

Reduction of Hepatotoxicity Induced by Doxorubicin

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Abstract

Doxorubicin (DOX) has been used in the treatment of variety of cancers but its administration is limited by a dose-dependent toxicity. Its cytotoxic effects on malignant cells have shown an increase in the risk of cardiotoxicity, hepatotoxicity, renal insufficiency.

Antioxidants have been explored for both their cancer preventive properties and chemomodulatory of DOX toxicity. Resveratrol (RSV) is a polyphenolic constituent of several dietary mainly of grapes and wine origin recently its anti-cancer potential has been extensively explored, revealing its anti-proliferative effect on different cancer cell lines, both *in vitro* and *in vivo*. RSV is also known to have modulatory effects on cell apoptosis, migration and growth via various signaling pathways. Though, RSV possesses great medicinal value, its applications as a therapeutic drug is limited. Problems like low oral bioavailability and poor aqueous solubility make RSV an unreliable candidate for therapeutic purposes. Additionally, the rapid gastrointestinal digestion of RSV is also a major barrier for its clinical translation. Hence, to overcome these disadvantages RSV-based nanodelivery systems have been considered in recent times. Nanodelivery systems of RSV have shown promising results in its uptake by the epithelial system as well as enhanced delivery to the target site. Herein we have tried to bring new new insights into the molecular mechanisms of DOX toxicity with respect to DNA damage, free radicals and whether RSV can be a playmaker as chemomodulatory of DOX.

Keywords: Doxorubicin; Hepatotoxicity; Chemotherapeutic drug; Cardiovascular disease

Introduction

Doxorubicin (DOX) is an anthracycline, also known as Adriamycin, has been used in oncologic practice since the late 1960s. It can be contributed as a simple agent or in merger with other chemotherapeutic drug in the treatment of cancers, including leukemias, lymphomas, and solid tumors but its administration is limited by a dose-dependent, irreversible, and progressive toxicity [1]. Cytotoxic effects on malignant cells, but, are confused by an improve in the risk of cardiotoxicity, hepatotoxicity, renal insufficiency [1,2]. Hepatic disfunction occasionally necessitates the withdrawal of or dose adjustments to not only DOX but also the coadministered medicines used to treat disease complications and improve unpleasant symptoms, thereby increasing the risk for chemotherapy failure [3]. Therefore, it is considered to be of critical importance to explore strategies for reducing the incidence and severity of the hepatic toxicity of anthracyclines. Changes in the expression and activity of various biomolecules have been regarded as a likely mechanism for the cardiotoxicity of DOX [3]. In contrast, oxidative stress is generally considered to be a major cause of DOX hepatotoxicity [4]; however, the mechanisms underlying DOX-induced hepatic impairments have not yet been elucidated in detail. It has been reported that the extrinsic

and intrinsic apoptotic responses mediated by Fas and Bax, respectively, were both associated with DOX-induced acute hepatic damage [4], which may be helpful for establishing a rational strategy to alleviate organ toxicity.

Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a polyphenol of the stilbene class found in some fruits such as grapes, blackberries or peanuts. Its name would come from *Veratrum album* L. var *grandiflorum*, the white verrato, from which it was first extracted in 1939 by Japanese, Takaoka. It was also identified in 1959 in a eucalyptus and then in 1963 in the root of *Fallopia japonica* (or *Polygonum cuspidatum*) [5], demonstrates that it is wine resveratrol which, by inhibiting the oxidation of LDL, must be responsible for its cardioprotective effect. Resveratrol is a good candidate for the use of the French paradox, an expression that refers to the surprising situation in the south-western region, where still a high consumption of animal fats, is observed a clever cardiovascular disease Countries of northern Europe.

Resveratrol (RSV) is a phytoalexin (antimicrobial) and It has been discovered that it has many Apart from the prevention of coronary artery disease, the list of well-documented beneficial effects of this compound has continued to grow: it involves inflammation, platelet activation, angiogenesis, maintenance of bone mass, Reduction of adipose mass, neuroprotection, photoprotection, anti-tumorigenic, cardioprotective, anti-aging and antiviral effects [6]. RSV activities are

mediated by the modulation of several molecules of cell signaling regulating cell cycle progression, inflammation, proliferation, apoptosis, invasion, metastasis and angiogenesis of tumor cells [7-9]. It has been proved that RSV can sensitize resistant cells to chemotherapeutic agents by overcoming one or more mechanisms of chemoresistance. In some tumor cells, however, RSV has been shown to act as chemoprotector. The potential mechanisms underlying this dual effect are described in this review.

Doxorubicin

Mechanism of action and toxicity

Doxorubicin (DO_x) like other anthracyclines, is an intercalating agent which enters the space between the base pairs of the DNA agent. Furthermore these molecules are inhibitors of type II DNA topoisomerases, enzymes involved in the maintenance of the three dimensional structure of the DNA during transcription and of the phenomena of replication [10]. Complex DNA /topoisomerase strands are stabilized thereby preventing DNA replication [10].

DOX provokes a drastic oxidative-stress status within erythrocytes and plasma as assessed by high malondialdehyde (MDA) and carbonyl protein, elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within plasma and a drastic depression in

some of the antioxidant enzymes in both compartments [11]. DO_x also increases free iron and H₂O₂ and depressed Ca²⁺ within erythrocytes as well as into plasma and it likely induces the highly toxic hydroxyl radical which in turn affected Ca²⁺ homeostasis.

Doxorubicin-induced hepatotoxicity

Oxygen-free radicals produced during the metabolic activation of DOX may have toxic effects on heart muscle [11], which is provided with poor mechanisms of detoxification of such species DOX is likely to have toxic effects on liver [12] by increasing levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx) enzyme in liver tissue of guinea pigs [13]. Nevertheless, distribution of a unique dose of DO_x decreases the content of cytochrome P-450 and glutathione in rat liver [14] and high levels of glutathione have been found to protect isolated hepatocytes from DOX toxicity [15].

DO_x has showed cardiotoxic and hepatotoxic effects in animals. It is known that DOX cause weight loss [13,16]. Thus, their prolonged use and over dosage cause death. These drugs cause disruption in basal metabolism by showing toxic effect especially in liver and hearth tissues [16]. The biochemical and electron microscopic findings showed that DO_x causes he patotoxic effect. Dose effect of DO_x show different effects among types of cancer disease. Besides, the administration time length is also important [16].

Delivery system	Purpose	Output	References
Dextran-doxorubicin (Dex- DO _x) conjugate	Antitumor efficacy	A higher molecular weight endowed the Dex- DO _x conjugate with a better antitumor efficacy in a limited number of tested samples	[30]
Zinc oxide nanoparticles (n-ZnO)	Antitumor efficacy	Blends of the nanocrystalline zinc oxide nanoparticles (n-ZnO) and triblock copolymer treated from the solution have been used to make the hybrid polymer-metal oxide for the preparation of the drug loaded nanocomposite	[31]
Gold nanoparticles	Anti-cancer efficacy	Delivery of three cytotoxic drugs with a newly designed PEGylated gold NPs may provide promising and novel prospect in cancer therapy	[32]
Polyethylene sebacate (PES)-Gantrez® AN 119 DO _x NPs	Anti-cancer efficacy	The high efficacy coupled with greater safety portrayed Pul DO _x NPs as a promising nanocarrier for improved therapy of HCC	[33]
Porous Silicon Nanoparticles	Inhibition of Doxorubicin-Resistant Cancer Cells	A synergistic effect with the presence of AmQu is observed in both normal MCF-7 and DO _x -resistant MCF-7 breast cancer cells. Due to the similar structure as dopamine, AmQu may facilitate both the interaction and internalization of PSi into the cells	[30]
Doxorubicin loaded Galactose conjugated poly(d,l-lactide-co-glycolide)(PLGA) nanoparticles	Hepatocyte-targeting drug Carrier	Cell cytotoxicity tests showed that unloaded NPs are non-toxic and that doxorubicin-loaded NPs caused a cellular viability decrease of around 80%, therefore representing a promising approach to improve liver-specific drug delivery	[34]
Poly(ethylene glycol)-b-poly(L-lactide-co-2-methyl-2(2-dicarba-closo-dodecarborane) propyloxycarbonyl-propyne carbonate) (PLMB)	Anti-cancer efficacy	It was demonstrated that DO _x @PLMB nanoparticles could selectively deliver boron atoms and DO _x to the tumor site simultaneously <i>in vivo</i>	[35]
Dextran-doxorubicin (Dex- DO _x) conjugate	Antitumor efficacy	The <i>in vivo</i> efficacy of these nanoparticles as antitumor drug carriers was determined by tumor regression and increased survival time as compared to drug conjugate and free drug	[36]

Table 1: Composition of delivery systems for DO_x.

Two ways of free radical formation by DO_x have been demonstrated [17]. The first pathway causes the formation of a free radical of semiquinone by the formation of several semi-inhibitors of DO_x. In the

presence of oxygen, redox cycling of DO_x-derived quinone-semiquinone yields superoxide radicals [18]. In the second pathway, the DO_x free radicals originate from a non-enzymatic mechanism

involving reactions with iron. For example, Fe^{3+} reacts with DO_x in a redox reaction after which the iron atom accepts an electron and a Fe^{2+} DO_x free radical complex is produced. This iron- DO_x complex can reduce oxygen to hydrogen peroxide and other active oxygen species [18]. DO_x generate superoxide anion radicals, H_2O_2 and hydroxyl radicals as a result of oxidative metabolism in rats [19].

Delivery Systems for Doxorubicin

Effect of delivery systems on pharmacokinetics and biodistribution

The pharmacokinetic studies of DO_x have shown that doxorubicin is made up of several phases after intravenous injection, whereas intravenous infusion is often followed by three-phase plasma clearance. The administration of DO_x is 3 to 5 minutes, shows the rapid absorption of the drug by the cells. It is the terminal half-life of 24 to 36 h. This shows to remove the tissue from its absorption [20]. An equilibrium distribution of the drug is unavoidable to reduce the risk of toxicity. The range of constant distribution varies from 500 to 800 l/m^2 , allowing body tissues to take a sufficient amount of doxorubicin [20]. DO_x binds to plasma proteins and most drugs, DO_x enters the cell by passive diffusion, general accumulating at intracellular concentrations [21]. DO_x acts in a non-specific manner, acting on normal a cell which proves the side effects in the cancer patient due to DO_x unpredictable cytotoxic properties. Several studies have been carried out to develop specific DO_x distribution systems capable of reducing their toxicity to target its effects directly on tumor cells. The use of these drug delivery systems is constantly tested and improved to increase the efficacy, selectivity and total effect of antineoplastic drugs. As we can see in Table 1, most promising DO_x delivery systems include the entrapment of the chemotherapeutic drug into polymeric drug carriers, such as liposomes, and nanoparticles [21].

Liposomal

Doxil® (doxorubicin HCl liposome injection [PLD]); distributed in the United States by Tibotec Therapeutics, a division of Ortho Biotech Products, L.P., Bridgewater, NJ; Caelyx® is distributed outside the United States by Schering-Plough, Kenilworth, NJ).

It is doxorubicin encapsulated in a pegylated liposome composed of hydrogenated soy phosphatidylcholine, N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (mPEG-DSPE), and cholesterol DO_x [22].

Is loaded into liposomes at a high concentration under the influence of an ammonium sulfate gradient and is suspended in a non-electrolytic solution [23]. Each liposome contains 9,000 to 16,000 molecules of doxorubicin. More than 91% of the drug is encapsulated in the liposomes [22]. The liposomes are 80 to 100 nm in diameter, with linear segments of surface-grafted PEG extending in a brush formation about 5 to 6 nm from the surface [24].

In pegylated liposomal DO_x (PLD), release of DO_x from the carrier is slow (half-life is several days). Because the DO_x stays associated with the carrier, it has pharmacokinetics similar to that of the carrier, which results in significantly altered plasma pharmacokinetics for PLD when compared with conventional DO_x . This includes significantly higher area under the plasma-concentration-time curve (AUC), increased

distribution half-life, lower rate of clearance, smaller volume of distribution, and 15- to 40-fold higher peak concentrations [25-27]. Tissue concentrations of DO_x are also significantly higher, and remain higher for significantly longer periods of time following delivery via pegylated liposomes, compared with free drug. Alterations in the pharmacokinetics and biodistribution of DO_x , when delivered in pegylated liposomes, reduce cardiotoxicity and the myelotoxicity and increase drug efficacy [28]. By comparison, non-pegylated liposomal doxorubicin (NPLD) is approximately 160 nm in diameter [29] although both pegylated and conventional liposomes may be formulated in a variety of sizes [24]. The PEG coating on pegylated liposomes is the unique feature that distinguishes them from conventional liposomes. In addition to preventing opsonization by plasma proteins, leading to reduced RES uptake of liposomes, PEG provides a steric stabilizing effect that results in reduced binding to cells, blood vessel walls and other surfaces. The steric stabilization effect leads to the reported colloidal stability for pegylated liposome suspensions.

DO_x -loaded PLD do not aggregate or out of suspension over a 2-year period. These sterile stabilized liposomes can circulate for several hours and, if they are very small, pass through the capillaries that have increased permeability, such as capillaries in diseased tissues or in the liver and certain other organs. Such long-circulating liposomes distribute as intact particles to normal tissues as well, perhaps by a process of transcytosis and/or extravasation at the level of postcapillary venules [28]. The liposomes are slowly removed from circulation by a combination of RES uptake and extravasation in normal tissues [28].

Nanoparticle

In the past, polymers such as dextran and polyethyleneimine have been used as potential chemotherapeutic delivery carriers (Table 1). Antitumor efficacy of Dextran-doxorubicin (Dex- DO_x) conjugate increased with the molecular weight of dextran [30]. Blends of the nanocrystalline zinc oxide nanoparticles (n-ZnO) and a copolymer consisting of three blocks was used to form the polymer-hybrid metal oxide for the preparation of DO_x loaded nanocomposite [31]. Pegylated gold nanoparticles (NPs) have been delivered DO_x successfully with anti-cancer efficacy [32].

DO_x was encapsulated into Polyethylene sebacate (PES)-Gantrez® AN 119 NPs and its high efficacy coupled with greater safety portrayed those NPs as a promising carrier for improved therapy of HCC [33].

DO_x -loaded galactose-conjugated poly(D,L-lactide-co-glycolide) (PLGA) NPs aimed at targeting hepatocytes revealed that unloaded NPs are non-toxic while DO_x -loaded NPs caused a cellular viability decrease of around 80% [34].

Poly(ethylene glycol)-b-poly(L-lactide-co-2-methyl-2(2-dicarbasilo-dodecarborane) propyloxycarbonyl-propyne carbonate) (DO_x @PLMB) NPs could selectively deliver boron atoms and DO_x to the tumor site simultaneously *in vivo* [35].

When the MG-63 and SaOS-2 cell lines were treated with the dextran- DO_x -PEI nanoparticles, the cell numbers were reduced from 5 to 10% at a concentration of 1 to 10 mg/l relative to free doxorubicin on 24-48 h [37]. This can be explained by the fact that the nanoparticles penetrate through a passive diffusion. Also, the cytotoxicity may be reduced due to slow-coupled DO_x release from the nanoparticles. These DO_x -coupled nanoparticles also showed selective delivery to tumors. This increases the margin of safety as well as reduces side-effects associated with DO_x -loaded dextran-PEI

nanoparticles. The use of nanoparticles as a drug delivery system is that they can be manufactured with ease, with the added benefits of low cytotoxicity and biodegradable properties.

A recent study implicated the encapsulation of DO_x using a chitosan drug delivery system by the complex coacervation process with dextran sulfate [37]. A level of encapsulation of more than 99% has been achieved, and the study proved that osteosarcoma cell death with these nanoparticles occurred through apoptosis, necrosis and autophagic cell death. Treatment with doxorubicin nanoparticles in mice having orthotopic osteosarcoma reduced tumor growth, decreased tumor-associated osteolysis and reduced metastasis to the lungs. These nanoparticles did not cause side effects in mice – specifically to the heart or skin. However, doxorubicin nanoparticles may prove to be useful clinically provided further studies are performed to validate such formulations in clinically relevant animal tumor models.

The *in vivo* antitumor of NPs, determined by tumor regression, showed an increased survival time as compared to drug conjugate and free DO_x [36].

Antioxidants as Drugs Decreasing Toxicity-Resveratrol

Antioxydants

Antioxydants have been explored for both their cancer preventive properties and chemodulatory of DO_x toxicity. In many studies

Vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect [18,38]. But some investigators have previously stated these effects of vitamin E have been found at 200 mg/kg/day dose levels [38,39].

Vitamin E protects cells and subcellular structures from the oxidative damage by decreasing MDA levels. There were no toxic effects of vitamin E on prescribed doses. Vitamin E was shown to decrease MDA level or keep its original value while increase SOD, GSHPx and CAT enzyme levels or keeping their original value. This shows that vitamin E used single oxygen formation pathway. Vitamin E alone or in combination with DO_x treatment may inhibit the toxic and hepatotoxic effects of DO_x [40].

Dose and time schedules of catechin treatment were chosen so that high levels of scavenging activities were met during early stages of the presence of DO_x in the cells. Additionally, *in vitro* experiments on an iron loaded rat heart impairment model which was made atonous helped us to select dose of catechin because high and long-term catechin therapy caused toxic effects on cardiac cells [39].

Recent studies proved that the antioxidant effect of catechin was related to the free radical scavenging activity [39,41]. the catechin caused an increase in the levels of, CAT, GSHPx and SOD enzymes. In the same way, catechin with DXR also caused a slight increase in these enzymes [40].

RSV form (dose)	Model	Study	Output	References
RSV: 20 and 40 µM	<i>In vitro</i> : hepatic cell lines (QSG-7701 and LO2) Hepatocellular carcinoma (HCC) CHC-LM3, SMMC-7721 Bel-7402 and HepG 2	Antitumor efficacy	The reduction of hexokinase 2, by resveratrol provides a new dimension to clinical HCC therapies aimed at preventing disease progression	[109]
RSV: 10, 20, 50 and 100 µM.	<i>In vitro</i> : HepG2 cells	Anti-cancer efficacy	He combination treatment of resveratrol and matrine is a promising novel anticancer strategy for liver cancer; it also provides new insights into the mechanisms of combined therapy	[110]
RVS: 100 mg / kg	<i>In vivo</i> : Mice BALB/c	Hepatotoxicity and neurotoxicity effects	RSV, rutin, quercetin, and quercetin, would be useful for the clinical treatment of neuro- and hepatotoxicity induced by oxaliplatin	[42]
RSV: 12.5, 25, 50 and 100 µM.	<i>In vitro</i> : HepG2	Cytotoxic effects of RSV	The RSV exerts differential synergistic effect with etoposide on proliferation of cancer cells from different origin which is mainly accompanied by p53 activation	[43]
RSV: 10, 30, 50, 100, 150 µM.	<i>In vitro</i> : HepG2 cells	Antiproliferative effects of RSV	RSV added to the culture medium decreased Hepatoma proliferation	[44]
RSV: 100 and 200 µM.	<i>In vitro</i> : AH109A rat ascites hepatoma cells	Antiproliferative effects of RSV	RSV inhibited both the proliferation and invasion of AH109A rat ascites hepatoma	[45]
RSV: 25, 50, 100 µM.	<i>In vitro</i> : AH109A	Antiproliferative effects of RSV	RSV inhibited vascular endothelial growth factor gene expression via hypoxia-inducible factor-1a inhibition	[46]
RSV: 10 µM.	<i>In vitro</i> : HepG2 and Hep3B	Antiproliferative effects of RSV	RSV inhibited cell growth only in p53- positive HepG2 cells	[47]

Table 2: RSV effects on hepatic cancer.

Resveratrol

Several *in vitro* and *in vivo* studies have shown the potential role of RSV as an anti tumoral hepatic agent. These studies are summarized in Table 2. In hepatocellular carcinoma cells (HCC) RSV reduced hexokinase 2, which provides a new dimension to clinical HCC therapies aimed at preventing disease progression. In some studies in which RSV was tested with another compounds in HepG2 cells, RSV and matrine revealed anticancer potential for liver. *In vivo* hepatotoxicity studies performed in BALB/c mice revealed that RSV could be useful for the treatment of hepatotoxicity induced by oxalidiplatin [42] whilst *in vitro* cytotoxic effects of RSV showed that phytoalexin exerts differential effect with etoposide on proliferation of cancer cells which is mainly accompanied by p53 activation [43].

The differentiation of human hepatoblastoma HepG2 cells and ao rat hepatoma F were negatively impacted by the addition of RSV to the culture medium in both dose and time-dependent manner [44]. The polyphenolic compound is increasing the number of cells arrested in the S and G2/M phase and likely delay or to prevent the cells from entering mitosis [44]. Hepatic growth factor (HGF) has been implicated in the ability of primary hepatic tumors to proliferate and invade adjacent tissue [44]. The effects of RSV on Hepatic growth factor mediated invasion determined in HepG2 cells revealed that RSV decreased invasion of liver cancer cells and hepatic growth factor induced scattering with concurrent inhibition of cell proliferation possibly due to a post-receptor mechanism rather than apoptosis [44].

RSV inhibited both invasion of AH109A rat ascites hepatoma cells and the proliferation at higher concentrations but suppressed only the invasion at lower concentrations, and that RSV-loaded rat serum restrained only the invasion, which suggested that anti invasive activity of RSV is independent of its antiproliferative activity but a relationship to its antioxidative property was found [45]. Subsequent studies confirmed the implication of the antioxidant property of RSV as sera from rats orally given RSV were found to suppress ROS potentiated invasion of AH109A cells [46]. Another study focused on factors involved in angiogenesis of expanding tumors proposed a mechanism of RSV's ability to curtail hepatoma cell invasion hails [47]. Though it had been known RSV had antiangiogenic abilities, it was found that RSV had an inhibitory effect on vascular endothelial growth factor gene expression via hypoxia-inducible factor-1 α inhibition [48].

The results proved that RSV inhibited cell growth only in p53-positive HepG2 cells, which was a result of cellular apoptotic death via p53 dependent pathway. It was shown that RSV treated cells were arrested in G1 phase and were associated with an increase in p21 and expression. The cytotoxic effects of RSV in HepG2 cells were confirmed by Kim et al. [49], who isolated the stilbene from the seeds of *Paeonia lactiflora*, a plant widely used in Chinese traditional medicine. An observation regarding time-dependent and concentration effects of RSV on cytotoxicity, apoptosis and cell proliferation activity in HepG2 cells has been reported [50]. Cytotoxicity became evident at RSV concentration of 50 or 100 μ M at treatments longer than 48 h. Cell cycle analysis showed an increment of Sphase cells at low concentrations of RSV (10–50 μ M) and a decrement at high concentrations (100–200 μ M). The synergistic antitumor effects of RSV with current anticancer drug 5-fluorouracil (5-FU) increased to a greater extent than for H22 cells exposed to 5-FU alone. A direct evidence of apoptosis was presented as the mechanism of antihepatoma activity of RSV [50]. Another study has found the time- and dose-dependent effects of RSV on cell proliferation in HepG2 cell lines [52].

General pharmacokinetics

The pharmacology of RSV during the past decade has been studied; its pharmacokinetics has also been investigated in preclinical models as well as in humans [53-54]. In the next subsections, the pharmacokinetics of RSV will be presented in more detail.

Absorption and bioavailability

This polyphenol has high oral absorption but extensive and rapid metabolism without adverse effects in both rodents [55,56] and humans [57,58]. In humans, about 70% of orally administered RSV (25 mg) is rapidly (<30 min) absorbed and metabolized with a peak plasma level of \approx 2 μ M of RSV metabolites and a half-life of 9-10 h [57-60]. The extent to which the human colon can absorb and metabolize RSV depends on the metabolic activity and on the hepatic function of the local intestinal microflora.

Metabolism

RSV undergoes extensive phase I (oxidation, reduction, and hydrolysis) and phase II (glucuronic acid, sulfate, and methyl conjugations) biochemical changes immediately after ingestion [60,61], being metabolized into both glucuronic acid and sulfate conjugations of the phenolic groups in liver and intestinal epithelial cells [60-63]. Hydrogenation of the aliphatic double bond is also present [64]. While presystemic and systemic conversion to major metabolites (glucuronic and sulfate conjugations) occur in the intestine and liver very fast and efficiently in the so called enterohepatic recirculation [61], other metabolites such as dihydro-resveratrol and piceatannol, are probably mediated by microbial fermentation of trans-resveratrol in the gastrointestinal tract. The sulfation of resveratrol in human liver by sulfotransferases (SULTs) has been examined [63] and three metabolites were identified: trans-resveratrol-3-O-4'-O-disulfate (S1), transresveratrol- 4'-O-sulfate (S2), and trans-resveratrol-3-O-sulfate (S3). The glucuronidation by uridine 5'-diphosphoglucuronosyltransferases (UGTs) on intestinal absorption of resveratrol was also investigated [64] and it was possible to identify two metabolites, namely, trans-resveratrol-4'-O-glucuronide (G1), and trans-resveratrol-3-O-glucuronide (G2). Modifications such as glucuronidation and sulfation typically reduce the cell permeability to drugs and aid in their excretion.

However, the *in vivo* efficacy of the administered PEG, despite its low bioavailability, has led some researchers to interrogate whether metabolites would be the bioactive forms of the parent compound [59,60]. Nevertheless, some studies show less pharmaceutical impact of the metabolites and indicate a decrease in the expression of SULT enzymes in certain types of cancer cells, which indicates an overall low sulfation activity in these cells comparing to the normal ones. Therefore, the reduction of SULT expression may be a favorable factor for achieving better therapeutic effects while an effective dose of trans-RSV is maintained in cancer cell.

Distribution

Blood transport: The efficiency of a therapeutic substance is often related to its affinity to bind to protein transporters [65]. RSV has poor water solubility and thus has to be bound to plasma proteins to assure its body distribution and bioavailability [66]. Indeed, in its transport, RSV can bind to serum proteins [67] such as lipoproteins, hemoglobin, and albumin which facilitate its carrier mediated cellular uptake and then it can passively diffuse through the plasma membrane [68,69]

investigated the binding properties of RSV to plasma proteins, such as human serum albumin (HSA) and hemoglobin (Hb) and confirmed that both complexes formed are spontaneous and exothermic. The binding constant of RSV–HSA complex is larger than that of RSV–Hb, which indicates the higher affinity of HSA to RSV. Hydrophobic interactions seem to play a major role in the binding of RSV to the hydrophobic cavity of HSA, and hydrogen bonding is the main force involved in the binding of RSV to the central cavity of Hb where some residues interact directly with the hydroxyl groups of the compound. Electrostatic interactions can also be involved in the formation of both complexes since residues with positive charge are in the proximity of the binding compound [69].

Liver uptake: It is known that liver plays a key role in the bioavailability of RSV. Some studies show the highest accumulation of RSV in the liver of rats and mice after oral administration [70,71]. Nevertheless, no toxicity or hepatocyte lysis was observed after treatment with high doses of RSV, which is relevant because certain antineoplastic agents cause hepatotoxicity, limiting their efficacy in anticancer therapy [72]. Moreover, the large uptake of RSV by liver cells, along with its weak toxicity suggests its important role in the prevention of liver diseases. Besides a passive diffusion influx, it was also shown that RSV enters the liver cells by an active process involving transporters, accounting for more than half of the total hepatic uptake [68]. This active process involves members of the family of uptake [68]. This active process involves members of the family of organic anion-transporting polypeptides (OATPs) which are multispecific transporters or albumin binding proteins that bind RSV–albumin complexes and then deliver RSV in a similar way to the fatty acid uptake [73].

RSV Delivery Systems

RSV has low bioavailability, and this has been associated with its low stability against environmental stress its inability to reach a target site and its poor water solubility in the body to exert the desired health effect. Nano-encapsulation allows improving the solubility of RSV, stabilizing it against trans to cis isomerization, and improving its bioavailability [74]. A range of encapsulant materials, technologies and formulations have been examined for enhancing the delivery of RSV [74].

Nanoparticles (NP) are an ideal way to deliver drugs because of their high loading efficiency and superior ability to penetrate cell membranes [75]. Regarding the loading efficiency, it depends mainly on the lipophilicity of RSV and its affinity to the hydrophobic core (PCL). The highest drug loading content of RSV into polymeric nanoparticles was about 20% and the encapsulation efficiency was 90% [75,76]. The cellular uptake of the polymeric nanoparticles occurs by endocytosis, which enhances the drug penetration in cells and demonstrates higher therapeutic efficiency than the obtained with free drug administration.

Solid lipid nanoparticles (SLN) are composed of solid lipids and surfactants and can be used as carriers for RSV because of their size, hydrophobic core with hydrophilic surface, biocompatibility, and also because drug loaded SLN protect drugs from hydrolysis and thus increase drug stability [77,78]. RSV loaded-SLN was studied regarding the release profile of RSV from SLN and also regarding the cellular uptake of the nanoparticles [79]. The controlled release profile of RSV-loaded SLN presents two phases. The first phase corresponds to an initial burst of RSV that is associated with the particle shell. This phase

is followed by a prolonged release of the remainder RSV that is located in the lipid matrix [79]. The efficiency of the SLN cellular uptake depends on the surface properties of these nanocarriers [80]. Several molecular interactions are expected when SLN encounters the cell membrane and these interactions may be dependent on several factors such as the biological membrane organization and the existence of lipid rafts [81], as well as the formation of actin cytoskeleton invaginations for the receptor mediated entrance [82].

SLN may also be considered as a physically stable and well tolerated nanocarrier system. Indeed, with sizes below 180 nm, SLN are able to pass rapidly through membranes causing no significant changes in cell morphology, metabolic activity or cell cycle [83]. Furthermore, SLN have been reported as excellent delivery systems for RSV being able to carry this bioactive compound until the nuclear target site. Indeed, on the one hand, RSV has multiple actions in cell environment, especially around the nuclear membrane and, on the other hand, SLN suffer intracellular trafficking [84]. Therefore, it is possible to conclude that SLN can be used as carrier systems to enhance the intracellular delivery of RSV.

Protective Effect of Antioxidants

General

Polyphenols are compounds widely distributed in the plant kingdom, generally involved in the defense against UV radiation or aggression by pathogens [85]. The most abundant are flavonoids, phenolic acids, lignans, and stilbenes; of these, phenolic acids and flavonoids account for 30% and 60%, respectively of total polyphenols in the diet, approximately 1 g/day [86]. The derivatives of the abundant phenolic acids in plants are hydroxycinnamic acids and hydroxybenzoic [86].

Berberine can be isolated from the stems and roots of several plants, such as *Berberis vulgaris* and *Coptis chinensis* [87-89]. Berberine is a nitrogenous cyclic compound with a structure that is highly similar to that of intercalating agents (e.g., ethidium) [89]. Intercalating agents are often used as nucleic acid dyes to study cell functions, and berberine is a well-known alkaloid drug that is commonly used as a fluorescent dye.

Berberine induces inhibits cell proliferation and apoptosis in various cell lines derived from breast, lung, colon, and liver cancer. However, berberine has been shown to have synergistic effects on cells treated in combination with more toxic drugs, including vincristine and irinotecan [90,91].

Resveratrol

RSV provides protection against the development of diseases caused by oxidative stress, such as cardiovascular disease, inflammation, neurological diseases and liver toxicity [92]. Also, a RSV reduced the oxidative stress created by the chemotherapy. Literature data reveals multiple cellular targets of RSV affecting apoptosis, growth and cellular proliferation, invasion, inflammation, metastasis and angiogenesis [93]. During the past decade, the amount of research on this phytoalexin has soared, and there exists strong evidence which supports RSV as a potent chemopreventive and chemotherapeutic agent [92].

Jang et al. [94] demonstrated the chemo-preventive effects of RSV in inhibiting multi-stage carcinogenesis. Also, RSV has been shown to

suppress proliferation of a variety of human tumor cells *in vitro* [95,96] which have led to numerous preclinical animal studies to evaluate the cancer chemotherapeutic and chemopreventive potential of RSV [97,98]. Several clinical trials, including one sponsored by the National Cancer Institute, are currently underway to investigate the use of both RSV and RSV-rich products, for prevention and treatment of colon cancer.

A significant amount of RSV accumulates and is retained in the liver [99,100]. RSV has been shown to inhibit the hepatic carcinogen-activating enzymes, including cytochrome CYP3A/2 P450 and 1A1 (CYP1A1) and induce hepatic phase 2 conjugating enzymes, namely glutathione S-transferase (GST), UDP-glucuronosyl transferase and NAD(P)H: quinone oxidoreductase, *in vivo* and *in vitro* [101,102]. The effects of these enzyme modulations by RSV could be the reduced exposure of cells to carcinogens due to inhibition of carcinogen activation, elevated carcinogen detoxification and elimination [102]. The RSV, with regards to liver cancer, is its strong antioxidant properties and anti-inflammatory, as both inflammation and oxidative stress have been strongly implicated in the progression and occurrence

of HCC [103-105]. However, despite its great promise, the effects of RSV on liver cancer have not been systematically studied until recently.

RSV is used for the hydroxyl radical induced lipid peroxidation with the aim of preventing in living cells [96]. *In vitro* and *in vivo* results showed that RSV make the direct scavenging effect *in vitro* and may disclose indirect antioxidant effect by an increase in the expression of intracellular antioxidant enzymes [105,106]. RSV was proved to protect hepatic antioxidant systems against ischemia reperfusion-induced oxidative stress, protect from peroxynitrite and oxidative DNA damage caused by hydrogen peroxide [107]. Malondialdehyde (MDA), that is a lipid peroxidation end-product, connect to the cross and Generate to modification in ion permeability and enzyme activity [108-110]. The oxidative stress and lipid peroxidation are evaluated by MDA level. According to studies realized *in vivo*, in the RSV treated rats, levels of MDA were lower than in the AOM group. Glutathione (GSH) plays important role in the protection against oxidative stress, as a cofactor of GSH peroxidases attend in the elimination of hydrogen peroxide and lipid hydroperoxides [107].

RSV, DO _x form	Cells	Study	Output	References
DO _x : 10 µM, RSV: 100 µM	Colorectal cancer cells HCT 116	Chemodulatory effects of RES and DID to DO _x in colorectal cancer cells	RSV sensitized colorectal cancer cells to DO _x via facilitating apoptosis and enhancing intracellular entrapment of DO _x	[118]
RSV: 10 mg / kg b.wt DO _x 5 mg /kg b.wt.	<i>In vitro</i> : MCF-7 et MDA-MB-231 <i>In vivo</i> : Albino Mouse	Anti-cancer effects of RSV	RSV chemosensitizes DO _x in combination, through inhibiting breast cancer cells proliferation and invasion, and inducing apoptosis via suppression of chronic inflammation and autophagy	[119]
DO _x : 5 µM RSV: 25 µM	<i>In vitro</i> : H ₉ C ₂ cardiac cells	Assess the effects of RSV on sirtuin 1 (SIRT1) activation in mediating DO _x -induced cytotoxicity	RSV protected H9c2 cells against DO _x -induced injuries via SIRT1 activation	[120]
DO _x : 5 µM RSV: 25 µM	<i>In vitro</i> : H ₉ C ₂	Assess the effects of RSV on AMPK/P53 activation in mediating DO _x -induced cytotoxicity	RSV protected H9c2 cells from DO _x -induced apoptosis via the AMPK/P53 pathway	[125]
RSV: 1-100 µM DO _x : 1 µM	<i>In vitro</i> : hematological malignancies K562/ADR	Antiproliferative effects of RSV	RSV increased anti-proliferative activity of bestatin through downregulating P-Glycoprotein expression via inhibiting PI3K/Akt/mTOR pathway in K562/ADR cells	[126]
RSV: 10/25/50/100 µM	HepG2, Hep3B cells and non-cancerous primary human hepatocytes	Anti-cancer effects of RSV	Nicotinamide phosphoribosyltransferase (NAMPT) and SIRT1 are differentially regulated by RSV in hepatocarcinoma cells and primary hepatocytes and that RSV did not act as a SIRT1 activator in hepatocarcinoma cells	[122]
RSV: 40 µM	<i>In vitro</i> : Hepa1-6 cells	The anticancer effect of curcumin combined with resveratrol in hepatocarcinoma cells	Combination of curcumin and RSV upregulated intracellular reactive oxygen species (ROS) levels in Hepa1-6 cells	[123]
RSV: 50, 100, 300 mg /kg	<i>In vivo</i> / <i>in vivo</i>	Cytotoxic effects of RSV	RSV-mediated chemoprevention of rat liver carcinogenesis is devoid of any adverse cardiovascular events	[124]

Table 3: RSV chemodulatory effects of RSV to DO_x in cancer cells.

Co-Delivery of DO_x and Antioxidants

DO_x and berberine

An animal and clinical investigation showed that berberine is beneficial in combating against reactive oxygen species (ROS) formation [111,112]. Doxorubicin mainly causes liver injury via the generation of activation of the nuclear factor kappa B and the free radicals. There are not many studies has been carried out concerning the protective effects of berberine on DO_x-induced hepatotoxicity.

In a study aimed at investigate whether berberine, a natural product alkaloid, can reduce the liver injury induced by DO_x. Mice of either gender were randomly divided into four groups: the control group, DO_x group, berberine group, and berberine+doxorubicin group. In the tests, body weight, general condition and mortality of the mice were observed, and serum alanine aminotransferase (ALT) and aspartate transaminase (AST) levels were determined to evaluate liver function. DO_x caused a series of side effects including decrease of the body weight and liver weight, elevation of serum ALT and AST levels, infiltration of inflammatory cell, and necrosis of hepatocytes. These biochemical and pathological alterations in the mouse model mostly resemble acute liver failure in human.

The higher serum aminotransferase activities could be the result of leakage from damaged liver cell membranes after DO_x treatment [113]. This pretreatment of berberine provided dramatic suppress from damage of mice and ameliorated liver function by lowering serum ALT and AST levels. The protective effect of berberine against DO_x was also evaluated in the livers from a histopathologic point of view. The histological damages such as inflammatory cell infiltration and necrosis of hepatocytes induced by DO_x were also remarkably attenuated by berberine. In agreement with clinical trials on DO_x-induced hepatotoxicity, the present data showed that the significant increase in the activities of AST and ALT and histopathological changes in the liver were partially due to DO_x therapy. Berberine is an alkaloid extracted from *Coptis*, *Cork* and other traditional Chinese medicines that possesses a broad spectrum of pharmacological and therapeutic activities [114,115].

Before and simultaneously treatment of berberine with DO_x protected the liver damage, as indicated from the significant attenuated in body weight and liver weight. Moreover, the elevated ALT and AST levels in the serum were significantly suppressed. The protective effect of berberine against DO_x was further confirmed in the liver by the histopathological examinations. The comparative histopathological studies revealed severe destruction of hepatic architecture, hemorrhage, necrosis, infiltration of mixed inflammatory cells around the necrotic hepatocytes induced by DO_x, which are reduced in mice treated with berberine. Administration with berberine significantly reduced the liver injury, especially restricted the inflammation and necrosis to a minor degree. Meanwhile, berberine showed a potency to decrease the increased histopathological grade values of the hepatocyte necrosis and inflammatory cell infiltration into DO_x-injured liver. These data suggest that berberine has the potential to protect against toxicity induced by DO_x.

DO_x and RSV

There is growing evidence demonstrating the RSV modulating effect on the cytotoxic profile of different anticancer agents and protection from their toxic effects [116,117]. Most recent and significative studies are summarized in Table 3. RSV sensitized colorectal [118] and breast

[119] cancer cells to DO_x via facilitating apoptosis and enhancing intracellular entrapment of DO_x and through inhibiting breast cancer cells proliferation and invasion, respectively. DO_x-induced either injuries [120] or apoptosis [121] H9c2 cardiac cells were protected by RSV via sirtuin 1 (SIRT1) activation and the AMPK/P53 pathway, respectively.

In hematological malignancies K562/ADR cells RSV increased anti-proliferative activity of bestatin through downregulating P-Glycoprotein expression via inhibiting PI3K/Akt/mTOR pathway. While studying anti-cancer effects of RSV, Nicotinamide phosphoribosyltransferase (NAMPT) and SIRT1 were found to be differentially regulated by RSV in hepatocarcinoma cells and non-cancer primary hepatocytes and that RSV did not act as a SIRT1 activator in hepatocarcinoma cells [122]. Combination of curcumin and RSV upregulated intracellular reactive oxygen species (ROS) levels in Hepa1-6 cells [123]. *In vivo* studies of cytotoxic effects revealed that RSV-mediated chemoprevention of rat liver carcinogenesis is devoid of any adverse cardiovascular events [124-126].

Conclusion and Future Directions

Epidemiological data have shown an alarming trend in an increased prevalence of cancer. This tendency, compiled with the disease's high rate of mortality due to limited efficiency of treatment methods. The main therapy and chemotherapy for cancer, but it is a non-specific manner, healthy cells and destroy the tumor cell, has forced researchers to examine preventive approaches as well as alternate routes to treatment.

DO_x is one of the most frequently used anticancer drugs and the front-line chemotherapeutic options for treating patients with HCC. The clinical applications of DO_x are restricted to specific tissue specificity, and especially serious cardio-toxic effects resulted from lipid peroxidation and the generation of free radicals.

Over the past decades, nanotechnology has made great contribution to the development of drug delivery systems. Encapsulation of DO_x by polymeric nanoparticles (NPs) facilitates drug distribution in tumor tissues through an enhanced permeability and retention (EPR) effect that shows better pharmacokinetics profiles *in vivo* and reduces multidrug resistance of malignances, leading to enhanced anticancer effects co-delivery of nanoencapsulated DO_x plus other drugs within cancer treatment using naturally occurring compounds, including those derived from fruits, vegetables and herbs, as potential cancer preventive and therapeutic agents has become a fascinating strategy.

RSV stands out as the molecule with the most potential for stifling the disease's consequent mortality rate and growing incidence. From studies using various liver cancer cell lines and chemically-induced tumors as well as implanted cancers in animal models as described in this review, it becomes apparent that RSV may play an important role not only in the prevention but also in the therapy of metastatic disease of the liver and reduces the toxic effect induced by chemotherapy. All these studies largely establish that RSV has great promise for battling cancer and more exactly the liver cancer. Many studies have indicated that RSV suppresses the growth of HCC cells and prevents hepatocarcinogenesis by mitigating oxidative stress. Future research should deal with further characterizing the exact mechanism by which RSV possesses its effects on the cell cycle, apoptosis and redox signaling. However, RSV scavenges modulates activities of antioxidant enzymes and ROS.

Future studies should explore these and other possible mechanisms of RSV action to understand the full potential of this dietary agent in the prevention and treatment of HCC. RSV is currently investigated for the prevention and treatment of human colon cancer. It is expected that additional research would lay the foundation for clinical trials with RSV in the prevention of HCC in high-risk patients predisposed with viral hepatitis, other liver diseases and environmental carcinogens. And RSV has effect against the toxicity induced by chemotherapy, several delivery systems described for RSV such as solution; suspension nanoparticles, nano emulsion RSV and compare it is the form more effective. And see the influences ranging dose of RSV, all this research will be realized on different models: cell cultures, whole animal and isolated organ.

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