REVIEW

Role of membrane dynamics processes and exogenous molecules in cellular resveratrol uptake: Consequences in bioavailability and activities

Dominique Delmas[1,2] *and Hung-Yun Lin*[3]

[1] Université de Bourgogne, Dijon, France
[2] INSERM U866 Research Center ''Lipids, Nutrition, Cancers'', Dijon, France
[3] Ordway Research Institute, Albany, NY, USA

In the fields of nutrition prevention and therapy treatment, numerous studies have reported interesting properties of *trans*-resveratrol (RSV), a natural polyphenol against pathologies such as vascular diseases, cancers, viral infections and neurodegenerative processes. These beneficial effects are supported by more studies showing the pleiotropic actions of RSV. Nevertheless, a crucial question concerning these effects is how the polyphenol, when applied to an organism, gains access to its targets. In this review, we focus on the biochemical and biological parameters involved in RSV transport, particularly the role of the phospholipid bilayer in RSV uptake (passive diffusion, carrier-mediated transport) and of exogenous molecules modulating RSV transport and effects. The dynamic processes of the plasma membrane reveal the importance of the role of lipid composition in the fluidity, the lipid rafts in RSV endocytosis and the ATP-binding cassette transporters in RSV efflux. Specific membrane receptors such as integrin αvβ3 contribute to RSV uptake and to activate signalling pathways involved in apoptosis. We discuss the role of intracellular receptors (i.e. aryl-hydrocarbon and estrogen receptors). In addition, circulating molecules (i.e. albumin, haemoglobin, fatty acids, lipoproteins) play a role as RSV carriers. Finally, we developed a hypothesis concerning the relation between RSV uptake and its biological activities.

Received: January 28, 2011
Revised: March 16, 2011
Accepted: March 25, 2011

Keywords:
Bioavailability / Integrin αvβ3 / Lipid rafts / Resveratrol / Uptake

1 Introduction

A wide variety of plant-derived compounds, including polyphenols, are present in the human diet and may protect against vascular diseases, cancers, viral infections, neurodegenerative processes and associated anti-inflammatory effects. The impetus sparking this scientific inquiry was the result of many epidemiologic studies that showed protective effects of plant-based diets on cardiovascular disease and cancer. Among these bioactive compounds, several studies [1] revealed that resveratrol (*trans*-3,4′,5-trihydroxystilbene; RSV) may be one of the main wine microcomponents responsible for health benefits. Indeed, RSV possesses a myriad of beneficial effects and can act at multiple levels, such as cellular signalling, enzymatic pathways, apoptosis and gene expression [2–4]. Although the biological positive effects of RSV are largely elucidated, little is known about the transport and the distribution of RSV throughout the body. The present review will discuss the uptake and efflux of RSV, the modulation of its transport by interaction with plasma circulating molecules, membranes and intracellular receptors, and binding sites of RSV. Lastly, we discuss the importance of dynamics of membrane events in early

Correspondence: Dr. Dominique Delmas, INSERM U866 Research Center ''Lipids, Nutrition and Cancer'', University of Burgundy, 7 Bd Jeanne d'Arc 21 000 Dijon, France
E-mail: ddelmas@u-bourgogne.fr
Fax: +33-380393434

Abbreviations: ABC, ATP-binding cassette; **AhR,** aryl hydrocarbon receptor; **BCRP,** breast cancer resistance protein; **Cav-1,** caveolin-1; **ER,** estrogen receptor; **ERK,** extracellular signal-regulated kinase; **Hb,** haemoglobin; **LDL-R,** LDL receptor; **MRP,** multidrug resistance-associated protein; **RSV,** resveratrol

biological effects, bioavailability of RSV and the current strategies to improve its activity or its site delivery by synthesis of RSV derivatives or use of colloidal/nanoparticle carriers.

2 Role of membrane dynamic processes

The permeability of cellular membranes is one of the most important determinants of the drug uptake. A molecule's ability to cross the phospholipid bilayer is essential for both absorption of xenobiotic nutrients and its entry into the cell or into intracellular organelles. Among the different mechanisms of cellular drug uptake, passive diffusion across the membranes, carrier-mediated transport and/or active transport are often the main processes of drug uptake. Moreover, recent studies reveal the importance of the lipid composition and most particularly specific structures called ''lipid rafts'' in mediated transports for various molecules such as nutrients. At the inverse of uptake, the efflux of molecules is, in majority of cases, a carrier-mediated transport, which involves ATP-binding cassette (ABC) transporters that translocate solutes across cellular membranes

2.1 Passive transport

Passive transport is usually not saturable. As the centre of the lipid bilayer is highly hydrophobic, a compound can diffuse across the lipid bilayer portion mainly as an uncharged species, depending on its molecular size and affinity to the centre of the lipid bilayer.

To put forward knowledge on RSV's access to cells, methods using the intrinsic fluorescence properties of RSV are developed [5, 6]. Indeed, based on the spontaneous fluorescence upon UV excitation [5], we have demonstrated by fluorescence microscopy that this polyphenol is essentially present in cytoplasm and in the nucleolus region [6]. Furthermore, ε-viniferin (an RSV dimer), which has a lower antiproliferative effect toward cancer cells, presents a similar uptake as RSV. An intestinal perfusion method conducted by Juan et al. with recirculation in vivo in rats shows that RSV uptake occurs by simple diffusion without the participation of a mediated transport in jejunal loops [7].

To quantify more accurately RSV uptake, we have labelled the polyphenol with tritium in the ortho- and para-positions of the benzenic rings. The time-, dose- and temperature-dependencies of tritiated RSV influx show a passive diffusion (50%) and a carrier-mediated process (50%) in hepatoblastoma HepG2 cells (Fig. 1) [6]. Interestingly, the RSV uptake kinetics is similar in human normal hepatocytes without any toxicity. Recently, we have obtained similar results in leukaemia cells versus normal monocytes (submitted for publication). The extent of passive RSV uptake could be dependent on the lipid composition of membranes.

2.2 Lipid composition and fluidity of membranes

Membrane fluidity can influence the passive diffusion of drugs and is mainly determined by the membrane lipid composition: cholesterol, the degree of saturation and chain length of the fatty acids, and the proportion of sphingomyelin as well as protein–lipid interactions [8]. Modification in fluidity affects a number of cellular functions, including passive diffusion, carrier-mediated transport, receptors, phagocytosis, endocytosis and cell growth.

Studies conducted with liposomal membranes have reported that flavonoids and isoflavones could be localized into the hydrophobic core of the membrane leading to an important decrease in lipid fluidity in this membrane region [9]. This disturbance of the membrane fluidity could sterically hinder diffusion of free radicals and thereby decrease the kinetics of free radical reactions. In a similar manner, various studies have reported the modulation of membrane fluidity with RSV, but the results obtained are controversial. Indeed, by using fluorescence anisotropy methods, RSV increases fluidity (~25%) in liposomes and in rabbit brain synaptosomes, shown by the decrease of diphenylhexatriene fluorescence polarization (with 25 and 10 μM of RSV, respectively) (Fig. 1) [10, 11], and contrarily RSV can induce rigidization on liposomal membranes [12]. We can propose two hypotheses between the modulation of membrane fluidity by RSV and its uptake. First, in many cases, RSV is associated with ethanol (final concentration of 0.1% in the culture medium), used as a solvent to increase RSV solubility, which lowers the threshold of RSV's antiproliferative effect as compared WITH that in DMSO (0.1%) [13]. In fact, ethanol can induce expansion of the membrane, accompanied by a drop in the membrane thickness as well as disordering and enhanced interdigitation of lipid acyl chains [14]. For example, the addition of low concentrations (0.02 or 0.04 M) of ethanol in vitro increased fluidity in erythrocyte, mitochondrial and synaptosomal membranes [15]. This modulation of fluidity by RSV–ethanol combination could contribute to its uptake increases. So far, the action mechanism of ethanol is not elucidated. In addition, RSV can modulate the synthesis of various lipids such as cholesterol or fatty acids, and consequently this effect could contribute to increase the fluidity. Indeed, we and others have been shown that RSV is able to deplete into lipid rafts in cholesterol and sphingomyelin [16] contributing to the fluidization of membranes. Further investigations are required to probe whether such a depletion could be due to the activation of a sphingomyelinase that induces ceramide generation and increases plasma membrane fluidity or not. Secondly, RSV could be incorporated in the plasma membrane, particularly in lipid rafts, and consequently this incorporation could lead to a transient rigidization of the membrane.

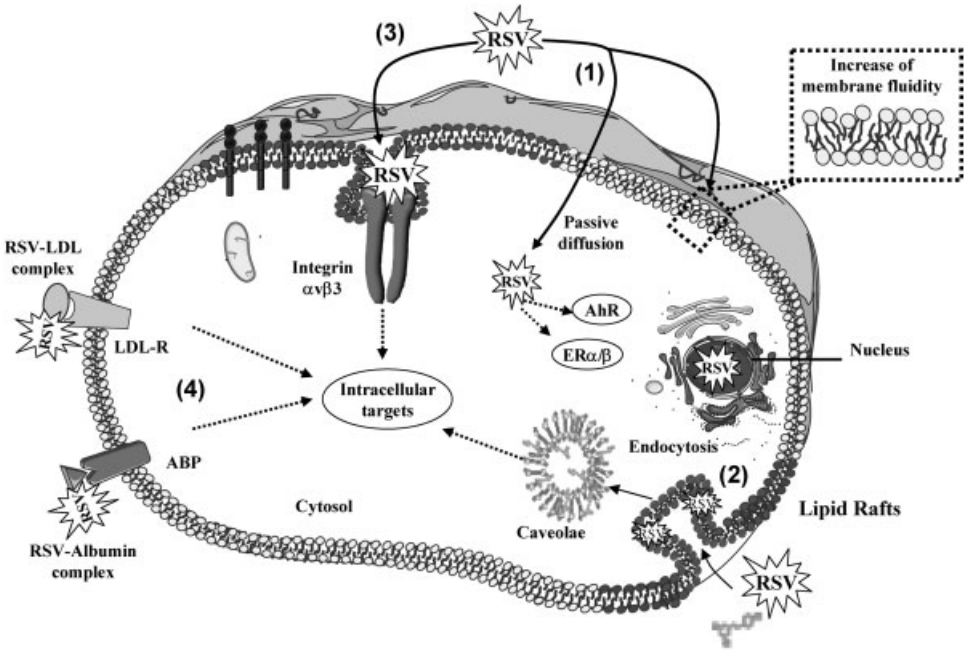

Figure 1. RSV uptake and binding to extracellular and intracellular receptors. The uptake of RSV could be due to passive diffusion (1), endocytosis via lipid rafts (2) or by binding to receptors such as integrin αvβ3 into rafts (3). HSA and LDL are important carriers (4) for RSV and are very likely to play essential role in its distribution. Consequently, both passive diffusion and carrier–mediated transport (2–4) occur in RSV uptake. RSV can also bind to intracellular target such as AhR or ERs.

2.3 RSV endocytosis uptake, a lipid raft pathway

For many drugs, there coexist passive diffusion and carrier-mediated transport, the latter being an active or a facilitated transport that has a limited capacity and thus is saturable and subject to inhibition. When carrier-mediated transport is not energy driven, it is defined as a facilitated transport process and relies on a concentration gradient of a substrate as well as a transporter protein. In fact, RSV uptake involves on one part a passive transport and on another part an active process based on two experimental observations:

(i) RSV uptake in haepatoblastoma cells is significantly higher (around 50%) at 37°C than at 4°C. Indeed, energy consumption and active transport processes are minimal at 0–4°C, whereas energy consumption is normal and active transport processes are functioning at 37°C. Consequently, it has often been assumed that when a compound is taken up at 37°C but not at 0–4°C, the transport is carrier-mediated, but when the compound is taken up at both temperatures then transport is thought to be passive.

(ii) *Cis*-inhibition experiments in which unlabelled RSV exerts an inhibition of the total tracer uptake [6].

This last result agrees with the involvement of a process more specific than passive diffusion in the transport of one part of RSV. By using a pan inhibitor of endocytosis, which disrupts the membrane electropotential and the pH [17], we show that intracellular accumulation of RSV is decreased. Interestingly, among the specific inhibitors of clathrin-mediated endocytosis, of macropinocytosis [18], of dynamin-dependent endocytosis and of lipid rafts-mediated endocytosis, only lipid raft inhibitors decrease the RSV uptake in colon carcinoma cell lines (submitted for publication). A variety of endocytic pathways that do not utilize clathrin are responsible for taking up either large particles or small solutes, together with membranes into cells. One clathrin-independent way of endocytosis depends on lipids rafts and caveolae [19]. These lipid microdomains result from preferential packing of complex sphingolipids and cholesterol in ordered plasma membrane structures and contain a variety of lipid-anchored and transmembrane proteins. They are resistant to cold lysis using nonionic detergents, and they are currently designated as detergent-resistant microdomains, lipid rafts or simply microdomains. Various molecules such as the B subunit of cholera toxin, after binding to a typical raft lipid, GM1, is internalized in part directly via these microdomains in a dynamin-mediated manner [20]. Additional non-clathrin pathways have been described for the endocytosis of glycosylphosphatidylinositol(GPI)-anchored proteins [21] and interleukin-2 by its receptors [22] and for several other lipids [23] occurs via lipid rafts. Based on their detergent insolubility property, we have isolated lipid rafts into fractions by ultracentrifugation on a sucrose gradient and we have demonstrated an accumulation of RSV into lipid rafts in colon cancer cell lines (submitted for publication). It is therefore likely that RSV, after initial insertion in the outer leaflet of the plasma membrane lipid bilayer, accumulates in lipid rafts and from there, is taken up by raft-mediated endocytosis (Fig. 1). Lipid raft involvement in RSV uptake is reinforced by another study conducted by Yang et al. which focuses on caveolin-1 (Cav-1) involvement [24]. Indeed, Cav-1 protein is a principal component of caveolae, which are plasma membrane invaginations and that are implicated in various functions including endocytosis, transcytosis and potocytosis [25]. Yang et al. show that RSV increases Cav-1

expression and a fluorescent RSV derivative (dansyl-chloride-derived RSV) colocalizes with Cav-1 [24]. In addition, RSV endocytosis is not mediated by estrogen receptor (ER) α and β, as suggested by lack of competitive inhibition by estrogen or Tamoxifen [24]. Recently, results from our group also indicate that RSV and ceramide uptake by ovarian cancer cells is via endocytosis, the former is integrin αvβ3-dependent and the latter is integrin αvβ3-independent (submitted for publication).

2.4 Integrin αVβ3 bears a receptor for RSV

Integrins are a family of transmembrane glycoproteins that form noncovalent heterodimers. Extracellular domains of the integrins interact with a variety of ligands [26], including extracellular matrix glycoproteins and the intracellular domain that is linked to the cytoskeleton [27]. The intracellular domain of certain integrins, such as integrin αvβ3 may activate extracellular signal-regulated kinase (ERK1/2) [28]. A structural protein of the plasma membrane that is essential to the interactions of cancer cells and blood vessel cells with specific extracellular matrix proteins, integrin αvβ3, has been shown by us to be a receptor for several small molecules, such as thyroid hormone analogues [28] and dihydrotestosterone [29] as well as RSV [30]. Identification of a putative receptor site for RSV in cancer cells, one that initiates transduction via ERK1/2 of the RSV signal downstream into p53-dependent apoptosis, supports the credibility and specificity of the compound as a potential therapeutic agent. The receptor may also be a vehicle for in vitro structure-activity studies of RSV analogues. Dysregulation of the β3 integrins has been implicated in cancer pathogenesis. Tumour growth and associated angiogenesis, particularly as mediated by vascular endothelial growth factor, are enhanced in β3-null mice. Integrin β3 overexpression, in contrast, can suppress tumour growth of a human glioma model in rats. Such results suggest that promotion of integrin β3 expression in cancer cells may be a therapeutic goal in the setting of cancer, but this topic is beyond the scope of the current review. Using radio-labelled RSV, we recently determined that the stilbene binds dissociably to integrin $\alpha v\beta_3$ [30], leading to activation of the mitogen-activated protein kinase (ERK1/2) signal transduction cascade. Blocking the integrin αvβ3-binding site by either an integrin antagonist peptide arginine–glycine–aspartate or anti-integrin $\alpha v\beta_3$ antibody, we are able to inhibit RSV-induced apoptosis in different cancer cell lines [30–32]. Recently, we have shown the importance of integrin αvβ3 in RSV uptake and consequently in early biological events leads by the polyphenol. Indeed, it appears that RSV induces the redistribution of integrin $\alpha_V\beta_3$ into lipid rafts as well as signalling molecules of the complex membrane (e.g. focal adhesion kinase, Fyn, Grb2, Ras, SOS) and various mitogen-activated protein kinase such as ERK1/2, c-Jun NH2-terminal Kinases involved in RSV-induced apoptosis. RSV treatment favours the formation of the integrin complex and it appears that occlusion of the arginine–glycine–aspartate-binding site in the integrin extracellular domain diminishes RSV uptake (submitted for publication). Analysis by computer modelling (DOCK6.3 program) shows that RSV forms hydrogen bonds to the Arg248 on the αv-subunit [33]. It is noteworthy that the 4′-OH group of RSV found to be required for its antioxidant activity and inhibition of cell proliferation [34] also binds to the Glu220 residue on the β3-subunit by hydrogen bonding [33].

2.5 Potential role of intracellular receptors in RSV transport

Transmembrane receptors cannot explain the pleiotropic RSV effects since a part of polyphenol uptake involves a passive process that could precede intracellular specific interaction. Little is known about the intracellular targets of RSV. Aryl hydrocarbon receptor (AhR) could be a good candidate for RSV intracellular transport. RSV is shown to be a competitive antagonist of dioxin binding to AhR, and promotes the AhR translocation to the nucleus [35], but RSV's ability to bind AhR remains controversial. Furthermore, due to its structural resemblance with human estrogens, RSV is able to bind ER-α and β with comparable affinity but with a 7000-fold lower affinity than estradiol [36]. Molecular dynamics studies have shown that the binding of RSV to ER-α is stereoselective, with a weaker binding of the *cis* form as compared with the *trans* isomer [37]. Furthermore, it has been revealed that ER-α is a positive regulator of GLUT4 (a glucose transporter) expression, whereas ER-β has a suppressive role [38]. RSV increases interaction between ER-α, Cav-1 and c-Src, and increased phosphorylation of Cav-1, c-Src and endothelial nitric oxide synthase (eNOS). Depletion of endogenous ER-α, not ER-β, by siRNA attenuates RSV- and E_2-induced ERK1/2, Src and eNOS phosphorylation. Nanomolar RSV induces ER-α–Cav-1–c-Src interaction, resulting in NO production through a Gα-protein-coupled mechanism in endothelial cells [39].

2.6 RSV efflux, a carrier-mediated process

In RSV uptake studies, observations reveal that the steady state is reached in a few minutes in hepatocytes such as HepG2 cells, indicating that RSV efflux occurs rapidly after the beginning of the uptake [6]. At 4°C, the initial rate of the incorporation of tritiated RSV is about four times lower than that estimated at 37°C, suggesting a carrier-mediated process involving transporters. Among transporters, ABC transporters translocate across cellular membranes diverse substrates ranging from chemotherapeutic drugs to naturally occurring biological compounds [40]. Various studies show that RSV efflux involves these transporters, such as multidrug resistance-associated protein (MRP) and the

breast cancer resistance protein (BCRP or ABCG2), playing an important role in the export of conjugated molecules (glucuronides or sulphates). Both in animal and human models, the main RSV metabolites are glucuronidated and sulphated [41–44]. Based on the known characteristics of MRP2 (ABCC2), this transporter is the candidate for intestinal and hepatic excretion of glucuronidated and sulphated conjugates of various polyphenols. Studies of RSV efflux across CaCo2 cells, which is a monolayer-forming cell line widely used in intestinal transport, reveal that RSV-sulphate is found to be exported to the apical side in Caco-2 cells, presumably by MRP2 [45–47]. Recently, hepatic MRP2 was shown to mediate the canalicular efflux of RSV-glucuronides and only partly of sulphates in the rat liver [48, 49]. In the rat enterocytes, the two apical transporters, MRP2 and BCRP, are involved in the efflux of RSV glucuronide and RSV sulphate (Fig. 2) [7]. Recently, MRP3 (ABCC3), which is located in the basolateral membrane of enterocytes, has been found to transport RSV-glucuronide [50]. The absence of MRP3 in mice results in an altered disposition of this glucuronide and RSV, leading to a reduced percentage of RSV being excreted via the urine in *Mrp3*$^{-/-}$ mice. Conversely, BCRP transports RSV-gucuronides and RSV-sulphates and its absence in mice results in high plasma levels of RSV-disulphate and in an increased disposal of RSV via the urine [50, 51].

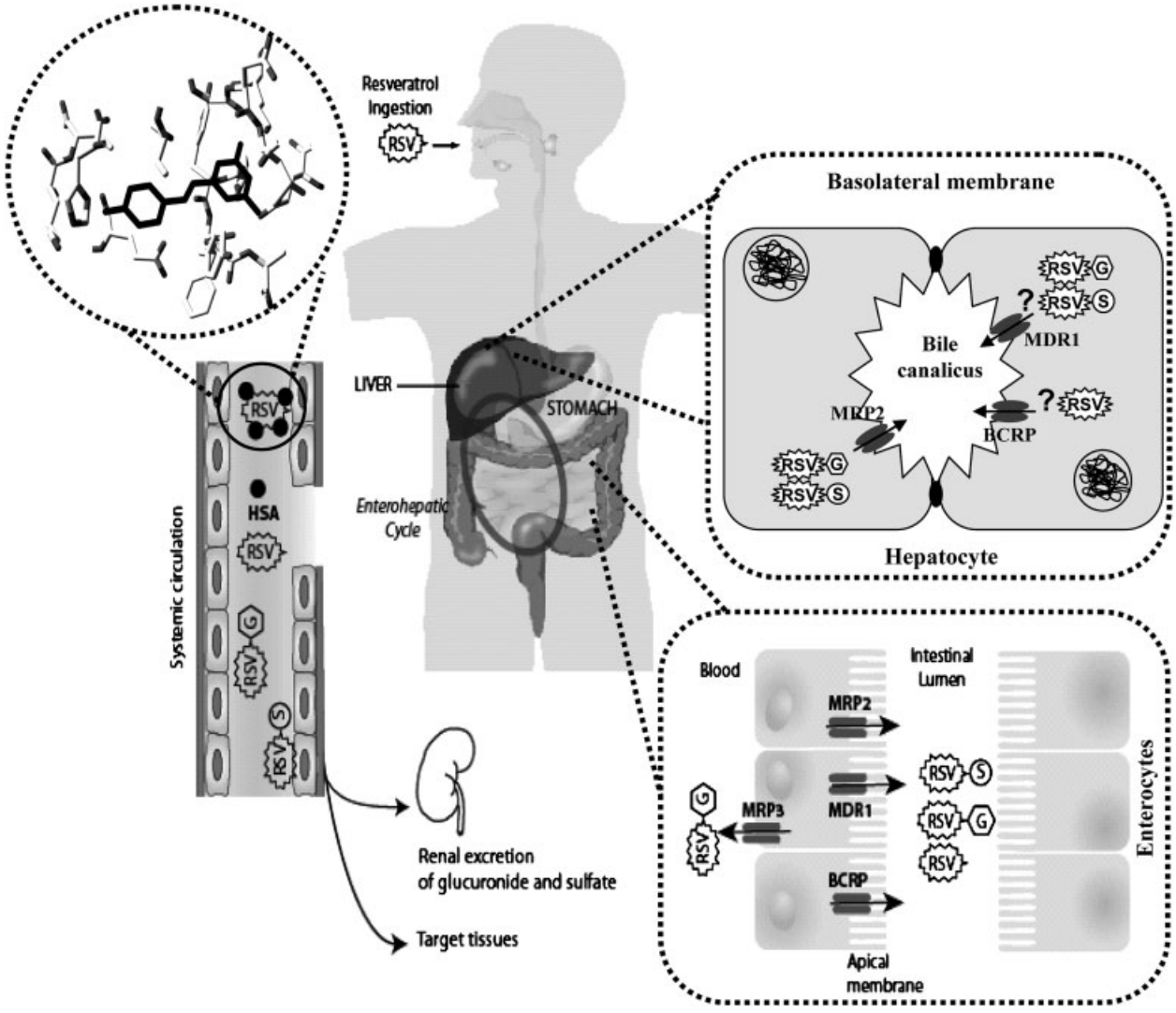

Figure 2. Bioavailability and efflux of RSV. RSV is absorbed upon oral administration and the hepatic metabolism produce RSV sulphate and glucuronide (RSV-S, RSV-G). RSV and metabolites can be release in intestinal lumen by various transporters present in the apical membrane of enterocytes or RS-G in blood by MRP3 at the basolateral membrane. In hepatocytes, MRP2 plays a role in excretion of glucuronidated and sulphated conjugates. Moreover enterohepatic recirculation releases compounds into the systemic circulation where RSV may be complexed with HSA molecular docking simulations of HSA-RSV show that RSV is located in the hydrophobic cavity between subdomain IB and IIA of HSA. In the kidney, RSV is present mainly in its native form, whereas in urine its conjugated forms are present.

3 Influence of exogenous molecules in RSV transport

Numerous events at multiple stages can be implicated in the decrease of RSV accessibility to cells, notably its binding with extracellular molecules, such as (i) serum proteins, (ii) fatty acids and (iii) lipoproteins.

3.1 Albumin

The efficiency of a therapeutic substance is related to its capacity (selectivity and affinity) to bind to protein transporters [52]. Among serum proteins that could play a plasmatic carrier's role, albumin is well known to bind and carry out a large number of amphiphilic molecules and could constitute as RSV's plasmatic carrier. The albumin involvement in RSV uptake was underlined by the alteration of RSV uptake by HepG2 cells. Indeed, as previously shown, we studied the tritiated-RSV uptake with BSA, and we show that the rate of passive transport of RSV is two-fold lower in serum-containing cell culture medium, compared with that obtained in serum-free medium [6]. Moreover, RSV uptake decreased with the increase of BSA concentration in the serum-free medium, suggesting a role of this major serum protein in its trapping ability of RVS. Various methods such as Fourier transform infrared spectroscopy, CD spectroscopy, ultrafiltration and fluorescence spectroscopic methods have been used to analyze the ligand binding mode, the binding constant, and the effects of complexation on BSA stability and conformation of the complexation of BSA with RSV, genistein and curcumin, at physiological conditions [53–55]. At a low polyphenol concentration (0.125 mM), an increase in intensity is observed for the protein amide I at 1656 cm^{-1} and amide II at 1545 cm^{-1}, in the difference CD spectra of the RSV–BSA. As the polyphenol concentration increased to 0.5 mM, a decrease in intensity of the amide I band is observed with features at 1658 cm^{-1} in the difference spectra of RSV–BSA complexes. Polyphenol binding alters BSA conformation with a major reduction in α-helices from 63% (free BSA) to 50% (RSV–BSA) and an increase in β-sheet and turn structures, indicating a partial protein unfolding at high polyphenol concentrations (0.5 mM) [53]. These results are consistent with the decrease in the intensity of the protein amide I band. Structural analysis shows that polyphenols bind BSA via hydrophilic and hydrophobic interactions with a different number of bound polyphenols (1.30 for RSV) and with a binding constant K (RSV–BSA) of 2.52 ($\pm$0.5) $\times$ 10^4 mol/L [53]. The affinity and the binding of RSV with BSA suggest that the polyphenol binds to HSA, the major plasma protein in humans [56]. Indeed, HSA is able to bind RSV and to maintain a high concentration in human serum [57]. First results obtained by N'soukpoe-Kossi et al. indicated a partial stabilization of protein secondary structure at high RSV content and show no major alterations at low RSV concentrations (0.125 mM), whereas at high pigment content (1 mM), major increases of α-helices from 57% (free HSA) to 62% and a decrease of β-sheets from 10% (free HSA) to 7% occurred in the RSV–HSA complexes [58]. On the contrary, Lu et al. show, as similar to the results obtained with BSA, a decrease in the α-helical structure of HSA (2 μM) with a range of RSV concentration (0.8–3.2 μM) [57]. In this study, it seems that HSA has only one binding site for RSV as similar to haemoglobin (Hb) [57]. The binding constant of RSV to HSA is greater than that of RSV to Hb indicating that the affinity of HSA toward the polyphenol is higher than that of Hb. [57]. Furthermore, thermodynamic analyses suggest that hydrophobic interactions play a major role in the binding of RSV to HSA with hydrogen binding being the main force allowing the binding of RSV to Hb [57].

3.2 Fatty acids

The role of fatty acids should be to ensure a lipophilic environment favourable to the binding of RSV. Indeed, during RSV association with BSA, fatty acids have a positive effect on the binding of RSV to BSA [54]. Fatty acids are usually used as vectors because they present a high affinity for the liver and have an efficient cellular uptake as a result of a specific interaction with the transmembrane transporter. As underlined by Bhattacharya et al., it is difficult to predict the effects of fatty acids on drug binding because both cooperative and competitive interactions are reported [59]. The drug-binding site named Sudlow's site I appears to be a primary site for medium-chain fatty acids, while Sudlow's drug site II is likely to have a high affinity for long-chain fatty acids [59]. Therefore, these two binding sites are not equally affected by plasmatic fatty acids that are essentially long-chain fatty acids. Recent studies show that the binding of fatty acids, the primary physiological ligand for the HSA protein, is shown to alter the polarity and increase the volume of drug site 1 [60]. In fact, upon binding of fatty acids, Y150 from subdomain IB moves to interact with the carboxylate moiety of the lipid bound to the site that straddles domains I and II (fatty acid site FA2) [61]. Interestingly, the synthesis of RSV derivatives such as RSV aliphatic acids can enhance the binding to albumin. For example, RSV hexanoic acid is found to be a much better ligand for BSA and HSA than RSV, and its solubility is improved in water, which consequently enhances its biological effect [62, 63].

3.3 Lipoproteins

Proteins other than albumin may also be implicated in high-affinity fixation for RSV. Indeed, the main function of lipoprotein particles is to transport lipids around the body in the blood, but these proteins have been also proposed as potential carriers for drugs, particularly chemotherapeutic agents. For example, LDLs are used to incorporate anti-

neoplastic lipophilic drugs, and consequently the resultant drug–LDL complex has been shown to have a better uptake of therapeutic drugs [64, 65] and a better cytotoxic effect towards tumour cells via a LDL receptor (LDL-R)-dependent pathway [66]. Indeed, the receptor-mediated assimilation of LDL by many cancer cells is much higher than that of normal cells [67]. This fact suggests that lipoproteins with incorporated xenobiotics or natural compounds such as polyphenols may be used as a carrier to cells and by extension to neoplastic cells.

In general, red wine antioxidants including RSV bind to human LDL and HDL and protect them from metal ion-dependent and -independent oxidation [68]. This protective effect of wine polyphenols might involve RSV, which might exert this effect by removing copper from LDL particles and arterial tissue and, thereby, delaying the consumption of flavonoids and endogenous antioxidants. Similar to other red wine antioxidants, RSV can interact with lipoproteins [69, 70]. In vitro assays show that the concentrations of *trans*-RSV added to plasma increase with the order of their lipid content, i.e. HDL < LDL < VLDL, and that RSV is more associated with lipoproteins than with lipoprotein-free proteins [69], mainly due to its lipophilic character. Burkon and Somoza reported that, in vitro, more than 90% of free *trans*-RSV is bound to human plasma lipoproteins in a non-covalent manner [71]. The RSV association could involve ionic interactions with charged residues on the surface of the particles, and the LDL complex binding to LDL-R could favour RSV uptake by endocytosis via LDL-R. Interestingly, RSV is able to increase the LDL-R in HepG2 cells [72], and primary observation in these cells and other tumour cell lines are in accordance with the involvement of lipoproteins in RSV transport. Moreover, the RSV–LDL binding also occurs in vivo since the presence of dietary polyphenolic compounds is detected in human LDL isolated from blood samples of healthy volunteers [73]. The involvement of LDL has been recently shown in 11 healthy male volunteers (aged 1850), non-smokers who have been consuming 250 mL of red wine [73]. The LDL samples obtained after 24 h reveal three metabolites (*trans*-RSV-3-*O*-glucuronide, *cis*-RSV-3-*O*-glucuronide, *cis*-RSV-3-*O*-glucoside) and free *trans*-RSV [73]. In a similar manner, when the piceid is administrated to healthy volunteers, the metabolites (*trans*-RSV-3-sulphate, *trans*-RSV-3,4′-disulphate, *trans*-RSV-3, 5-disulphate and *trans*-RSV-C/O-diglucuronides) were bound non-covalently to plasma protein [71].

4 Impact of RSV transport on its bioavailability and its biological activities

The different mechanisms of RSV transport (influx, efflux) and RSV binding to various molecules can modify its bioavailability and its biological activity. Pharmacokinetics studies show that oral absorption of RSV in humans is about 75% and is thought to occur mainly by transepithelial diffusion. Extensive metabolism in the intestine and liver results in an oral bioavailability considerably less than 1% [74–77]. These low levels in circulation could be explained by a rapid and extensive phase II metabolism, which generates conjugates and five distinct metabolites that are present in the urine: RSV monosulphate, two isomeric forms of RSV monoglucuronide, dihydroRSV monosulphate and dihydroRSV monoglucuronide [41–44]. The nature and quantity of metabolites may differ among subjects due to inter-individual variability [44, 74, 75]. Once in the bloodstream, metabolites can be subjected to phase II metabolism with further conversions occurring in the liver, where enterohepatic transport in the bile may result in some recycling back to the small intestine [78]. We have already shown in hepatic cells that RSV is highly conjugated after 4 h of incubation into mono- (3-sulphate-RSV and 4′-sulphate-RSV) and disulphate (3,4′-disulphate- RSV and 3,5-disulphate- RSV) derivatives but no glucuronide conjugates could be found [41]. RSV is also able to induce its own metabolism by increasing the activity of hepatic detoxifying enzymes of phase II [41]. Several Phase I/II clinical trials are currently underway for orally administrated RSV in humans, and these studies reveal that after RSV administration daily for 29 days in 40 healthy volunteers, no significant adverse effects were observed, only mild to moderate gastrointestinal symptoms with 2.5 and 5.0 g of RSV [77]. Other reports on trials of RSV in humans after single [44, 74] or multiple daily doses of up to 600 mg/day administered over 2–3 days [79, 80], show that RSV is safe under the tested conditions. In this study, circulating levels of major RSV metabolites, RSV-3-*O*-sulphate, RSV-4′-*O*-glucuronide and RSV-3-*O*-glucuronide, were much higher, particularly in the case of the sulphate. Interestingly, Patel et al. show that patients with confirmed colorectal cancer and who received RSV 0.5 and 1.0 g daily for 8 days before surgery, present high levels of RSV conjugates in the colorectum [76], and can generate levels in the human gastrointestinal tract that are sufficiently high for the polyphenol to exert its antiproliferative properties [76]. Enterohepatic recirculation, which releases the parent drug into the systemic circulation, may be associated with a delayed elimination of the drug from the body and a prolongation of its effect. Considering the concentration of HSA (40 g/L) and Hb (140 g/L) in plasma, both play important roles in drug transport. In general circulation, molecules can bind to plasmatic proteins to form complexes with a reversible connection. This interaction has an important physiological significance, because the concentration of the free form conditions the importance of the effect and the elimination kinetic. We can then put forth the assumption that the complex formed by HSA with RSV could constitute, in vivo, a plasmatic reserve allowing a prolonged release of the molecule towards its cellular targets and an enhancement of its stability (Fig. 2). It may be also hypothesized that RSV–albumin complexes should be retained by albumin membrane receptors and that RSV

should be then delivered to the cell membranes, as described for fatty acid transport. Furthermore, the accumulation of albumin in solid tumours forms the rationale for developing albumin-based drug delivery systems for tumour targeting. Recent studies show antitumour activity of RSV–BSA nanoparticles in different tumour models [81, 82]. Albumin nanoparticles constitute a promising drug carrier system for the transport of drugs and for specific target delivery [83–86]. Other incorporations of RSV into nanoparticles show a higher cell death compared with an equivalent dose of free RSV and an enhancement of RSV stability [87–89]. In these experiments, lipophilic RSV could be incorporated into the hydrophobic core of the nanosphere, while its hydrophilic outer shell still exists as a stabilizer for the system.

LDL can also influence RSV transport and its activity, since recent evidences suggest that LDL-associated photosensitizers are taken up by tumour cells largely via a receptor-mediated endocytotic process. RSV is able to increase LDL-Rs in HepG2 cells [72]. A consequence of RSV and RSV metabolites binding non covalently to plasma protein LDL [71, 73, 90] is a greater accessibility to lipid peroxyl radicals within LDL particles, which would likely exert their peroxyl scavenging activity in the arterial intima, where oxidation of LDL commonly occurs in microdomains sequestered from plasmatic antioxidants [91]. RSV efficiently decreases the accumulation of hydroperoxides in LDL promoted by ferrylmyoglobin, another potent oxidant formed by the reaction of metmyoglobin with hydrogen peroxide, promptly reducing the oxoferryl complex to metmyoglobin [92], and consequently preventing coronary heart diseases. Another possible contributory mechanism toward their antioxidant activities is their ability to stabilize membranes by decreasing membrane fluidity. RSV, through modifications in membrane fluidity, could control protein expression, receptor exposure on the cell surface and could thereby alter functional properties of cells including their susceptibility to apoptosis by clustering of death receptors [16, 93]. Moreover, the events at the surface of plasma membrane seem very important, particularly RSV accumulation into lipid rafts and its colocalization with caveolae, since a disturbance of these microdomains affect RSV-induced MAPK activation and apoptosis. In phamacodelivery, caveolae might constitute a useful transport pathway by improving tissue-directed drug and gene delivery in vivo [94]; this method could improve RSV access to solid tumour cells. Another way to achieve selective targeting to solid tumours is the use of polymeric micelles [95].

Finally, various RSV derivatives have been synthesized to improve their solubility, stability and/or intestinal absorption. For example, the methoxylation of RSV could enhance its activity since methylation increases lipophilicity and may enhance cell membrane permeability [96]. In a similar manner, we have produced a triacetyl-RSV that does not change the target compared with RSV [97, 98] but this esterification could improve RSV's intestinal absorption and cell permeability [99]. In this context, a recent series of patents have covered industrial production and new galenic forms to increase solubility, stability and/or intestinal absorption of RSV in the form of vectors. For example, Longevinex® is a RSV form that is micro-encapsulated in cyclodextrins to preserve it from degradation by light, heat and oxygen and it appeared that this formulation exerts a significant cardioprotection against ischaemia/reperfusion injury in a manner similar to RSV [100].

5 Concluding remarks

There are compelling evidences that RSV can act as a chemopreventive agent or a chemotherapeutic drug against atherosclerosis, neurodegenerative process and various cancers through the regulation of multiple therapeutic targets. The use of this polyphenol requires several important parameters such as pharmacokinetics, metabolism drug uptake, distribution and the identification of targets. This report highlights the mechanisms of RSV uptake, which involve several processes known for drugs transport such as diffusion, carrier-mediated transport, in particular, lipid rafts endocytosis, which seems a determinant process for the biological activities of RSV. This uptake pathway is associated with the involvement of integrin αvβ3 MAPK activation and p53-dependent apoptosis in a variety of cancer cells. However, cells that present less integrin αvβ3 may take up RSV through different routes. In the different uptake processes, various molecules can modify the transport and the bioavailability of the polyphenol. Consequently, if spatial targeting (drug targeting: active and passive targeting) is combined with temporally controlled release (controlled release of a drug such as sustained release, self-regulating release), an improved therapeutic index of RSV could be obtained. Actually, various RSV derivatives and delivery complexes are in the process of development to optimize RSV uptake and its delivery. Nevertheless, correct RSV localization within the membrane, how the biophysical properties of the membrane lipid bilayer are affected by RSV and what the activities/targets of RSV metabolites are remain aspects that need to be clarified and require further investigations.

The research described in this article was supported in part by the ''Conseil Régional de Bourgogne'', the BIVB and the Ligue Contre le Cancer, comité grand-est. The authors appreciate Dr. P. Andreoletti for BSA – resveratrol complex representation.

The authors have declared no conflict of interest.

6 References

[1] Renaud, S. C., Gueguen, R., Schenker, J., d'Houtaud, A., Alcohol and mortality in middle-aged men from eastern France. *Epidemiology* 1998, *9*, 184–188.

[2] Kroon, P. A., Iyer, A., Chunduri, P., Chan, V., Brown, L., The cardiovascular nutrapharmacology of resveratrol: pharmacokinetics, molecular mechanisms and therapeutic potential. *Curr. Med. Chem.* 2010, *17*, 2442–2455.

[3] Shih, A., Zhang, S., Cao, H. J., Boswell, S. et al., Inhibitory effect of epidermal growth factor on resveratrol-induced apoptosis in prostate cancer cells is mediated by protein kinase C-alpha. *Mol. Cancer Ther.* 2004, *3*, 1355–1364.

[4] Lin, H. Y., Shih, A., Davis, F. B., Tang, H. Y. et al., Resveratrol induced serine phosphorylation of p53 causes apoptosis in a mutant p53 prostate cancer cell line. *J. Urol.* 2002, *168*, 748–755.

[5] Colin, D., Lancon, A., Delmas, D., Lizard, G. et al., Antiproliferative activities of resveratrol and related compounds in human hepatocyte derived HepG2 cells are associated with biochemical cell disturbance revealed by fluorescence analyses. *Biochimie* 2008, *90*, 1674–1684.

[6] Lancon, A., Delmas, D., Osman, H., Thenot, J. P. et al., Human hepatic cell uptake of resveratrol: involvement of both passive diffusion and carrier-mediated process. *Biochem. Biophys. Res. Commun.* 2004, *316*, 1132–1137.

[7] Juan, M. E., Gonzalez-Pons, E., Planas, J. M., Multidrug resistance proteins restrain the intestinal absorption of *trans*-resveratrol in rats. *J. Nutr.* 2010, *140*, 489–495.

[8] Grunberger, D., Haimovitz, R., Shinitzky, M., Resolution of plasma membrane lipid fluidity in intact cells labelled with diphenylhexatriene. *Biochim. Biophys. Acta* 1982, *688*, 764–774.

[9] Arora, A., Byrem, T. M., Nair, M. G., Strasburg, G. M., Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. *Arch. Biochem. Biophys.* 2000, *373*, 102–109.

[10] Brittes, J., Lucio, M., Nunes, C., Lima, J. L., Reis, S., Effects of resveratrol on membrane biophysical properties: relevance for its pharmacological effects. *Chem. Phys. Lipids* 2010, *163*, 747–754.

[11] Fotiou, S., Fotiou, D., Alamanou, A., Deliconstantinos, G., Resveratrol activation of nitric oxide synthase in rabbit brain synaptosomes: singlet oxygen (1O2) formation as a causative factor of neurotoxicity. *In Vivo* 2010, *24*, 49–53.

[12] Tsuchiya, H., Nagayama, M., Tanaka, T., Furusawa, M. et al., Membrane-rigidifying effects of anti-cancer dietary factors. *Biofactors* 2002, *16*, 45–56.

[13] Delmas, D., Jannin, B., Malki, M. C., Latruffe, N., Inhibitory effect of resveratrol on the proliferation of human and rat hepatic derived cell lines. *Oncol. Rep.* 2000, *7*, 847–852.

[14] Gurtovenko, A. A., Anwar, J., Interaction of ethanol with biological membranes: the formation of non-bilayer structures within the membrane interior and their significance. *J. Phys. Chem. B* 2009, *113*, 1983–1992.

[15] Chin, J. H., Goldstein, D. B., Effects of low concentrations of ethanol on the fluidity of spin-labeled erythrocyte and brain membranes. *Mol. Pharmacol.* 1977, *13*, 435–441.

[16] Delmas, D., Rebe, C., Micheau, O., Athias, A. et al., Redistribution of CD95, DR4 and DR5 in rafts accounts for the synergistic toxicity of resveratrol and death receptor ligands in colon carcinoma cells. *Oncogene* 2004, *23*, 8979–8986.

[17] Tartakoff, A. M., Perturbation of vesicular traffic with the carboxylic ionophore monensin. *Cell* 1983, *32*, 1026–1028.

[18] Koivusalo, M., Welch, C., Hayashi, H., Scott, C. C. et al., Amiloride inhibits macropinocytosis by lowering submembranous pH and preventing Rac1 and Cdc42 signaling. *J. Cell. Biol. 188*, 547–563.

[19] Ikonen, E., Roles of lipid rafts in membrane transport. *Curr. Opin. Cell Biol.* 2001, *13*, 470–477.

[20] Henley, J. R., Krueger, E. W., Oswald, B. J., McNiven, M. A., Dynamin-mediated internalization of caveolae. *J. Cell Biol.* 1998, *141*, 85–99.

[21] Nichols, B. J., A distinct class of endosome mediates clathrin-independent endocytosis to the Golgi complex. *Nat. Cell Biol.* 2002, *4*, 374–378.

[22] Lamaze, C., Dujeancourt, A., Baba, T., Lo, C. G. et al., Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway. *Mol. Cell* 2001, *7*, 661–671.

[23] van der Luit, A. H., Budde, M., Ruurs, P., Verheij, M., van Blitterswijk, W. J., Alkyl-lysophospholipid accumulates in lipid rafts and induces apoptosis via raft-dependent endocytosis and inhibition of phosphatidylcholine synthesis. *J. Biol. Chem.* 2002, *277*, 39541–39547.

[24] Yang, H. L., Chen, W. Q., Cao, X., Worschech, A. et al., Caveolin-1 enhances resveratrol-mediated cytotoxicity and transport in a hepatocellular carcinoma model. *J. Transl. Med.* 2009, *7*, 22.

[25] Lajoie, P., Nabi, I. R., Lipid rafts, caveolae, and their endocytosis. *Int. Rev. Cell Mol. Biol.* 2010, *282*, 135–163.

[26] Plow, E. F., Haas, T. A., Zhang, L., Loftus, J., Smith, J. W., Ligand binding to integrins. *J. Biol. Chem.* 2000, *275*, 21785–21788.

[27] Calderwood, D. A., Shattil, S. J., Ginsberg, M. H., Integrins and actin filaments: reciprocal regulation of cell adhesion and signaling. *J. Biol. Chem.* 2000, *275*, 22607–22610.

[28] Bergh, J. J., Lin, H. Y., Lansing, L., Mohamed, S. N. et al., Integrin alphaVbeta3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology* 2005, *146*, 2864–2871.

[29] Lin, H. Y., Sun, M., Lin, C., Tang, H. Y. et al., Androgen-induced human breast cancer cell proliferation is mediated by discrete mechanisms in estrogen receptor-alpha-positive and -negative breast cancer cells. *J. Steroid Biochem. Mol. Biol.* 2009, *113*, 182–188.

[30] Lin, H. Y., Lansing, L., Merillon, J. M., Davis, F. B. et al., Integrin alphaVbeta3 contains a receptor site for resveratrol. *FASEB J.* 2006, *20*, 1742–1744.

[31] Lin, H. Y., Tang, H. Y., Keating, T., Wu, Y. H. et al., Resveratrol is pro-apoptotic and thyroid hormone is anti-apoptotic in glioma cells: both actions are integrin and ERK mediated. *Carcinogenesis* 2008, *29*, 62–69.

[32] Lin, H. Y., Sun, M., Tang, H. Y., Simone, T. M. et al., Resveratrol causes COX-2- and p53-dependent apoptosis

in head and neck squamous cell cancer cells. *J. Cell Biochem.* 2008, *104*, 2131–2142.

[33] Hsieh, T. C., Wong, C., Bennett, D. J., Wu, J. M., Regulation of p53 and cell proliferation by resveratrol and its derivatives in breast cancer cells: An in silico and biochemical approach targeting integrin alphavbeta3. *Int. J. Cancer.* 2011, in press, DOI:10.1002/ijc.25930.

[34] Stivala, L. A., Savio, M., Carafoli, F., Perucca, P. et al., Specific structural determinants are responsible for the antioxidant activity and the cell cycle effects of resveratrol. *J. Biol. Chem.* 2001, *276*, 22586–22594.

[35] Casper, R. F., Quesne, M., Rogers, I. M., Shirota, T. et al., Resveratrol has antagonist activity on the aryl hydrocarbon receptor: implications for prevention of dioxin toxicity. *Mol. Pharmacol.* 1999, *56*, 784–790.

[36] Bowers, J. L., Tyulmenkov, V. V., Jernigan, S. C., Klinge, C. M., Resveratrol acts as a mixed agonist/antagonist for estrogen receptors alpha and beta. *Endocrinology* 2000, *141*, 3657–3667.

[37] Abou-Zeid, L. A., El-Mowafy, A. M., Differential recognition of resveratrol isomers by the human estrogen receptor-alpha: molecular dynamics evidence for stereoselective ligand binding. *Chirality* 2004, *16*, 190–195.

[38] Barros, R. P., Machado, U. F., Warner, M., Gustafsson, J. A., Muscle GLUT4 regulation by estrogen receptors ERbeta and ERalpha. *Proc. Natl. Acad. Sci. USA* 2006, *103*, 1605–1608.

[39] Klinge, C. M., Wickramasinghe, N. S., Ivanova, M. M., Dougherty, S. M., Resveratrol stimulates nitric oxide production by increasing estrogen receptor {alpha}-Src-caveolin-1 interaction and phosphorylation in human umbilical vein endothelial cells. *FASEB J.* 2008, *22*, 2185–2197.

[40] Higgins, C. F., ABC transporters: from microorganisms to man. *Annu. Rev. Cell Biol.* 1992, *8*, 67–113.

[41] Lancon, A., Hanet, N., Jannin, B., Delmas, D. et al., Resveratrol in human hepatoma HepG2 cells: metabolism and inducibility of detoxifying enzymes. *Drug Metab. Dispos.* 2007, *35*, 699–703.

[42] Wenzel, E., Soldo, T., Erbersdobler, H., Somoza, V., Bioactivity and metabolism of trans-resveratrol orally administered to Wistar rats. *Mol. Nutr. Food Res.* 2005, *49*, 482–494.

[43] Wenzel, E., Somoza, V., Metabolism and bioavailability of trans-resveratrol. *Mol. Nutr. Food Res.* 2005, *49*, 472–481.

[44] Boocock, D. J., Faust, G. E., Patel, K. R., Schinas, A. M. et al., Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiol. Biomarkers Prev.* 2007, *16*, 1246–1252.

[45] Vaidyanathan, J. B., Walle, T., Transport and metabolism of the tea flavonoid (−)-epicatechin by the human intestinal cell line Caco-2. *Pharm. Res.* 2001, *18*, 1420–1425.

[46] Kaldas, M. I., Walle, U. K., Walle, T., Resveratrol transport and metabolism by human intestinal Caco-2 cells. *J. Pharm. Pharmacol.* 2003, *55*, 307–312.

[47] Henry, C., Vitrac, X., Decendit, A., Ennamany, R. et al., Cellular uptake and efflux of trans-piceid and its aglycone trans-resveratrol on the apical membrane of human intestinal Caco-2 cells. *J. Agric. Food Chem.* 2005, *53*, 798–803.

[48] Maier-Salamon, A., Hagenauer, B., Reznicek, G., Szekeres, T. et al., Metabolism and disposition of resveratrol in the isolated perfused rat liver: role of Mrp2 in the biliary excretion of glucuronides. *J. Pharm. Sci.* 2008, *97*, 1615–1628.

[49] Maier-Salamon, A., Hagenauer, B., Wirth, M., Gabor, F. et al., Increased transport of resveratrol across monolayers of the human intestinal Caco-2 cells is mediated by inhibition and saturation of metabolites. *Pharm. Res.* 2006, *23*, 2107–2115.

[50] van de Wetering, K., Burkon, A., Feddema, W., Bot, A. et al., Intestinal breast cancer resistance protein (BCRP)/Bcrp1 and multidrug resistance protein 3 (MRP3)/Mrp3 are involved in the pharmacokinetics of resveratrol. *Mol. Pharmacol.* 2009, *75*, 876–885.

[51] Alfaras, I., Perez, M., Juan, M. E., Merino, G. et al., Involvement of breast cancer resistance protein (BCRP1/ABCG2) in the bioavailability and tissue distribution of trans-resveratrol in knockout mice. *J. Agric. Food Chem.* 2010, *58*, 4523–4528.

[52] Khan, M. A., Muzammil, S., Musarrat, J., Differential binding of tetracyclines with serum albumin and induced structural alterations in drug-bound protein. *Int. J. Biol. Macromol.* 2002, *30*, 243–249.

[53] Bourassa, P., Kanakis, C. D., Tarantilis, P., Pollissiou, M. G., Tajmir-Riahi, H. A., Resveratrol, genistein, and curcumin bind bovine serum albumin. *J. Phys. Chem. B* 2010, *114*, 3348–3354.

[54] Jannin, B., Menzel, M., Berlot, J. P., Delmas, D. et al., Transport of resveratrol, a cancer chemopreventive agent, to cellular targets: plasmatic protein binding and cell uptake. *Biochem. Pharmacol.* 2004, *68*, 1113–1118.

[55] Xiao, J. B., Chen, X. Q., Jiang, X. Y., Hilczer, M., Tachiya, M., Probing the interaction of trans-resveratrol with bovine serum albumin: a fluorescence quenching study with Tachiya model. *J. Fluoresc.* 2008, *18*, 671–678.

[56] Yang, F., Bian, C., Zhu, L., Zhao, G. et al., Effect of human serum albumin on drug metabolism: structural evidence of esterase activity of human serum albumin. *J. Struct. Biol.* 2007, *157*, 348–355.

[57] Lu, Z., Zhang, Y., Liu, H., Yuan, J. et al., Transport of a cancer chemopreventive polyphenol, resveratrol: interaction with serum albumin and hemoglobin. *J. Fluoresc.* 2007, *17*, 580–587.

[58] N'soukpoe-Kossi, C. N., St-Louis, C., Beauregard, M., Subirade, M. et al., Resveratrol binding to human serum albumin. *J. Biomol. Struct. Dyn.* 2006, *24*, 277–283.

[59] Bhattacharya, A. A., Grune, T., Curry, S., Crystallographic analysis reveals common modes of binding of medium and long-chain fatty acids to human serum albumin. *J. Mol. Biol.* 2000, *303*, 721–732.

[60] Ghuman, J., Zunszain, P. A., Petitpas, I., Bhattacharya, A. A. et al., Structural basis of the drug-binding specificity of human serum albumin. *J. Mol. Biol.* 2005, *353*, 38–52.

[61] Curry, S., Mandelkow, H., Brick, P., Franks, N., Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. *Nat. Struct. Biol.* 1998, *5*, 827–835.

[62] Chen, L., Zhang, Y., Sun, X., Li, H. et al., Synthetic resveratrol aliphatic acid inhibits TLR2-mediated apoptosis and an involvement of Akt/GSK3beta pathway. *Bioorg. Med. Chem.* 2009, *17*, 4378–4382.

[63] Jiang, Y. L., Design, synthesis and spectroscopic studies of resveratrol aliphatic acid ligands of human serum albumin. *Bioorg. Med. Chem.* 2008, *16*, 6406–6414.

[64] Reddi, E., Role of delivery vehicles for photosensitizers in the photodynamic therapy of tumours. *J. Photochem. Photobiol. B* 1997, *37*, 189–195.

[65] Jori, G., Reddi, E., The role of lipoproteins in the delivery of tumour-targeting photosensitizers. *Int. J. Biochem.* 1993, *25*, 1369–1375.

[66] Vitols, S., Uptake of low-density lipoprotein by malignant cells–possible therapeutic applications. *Cancer Cells* 1991, *3*, 488–495.

[67] Vitols, S., Gahrton, G., Ost, A., Peterson, C., Elevated low density lipoprotein receptor activity in leukemic cells with monocytic differentiation. *Blood* 1984, *63*, 1186–1193.

[68] Ivanov, V., Carr, A. C., Frei, B., Red wine antioxidants bind to human lipoproteins and protect them from metal ion-dependent and -independent oxidation. *J. Agric. Food Chem.* 2001, *49*, 4442–4449.

[69] Belguendouz, L., Fremont, L., Gozzelino, M. T., Interaction of transresveratrol with plasma lipoproteins. *Biochem. Pharmacol.* 1998, *55*, 811–816.

[70] Blache, D., Rustan, I., Durand, P., Lesgards, G., Loreau, N., Gas chromatographic analysis of resveratrol in plasma, lipoproteins and cells after in vitro incubations. *J. Chromatogr. B Biomed. Sci. Appl.* 1997, *702*, 103–110.

[71] Burkon, A., Somoza, V., Quantification of free and protein-bound trans-resveratrol metabolites and identification of trans-resveratrol-C/O-conjugated diglucuronides - two novel resveratrol metabolites in human plasma. *Mol. Nutr. Food Res.* 2008, *52*, 549–557.

[72] Pal, S., Ho, N., Santos, C., Dubois, P. et al., Red wine polyphenolics increase LDL receptor expression and activity and suppress the secretion of ApoB100 from human HepG2 cells. *J. Nutr.* 2003, *133*, 700–706.

[73] Urpi-Sarda, M., Zamora-Ros, R., Lamuela-Raventos, R., Cherubini, A. et al., HPLC-tandem mass spectrometric method to characterize resveratrol metabolism in humans. *Clin. Chem.* 2007, *53*, 292–299.

[74] Walle, T., Hsieh, F., DeLegge, M. H., Oatis, J. E., Jr., Walle, U. K., High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab. Dispos.* 2004, *32*, 1377–1382.

[75] Cottart, C. H., Nivet-Antoine, V., Laguillier-Morizot, C., Beaudeux, J. L., Resveratrol bioavailability and toxicity in humans. *Mol. Nutr. Food Res.* 2010, *54*, 7–16.

[76] Patel, K. R., Brown, V. A., Jones, D. J., Britton, R. G. et al., Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. *Cancer Res.* 2010, *70*, 7392–7399.

[77] Brown, V. A., Patel, K. R., Viskaduraki, M., Crowell, J. A. et al., Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer Res.* 2010, *70*, 9003–9011.

[78] Crozier, A., Jaganath, I. B., Clifford, M. N., Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.* 2009, *26*, 1001–1043.

[79] Almeida, L., Vaz-da-Silva, M., Falcao, A., Soares, E. et al., Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. *Mol. Nutr. Food Res.* 2009, *53*, S7–15.

[80] Nunes, T., Almeida, L., Rocha, J. F., Falcao, A. et al., Pharmacokinetics of trans-resveratrol following repeated administration in healthy elderly and young subjects. *J. Clin. Pharmacol.* 2009, *49*, 1477–1482.

[81] Guo, L., Peng, Y., Yao, J., Sui, L. et al., Anticancer activity and molecular mechanism of resveratrol-bovine serum albumin nanoparticles on subcutaneously implanted human primary ovarian carcinoma cells in nude mice. *Cancer Biother. Radiopharm.* 2010, *25*, 471–477.

[82] Guo, L. Y., Peng, Y., Li, Y. L., Yao, J. P. et al., [Mechanisms of resveratrol bovine serum albumin nanoparticle-induced cell death in human ovarian cancer SKOV3 cells.]. *Nan Fang Yi Ke Da Xue Xue Bao.* 2010, *30*, 2440–2442.

[83] Conlin, A. K., Seidman, A. D., Bach, A., Lake, D. et al., Phase II trial of weekly nanoparticle albumin-bound paclitaxel with carboplatin and trastuzumab as first-line therapy for women with HER2-overexpressing metastatic breast cancer. *Clin. Breast Cancer* 2010, *10*, 281–287.

[84] Kufleitner, J., Worek, F., Kreuter, J., Incorporation of obidoxime into human serum albumin nanoparticles: optimisation of preparation parameters for the development of a stable formulation. *J. Microencapsul.* 2010, *27*, 594–601.

[85] Petrelli, F., Borgonovo, K., Barni, S., Targeted delivery for breast cancer therapy: the history of nanoparticle-albumin-bound paclitaxel. *Exp. Opin. Pharmacother.* 2010, *11*, 1413–1432.

[86] Kratz, F., Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. *J. Control Release* 2008, *132*, 171–183.

[87] Shao, J., Li, X., Lu, X., Jiang, C. et al., Enhanced growth inhibition effect of resveratrol incorporated into biodegradable nanoparticles against glioma cells is mediated by the induction of intracellular reactive oxygen species levels. *Colloids Surf. B Biointerface.* 2010, *72*, 40–47.

[88] Teskac, K., Kristl, J., The evidence for solid lipid nanoparticles mediated cell uptake of resveratrol. *Int. J. Pharm.* 2009, *390*, 61–69.

[89] Lu, X., Ji, C., Xu, H., Li, X. et al., Resveratrol-loaded polymeric micelles protect cells from Abeta-induced oxidative stress. *Int. J. Pharm.* 2009, *375*, 89–96.

[90] Urpi-Sarda, M., Jauregui, O., Lamuela-Raventos, R. M., Jaeger, W. et al., Uptake of diet resveratrol into the human low-density lipoprotein. Identification and quantification of resveratrol metabolites by liquid chromatography coupled with tandem mass spectrometry. *Anal. Chem.* 2005, *77*, 3149–3155.

[91] Witztum, J. L., The oxidation hypothesis of atherosclerosis. *Lancet* 1994, *344*, 793–795.

[92] Brito, P., Almeida, L. M., Dinis, T. C., The interaction of resveratrol with ferrylmyoglobin and peroxynitrite; protection against LDL oxidation. *Free Radic. Res.* 2002, *36*, 621–631.

[93] Reis-Sobreiro, M., Gajate, C., Mollinedo, F., Involvement of mitochondria and recruitment of Fas/CD95 signaling in lipid rafts in resveratrol-mediated antimyeloma and antileukemia actions. *Oncogene* 2009, *28*, 3221–3234.

[94] Schnitzer, J. E., Caveolae: from basic trafficking mechanisms to targeting transcytosis for tissue-specific drug and gene delivery in vivo. *Adv. Drug Deliv. Rev.* 2001, *49*, 265–280.

[95] Kristl, J., Teskac, K., Caddeo, C., Abramovic, Z., Sentjurc, M., Improvements of cellular stress response on resveratrol in liposomes. *Eur. J. Pharm. Biopharm.* 2009, *73*, 253–259.

[96] Sarpietro, M. G., Ottimo, S., Giuffrida, M. C., Spatafora, C. et al., beta-Cyclodextrins influence on E-3,5,4′-trimethoxystilbene absorption across biological membrane model: a differential scanning calorimetry evidence. *Int. J. Pharm.* 2010, *388*, 144–150.

[97] Colin, D., Gimazane, A., Lizard, G., Izard, J. C. et al., Effects of resveratrol analogs on cell cycle progression, cell cycle associated proteins and 5fluoro-uracil sensitivity in human derived colon cancer cells. *Int. J. Cancer.* 2009, *124*, 2780–2788.

[98] Marel, A. K., Lizard, G., Izard, J. C., Latruffe, N., Delmas, D., Inhibitory effects of trans-resveratrol analogs molecules on the proliferation and the cell cycle progression of human colon tumoral cells. *Mol. Nutr. Food Res.* 2008, *52*, 538–548.

[99] Riva, S., Monti, D., Luisetti, M., Danieli, B., Enzymatic modification of natural compounds with pharmacological properties. *Ann. N. Y. Acad. Sci.* 1998, *864*, 70–80.

[100] Mukhopadhyay, P., Mukherjee, S., Ahsan, K., Bagchi, A. et al., Restoration of altered microRNA expression in the ischemic heart with resveratrol. *PLoS One* 2011, *5*, e15705.

