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Critical review of resveratrol in xenobiotic-induced hepatotoxicity

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Abstract

Use of natural products is increasingly popular. In fact, many patients with liver diseases self-medicate with herbal supplements. Resveratrol (RSV), in particular, is a common natural product that can reduce injury in experimental models of liver disease. Xenobiotic hepatotoxicity is a particularly important area-of-need for therapeutics. Drug-induced liver injury, for example, is the most common cause of acute liver failure (ALF) and ALF-induced deaths in many countries. Importantly, RSV protects against hepatotoxicity in animal models *in vivo* caused by several drugs and chemicals and may be an effective intervention. Although many mechanisms have been proposed to explain the protection, not all are consistent with other data. Furthermore, RSV suffers from other issues, including limited bioavailability due to extensive hepatic metabolism. The purpose of this article is to summarize recent findings on the protective effects of RSV in xenobiotic-induced liver injury and other forms of liver injury and to provide a critical review of the underlying mechanisms. New mechanisms that are more consistent with data emerging from the toxicology field are suggested. Efforts to move RSV into clinical use are also considered. Overall, RSV is a promising candidate for therapeutic use, but additional studies are needed to better understand its effects.

Keywords

Acetaminophen; Liver injury; Mitochondria; Inflammation; Oxidative stress; Natural products

1. INTRODUCTION

Hepatotoxicity is a major problem in the development and use of drugs. Before a drug reaches the market, the potential for intrinsic hepatotoxicity is thoroughly tested. In fact,

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts.

liver injury is one of the major reasons for the discontinuation of a drug at multiple stages in the discovery and development pipeline (US FDA, 2009). Clinically, drug-induced liver injury is the most frequent cause of acute liver failure (ALF) in the West (Lee, 2008). Although acetaminophen (APAP) overdose accounts for most of these cases, idiosyncratic hepatotoxicity caused by other drugs is also a significant problem (Lee, 2008). Hepatotoxicity can also be caused by a number of non-drug xenobiotics as well.

Many traditional medicines and other natural products are thought to have beneficial effects in the liver and to protect the liver against various insults (Girish and Pradhan, 2011; Zhao et al., 2014; Seeff et al., 2015). Despite the fact that clinical trials of many such compounds in the U.S. have been unsuccessful (Seeff et al., 2015), self-medication with herbal products is common among patients with chronic liver diseases (Strader et al., 2009; Seeff et al., 2008). Among the natural products that have been studied for their potential hepatoprotective effects, one of the most popular is 3,5,4'-trihydroxy-trans-stilbene, otherwise known as resveratrol (RSV) (Bishayee et al., 2010a). RSV is a naturally-occurring polyphenol that was first isolated from *Veratrum grandiflorum* (Takaoka, 1939). It was initially characterized as a phytoalexin produced by plants (particularly berries) in response to injury or stress (Langcake et al., 1976; Burns et al., 2002). It was postulated to explain the French Paradox, or the cardiovascular effect of red wine, in the early 1990s (Renaud et al., 1992). Since then, numerous biological effects of RSV have been reported, including anti-oxidant, anti-inflammatory and anti-tumorigenic effects (Baur and Sinclair, 2006). It has been shown to be beneficial in many models of disease, including cell culture and multiple *in vivo* rodent models of cardiovascular diseases (Bradamante et al., 2004), cell culture and multiple *in vivo* rodent models of neurodegenerative diseases (Sun et al., 2010), ischemic injury in the brain in gerbils (Wang et al., 2002; Sun et al., 2010), metabolic changes associated with aging in cultured cells (Park et al., 2012) and in both *in vitro* and *in vivo* mouse models of cancer (Jang et al., 1997).

Importantly, RSV has been shown to protect against numerous *in vitro* and *in vivo* rodent models of liver injury, including hepatotoxicity caused by drugs and other xenobiotics (Bishayee et al., 2010a). However, the mechanisms that have been proposed for some of the beneficial effects of RSV in xenobiotic hepatotoxicity are questionable due to poor study design or the failure to account for fundamental aspects of the known pathophysiology induced by these chemicals. Thus, the purpose of this review is to summarize the major effects of RSV in the liver and to discuss the mechanisms of RSV-mediated protection in drug hepatotoxicity. In addition, the effects of RSV in other forms of acute liver injury are summarized and the clinical use of RSV is discussed.

2. KNOWN EFFECTS OF RESVERATROL IN THE LIVER AND DURING LIVER INJURY

The most commonly proposed mechanism of RSV-mediated protection in liver injury is that RSV acts as an anti-oxidant. It is well known that reactive oxygen species (ROS) have a critical role in the initiation and progression of a number of liver pathologies, such as those caused by hepatitis C, alcohol, drugs or endotoxemia (Jaeschke et al., 2002; Muriel, 2009). RSV appears to decrease oxidative stress by directly scavenging free radicals or by

upregulating cellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidases.

Data supporting the latter have been reported from several *in vivo* rodent models (Bishayee et al., 2010a), including hepatic ischemia-reperfusion, ethanol toxicity, and carbon tetrachloride toxicity, and these mechanisms may account for some of the protective effects of RSV in liver disease (Bishayee et al., 2010a).

Another commonly cited mechanism is an anti-inflammatory effect of RSV. Inflammation often accompanies the liver injury process, and in some cases, may exaggerate the liver damage (Jaeschke et al., 2002; Jaeschke, 2011). For example, it has been shown in naphthalene hepatotoxicity in mice that pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6 are greatly increased (Chirli et al., 2008). It is known from the galactosamine/LPS model in mice that TNF- α is responsible for directly inducing apoptotic cell death (Leist et al., 1994; 1995) and neutrophil activation and recruitment (Schayler et al., 1988), which causes additional liver injury (Jaeschke et al., 1998). RSV administration appeared to prevent these effects in mice and protected against the liver damage caused by naphthalene (Chirli et al., 2008).

RSV could also have an effect on metabolism in the liver, which is the effect that has received the most attention of late (Kulkarni and Cantó, 2015). The AMP-related kinase (AMPK) and sirtuin 1 (SIRT1) are 2 key targets of RSV (Kulkarni and Cantó, 2015). As fuel sensors, they can activate the peroxisome-proliferator-activated receptor γ co-activator-1 α (PGC-1 α), and diverse downstream transcriptional regulators, such as peroxisome proliferator-activated receptors (PPARs), estrogen-related receptors (ERRs) and nuclear respiratory factors (NRFs), all of which have been shown to be critical in regulation of liver function by modulating mitochondrial biogenesis, mitophagy, gluconeogenesis or lipid metabolism (Kulkarni and Cantó, 2015; Jornayez and Shulman, 2010). Indeed, it has been shown that RSV can improve energy metabolism in alcohol- or high fat diet-induced liver disease in mice *in vivo* by targeting metabolism in the liver (Lagouge et al., 2006; Baur et al., 2006; Heebøll et al., 2014).

Beside these mechanisms, RSV also affects both Phase I and II drug metabolizing enzymes. RSV itself is primarily eliminated through phase II metabolism, forming multiple glucuronide and sulfate conjugates (Wenzel and Somoza, 2005). There is limited evidence for biotransformation of RSV by cytochrome P450 enzymes *in vitro* (Steenwyk and Tan, 2010). Furthermore, RSV seems to inhibit CYP3A4 in a mechanism-based fashion (Chan and Delucchi, 2000). However, there is no evidence for oxidation products in humans so it is not yet clear if P450s play a role in metabolism of RSV *in vivo* (Walle, 2011), and even the positive *in vitro* results have been challenged in other studies (Yu et al., 2002). Nevertheless, both *in vitro* studies and animal models have demonstrated that RSV inhibits the activity of various CYP enzymes as well as their expression through various nuclear factors (Baur and Sinclair, 2006). In humans, RSV induced CYP1A2 activity and inhibited CYP3A4, CYP2D6, and CYP2C9 (Wenzel and Somoza, 2005). These results suggest the possibility that RSV may alter the metabolism of other drugs in the liver, and also affect activation or

detoxification of xenobiotics and carcinogens. Phase II enzymes have also been shown to be induced or inhibited by RSV (Baur and Sinclair, 2006; Chow et al., 2010).

Overall, RSV has been suggested to have a large number of biological effects. In particular, RSV is commonly believed to have anti-inflammatory and anti-oxidant effects. However, the mechanisms by which RSV has been proposed to protect in certain models of hepatotoxicity and other liver diseases are not always consistent with fundamental data from the experimental models used. A summary of our conclusions is available in table 1.

3. RESVERATROL AND DRUG HEPATOTOXICITY

3.1 Resveratrol in acetaminophen hepatotoxicity

Among the hepatotoxicants that RSV has been shown to protect against, APAP is arguably the most important. As mentioned, APAP overdose is the most common cause of acute liver failure in many Western countries (Larson et al., 2005; Lee, 2008). In mice, the liver injury is initiated by the formation of a reactive metabolite that depletes liver glutathione (GSH) and binds to proteins (Jollow et al., 1973; Mitchell et al., 1973; McGill et al., 2013) (Fig. 1A). Protein binding appears to cause mitochondrial dysfunction and oxidative stress. Mitochondrial respiration is decreased (Meyers et al., 1988) and evidence of oxidative stress is dramatically increased in the livers of mice treated with toxic doses of APAP (Jaeschke, 1990). The major ROS in APAP toxicity is thought to be superoxide (O_2^-). There is strong evidence that O_2^- production within damaged mitochondria is increased and that it reacts with nitric oxide (NO) to form peroxynitrite ($ONOO^-$). $ONOO^-$ can then react with amino acid residues in proteins, particularly tyrosine. Increased nitrotyrosine adducts have repeatedly been measured in the livers of mice after APAP overdose (Hinson et al., 2000; Knight et al., 2001; Cover et al., 2005; Ishii et al., 2006; Burke et al., 2010). Furthermore, scavenging $ONOO^-$ with GSH reduces APAP-induced liver injury (Knight et al., 2002; Bajt et al., 2003), and a $ONOO^-$ decomposition catalyst has also been shown to protect against APAP (LoGuidice and Boelsterli, 2011). The initial oxidative stress appears to activate the c-Jun N-terminal kinases (JNK) 1/2 via several other kinases (Nakagawa et al., 2008; Sharma et al., 2012; Ramachandran et al., 2013; Xie et al., 2015). The activated JNK then translocates to mitochondria (Hanawa et al., 2008; Win et al., 2011), where it enhances mitochondrial dysfunction. Eventually, the mitochondrial membrane permeability transition (MPT) pore forms and mitochondrial membrane potential is lost (Kon et al., 2004; Reid et al., 2005; Masubuchi et al., 2005; Ramachandran et al., 2011).

Swelling of the mitochondria and lysis of the outer mitochondrial membrane results in release of endonucleases that translocate to the nucleus and damage nuclear DNA (Bajt et al., 2006). Mitochondrial Bax also plays a role in endonuclease release at early time points (Bajt et al., 2008). The final result is oncotic necrosis of the hepatic parenchyma (Gujral et al., 2002). Importantly, the mechanisms of toxicity in humans seem to mimic these mechanisms in mice (Antoine et al., 2012; McGill et al., 2012; 2014; Xie et al., 2014).

Three studies of the mechanism of protection of RSV against APAP hepatotoxicity have been published. In the first study, the authors reported that malondialdehyde (MDA), a marker of lipid peroxidation (LPO), and TNF- α were increased in mice after APAP

treatment and that these effects were attenuated by co-treatment with RSV (Sener et al., 2006). Based on these data, the authors concluded that RSV protected against APAP hepatotoxicity by reducing LPO and inflammation. In the second study, it was found that C57Bl/6 mice had higher levels of TNF- α in the liver and lower levels of the anti-inflammatory cytokine IL-6 than BALB/c mice, which are less susceptible to APAP-induced liver injury (Masubuchi et al., 2009). They also showed that that RSV treatment could protect against APAP in C57Bl/6 mice and that this protection was associated with a shift toward lower TNF- α expression and higher IL-6 expression (Masubuchi et al., 2009). The authors concluded that inflammation plays an important role in APAP hepatotoxicity and that RSV protected by reducing inflammation. However, the mechanistic conclusions from these two studies are questionable. First, neither study included a detailed analysis of NAPQI formation and protein binding, which is necessary to preclude an upstream effect on metabolism (Jaeschke et al., 2013). Any effect on formation of the reactive metabolite of APAP and its binding to proteins will alter the toxicity (Jaeschke et al., 2013). Moreover, it has been shown that the amount of LPO that occurs during APAP-induced liver injury is not sufficient to cause cell death, and a diet high in vitamin E does not protect against APAP (Knight et al., 2003). Finally, although early studies found that pre-treatment of mice with antisera against TNF- α protected against APAP toxicity (Blazka et al., 1995), there is strong evidence that treatment with antibodies can cause significant off-target effects (Jaeschke and Liu, 2007). Importantly, TNF-deficiency does not protect against APAP-induced liver injury (Boess et al., 1998) and it has also been extensively demonstrated that sterile inflammation does not aggravate APAP hepatotoxicity in mice or humans (Jaeschke et al., 2012), though it may be important for regeneration (Dambach et al., 2002; You et al., 2013; Williams et al., 2014). Thus, it is clear that the RSV-mediated reduction of LPO and TNF expression during APAP toxicity must be the consequence of protection instead of the cause. Rather, emerging evidence suggests that RSV protects against APAP-induced liver injury by reducing protein nitration and inhibiting release of endonucleases from mitochondria (Du et al., 2015) (Fig. 1A). It was recently reported that post-treatment with RSV does not interfere with protein binding, JNK activation or mitochondrial damage in the livers of mice treated with a hepatotoxic dose of APAP, but it does reduce nitrotyrosine adducts and nuclear DNA damage caused by mitochondrial endonucleases (Du et al., 2015). Consistent with this, it has been shown that RSV can directly scavenge ONOO⁻ and prevent nitrotyrosine formation in renal tubule cells treated with a ONOO⁻ donor (Holthoff et al., 2010). Although the reason for the reduced endonuclease release from mitochondria is unclear, it is known that RSV can partition into lipid membranes and it is possible that it stabilizes the membrane structure and prevents release in that way (Fabris et al., 2008).

3.2 Resveratrol in carbon tetrachloride hepatotoxicity

Unlike APAP, toxic doses of carbon tetrachloride (CCl₄) do cause LPO in the liver (Weber et al., 2003). CCl₄ is converted to trichloromethyl radical (CCl₃·) and trichloromethyl peroxy radical (CCl₃OO·) (Fig. 1B), which can abstract hydrogen from polyunsaturated fatty acids thereby initiating the chain reaction of LPO that destroys lipid membranes and disturbs lipid homeostasis in cells (Weber et al., 2013). RSV has been shown to protect against CCl₄-induced liver injury (Rivera et al., 2008; Vitaglione et al., 2009; Chan et al., 2014). Similar to the case of APAP, it has occasionally been suggested that the mechanism of RSV-

mediated protection against CCl₄ is through its anti-inflammatory effects (Chávez et al., 2008; Roy et al., 2011); however, this is usually based on a reduction in expression of one or more pro-inflammatory cytokines in the liver at a single time point. As such, it is difficult to determine whether or not the reduced inflammation is a reason for or simply a result of the protection. Nevertheless, an effect on inflammation cannot be entirely ruled out at this point, particularly in models of chronic CCl₄ treatment. RSV has been shown to inhibit NFκB activity (Chávez et al., 2008). It has also been shown to reduce activation and function of stellate and Kupffer cells in vitro (Kawada et al., 1998) and to prevent fibrosis in vivo (Chávez et al., 2008). The fact that RSV tends to partition into lipid membranes and can directly inhibit LPO and membrane damage in simplified model systems (Tadolini et al., 2000; Megli et al., 2004; Fabris et al., 2008) suggests that RSV also acts as a direct chain breaking antioxidant in membranes, similar to vitamin E. However, Knockaert et al. (2012) reported that inhibition of LPO in mice by known antioxidants did not fully protect against CCl₄ hepatotoxicity, despite the fact that it prevented the early mitochondrial damage caused by the xenobiotic. Overall, the mechanism of RSV-mediated protection against CCl₄ is not well understood. Additional research is needed.

3.3 Resveratrol in alcohol-induced liver injury

RSV has also been shown to protect against alcohol-induced liver injury. Kasdallah-Grissa et al. (Kasdallah-Grissa et al., 2006) initially reported that ethanol feeding modestly increased MDA in the liver and RSV ameliorated this effect. Although they did not measure ALT or perform any histological analysis, follow-up studies showed that RSV could reduce liver injury and mortality caused by chronic ethanol exposure (Bujanda et al., 2006). Together, these data suggested that RSV protected against ethanol by inhibiting LPO. Interestingly, it was later demonstrated that RSV-mediated SIRT1 activation results in increased deacetylation and degradation of SREBP1 and activation of PGC-1α in vitro, leading to decreased expression of lipogenic genes and increased fatty acid oxidation in a mouse model of chronic ethanol feeding (You et al., 2008). It was also shown that RSV co-feeding reduces steatosis caused by ethanol in mice (Ajmo et al., 2008). In light of this, it was surprising that another group found that RSV enhances fibrosis in a continuous gastric infusion rat model of ethanol exposure (Oliva et al., 2008), since steatosis is an important part of the pathogenesis and may contribute to the development of fibrosis. Unfortunately, the relevance of either model of alcohol consumption (feeding or continuous infusion) is unclear. While continuous gastric infusion results in more severe changes in liver pathology than ad libitum ethanol feeding, and certain pathological features of the model more closely resemble what is observed in humans with advanced alcoholic liver disease, it is an extreme approach that generally does not reflect human patterns of regular but intermittent alcohol consumption. It seems likely that ethanol feeding is a better model, at least for the early stages of alcoholic liver disease. Thus, RSV probably protects against ethanol hepatotoxicity by reducing steatosis through increased fatty acid oxidation and decreased lipogenesis (Fig. 1D). RSV has also been shown to enhance autophagy in primary human hepatocytes exposed to ethanol, possibly through SIRT1-mediated deacetylation and activation of FoxO3a (Ni et al., 2013). However, the significance of the latter effect of RSV has not been tested in vivo.

3.4 Resveratrol in hepatotoxicity caused by other xenobiotics

RSV has also been shown to protect against hepatotoxicity caused by naphthalene in mice (Chirli et al., 2008), methotrexate in rats (Tunali-Akbay et al., 2010) and dimethylnitrosamine in rats (Lee et al., 2010; Ahmad and Ahmad, 2014). Various mechanisms were proposed in these studies to explain the protection afforded by RSV in these models, including antioxidant, anti-inflammatory, and antifibrotic signaling (Fig. 1C). However, each study included only one or a few late time points, making it difficult to differentiate between what caused the protection and what was merely a consequence of it.

4. RESVERATROL AND OTHER LIVER DISEASES

4.1 Resveratrol in liver cancer

Liver cancer ranks among the most common types of cancer worldwide and approximately 90% of all liver cancers are hepatocellular carcinoma (HCC) (EASL-EORTC, 2012). The potential beneficial effect of RSV on liver cancer has been studied extensively and it appears that RSV may have benefits at multiple stages of carcinogenesis. Several *in vitro* studies have shown that RSV can affect growth and proliferation of hepatoma cell lines, such as HepG2 (Delmas et al., 2000; Kozuki et al., 2001; Kuo et al., 2002). Some rodent studies suggest that RSV also protects against liver cancer *in vivo* (Bishayee and Dhir, 2009; Bishayee et al., 2010b). Limited data from induced tumorigenesis studies in rodents suggest that RSV limits cancer through its anti-inflammatory effects (Bishayee et al., 2010c; Mbimba et al., 2012). However, the latter is largely based on the fact that treatment with RSV reduces expression of inflammatory markers. Without more detailed mechanistic investigation, the possibility that the reduction in inflammation markers was the result of protection by RSV rather than the cause of protection cannot be ruled out.

The most common causes of liver cancer in humans are chronic liver diseases including viral hepatitis, alcohol hepatotoxicity, non-alcoholic fatty liver disease (NAFLD), and exposure to carcinogens such as aflatoxin (Bishayee et al., 2010b). Interestingly, there is some evidence that RSV can protect against these insults as well. Alcohol hepatotoxicity and fatty liver disease are discussed elsewhere in this manuscript. Like alcohol and high-fat diet, transgenic expression of hepatitis C virus core protein in mice has been shown to cause steatosis and this can be reduced by RSV treatment (Jiang et al., 2012). It is well known that many carcinogens, particularly aryl hydrocarbons, require metabolic activation for their carcinogenic properties, and one of the most important mechanisms of this activation is via P450-mediated metabolism. It has been documented that RSV can act as a chemopreventative agent against liver cancer by inhibiting aryl hydrocarbon-mediated induction of CYP1A1 expression and activity (Ciolino et al., 1998). The mechanism behind this seems to be inhibition of the recruitment of both the aryl-hydrocarbon receptor and RNA-polymerase complex to the regulatory region of the CYP1A1 gene, ultimately leading to decreased expression (Beedanagari et al., 2009). Thus, RSV may play a preventative role in the initiation of tumorigenesis caused by carcinogens by modulating expression of drug metabolizing enzymes.

Once a tumor is established, malignant cancers are characterized by unregulated cell growth, tissue invasion, and metastasis. In previous studies, RSV has been shown to be protective against these processes, leading to a dual inhibitory effect of RSV on cell growth as a result of G1 cell-cycle phase arrest and induction of cell death, ultimately leading to decreased proliferation and decreased cell survival, both in cell culture and in rats (Kuo et al., 2002; Notas et al., 2006; Bishayee and Dhir, 2009). Finally, RSV has been shown to cause decreased matrix metalloproteinase-9 (MMP-9) expression by inhibition of NF- κ B, leading to decreased migration of HepG2 cells in culture (Yu et al., 2008). Interestingly, RSV inhibition of NF- κ B was also shown to decrease VEGF expression and angiogenesis in both HepG2 cell lines and mice (Yu et al., 2010; Zhang et al., 2014), a process critical for metastasis (Stetler-Stevenson, 1999). That RSV has been shown to be protective at both early and late stages of carcinogenesis (Rajasekaran et al., 2011) underscores the complexity of the effects RSV in HCC.

4.2 Resveratrol in cholestatic liver disease

Cholestasis is defined as a reduction of bile flow and can have several etiologies. In patients with end-stage liver disease secondary to cholestatic liver disease, transplantation is required (Carrion and Bhamidimarri, 2013). During acute cholestasis, damage to the bile ducts leads to release of osteopontin and subsequent initiation of an inflammatory cascade (Yang et al., 2014; Woolbright and Jaeschke, 2012). Over the past decade, several studies have demonstrated a beneficial effect of the antioxidant properties of RSV on the pathophysiology of cholestasis. To date, most studies demonstrate a less robust inflammatory response following RSV treatment. In both the bile duct ligation and ethinylestradiol models of cholestasis, RSV treatment led to decreased expression of pro-inflammatory molecules such as TNF α , IL6, IL-1 α , and NO (Ara et al., 2005; Chan et al., 2011; Hussein, 2013) with a concurrent increase in anti-inflammatory molecules such as SOD, GR, GPx, and catalase (Hussein, 2013). In addition, these findings correlated with decreased KC activation and leukocyte infiltration of the liver (Chan et al., 2011), decreased liver injury, and a decrease in ductal proliferation and fibrogenesis (Chan et al., 2011; Hussein, 2013). These data confirm the importance of inflammation in the progression of cholestatic liver injury and are in line with antioxidant properties of RSV. Perhaps a more interesting finding, however, is that by Lin, et al. (2012) demonstrating that the protective effects of RSV stem from a combination of anti-apoptotic activity, mitochondrial biogenesis, and induction of autophagy. However, it is unclear whether the involvement of these processes is a direct effect of RSV activity or secondary to other effects of RSV. In addition, recent studies indicate that necrosis, rather than apoptosis, predominates during cholestasis (Gujral et al., 2004; Fickert et al., 2005; Woolbright et al., 2013) thus, the mechanisms of RSV-mediated protection against cholestasis need further investigation.

4.3 Resveratrol in ischemia-reperfusion-induced liver injury

Ischemia-reperfusion injury (IRI) is the process by which re-introduction of oxygen to a previously ischemic organ, leads to exacerbation of injury to that organ. Clinically, IRI is observed during resection surgery (due to the Pringle maneuver), transplantation, or periods of severe hypotension followed by fluid resuscitation (Eltzschig and Eckle, 2011). In this context, a protective role of systemic administration of RSV following IRI has been

described on the basis of decreased plasma ALT and AST activities and reduced necrosis early (3 h) after reperfusion in rats (Hassan-Khabbar et al., 2008; 2010; Nivet-Antoine et al., 2010). However, these studies examining the role of RSV during IRI have neglected to take into account the late stage, which is mediated by the influx of neutrophils to the hepatic parenchyma (Jaeschke et al., 1990). These studies focus only on the early reperfusion period, a time point at which RSV has been shown to ameliorate the oxidative stress responsible for initial injury, decrease neutrophil recruitment, and down-regulate thioredoxin-interacting protein, a protein that inhibits the function of thioredoxin (Hassan-Khabbar et al., 2008; 2010; Nivet-Antoine et al., 2010). Kupffer cells but not neutrophils are responsible for the early oxidant stress and cell death during reperfusion (Jaeschke and Farhood, 1991). However, this early reperfusion injury determines the degree of neutrophil recruitment during the later stages of the pathophysiology (Jaeschke, 2003). Neutrophil-induced oxidant stress is the main cause of neutrophil cytotoxicity (Hasegawa et al., 2005). It is therefore likely that RSV attenuated the effect of the early Kupffer cell- and later neutrophil-induced oxidant stress by either acting as antioxidant and/or modulated the capacity of the inflammatory cells to generate these reactive oxygen species. Thus, more detailed mechanistic studies involving prolonged reperfusion time points are necessary before drawing any conclusions about the possible beneficial effect of RSV during IRI.

5. CLINICAL USE OF RESVERATROL FOR LIVER DISEASES

The mounting preclinical evidence of beneficial effects of RSV has prompted a few clinical trials of its use in human diseases. Preclinical and clinical studies have demonstrated that RSV is relatively safe, well-tolerated and lacks serious adverse effects (Novelle et al., 2015). Importantly, promising results have been achieved in some clinical trials for diabetes, obesity and cardiovascular diseases, and cancer (Smoliga et al., 2011; Novelle et al., 2015).

Although the effect of RSV in drug hepatotoxicity has not been tested in humans, it has been tested in certain liver diseases. In particular, RSV may be beneficial in the treatment of fatty liver in humans. Daily treatment with RSV has been shown to modestly reduce hepatic lipid content and other evidence of liver stress in obese men (Timmers et al., 2011), although these results were not reproducible in a non-obese female population (Yoshino et al., 2012). Another study found that RSV decreased plasma levels of hepatic lipoproteins in overweight subjects (Dash et al., 2013). RSV has also been shown to decrease evidence of apoptosis in patients with NAFLD (Faghihzadeh et al., 2014), though another clinical study failed to reproduce these health benefits (Chachay et al., 2014). Currently, more than ten clinical trials investigating the effects of RSV on liver diseases, NAFLD (and related metabolic diseases) and liver cancer are ongoing, according to the US federal database of clinical trials. Nevertheless, the number and scope of human clinical trials regarding the therapeutic benefits of RSV in liver disease are still very limited, especially when compared to the >7000 publications on RSV listed in Pubmed and the various benefits in preclinical studies for liver diseases (Bishayee et al., 2010a).

Several factors may account for the small number of clinical trials of RSV for liver diseases. The most important might be the limited decisive evidence of health benefits in humans. Some completed clinical trials on RSV have obtained compelling results with a focus on its

physiologic benefits. Although physiologic effects of RSV might not reflect its true potential as a therapeutic agent for pathologies, a neutral or negative result from such trials often prevents its further use into clinical patients (Smoliga et al., 2011; 2012). The second important factor might be the nature of RSV. The compound appears to have multiple molecular targets, which renders the interpretation of clinical data difficult. This is further complicated by the use of different formulations of RSV in different trials (Smoliga et al., 2012). Another issue is that clinical trials are expensive and most natural products like RSV cannot be protected by patents. Finally, RSV has poor bioavailability, undergoing rapid and extensive metabolism in the liver (Smoliga and Blanchard, 2014). Only trace amounts of free RSV (<5 ng/ml) could be detected in plasma after a 25 mg oral dose and most of the compound was converted to sulphate and glucuronide conjugates (Walle et al., 2004). Therefore, new formulations with improved RSV bioavailability will be needed for clinical trials (Amri et al., 2012). A number of strategies have been tried in animal models to improve the bioavailability of RSV, such as applying nano-emulsifying drug delivery systems, controlled release devices, or consumption with other phytochemicals (Amri et al., 2012; Smoliga and Blanchard, 2014). Interestingly, co-administration of vinegar-baked *Radix bupleuri* has been shown to selectively improve hepatic distribution of RSV in mice (Zhao et al., 2009), though its use in clinical studies needs further evaluation.

Another critical factor for therapeutic use of RSV is the determination of its clinical dosage, duration and route of administration. Species differences, inter-individual variation in human subjects and the type and severity of liver disease could all render the translation of data from in vitro and animal studies into clinical use difficult, and the potential confounding variables could affect the accurate interpretation of clinical studies (Novelle et al., 2015). It was also suggested that most studies investigating RSV in humans so far have been limited by their sample sizes (Novelle et al., 2015). Therefore, well-designed clinical studies are needed to bridge the gap between the large number of published preclinical studies and the paucity of information concerning the use of RSV as a therapeutic agent in liver disease.

6. CONCLUSIONS

A large number of studies exploring the effects of RSV in various liver diseases have been published. As discussed, there is compelling evidence from preclinical studies in rodents that RSV can protect against xenobiotic-induced hepatotoxicity in particular. However, the mechanisms by which this protection occurs are poorly understood. Most of the mechanisms that have been proposed to date are, at best, questionable due to poor study design. An improved understanding of the effects of RSV in liver disease and hepatotoxicity will help us to identify human diseases that might benefit from RSV therapy. The poor bioavailability of RSV also continues to be a major obstacle to clinical use. Overall, while RSV appears to be a promising therapeutic candidate, additional work is clearly needed before it is likely to be clinically useful.

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RESEARCH HIGHLIGHTS

- Resveratrol has been shown to protect against many hepatotoxicants
- The most commonly proposed mechanisms are antioxidant and anti-inflammatory effects
- The proposed mechanisms are not always consistent with data from related studies
- Where possible, we suggest the most likely mechanisms of protection based on all available data
- Resveratrol may be a promising candidate for clinical use to treat liver disease, but the mechanisms are not always clear **and bioavailability is an issue**

Figure 1.

Possible mechanisms of RSV-mediated protection in xenobiotic hepatotoxicity. (A) Acetaminophen (APAP) hepatotoxicity involves cytochrome P450 enzyme (P450s)-mediated conversion of APAP to a reactive intermediate that binds to proteins. Binding to mitochondrial (Mito.) proteins leads to oxidative stress that is exacerbated by the c-Jun N-terminal kinases (JNK) 1/2. Protein nitration by peroxynitrite contributes to the mitochondrial injury. Eventually, the mitochondria release endonucleases (AIF, EndoG) that damage DNA. RSV appears to prevent both protein nitration and endonuclease release. (B)

Carbon tetrachloride is converted to a reactive intermediate that initiates lipid peroxidation (LPO), which may lead to inflammation. RSV may inhibit both LPO and inflammation. (C) Various xenobiotics seem to cause inflammation, oxidative stress, and even fibrosis. RSV appears to interfere with all of those processes. (D) Alcohol is metabolized to acetaldehyde by alcohol dehydrogenase (ADH). Acetaldehyde is further metabolized by aldehyde dehydrogenase (ALDH2), but can also stimulate sterol regulatory binding element protein (SREBP) to induce expression of lipogenic genes. Furthermore, production of NADH in alcohol metabolism may also stimulate lipogenesis. Accumulation of lipids seems to lead to oxidative stress, injury and inflammation. RSV has been shown to inhibit SREBP and activate peroxisome proliferator-activated receptor gamma-coactivator 1 α (PGC-1 α). The latter leads to enhanced fatty acid oxidation to reduce lipids and downstream oxidative stress and inflammation.

Table 1

Proposed and likely mechanisms of RSV-mediated protection in various models.

Model	Proposed Mechanisms	Refs.	Likely Mechanisms [*]
<i>Xenobiotic hepatotoxicity</i>			
Acetaminophen overdose	Anti-inflammatory, anti-LPO, anti-ONOO ⁻ , prevents endonuclease release	Sener et al., 2006; Masubuchi et al., 2009; Du et al., 2015	Anti-ONOO ⁻ and prevents endonuclease release
Carbon tetrachloride	Anti-inflammatory	Chávez et al., 2008; Roy et al., 2011;	Unclear; anti-LPO?
Chronic alcohol	Anti-LPO, increased β -oxidation, decreased lipogenesis, increased autophagy	Kasdallah-Grissa et al., 2006; You et al., 2008; Ajmo et al., 2008; Ni et al., 2013	Increased β -oxidation and decreased lipogenesis
Other xenobiotics	Anti-inflammatory, anti-LPO	ehirli et al., 2008; Tunali-Akbay et al., 2010; Lee et al., 2010; Ahmad and Ahmad, 2014	Unclear
<i>Other liver diseases</i>			
Cancer	Reduced P450 expression, cell cycle arrest, cell death, decreased metastasis	Ciolino et al., 1998; Kuo et al., 2002; Notas et al., 2006; Bishayee and Dhir, 2009; Yu et al., 2008; 2010; Zhang et al., 2014	Unclear; possibly all proposed mechanisms?
Cholestasis	Anti-inflammatory, increased mitochondrial biogenesis, increased autophagy, decreased cell death	Chan et al., 2011; Hussein, 2013; Ara et al., 2005; Lin et al., 2012	Unclear
Ischemia-reperfusion	Anti-inflammatory, anti-oxidant	Hassan-Khabbar et al., 2008; 2010; Nivet-Antoine et al., 2010	Anti-inflammatory, anti-oxidant, neutrophil inhibition?
<i>Most commonly proposed protective effects in all models</i>			
Anti-inflammatory, anti-LPO, anti-oxidant, mitochondrial effects			

LPO, lipid peroxidation. ONOO⁻, peroxynitrite.

^{*} The most likely mechanism(s) of RSV-mediated protection based on the relevance of the models used and the quality of the studies reviewed here. Question marks indicate that these mechanisms are plausible, but have not yet been investigated.