nitrilotriacetate (Fe-NTA) was attenuated by antioxidants such as α-tocopherol (Iqbal and Athar, 1998), 2-mercaptoethanesulphonate, and *N*-acetylcysteine (Umemura et al., 1996). Also, gentamicin-induced nephrotoxicity was ameliorated with

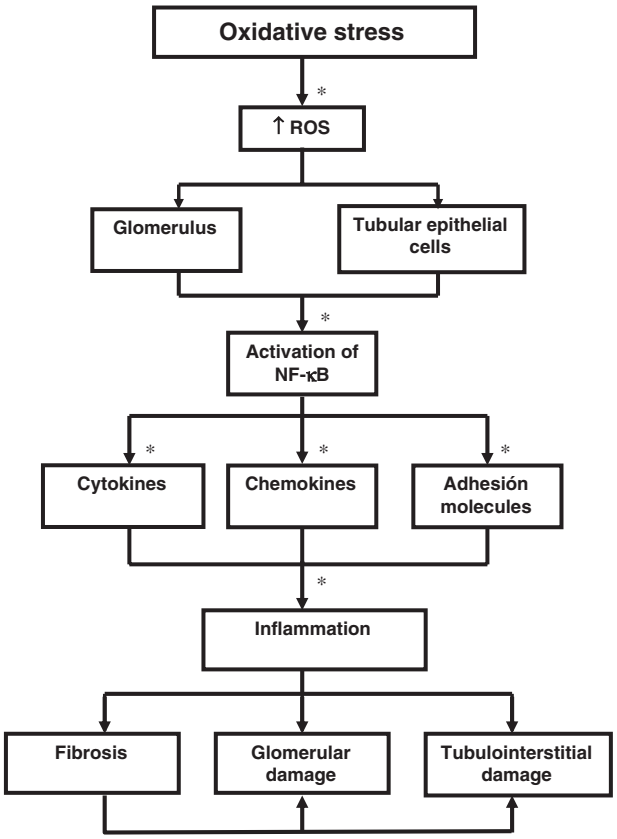

Fig. 1. Hypothesis to explain the glomerular and tubulointerstitial damage caused by oxidative stress and the levels of renoprotective effects of polyphenols. * sites for potential effects of polyphenols.

garlic, rich in polyphenols (Pedraza-Chaverri et al., 2000). Alpha G-rutin, a wine polyphenol that works as an antioxidant in vivo either by scavenging ROS or by chelating ferric ions, served to prevent oxidative renal damage in mice treated with Fe-NTA (Shimoi et al., 1997). A hypothesis to explain the renoprotective effects of polyphenols against oxidative stress is depicted in Fig. 1.

4. Integrated renoprotective effects of wine polyphenols

Most studies of the renoprotective effects have been performed in rodents, mainly rats and mice, but the improvement on the knowledge of the mechanisms causing oxidative injury of the kidney has also support the view of a therapeutic application in humans. Myoglobinuria plays a key role in the pathophysiology of acute renal failure both in clinical settings characterized by muscle tissue injury (Vanholder et al., 2000) and in a widely used animal model of glycerol-induced rhabdomyolysis. The intratubular degradation of myoglobin results in the generation of ROS that are implicated in the pathogenesis of renal damage. Although the kidney possesses an antioxidant defense system that scavenges ROS, the capacity of this system can be overwhelmed during myoglobinuria. Naturally occurring antioxidants act to reinforce the endogenous antioxidant systems that depurate ROS and this contributes to their beneficial health effects (Nijveldt et al., 2001). Although the mechanism of action of antioxidants has not yet been fully established, they may attenuate the effects of oxidative challenges to the kidney. Polyphenols, that are particularly abundant in Chilean red wine (McDonald et al., 1998) may strengthen the antioxidant mechanisms, and this action would support the hypothesis that moderate red wine consumption is protective, in agreement with previous studies (Shimoi et al., 1997; Iqbal and Athar, 1998; Avramovic et al., 1999; Stefanovic et al., 2000; Giovannini et al., 2001). Recently, a model of chronic exposure to red wine was used to characterize the response of the antioxidant system in the rat (Rodrigo et al., 2002a). Red wine enhanced the antioxidant capacity of plasma, a finding also reported in humans (Duthie et al., 1998; Durak et al., 1999). The major role of polyphenols in this effect was supported through the administration of alcohol-free red wine in humans (Serafini et al., 1998). This effect was associated with higher activities of two antioxidant enzymes (CAT and GSH-Px) and a higher GSH/GSSG ratio in the kidney of the rats receiving red wine, in agreement with previous studies (Roig et al., 1999). Also, lipid peroxidation of kidney, liver and lung was negatively correlated with the polyphenol concentration of the administered red wine (Rodrigo et al., 2005), partly in agreement with the renoprotective effect previously reported for resveratrol (Bertelli et al., 2002). Indeed, renal polyphenols preserved the composition of kidney lipids (Araya et al., 2003). Finally, wine polyphenols could also exert a modulation at the level of superoxide anion production, because they diminish the activity of cytochrome *P*450 (CYP) in the kidney of rats chronically exposed to alcohol-free red wine (Orellana et al., 2002a), an effect also reported in rat liver (Orellana et al., 2002b). Recently, it was reported that the wine polyphenol resveratrol and particularly epsilon-viniferin, a dimer of resveratrol, display a potent inhibitory effect for at least 8 isoforms of human CYPs activities, including CYP2E1 (Piver et al., 2003).

These data indicate that red wine increased the pro-oxidant status. Therefore, the vulnerability of the kidney to oxidative challenges should be expected to decrease following chronic red wine exposure. To corroborate this assumption, an experimental model of acute renal failure was applied to rats through rhabdomyolysis induced by glycerol injection (50%, 10 mL/kg, i.m.), causing myoglobinuria, and the kidney was subjected to biochemical, functional and ultrastructural studies. The effects of red wine (12.5% ethanol, v/v), ethanol 12.5% (v/v) and alcohol-free red wine were compared with a control group drinking water. These studies are detailed below.

4.1. Functional studies

The experiments showed that rhabdomyolysis caused a diminution of glomerular filtration rate, as assessed by increased plasma levels of creatinine and blood urea nitrogen, but this effect was significantly attenuated by red wine and alcohol-free red wine treatments. As expected, at 6 h following glycerol injection, serum creatinine and blood urea nitrogen were significantly increased, and reached levels that were 3.2 and 1.8 times greater than basal values, respectively. Red wine

Table 1
Effects of glycerol-induced rhabdomyolysis on creatinine clearance (mL/min/100 g BW), fractional excretion of sodium (FE_{Na}) and renal (Na+K)-ATPase activity

Parameter	Group	Basal	Glycerol
Creatinine clearance (mL/min/100 g BW)	Control	0.60±0.02	0.19±0.09[a]
	Alcohol-free red wine	0.63±0.08	0.33±0.05[a,b,d,e]
	Ethanol	0.56±0.05	0.22±0.04[a,c,e]
	Red wine	0.62±0.09	0.49±0.03[a,b,c,d]
FE_{Na} (%)	Control	0.43±0.03	2.10±0.11[a]
	Alcohol-free red wine	0.51±0.12	0.53±0.10[b,d]
	Ethanol	0.35±0.05	0.94±0.08[a,b,c,e]
	Red wine	0.37±0.09	0.51±0.13[b,c,d]
(Na+K)-ATPase (μmol/Pi/mg protein/h)	Control	13.8±0.5	10.3±0.4[a]
	Alcohol-free red wine	12.8±0.3	11.3±0.3[a,b,d,e]
	Ethanol	17.5±0.7	14.4±0.7[a,b,c,e]
	Red wine	13.2±0.3	12.6±0.4[b,c,d]

Values are means±S.E.M. (n=10–12). Pi, inorganic phosphate; BW, body mass. Statistically significant differences, at $P<0.05$ assessed by ANOVA, followed by Bonferroni post-hoc test, are indicated by superscript letters: [a]vs. basal, [b]vs. control-glycerol, [c]vs. alcohol-free red wine-glycerol, [d]vs. ethanol-glycerol and [e]vs. red wine-glycerol. From Rodrigo et al. (2004), with permission.

administration diminished these increases to 1.5 and 1.2 times, respectively, indicating a renoprotective effect. This effect was also observed in the ethanol and alcohol-free red wine groups, although to a lesser extent. The renoprotection was confirmed by the creatinine clearance data, which showed a 68% decrease from control levels following glycerol-injection, whereas this diminution was 60%, 47% and 21% in ethanol, alcohol-free red wine and red wine groups, respectively. Also, rhabdomyolysis elevated the fractional excretion of sodium in the control and ethanol groups by 4.9 and 2.7 times, respectively, but these values were not changed in the red wine and alcohol-free red wine groups. In the control, ethanol and alcohol-free red wine groups, this effect was paralleled by a diminution in activity of (Na+K)-ATPase to 75%, 82% and 88% of basal values, respectively. In contrast, kidneys from the red wine group showed no change in (Na+K)-ATPase activity following glycerol injection (Rodrigo et al., 2004). These data are shown in Table 1. At basal conditions it is noticeable that the kidneys of ethanol group shows a significantly higher (Na+K)-ATPase activity, an effect that has previously been attributed to up-regulation by chronic exposure to ethanol and blunted by polyphenols. (Rodrigo et al., 2002a). Accordingly, it was reported that polyphenols cause restoration of the affinity of ATP and Na^+ binding sites and of Vmax of the enzyme when its kinetics properties are altered by the administration of the nitric oxide inhibitor L-NAME (Javorkova et al., 2003), although these compounds fail to restore normal NO synthesis (Javorkova et al., 2004). Although chronic exposure to moderate doses of ethanol results in up-regulation of (Na+K)-ATPase

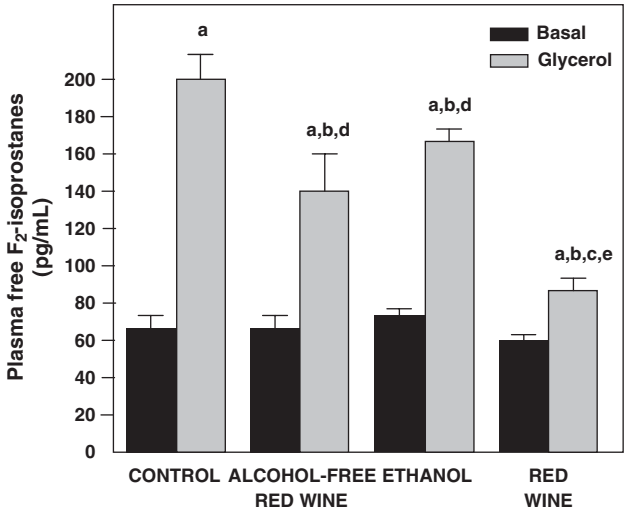

Fig. 2. Effects of rhabdomyolysis on plasma levels of free F2-isoprostanes in control, ethanol, red wine and alcohol-free red wine groups. Values are means ±S.E.M. (n=20). Statistically significant differences, at $P<0.05$, are indicated by superscript letters: [a]vs. basal, [b]vs. control-glycerol, [c]vs. ethanol-glycerol, [d]vs. red wine-glycerol and [e]vs. alcohol-free red wine-glycerol. From Rodrigo et al. (2004), with permission.

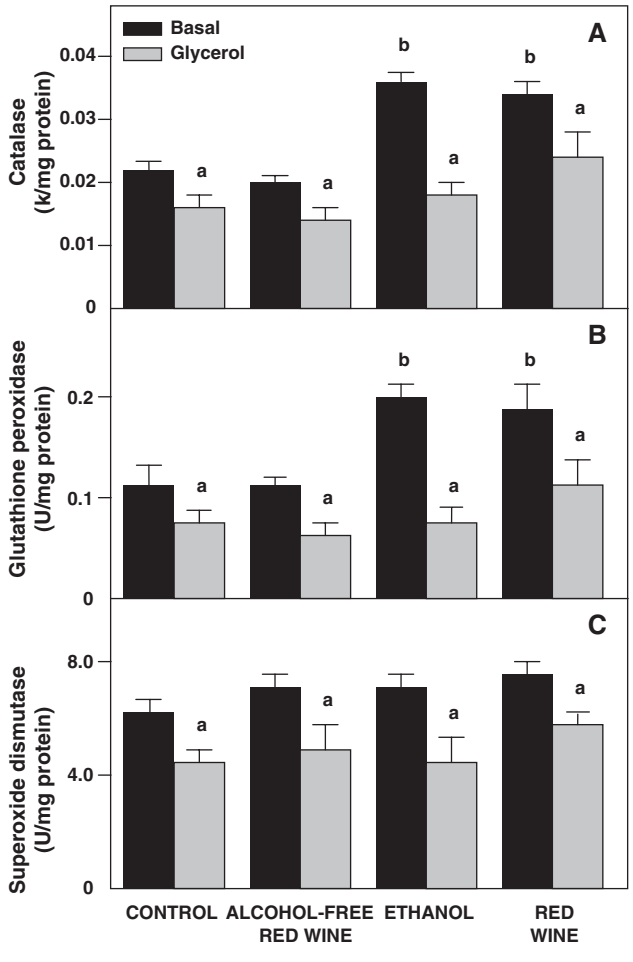

Fig. 3. Effects of rhabdomyolysis on the activity of the antioxidant enzymes catalase (A), glutathione peroxidase (B) and superoxide dismutase (C) in renal cortex in control, ethanol, red wine and alcohol-free red wine groups. Values are means±S.E.M. (n=20). Statistically significant differences, at $P<0.05$, are indicated by superscript letters: [a]vs. basal and [b]vs. control-glycerol. k, catalase first-order kinetic constant for breakdown of hydrogen peroxide (M^{-1} s^{-1}). U, units. From Rodrigo et al. (2004), with permission.

(Rodrigo et al., 2002a) and its functional involvement (Rodrigo et al., 1998), tissue damage has been reported in kidney and lung during acute ethanol intoxication (Rodrigo et al., 2002b). Nevertheless, following chronic exposure to red wine, the deleterious effects of ethanol seem to be blunted by the presence of polyphenols (Rodrigo et al., 2002a).

4.2. Biochemical effects

Lipid peroxidation, assessed by plasma levels of free F2-isoprostanes at basal conditions, was not different between the groups, and glycerol injection increased these levels by 3, 2.3, 1.5 and 2 times in the control, ethanol, red wine and alcohol-free red wine groups, respectively (Fig. 2). Similar data were obtained by measurements of both malondialdehyde levels and protein carbonylation in renal tissues (data not shown). The activity of catalase and glutathione peroxidase, two antioxidant enzymes, was higher than control in the kidneys of the ethanol and red wine groups at basal conditions. The activity of superoxide dismutase (SOD), another antioxidant enzyme was similar in all groups. Glycerol injection caused decreased antioxidant enzyme activity in the four groups, and in the red wine group this decrease produced levels that were not different from basal values in the control group (Fig. 3).

4.3. Morphological studies

The kidney of control rats following glycerol injection showed a morphological impairment characterized by tubular necrosis (vacuolar and hydropic cell degeneration) and tubulorhexis (light microscopy, data not shown). Also, the glomeruli from this group presented abundant accumulation of electron dense material in the endothelium, glomerular basement membrane (GBM) and foot processes of the podocytes causing the GBM to protrude into the lumen. In contrast, this effect was appreciably less severe in glomeruli from the red wine group, which had a normal filtration barrier without accumulation of electron dense material (electron microscopy, Fig. 4).

The renoprotective effects of red wine polyphenols have been also attributed to the release of endothelial nitric oxide (NO), a pivotal vasoprotective molecule, since increases in NO synthase expression have been observed (Leikert et al., 2002). These data are in agreement with the increased bioavailability of NO in the kidneys of rats receiving a dose of resveratrol designed to cause plasma levels that are similar to those found in moderate wine consumers, which was given prior to ischaemia–reperfusion induced oxidative stress (Giovannini et al., 2001), a finding also reported for quercetin (Kahraman et al., 2003). However, oxidative stress should cause NO

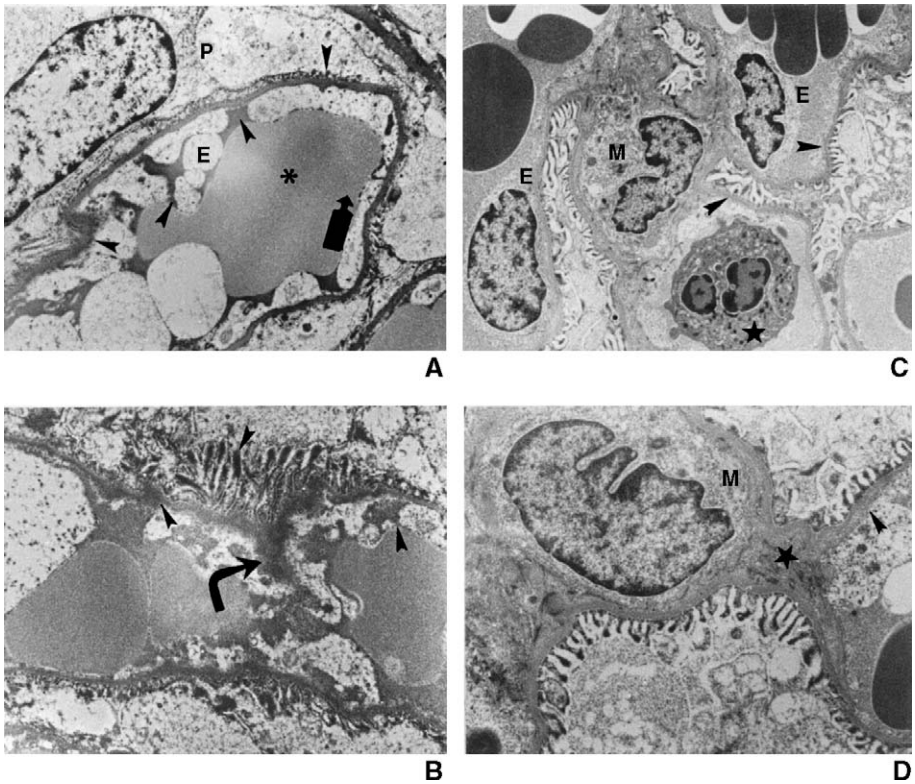

Fig. 4. Electron micrographs of the kidneys of rats from the control (A and B) and red wine (C and D) groups showing the ultrastructural characteristics of the glomerulus of a nephron, after 6 h of glycerol injection (A). The swelling of the endothelium (E), electron-dense deposits within the endothelial cells, the foot processes and the GBM (arrowheads) and glomerular capillary lumen (thick arrow) with dark plasma (*) (original × 25 000) are shown (B). The electron-dense deposits causing the GBM to protrude into the capillary lumen (arrow). The same deposits were present in the cytoskeleton of the foot processes, the endothelial cells, and the GBM (arrowheads). The foot processes were all intact and detachments from the GBM were not seen (original × 35 000) (C). The normal endothelium (E), mesangium (M) and filtration barrier (arrowheads). Presence of a neutrophil in the capillary lumen (star) (original × 35 000) (D). A normal mesangium (M) and filtration barrier (arrowheads) and mesangial matrix (star) are shown. Electron-dense deposits were not seen (original × 38 500). From Rodrigo et al. (2004), with permission.

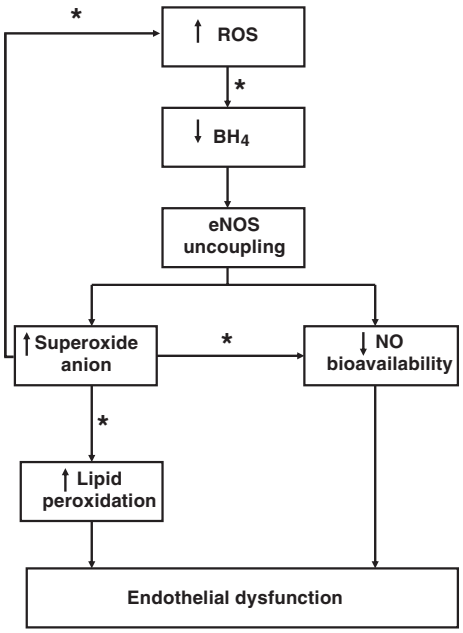

Fig. 5. Interaction of ROS with NO and the sites for potential effects of polyphenols. ROS, reactive oxygen species; BH4, tetrahydrobiopterin; NO, nitric oxide; eNOS, endothelial nitric oxide synthase. * sites for potential effects of polyphenols.

consumption via its reaction with the superoxide anion to form peroxynitrite, a highly peroxidant molecule. Interestingly, NO production by endothelial nitric oxide synthase (eNOS) is dependent on dimerization of the enzyme stimulated by cofactor tetrahydrobiopterin (BH_4). Augmented ROS cause oxidation of BH_4 and consequent uncoupling of eNOS, further contributing to ROS production, since uncoupled eNOS generates superoxide anion. Hence increases of antioxidant availability, such as that provided by polyphenols, are expected to decrease superoxide generation by increasing levels of BH_4 (Brown and Hu, 2001). Therefore, it is very likely that the antioxidant effects of red wine contribute to the increased bioavailability of NO. In agreement with the present data, other studies have shown that bioflavonoids from the seeds of grapes exert morphological and functional protection against renal damage by experimental myoglobinuric acute renal failure (Stefanovic et al., 2000). Also, a renoprotective effect by the wine bioflavonoid proanthocyanidin-BP1 was reported in a study examining the same glycerol-induced rhabdomyolysis animal model used in the present experiments (Avramovic et al., 1999). The interaction of ROS with NO and the sites for potential effects of polyphenols are summarized in Fig. 5.

5. Concluding remarks

Data collected could lead to the suggestions that the renoprotective effects of polyphenols, mainly found in red wine and grapes, could be partly attributed to their properties causing an enhancement of the antioxidant defense system and to an increased release of NO by endothelial cells. In rats, up-regulation of at least two antioxidant enzymes (catalase and glutathione peroxidase) could be attributed to ethanol. Although acute exposure to ethanol could result in oxidative damage of the kidney, polyphenols could counteract these deleterious effects. Once absorbed into the bloodstream, polyphenolic compounds may undergo metabolic modifications, but their availability and capability to exert biological activity still remain. Both in rodents and humans, red wine administration is associated with an increased antioxidant capacity of plasma. In addition, down-regulation of rat kidney cytochrome *P*450 found in chronic exposure to alcohol-free red wine could account for a diminished production of free radicals. This renal response to red wine exposure could contribute to an amelioration of the effects resulting from oxidative challenges, as shown by the decreased functional, biochemical and morphological renal damage induced by models such as ischemia–reperfusion and myoglobinuria known to cause acute renal failure. There are few experimental studies of polyphenols in humans; however, the known similarity with rodents, mainly rats, in the pathophysiological mechanism of renal damage, could give rise to support the view that the maintained exposure to these compounds not solely from red wine but also from vegetables, fruits or tea, could protect the kidney against various acute or chronic deleterious effects.

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