Expert Opinion

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New delivery systems to improve the bioavailability of resveratrol

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Introduction: Resveratrol (RSV) has been one of the most extensively studied polyphenols in the last 10 years, owing to its numerous and potent therapeutic activities, namely its high antioxidant properties. However, RSV's bioavailability is compromised by its physicochemical properties, such as low stability, increased oxidation on heat and light exposure, low water solubility and also its high hepatic uptake. Moreover, results obtained in human pharmacokinetic studies have shown a low amount of intact RSV in the systemic circulation, which does not justify its therapeutic activities, raising doubts about RSV's potential. RSV is already available as a nutritional supplement, although its translation to the clinic is not straightforward, owing to the lack of clinical data.

Areas covered: In this review, formulations that are being used for delivery of RSV are discussed. New delivery systems are presented as valid alternatives to circumvent the limitations of the physicochemical characteristics and pharmacokinetics of RSV. In this way, they are compared with classical formulations with regard to improving RSV protection and bioavailability.

Expert opinion: Despite promising results in preclinical settings, the applicability of RSV to humans has met with only limited success, largely owing to its inefficient systemic delivery and consequently its low bioavailability. To achieve an optimal response of RSV, new strategies are still required to enhance its bioavailability and reduce its perceived toxicity.

Keywords: bioavailability, new delivery systems, pharmacokinetics, resveratrol, stability

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1. Introduction

Phenolic compounds are a large group of substances that derive from the shikimic acid or acetic metabolic pathways, belonging to secondary metabolism of plants. These polyphenols, known to grant sensorial properties to plants (ton color, astringency and some fruits' flavor), have the quality of phytoalexins, compounds that are synthesized by plants under environmental stress, such as injury, microbial (fungal), infection or UV-irradiation conditions, which are responsible for growth, reproduction and confer disease resistance [1]. Their chemical constitution consists of at least one aromatic ring substituted with at least one hydroxyl group, ranging from simple structures, such as phenolic acids, to complex ones, such as tannins. Within the group lie stilbenes, and more specifically resveratrol (RSV), a triphenol non-flavonoid and atoxic phytoestrogen [1]. RSV is produced in higher plants, through the stilbene synthase [2], which is present in at least 70 vegetal species [3] and has been identified as the principal active compound of stilbene phytoalexins [4].

Like other stilbenes, RSV (3,4′,5-trihydroxy-*trans*-stilbene) is presented in nature by two isomeric forms: *trans*-RSV (*t*-RSV, **Figure 1A**) and *cis*-RSV (*c*-RSV, **Figure 1B**). In plants a major form of RSV is the glucosidic form called piceid (RSV-3-*O*-β-D-glucoside) or polydatin (PCD, **Figure 1C**) in the isomeric form *trans*-piceid [5,6]. Piceid can exist as *cis*-piceid [7].

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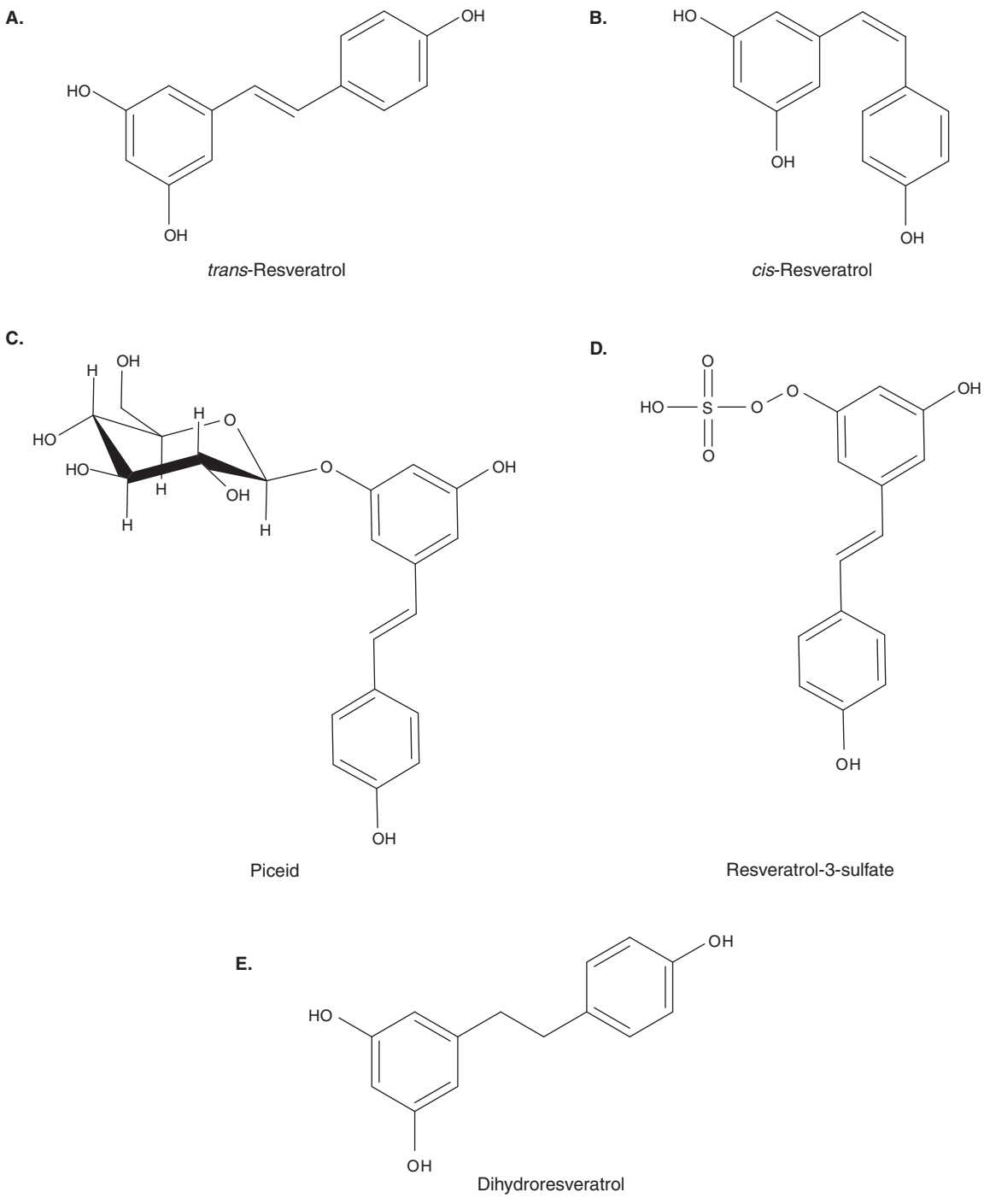

Figure 1. **A.** *trans*-Resveratrol. **B.** *cis*-Resveratrol. **C.** Piceid. **D.** Resveratrol-3-sulfate. **E.** Dihydroresveratrol.

RSV in solution shows photosensitivity (*trans*- to *cis*-isomerization is facilitated by UV exposure) and pH-sensitivity, being easily oxidizable [1,2]. When protected from light, *t*-RSV is stable for months, except in high-pH buffers, and *c*-RSV is stable only near neutral pH. When RSV and RSV glucosides are in solution, the primary degradant is the *cis*-isomer [5]. Besides this conclusion, it has been considered that *t*-RSV is not particularly sensitive to UV/fluorescent light, elevated humidity and temperature, or atmospheric oxidants at ambient concentrations [5]. Like RSV, PCD is a stable solid [5]. On the contrary, Shi *et al.*'s results [8] claim that solid RSV is unstable when exposed to light and elevated humidity.

RSV's chemical structure, related to the synthetic lipophilic estrogen diethylstilbestrol [1], enables it to interact with

receptors and enzymes, giving rise to important biological effects (see **Table 1**) [2,9], namely prevention of many disease states that are a consequence of cell damage or death triggered by severe oxidative stress (Alzheimer's [10] and Parkinson's diseases [11], cancer [12,13], cardiovascular disease [14], multiple sclerosis [15], colitis [16], severe acute pancreatitis [17] and diabetic neuropathy [18]), which makes it an important target of interest. Concerning mode of action, RSV reveals dual actions – cell protection or cell apoptosis – which might depend on cell type, concentration, cytosolic redox status and duration of contact [19]. More precisely, it shows antioxidant activity in low oral doses (10 μM), and cytotoxic action, when administrated at higher concentrations (100 μM), is seen as changes in cell shape, detachment and apoptotic features [3,20]. After comparing some of the stilbenoids, it has been concluded that the presence of different functional groups may result in derivates with different antioxidative properties, targeting mainly extracellular radical oxygen species (ROS) [21].

It is not yet clear whether RSV itself or derivates or metabolites have therapeutic *in vivo* effects. Recognition of the role of RSV's active metabolites will make possible correct interpretation of the pharmacodynamic data observed in preclinical studies and extrapolation of the data to humans. Besides, the existence of RSV metabolites and quantification of their level of activity will allow a better delivery system for RSV to be designed.

There are still questions about safety, dosing and clinical efficacy of RSV. The design of delivery systems requires a thorough understanding of the physicochemical principles of RSV, namely its low water solubility and high instability in solution, which may affect laboratory assays and formulations containing dissolved RSV. With the aim of contributing to developing a delivery system for RSV, the pharmacokinetics of this compound as well as the experimental conditions used during formulation and assays are discussed. Furthermore, new drug delivery systems – such as cyclodextrins (CDs), nanoparticles (NPs) and liposomes – are described and compared with classic formulations of RSV, focusing on their advantages in overcoming RSV limitations, such as solubility and stability, by also increasing potential for targeting and sustained delivery. A better understanding of the strength of the evidence on delivery systems' effectiveness may contribute to increasing RSV's bioavailability.

2. Pharmacokinetics of resveratrol

After oral administration, once in the small intestine RSV and PCD are directly absorbed in the intestinal lumen, as can be seen in **Figure 2**, which is an adaptation from Ros [22]. According to studies with Caco-2 cells, the velocity of PCD absorption is four times lower than RSV's [23]; however, PCD can be hydrolyzed before its absorption by lactase phlorizin hydrolase (LPH), a β-glucosidase present in the intestinal lumen. *t*-RSV crosses the intestinal epithelium by rapid transepithelial passive diffusion [24,25], whereas PCD uses the sodium-dependent active transporter SGLT1 (**Figure 2**) [23]. RSV and PCD are subject to a presystemic metabolism through first-pass glucuronidation and sulfate conjugation (Phase II conjugation reactions). This metabolization leads to various metabolites, fundamentally glucuronides and sulfates, described as G and S compounds, respectively, in **Figure 2**. This metabolism has been described as the main cause of the trace amounts of RSV found in the systemic circulation [26] and consequently its low oral bioavailability [24,25,27]. Once absorbed, PCD in the enterocytes' cytoplasm may be hydrolyzed into RSV by the local cytosolic-β-glucosidase (cβG, **Figure 2**) [28].

The remaining RSV and derivates are transported via port towards the liver and, in hepatic microsomes, RSV is once more promptly metabolized in glucuronides and sulfates. Some results indicate that glucuronidation is preferred for the two RSV isomers, occurring in only small amounts of RSV sulfates. Other authors maintain that RSV is a better substrate for sulfotransferases (ST, **Figure 2**) [29,30]. Sulfation of RSV in human liver cytosol in the presence of 3′-phosphoadenosine-5′-phosphosulfate reveals that RSV is converted into *t*-RSV-3-*O*-sulfate (**Figure 1D**), *t*-RSV-4′-*O*-sulfate and *t*-RSV-3-*O*-4′-*O*-disulfate metabolites, suggesting that these are possible sulfated metabolites, derived from the liver's *in vivo* metabolism [30].

After hepatic metabolization, liver is exposed again to RSV during enterohepatic recirculation, in which RSV conjugates are excreted in bile and reabsorbed in its aglycone and/or conjugated forms, after undergoing enzymatic cleavage by the β-glucuronidase enzyme in the small intestine [29,31-32]. The non-absorbed RSV in the small intestine addresses the colon. RSV can pass the gut without metabolic conversion, yet it can be metabolized (reduction reactions) by bacterial enzymes of the intestinal microflora (β-glucuronidases) through hydrogenation of the aliphatic double bound, resulting in dihydroresveratrol (dihRSV, **Figure 1E**) [25]. DihRSV is excreted in both the glucuronide and sulfate forms [29], may be absorbed, and also then be combined in their glucuronide and sulfate conjugates, which agrees with their identification in plasma [33] and in urine [25]. Monitoring of RSV and its metabolites in human feces indicates the existence of RSV sulfates [25] in a small quantity (< 1%) [32].

After the liver stage, the remaining RSV and its conjugates are absorbed to the systemic circulation, where they can be transported along with the blood cells (red blood cells and platelets) and lipoproteins (low-density lipoprotein [LDL] *in vitro* and in humans) [34], but most travel attached to plasma proteins [7]. Pharmacokinetics of RSV has been studied mainly in animal models; however, recent studies have been performed in humans. The results obtained are summarized in **Table 2**. Formulations used as well their total content of RSV are discriminated. Plasma concentration of RSV represents 1 – 15% [35] and, in some cases, it was not detectable or quantifiable. Even when a very large dose of RSV (2.5 and 5.0 g) was given, its blood concentration failed

Table 1. Biological activities of resveratrol and respective physiological mechanisms.

Biological activities	Mechanism(s)
Antioxidant	ROS scavenger, influencing the cell redox-signaling pathways [1,6]
	Trigger apoptosis [2,24]
	Prevent apoptosis [24]
	Modulation of NO production [1] and cerebral blood flow variables [85]
	Inhibition of membrane lipid peroxidation [1]
Anti-inflammatory	Downregulation of pro-inflammatory mediators: inhibition of COX-1 [6], COX-2 [2,6], hydroxyperoxidase activities [31], MAP kinases [86], lipoxygenases [24] and iNOS [86]
Neuroprotection	Inhibition of β-amyloid polymerization [46]
	Increase of heme oxygenase-1 activity [1]
	Increase of glutamate uptake, glutathione content and trophic factor S100B secretion [87]
Cardiovascular protection	Modulation of lipoprotein metabolism (increase of HDL proteins and decrease of LDL and VLDL) [2,31]
	Inhibition of platelet aggregation [2,6,20,24,31]
	Regulation of vascular smooth muscle proliferation [86]
	Inhibition of TNF-α [88]
	Increase of endothelial mitochondrial biogenesis [89]
	Activation of Nrf2-driven antioxidant response [90]
Anticancer	Modulation of biological pathways involved in differentiation, transformation, cell cycle regulation and cell death induction [2]
	Cancer prevention at the initiation stage [2,25]
	Suppression of growth of pre-neoplasic lesions [2,25]
	Pro-antioxidant action (disruption of intracellular redox balance which leads to apoptosis) [1,3,31]
	Inhibition of cell proliferation (by blocking cell cycle progression in human cancer cell lines) [2,25]
	Induction of Phase II enzymes, such as quinone reductase [6]
	Agonist for the estrogen receptors [3,6,24]

HDL: High-density lipoprotein; LDL: Low-density lipoprotein; Nrf2: Nuclear factor-E(2)-related factor-2; ROS: Radical oxygen species; VLDL: Very low-density lipoprotein.

to reach the necessary level for systemic cancer prevention [32]. However, when given in a proprietary formulation (3.0 or 5.0 g) developed by Sirtris Pharmaceuticals (Cambridge, MA, USA), RSV reached 5 – 8 times higher blood levels, which approached the necessary concentration to exert effects *in vitro* and in animal experiments [36].

The most abundant metabolite in the systemic circulation was *t*-RSV-3,5-disulfate [7], followed by *t*-RSV-3-sulfate, which has been described previously by Boocock *et al.* [32] as the most abundant metabolite. However, Urpí-Sardà and co-workers [34,35] and Vitaglione *et al.* [37] have identified two glucuronides, namely *t*-RSV-3-*O*-glucuronide and RSV-4′-*O*-glucuronide, as the most abundant forms, yet in lower percentages than the disulfate compound. Following the addition of two new plasma metabolites of RSV, *t*-RSV-*C*/*O*-diglucuronide (18%) and *t*-RSV-3,4′-disulfate (11%), one RSV disulfate in plasma was identified, but it has not been quantified, leading to the assumption that it probably coincides with the previous one [38].

RSV glucosides (*t*-RSV-4-*O*-glucoside and *c*-RSV-3-*O*-glucoside) were detected in human LDL samples after oral intake of red wine. These RSV glucosides are absorbed from the intestine, possibly by the same glucose transport system of phenolic compounds in rats. Once in human liver microsomes, glucosidation may serve as an alternative detoxification pathway, as aglycones can preferentially undergo glucosidation [34].

RSV was identified in several organs, mostly in the small intestine, colon, kidney, liver, lungs and brain [22]. Most routes of excretion are via urine and feces; however, the percentage of excreted compounds varies according to experimental conditions used in the study. As can be seen in **Table 2**, most of the orally administered RSV is recovered in urine in highly variable amounts [25]. After oral administration of formulations containing an amount of RSV varying from 0.5 to 5.0 g, 1.0, 5.4 and 25 mg of RSV, the results of RSV quantification in urine revealed trace amounts, 17%, and 26 – 52% compared with initial RSV. Contrary to reports in animals [39], human studies reveal that sulfated forms are more abundant than glucuronidated ones and RSV is present in a much smaller proportion. The most abundant RSV derivate in urine is *c*-RSV-4′-sulfate (84%), followed by *c*-RSV-3-*O*-glucuronide (8%) and other glucuronides and sulfates [35]. According to Burkon and Somoza's studies [7], the most abundant metabolite is *t*-RSV-3,5-disulfate, followed by *t*-RSV-3-sulfate, where *t*-RSV-*C*/*O*-diglucuronide and *t*-RSV-3,4′-disulfate were also posted. In Boocock *et al.*'s [32] studies, RSV-3-sulfate was the predominant metabolite. RSV was detected in varying amounts in urine, from only trace amounts [32,38] up to 17% [34] and 53 – 85% [25].

To the best of the authors' knowledge, several RSV derivates were reported but total recovery in urine was ~ 1 – 52%, which is very different from the 53 – 85% reported by Walle *et al.* [25]. In this case, the identity of the radioactive

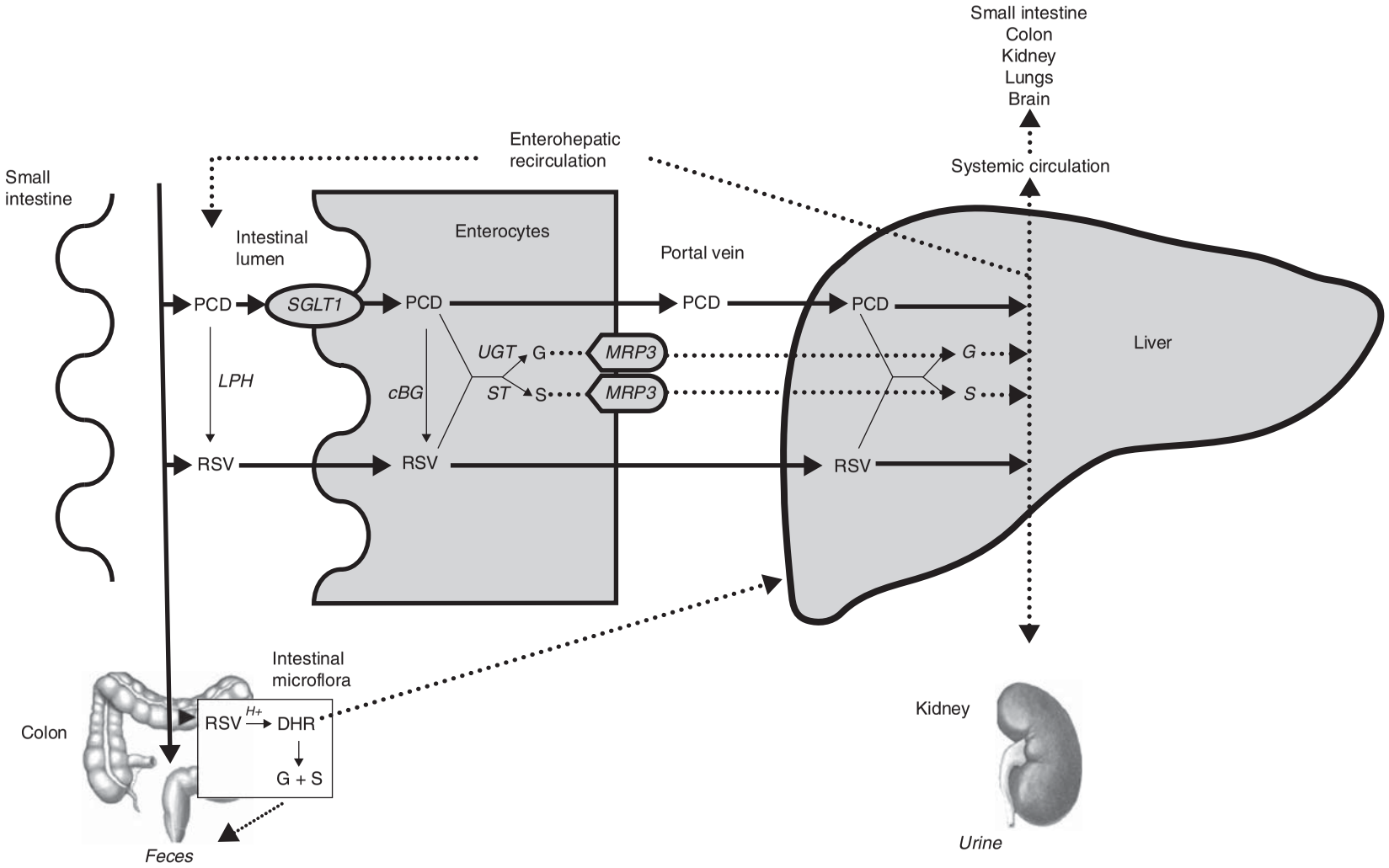

Figure 2. Oral pharmacokinetics of RSV, piceid (unbroken arrows) and their derivates (dotted arrows). In the intestinal lumen, PCD can be hydrolyzed to RSV, which is directly absorbed by diffusion, whereas PCD uses a specific transporter. In the enterocytes' cytoplasm, PCD can be again hydrolyzed to RSV. RSV and PCD are metabolized in G (derivated glucuronides) or S (derivated sulfates). G and S derivates cross enterocytes towards the liver through specific transport. Once in the liver, RSV and PCD can once more be metabolized into their G and S derivates, which can undergo enterohepatic recirculation and be reabsorbed in the small intestine. In the liver, compounds are delivered to the systemic circulation and distributed to organs. They can also be directly excreted in urine instead. In the intestinal tract, the non-absorbed RSV can reach the colon, where it is metabolized in DHR. DHR can be absorbed or excreted through G and S derivates in feces [23,60].

Adapted from [22].

cβG: Cytosolic-β-glucuronidase; DHR: Dihydroresveratrol; LPH: Lactase phlorizin hydrolase; MRP3: Multi-drug resistance-associated protein 3; PCD: Piceid; RSV: Resveratrol; SGLT1: Sodium-glucose transport proteins; ST: Sulfotransferase; UGT: UDP-glucuronosyltransferase.

Table 2. Results obtained in human pharmacokinetics studies following oral administration of RSV-based formulations (RSV administered amount (milligrams) was calculated with reference to the average adult human weight of 70 kg).

Formulations	RSV (mg)	RSV and metabolites							Ref.
		Plasma (%)		Urine (%)		Feces (%)		Overall recovery (%)	
		RSV	Metabolites	RSV	Metabolites	RSV	Metabolites		
Wine (with a standard meal)	0.25*	< LOD	*t*-4′-**G** > *t*-3-**G**	NA	NA	NA	NA	NA	[37]
Wine	1.92*	< LOQ	*t*-4′-**G** > *t*-3-**G**	NA	NA	NA	NA		
Wine (with a 'fat' meal)	0.48*	1 – 6 ng/ml*	*t*-3-**G**	NA	NA	NA	NA		
Wine with a 'lean' meal		1 ng/ml*	*t*-4′-**G**	NA	NA	NA	NA		
Wine	0.36	NA	NA	< LOD	*t*-3-*O*-**G**, *c*-3-*O*-**G**	NA	NA	72 nmol/g in urine‡	[48]
	2.56							350 nmol/g in urine‡	
	0.40							212 nmol/g in urine‡	
Wine	0.4§¶	2	*t*-3-*O*-**G** > *t*-4′-*0*-**G** > *c*-4′-**S** = *c*-3-*O*-**G** > *c*-3-**S**#** > *t*-3-**S** > *t*-4′-**S**	< LOD	*c*-4′-**S** > *c*- 3-*O*-**G** > *c*-4′-*O*-**G** > *t*-3-*O*-**G** > *c*-3-**S** < *t*-4′-*0*-**G** > *t*-3-**S** > *t*-4′-**S**	NA	NA	NA	[35]
Wine	5.4§	1 – 9‡‡	*t*-3-*O*-G ↑ > *t*-4-*O*-**Glucos.**§§ > *c*-3-*O*-**Glucos.**	26 – 52	NA	NA	NA	NA	[34]
Wine, grape juice and tablets	1*	*c*-RSV and *t*-RSV < LOD	dihRSV¶¶	< LOD	dihRSV (↑)	NA	NA	NA	[33]
Whisky	2	< LOQ	NA	52	**G**	NA	NA	NA	[91]
	35		NA	34					
	70	4	**G**	26					
Grape juice	1##	< LOD	< LOD	< LOQ					
	2			5					
Wine	25*	[Conjugates]/[RSV] = 30 – 50×		[Conjugates]/[RSV] = 20 – 30×		NA	NA	25* in urine	[92]
Wine	25*	10 – 15	NA	< 1	NA	NA	NA	89 – 97	[93]
Wine, grape and vegetable juices	25*	1.7 – 1.9	**G** and **S**	16 – 17	**G** and **S**	NA	NA	NA	[94]
Ethanolic solution	25	< 5 ng/ml	**G** and **S**	53 – 85	**S** > **G**	0 – 38	**S**	71 – 98	[25]
	100	NA	NA	< LOQ	Two **G**, dih**G*****, **S**, dih**S**‡‡‡	NA	NA	NA	
	0.2§§§	4 – 16 ng/ml	9 – 14 ng/ml **S**	42 – 83	**S**	1 – 23	**S**	54–91	
Capsules	25 – 150*	≤ 1 to < 10 ng/ml	NA	NA	NA	NA	NA	NA	[18]
Ethanolic solution	50*¶¶¶	< LOD	*t*-3,5-diS > *t*-3-**S** > *t*-***C/O***-di**G**### > *t*-3,4′-di**S** > *t*-4′-**G**> *t*-3-**G**	< LOD	*t*-3,5-di**S**, > *t*-3-**S** > 17 *t*-3-**G** > *t*-***C/O***-di**G** > *t*-3,4′-di**S** > *t*- 4′-**G**	NA	NA	14 – 36 in urine	[7]
Capsules	500 – 5000	8.4 – 52 ng/ml	3-**S** > **S**, di-**S**, **G**, **G**-**S**	< 0.04	3-**S**** and other **S**, 1 di-**S**, 2 **G**, **G**-**S**	0 – 23 μg/g‡‡‡	< 1%§§§§	77% in urine	[32]

Table 2. Results obtained in human pharmacokinetics studies following oral administration of RSV-based formulations (RSV administered amount (milligrams) was calculated with reference to the average adult human weight of 70 kg) (continued).

Formulations	RSV (mg)	RSV and metabolites							Ref.
		Plasma (%)		Urine (%)		Feces (%)		Overall recovery (%)	
		RSV	Metabolites	RSV	Metabolites	RSV	Metabolites		
Capsules	1000	< 1 ng/ml	**S**-**G**, **G**, di-**S**, **S**	< 1 ng/ml	**S**-**G**, **G**, di-**S**, **S**	NA	NA	58 in plasma and urine	[38]
Standard food	2000 twice daily*	1274 ng/ml	NA	NA	NA	NA	NA	NA	[95]
High-fat food		689 ng/ml	NA	NA	NA	NA	NA	NA	
Standard food and quercetin		1272 ng/ml	NA	NA	NA	NA	NA	NA	
Standard food, quercetin and alcohol		1296 ng/ml	NA	NA	NA	NA	NA	NA	
Wine	Moderate daily consumption	NA	NA	NA	*t*-3-*O*-**G**, *c*-4′-*O*-**G**, *c*-3-*O*-**G**, *t*-4′-*O*-**S**, *t*-3-*O*-**S**, *c*-4′-*O*-**S**, *c*-3-*O*-**G**¶¶¶¶	NA	NA	1243 nmol/g####	[49]

**Trans*-RSV.

‡Nanomoles of total metabolites per gram creatinine.

§Total RSV.

¶The mean amount of total RSV consumed was 5.4 mg, corresponding to 2.6 mg of *trans*-piceid, 2.0 mg of *cis*-piceid, 0.4 *trans*-RSV and 0.4 *cis*-RSV.

#Percentage values were calculated based on published results, with the expense of Phase I metabolites and microflora metabolites, which were detected but not quantified (< LOQ).

**Values report to blood LDL fraction.

‡‡Picomoles *trans*-RSV per milligram LDL protein.

§§RSV **Glucos**ide.

¶¶**Dih**ydroRSV.

##Although four different doses (200, 400, 600 and 1200 ml) of grape juice (0.16 mg RSV/100 ml) were administered, only the two largest were considered here regarding the lack of quantifiable results, which contain 1 and 2 mg of total RSV.

***Dihydro**G.**

‡‡‡Dihydro**S.**

§§§Intravenous administration.

¶¶¶Eighty-five point five milligrams of piceid.

###C/O conjugates.

****At 0.5 mg dose.

‡‡‡‡Of dry weight feces.

§§§§With respect to RSV.

¶¶¶¶Cumulative effect of RSV repeated intake (increased concentration of metabolites).

####Nanomoles total RSV per gram creatinine.

LDL: Low-density lipoprotein; LOD: Limit of detection; LOQ: Limit of quantitation; NA: Not available; RSV: Resveratrol.

moiety was not taken into consideration in the recovery calculation and RSV and all metabolites were presumed to participate in radioactivity recovery, suggesting that not all RSV metabolites have been identified yet. The same assumption is applied to feces recovery [40]. It must also be taken into account that sulfate conjugates have some technological limitations, namely chromatographic behavior, which prevent their correct identification [25].

3. Evaluation of RSV efficacy

t-RSV is the isomer most used in human pharmacological studies [33] because not much is known about *c*-RSV in comparison, for reasons of lack of stability. However, both isomers are biologically active [41]. The effectiveness of RSV is supported by *in vivo* testing and bioavailability studies in animals and humans. However, in most experiments RSV has been used at concentrations often 10 – 100 times greater than peak concentrations observed in human plasma after oral consumption [42] in a free form dissolved in different organic solvents (i.e., dimethyl sulfoxide [DMSO], acetone and ethanol) that are not suitable for drug delivery [43-46].

Given the influence of the matrix type on RSV yield and presumably its stability [33], the formulations must be analyzed before and during experiments. It is also important to take into account the contribution of RSV metabolites, to assess them as precisely as possible and to make use of highly sensitive analytical techniques. These techniques should be standardized so the results obtained may have a degree of comparison and consequently higher correlation, particularly with regard to the limit of detection (LOD) and the limit of quantitation (LOQ) values. Finally, it is also of relevance to evaluate the impact of RSV and metabolites' interactions with serum proteins on RSV activity to understand better the differences between *in vitro* and *in vivo* assays.

RSV formulations are available as nutritional supplements at doses ranging from 15 to 600 mg per capsule or tablet. Marketing websites recommend both low and high doses, and make claims of 'high potency' formulations, yet no evidence exists for a recommended dose of RSV. In fact, despite similar *t*-RSV doses being administered, its bioavailability from wine and grape juice was sixfold higher than from tablets [33]. The absence of a therapeutic dose for RSV is a result of an inability to translate to humans successful doses used in animal models [47] and incomplete knowledge about the biomarkers of RSV activity. Although cells that line the digestive tract are exposed to unmetabolized RSV, research in humans suggests that other tissues are exposed primarily to RSV metabolites. RSV urine metabolites have been proposed as biomarkers of moderate wine consumption [35,48-49], although noticeable inter-individual variability was observed [49].

The therapeutic potential of certain polyphenols is similar to or higher than that of the parent molecule [50,51] and it is still unclear whether RSV exerts its biological effects directly, through its metabolites [52], or whether there is an *in vivo* interplay between all of them. Therefore, the therapeutic potential of RSV conjugates should be considered in future investigations [39,40] and, ideally, all the RSV metabolites should be identified and linked to the administered RSV. Several studies have reported the activity for RSV and its metabolites found in *in vitro* or *in vivo* studies, as can be seen in **Table 3**. To determine whether RSV metabolites demonstrate any cytotoxic properties, three major human sulfated conjugates of RSV (*t*-RSV-3-*O*-sulfate, *t*-RSV-4′-*O*-sulfate and *t*-RSV-3-*O*-4′-*O*-disulfate) were synthesized and their anticancer activity was evaluated against three breast cancer cell lines. In contrast to *t*-RSV, its sulfated metabolites showed poor cytotoxicity in human malignant and non-malignant breast cancer cell lines. Conjugation of the phenolic groups with sulfuric acid strongly affects the cytotoxicity of all metabolites, which were reduced ~ 10-fold [53]. However, the *in vitro* activity of the metabolites may not necessarily reflect their *in vivo* function, given the fact that the existing human sulfatases [53] and β-glucuronidases [54] could convert the metabolites back to RSV in humans.

RSV oligomers have also been proposed as therapeutic compounds. A chemical theoretical approach has shown that oligomers of *t*-RSV, *t*-RSV-3-*O*-glucuronide and glucosides have remarkably higher antioxidant activity than *t*-RSV [55]. The structure–activity relationships obtained for the inhibitory effect of stilbene derivates, which are RSV oligomers ranging from monomer to tetramer, against murine tyrosinase activity suggests that the double bond in the stilbene skeleton is critical for the inhibition and also that molecular size is important for inhibitory potency. The effects of some resveratrol derivates were tested on the F-11 neuroblastoma cell line, which expresses sodium current (I_{Na}) and two different types of voltage-dependent potassium current: inactivating inward-rectifying current (I_{ERG}) and a slowly inactivating delayed rectifier current (I_{DR}). RSV derivatives, which have been implicated in neuronal apoptosis, modulate voltage-gated potassium channels similarly to the parent compound, and in some cases they show better activity and higher specificity towards I_{DR} than I_{ERG} currents [56].

To determine total plasma RSV or metabolites concentrations, it is also necessary to take into account LDL and protein-bound fractions. *In vitro* assays have shown that > 90% of *t*-RSV is bound to human plasma lipoproteins in a non-covalent manner [7], which is reinforced by a study that focused on the binding of RSV to LDL. RSV and its metabolites were recovered in the LDL fraction of healthy volunteers after consumption of 250 ml of Merlot wine [34]. It is also suggested that RSV levels in serum could be underestimated because of the amounts potentially contained in the cellular fraction, and therefore its effects may be a result of the RSV cellular fraction, which is not assessed [40].

As a rational path to overcome the discrepancy between the concentration of RSV required for activity *in vitro* and

Table 3. Results obtained in studies that reported: cytotoxic properties against three breast cancer cell lines; effect on membrane potential of F-11 neuroblastoma cells line; antioxidant activity; and inhibition of melanin production of RSV and its derivates.

Compounds	Activity		
	Anticancer properties [53]		
	Cells		
	MCF-7	ZR-75-1	MDA-MB-231
t-RSV	All three sulfated metabolites were less potent than *t*-RSV		
t-RSV-3-O-sulfate	(*t*-RSV-3-O-sulfate < *t*-RSV-4′-O-sulfate < *t*-RSV-3-O-4′-O-disulfate)		
t-RSV-4′-O-sulfate			
t-RSV-3-O-4′-O-disulfate			
	Potassium channels' activity [56]		
	Conc. (μM)	I_{ERG} blocked (%)	I_{DR} blocked (%)
t-RSV	90	28.9	42.9
DihydroRSV	90	39.2	50.0
	Antioxidant properties [55]		
t-RSV	++*		
t-RSV-3-O-glucuronide	+++		
PCD	+++		
	Inhibition of tyrosinase activity [96]		
	Inhibition (%)‡		
RSV	97		
DihydroRSV	60		
PCD	17		

*From lower (+) to higher (+++) activity.
‡Inhibitory effects on tyrosinase activity by samples at a concentration of 100 μM.
PCD: Piceid; RSV: Resveratrol.

the doses found to be efficacious *in vivo*, an adaptation of a previously strategy [42] is proposed:

- Further efficacy studies of RSV in rodents *in vivo* should, as a priority, include measurement of the parent compound and metabolites in the target tissues.
- To determine total plasma RSV or metabolite concentrations, including LDL- and protein-bound fractions.
- Mechanistic *in vitro* studies should explore the activity of RSV at nanomolar concentrations and focus on RSV metabolites, especially its conjugates and oligomers.
- Metabolites and oligomers of RSV, *c*-RSV and PCD should be characterized and quantified in humans.
- Bioavailability assays of RSV in humans need to be increased, preferably with longer testing periods and in larger groups.
- Make an allometric approach between the animal and the human RSV studies.
- Develop suitable delivery systems to provide the optimal state and concentration of RSV able to be delivered to target tissues.

4. Resveratrol delivery systems

RSV is targeted for long-term treatments, more properly for prevention. Oral administration of RSV is the preferred and only viable route, except for topical application. However, it is hypothesized that administering RSV through a biodegradable drug delivery system via injection might be a potential therapeutic tool [19] to transpose the step taken as limiting RSV's bioavailability: intestinal metabolism.

Buccal delivery of RSV-loaded lozenges, which consists of, without swallowing, direct absorption through the inside of the mouth, has revealed much higher blood levels of RSV than systemic-intended oral formulations. When 1 mg of RSV in 50 ml solution was retained in the mouth for 1 min before swallowing, 37 ng/ml of RSV were measured in plasma 2 min later, similar to values achieved with 250 mg of RSV taken in a pill form [57].

Administering higher oral doses to improve efficacy might be insufficient to elicit systemic levels commensurate with given therapeutic effects [32] and might not be possible as toxic effects have been observed at 1 g/kg (body weight), which would result, moreover, in very high costs [6]. In this sense, no dose effect in the absorption of RSV was found; therefore, it is not worthwhile increasing RSV content in oral formulations [25].

Glycosylation can represent an alternative soluble form for RSV administration [7,40], whereas it protects RSV from deleterious oxidation, increasing its solubility in the cell cytoplasm [58]. Another approach is to modify the RSV structural determinants, such as number and position of the hydroxyl groups, intramolecular hydrogen bonding, stereoisomerism and double bond [40]. Methylated RSV has been proposed to circumvent RSV's high degree of metabolism, as it is metabolized slower, a property that has been exploited in

drug development of RSV analogues [27,59]. However, methylation can often change the activity of polyphenols, so it cannot be a solution [60].

To achieve an optimum response, RSV should be delivered to its site of action at a rate and concentration that both maximize its therapeutic effects and minimize its side effects, which implies the development of an appropriate RSV delivery system. Strategies of formulations for RSV are presented and explained in terms of pharmaceutical dosage forms, which are: classical dosage forms and new delivery systems. Classical dosage forms are established drug delivery systems and new drug delivery systems are drug carriers that aim to deliver RSV to the target or receptor site in a manner that provides maximum therapeutic activity, prevents degradation or inactivation during transit to target sites, and protects the body from adverse reactions because of inappropriate disposition. Examples include macromolecular drug carriers (protein drug carriers), particulate drug delivery systems (e.g., microparticles, nanoparticles and liposomes), monoclonal antibodies and cells. Over the last two decades, a variety of nanoscale vehicles, including gelatin [61], ceramic [62], liposomes [3] and micelles [63], have been under development for therapeutic use.

4.1 Classical pharmaceutical dosage forms

These are formulations often based on uncontrolled processing procedures and sources of RSV, of which the most common are tablets, capsules and powders. The tablets and hard gelatin capsules, because of their greater accuracy of drug content, are the most popular. They are available as nutritional supplements, so data about accurate RSV content are often scarce.

Stability of polyphenols is a major concern, so powders, often micronized, are preferred to liquid RSV solutions, and their formulations include surface agents in order to improve RSV absorption following oral administration.

4.2 New delivery systems

The slow progress in the efficacy of the treatment of severe diseases suggested a growing need for new ideas on controlling the pharmacokinetics, pharmacodynamics, nonspecific toxicity, immunogenicity, biorecognition and efficacy of drugs. These new strategies, often called drug delivery systems, are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry and molecular biology.

Targeting of drugs to specific sites in the body can be achieved by linking particulate systems or macromolecular carriers to monoclonal antibodies or to cell-specific ligands (e.g., asialofetuin, glycoproteins or immunoglobulins), or by alterations in the surface characteristics of carriers so that they are not recognized by the reticuloendothelial system. This review intends to cover targeted delivery systems that have been proved for RSV, including a comparison of available results related to stability, solubility and pharmacokinetics of RSV, as shown in **Table 4**. However, it should be noted that details pertaining to these techniques are out of the scope of this review. Excellent reviews of micro- and nanoencapsulation technology have been published [64,65].

4.2.1 *Macromolecular drug carriers*

Both natural and synthetic water-soluble polymers have been used as macromolecular drug carriers. A casein–RSV complex makes RSV available in stable forms and formulations having long shelf-life and improved solubility, preferably in aqueous media. The complex is in the form of discrete powder particles, with an average particle diameter of 5 – 2000 μm, or in the form of dispersion. The compositions containing the complex are administered to human adult (body weight ~ 70 kg) in the form of a capsule, tablet or liquid formulation. Dosage is 0.5 – 2000 (preferably 5 – 500) mg/day [66].

4.2.2 *Particulate drug delivery*

4.2.2.1 *Microencapsulation*

Encapsulating RSV in a matrix or inside a capsule can reduce its degradation with light and heat and make possible a slow release pattern, which could improve its absorption. RSV was immobilized on polymeric microspheres and the antioxidant activity found to be preserved for aged samples in ethanolic media [67], thus demonstrating a strategy to stabilize RSV in solution. Recently, encapsulation within yeast cells was also described as a technique for stabilizing solid RSV [8].

When together, pectins and calcium divalent ions (Ca^{2+}) hardened with polyethyleneimine (PEI) constitute suitable carriers for a colon-specific delivery system, called a multiparticulate calcium-pectinate carrier. Pectins (natural polysaccharides) are resistant to the stomach and intestine enzymes, but sensitive to colonic bacterial enzymes, enabling them to transverse the stomach and be degraded in the colon. However, their solubility and swellability in the basic/neutral fluids (such as intestine) prevent them being used as efficient colon-specific drugs. These pectin microspheres can encapsulate > 80% of RSV without altering the RSV-retention pattern in simulated gastrointestinal (GI) conditions. The formation of a strong matrix and hard surface layer slows down the release of RSV, which is stable when stored at 4°C and room temperature. Indeed, microspheres in simulated conditions are able to prevent the release of RSV in simulated upper GI conditions and release in simulated colonic conditions. Multiple-unit dosage forms, such as multiparticulate calcium-pectinate bead formulations, seem to be better than single-unit dosage forms owing to their reproducible and predictable GI transit time, more reliable drug release profile and less local irritation than single-unit forms [68].

Chitosan is a natural polysaccharide derived from chitin, which has good properties of non-toxicity, good biocompatibility and mechanical film-forming ability. Its potential is exploited as a delivery system of active agents in the format of microspheres or nanospheres. To increase the timeframe

Table 4. Characteristics of new delivery systems for RSV, particularly with regard to stability and solubility of RSV.

Delivery system	Effect on RSV properties		
	Stability	**Solubility**	**Release**
Micellar solutions	NA	3,7,12-TCA showed the biggest RSV solubilization effect and the lowest membranolytical potential [63]	NA
Cyclodextrins	Stabilization at pH 5.5 – 8.5 HP-β-CD-*t*-RSV shows high stability [21]	High solubility of resulting complexes [21] Solubility of RSV–CD complex at least 100 times greater [97]	Controlled release of RSV [21]
Solid lipid nanoparticles	Partition of *t*-RSV into the SLN sphere [72] Physical stability (zeta potential: -38 mV), stable for at least 4 weeks [72]	Poloxamer 188 enhances RSV's solubility	Two-stage model controlled release of RSV [72] Fast delivery of RSV to the nuclear region [72]
Nanoparticles	Stable loaded biodegradable NPs [19]	NA	Controlled release dosage form [19,71] RSV-loaded NPs lead to higher cell death [71]
Liposomes	Protection from light and other degradative processes [3,20]	NA	Slow and sustained release of RSV [3,20] Targeting to multiple intracellular sites [3] Rapid cellular internalization [3]
Multiparticulate calcium-pectinate carrier	Resistance to stomach's acid-base [68] Stability at 4°C (> 99%) for 6 months, but poorly stable at 40°C (> 90%) [68]	NA	Gradual drug release [68] Colon-specific delivery [68]
Acoustically active lipospheres	12-h stability [73]	RSV's water solubility enhanced to 130- and 10-fold compared with pH 7.4 buffer and coconut oil, respectively [73]	Abrupt RSV release on ultrasound pressure [73] Retarded RSV-release profile (compared with aqueous one) [73]
Chitosan microspheres	More resistance after irradiation and higher thermal stability in relation to RSV [69] Stability of encapsulated RSV kept constant [98]	NA	Two-stage release: first burst release and the second, slower one, occurs at higher pH [69]
Casein–RSV complex	Long shelf-life [66]	Twofold increase of RSV's solubility [66]	NA

CDs: Cyclodextrins; NA: Not available; NPs: Nanoparticles; RSV: Resveratrol; SLNs: Solid lipid nanoparticles.

and controlled release property of active agents, chitosan microspheres usually need to be crosslinked. RSV-loaded chitosan microspheres showed high stability in relation to light and heat. Precisely, these microspheres showed > 16% of resistance after 60 min of irradiation and thermal stability rose from 72 to 84% at 60°C and from 48 to 75% at 70°C for 15 days, in relation to reference samples of RSV [69]. Peng *et al.* [69] used vanillin, a natural and non-toxic crosslinker, to obtain microspheres with a compacted and continuous network, however with many voids, the voids probably being related to the mechanisms of air bubbles or entrapped fluid formed during the crosslinking and solidification process. The encapsulation efficiency was very high, 94% [69], and exceeded the values obtained so far with liposomes (76%) [3,20], nanoparticles (91%) [19] and other tested RSV delivery systems. Release of RSV from chitosan or derivative microspheres involves three different mechanisms: release from the surface of particles, diffusion through the swollen rubbery matrix and release due to polymer erosion. Controlled release of RSV was dependent on the pH value of media conditions, with slower release kinetics at higher pH conditions. The release pattern of RSV from chitosan microspheres was divided into two stages. The first stage was initially rapid (burst release), which may be a result of the rapid diffusion of RSV onto the surface of microspheres from the initial swelling of the spheres. Later, the second stage of release from the microspheres was slow (controlled release). The burst release helps to reach the effective RSV concentration rapidly in plasma, whereas the controlled release maintains the effective concentration of RSV in plasma for a long time. These results suggest that the poor bioavailability of RSV could be supplemented by an encapsulation method, thus prolonging its biological half-life *in vivo* [69].

4.2.2.2 Cyclodextrins

Cyclodextrins (CDs) are a group of naturally occurring cyclic oligosaccharides composed of glucopyranose derived from starch, which are constituted by variable glucose residues linked by glycosidic bonds. These systems consist of a truncated cone structure with a hydrophobic cavity and are well known for their ability to form inclusion complexes with a wide range of guest molecules [31,70]. CDs are divided into two groups: naturals, obtained with higher yield, namely α-, β- and γ-CD, and chemically modified CDs. Unmodified or unsubstituted β-CD has poor water solubility and is unsafe due to its nefrotoxicity, therefore several modified and relatively safe semi-synthetic CDs with higher capacity of molecular recognition and aqueous solubility have been made, such as HP-β-CDs (hydroxypropyl-β-cyclodextrins) and sulfobutyl ether β-CD. In comparison, it was also found that HP-β-CDs have larger inclusion ability than β-CDs [70].

Cyclodextrins act as a controlled dosage reservoir that protects RSV against rapid oxidation by free radicals, increasing its antioxidant activity [31]. This effect may be due to the formation of inclusion complexes between RSV and HP-β-CDs, where intervenes the -OH group of the monophenolic ring of RSV. The RSV's antioxidant activity is prolonged in time and reaches its maximum when all of it is complexed [31]. In practice, RSV-HP-β-CD complex shows a higher scavenging capacity than RSV-β-CD complex. However, the inclusion process has little influence on the antioxidant activity of RSV [70]. It has also been concluded that *t*-RSV presents a higher stability when complexed by HP-β-CD, compared with pterostilbene and pinosylvin, two stilbenoid compounds [21].

Other possible alterations that may interfere in RSV's bioavailability are on RSV's dissolution capacity, stability, and the slowing of its rapid metabolism and elimination [31]. Besides the scavenging capacity of β-CD and HP-β-CD increasing with increasing concentration of CDs [70], RSV is 38% bioavailable after oral administration in a solution of HP-β-CD in rats, a significantly higher value than its bioavailability alone, which is shown to be insignificant [31]. The solubility of RSV increases with increasing CD concentration in the order β-CD < HP-β-CD, which implies that the cavity of modified CDs provides a better protective microenvironment [70]. However, it was suggested that RSV's poor solubility is not the main cause of its oral bioavailability while carried by RM-β-CDs (randomly methylated β-cyclodextrin) in suspension. RSV might be rapidly crashing out of the complexes following immediate dilution in the GI tract by binding to the plasma proteins (this explains why the enhanced solubility by means of CD complexation did not result in increased oral bioavailability of RSV). However, the poor solubility of RSV in aqueous environment coupled with its rapid occurrence in the plasma refutes such a possibility. Alternatively, it is possible that solubility of RSV is pH-dependent, its absorption in the GI tract no longer being just an issue of solubility, but a pH concern as well. The involved data clearly indicated a pH-dependent effect [26].

López-Nicolás *et al.* reported that HP-β-CDs, including the protonated form of pinosylvin, were more stable than the interaction with the deprotonated form. Therefore, it is necessary to control the pH in the RSV's inclusion formulations because its protonated structures (low pH) have important beneficial effects for human health to the detriment of higher pH values [21].

4.2.2.3 Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are endowed with a lipophilic nature, and consequently function as carrier systems for hydrophobic drugs, such as RSV. The affinity between the RSV and SLN is satisfied, leading to the preferential partitioning of RSV into the SLN sphere instead of remaining in the aqueous medium [19,71-72]. The release profile of RSV from the SLNs into dialysis medium, composed of a phosphate buffered saline (pH 7.4), correlates with the RSV loading distribution into the latter. The physicochemical characteristics of RSV favor its localization near the SLNs' shell, enabling its rapid release from the nanoparticles (5 h)

during the first stage [71,72]. This happens because, beyond RSV's lipophilic nature, it has three -OH groups with a tendency to localize at the interface in the SLNs' hydrophilic area. In the following stage, the adsorbed RSV on the particle surface is steadily released over a long period in a sustained manner [72].

SLNs have the capacity to be transdermally delivered by crossing the keratinocyte membrane (< 1 min), transversing the cytoplasm and concentrating near the nucleus. The rate and efficiency of uptake of RSV by these cells depend on the nanoparticles' surface properties [72].

4.2.2.4 Liposomes

Liposomes are small, spherical vesicles that consist of amphiphilic lipids enclosing an aqueous core. The constituent lipids are predominantly phospholipids that form bilayers similar to those found in biomembranes. In most cases, the principal component is phosphatidyl choline. Depending on the processing conditions and the chemical composition, liposomes are formed with one or several concentric bilayers.

Liposomes are considered to be an appropriate delivery system for RSV [3]. This molecule, once in liposomes, prefers localizing at the liposome surface, where it remains biologically effective in *trans*-conformation by the prevention of transformation into the *cis*-form [3,20]. The liposomal bilayers store RSV, preventing overloading of the cells' membranes by the slow and sustained release of RSV to the biological domains, avoiding cytotoxicity. For this, it is required that liposomes contain an amount of RSV that would be toxic in its unloaded state to maintain therapeutic-free concentration. These bilayers further promote stimulation of the cell-defense system and RSV long-term stability [3,20].

4.2.2.5 Acoustically active lipospheres

Microbubbles are a class of oily parenteral formulations constituted by spherical voids filled by a gas that functionate as lipophilic drug carriers sensitive to ultrasounds waves. Acoustically active lipospheres (AALs) constitute a kind of microbubble and comprise perfluorocarbons and coconut oil as the cores of the inner phase, stabilized with coconut and phospholipid coatings, and the co-emulsifier Pluronic F68 (PF68) (which lowers interfacial tension, adds rigidity, and impedes gas escape and coalescence). As ultrasound pressure waves interact with microbubbles, they begin to oscillate or resonate, which triggers collapse and abrupt drug release in a specific location. These precise locations can be determined by focusing ultrasound energy. On insonation of sufficient energy from ultrasound, however, they may convert to a gas, in turn increasing the acoustic reactivity and the potential for localized drug release. Cavitation of AALs with ultrasound can be used to reach the cardiovascular system and treat vascular thromboses by the drug's delivery. In AAL formulations, RSV shows a sustained release profile. This profile can be well modulated by the alteration of the microbubbles' oil and perfluorocarbon percentages. Formulations with high oil (18%) and perfluocarbon (32%) percentages have low drug release. On the other hand, ultrasound at 1 MHz showed a more efficient ability to accelerate the amount of drug delivered from the AALs with high oil and perfluoropentane, compared with the opposite concentrations. The larger droplet size may contribute to increasing the acoustic reflectivity, thus increasing the ultrasound efficacy. PF68 was shown to be effective at slightly but further slowing down RSV release [73].

The chosen microbubbles' components with high ratios in AALs may be feasible because of their small size, acceptable safety, sustained drug release and high sensitivity to ultrasound treatment. One of the possible applications of these systems is parenteral injection. As phospholipids are known to cause hemolysis, the formulation of AALs with high oil contents decreases this phenomenon substantially, making it negligible [73].

4.2.2.6 Nanoparticles

Recent progress in drug delivery has focused on improving it by nanomedicine and polymer techniques [74]. New drug delivery systems may be desirable and useful for the therapeutic use of antioxidants in human neurodegenerative diseases. The structure and tunable surface functionality of nanoparticulate systems allow them to encapsulate/conjugate single or multiple entities, either in the core or on the surface, rendering them ideal carriers for various drugs.

In a recent study, poly(lactide-*co*-epsiloncaprolactone) (PLCL) was successfully developed as epigallocatequin-3-gallate (EGCG) eluting polymeric stent, which could be utilized for preventing thrombosis, inflammation and in-stent restenosis [75]. In another study, Italia *et al.* also suggested the potential of biodegradable NPs in improving the therapeutic efficacy of EGCG [76]. Sahu *et al.* [77] and Thangapazham *et al.* [78], in two separate studies, have demonstrated that curcumin can be delivered by means of nanotechnology-based carriers for prevention and therapy of cancer. Curcumin was also nanoformulated with three biocompatible polymers – alginate (ALG), chitosan (CS) and pluronic – by ionotropic pre-gelation followed by polycationic crosslinking.

Pluronic F127 was used to enhance the solubility of curcumin in the ALG-CS NPs. This study demonstrated cellular internalization of curcumin-loaded composite NPs [79]. A study also demonstrated that curcumin-loaded poly(caprolactone) nanofiber matrix is bioactive and has potential as a wound dressing with inflammatory induction and increased rate of wound closure [80]. In a different study, PEGylated curcumin conjugate was demonstrated to have much more potent effects on pancreatic cancer cell growth inhibition than free curcumin [81].

Amphiphilic block copolymer-based polymeric micelles receive most attention because they can self-assemble into NPs with hydrophilic outer shells and hydrophobic inner cores, which capture hydrophobic drug in the cores and easily disperse in solution with the protection of the hydrophilic

shells. These drug-loaded polymeric micelles are not bigger than 100 nm, being easy to internalize by cells [74]. By incorporating in these NPs, drugs are prevented from being quickly degraded [82,83] and sustained release is enabled at the expected site. The hydrophobicity of RSV makes it possible, acting as a model drug, to develop RSV-loaded NPs. It also brings about the assumption that water-soluble, biocompatible, injectable and controlled release RSV-loaded nanoparticles can be used as a potential therapeutic tool for β-amyloid peptide (Aβ)-related oxidative stress.

RSV-loaded NPs at lower concentration were recently observed to lead to significantly higher cell death compared with an equivalent dose of free RSV, and this difference of cytotoxicity was not found to be abrogated by the antioxidant vitamin E [71]. In a separate study [19], a 12 h pre-incubation of RSV-loaded NPs was found to protect cells from Aβ-induced damage in a dose-dependent manner by attenuating intracellular oxidative stress and caspase-3 activity. Naraynan *et al.* [84] recently used liposome-encapsulated curcumin and RSV individually and in combination in male B6C3F1/J and prostate-specific PTEN knockout mice. *In vitro* assays using PTEN-CaP8 cancer cells were also performed to investigate the combined effects of curcumin with RSV. In this study, HPLC analysis of serum and prostate tissues showed a significant increase in curcumin level when liposome-encapsulated curcumin was co-administered with liposomal RSV. Combination of liposomal forms of curcumin and RSV significantly decreased prostatic adenocarcinoma *in vivo* in PTEN mice, and the *in vitro* studies revealed that curcumin plus RSV effectively inhibited cell growth and induced apoptosis. Findings from this study provided evidence for the first time that phytochemicals in combination enhance chemopreventive efficacy in prostate cancer.

The hydrophobic characteristics of nanocarriers based on polymeric micelles also enable the blood–brain barrier to be crossed, where these systems release the drug by water-soluble controlled release [19].

5. Conclusions

Resveratrol's therapeutic potential is hampered either by its physicochemical properties, mainly its low aqueous solubility and stability, or by its pharmacokinetics profile. Owing to a lack of relationship between the effective amount of RSV and/or its metabolites needed to perform a desired effect, appropriate techniques of extraction and quantification of them in samples collected during pharmacokinetic studies compared with the concentration of RSV initially administered are required. RSV interactions with serum proteins are also of relevance, as they may compromise RSV bioavailability; their lack of quantification is still a weakness of pharmacokinetics research.

The intravenous route is actually considered as a potential tool for RSV administration; however, oral administration seems to be the preferred means of administration. As classical pharmaceutical dosage forms are not efficient, an alternative for the development of a safe and effective RSV formulation is the use of drug delivery systems.

There are few studies available at present on RSV delivery systems. Multiparticulate calcium-pectinate carriers are able to prevent release of RSV in simulated upper GI conditions and simulated colonic condition. Chitosan microspheres showed a high stability against light and heat and provided a controlled release profile of RSV. RSV encapsulation in cyclodextrins increased solubility of the polyphenolic compound; however, it has been concluded that it may not be responsible for its poor bioavailability. Liposomes maintain a stable environment for RSV and promote its sustained release to the biological domains. New delivery systems have shown advantages over free drug in the assessment of its therapeutic activity. In general, these delivery systems have a great RSV encapsulation capacity (> 70%), increase RSV's stability and solubility contained therein, and in some cases are also biodegradable. In target sites, where RSV is released in a controlled manner, effective plasma concentrations may be achieved for required and therapeutic time intervals.

In spite of the fact that most of the available studies have been concerned with oral administration of RSV for systemic action, few carried out by buccal administration (direct absorption through the inside of the mouth, without swallowing) appear promising, insofar as it was possible rapidly to achieve therapeutic concentrations in bloodstream. Therefore, more studies involving buccal administration of RSV are needed in order to amplify its properties.

So far, only short duration treatments with RSV have been performed, necessitating the need for treatments to be carried out in the long run, so as to provide for their safety in the face of chronic administration.

6. Expert opinion

Resveratrol is a natural multitargeting compound, attractive for prevention or therapy where a magnitude of pathophysiological pathways is affected, such as cancer, diabetes and neurodegenerative diseases. RSV formulations, mainly available as nutritional supplements, are classic pharmaceutical dosage forms such as powders, tablets and hard gelatin capsules, to be administrated by the oral route, often produced under uncontrolled processing procedures and sources of RSV.

For several reasons, a commercial formulation of RSV developed for clinical proposal is still not available. First of all, the levels of RSV obtained in blood following its oral administration do not justify its therapeutic activities, raising doubts about the straight relationship between activity and RSV content claimed by formulations. Another reason, linked to the previous one, is the high variety of formulations being tested in several animal models, making the comparison of results very difficult and in consequence the translational research. Therefore, a systematic organization of RSV formulations according to their mode of preparation with

discrimination of their content on RSV as well an improvement in quality assurance and standardization of such formulations would make the task of comparing results easier. Finally, despite promising results in preclinical settings, the applicability of RSV to humans has met with only limited success, largely owing to inefficient systemic delivery of RSV and consequently low bioavailability. Some fundamental aspects of RSV's action need to be understood as follows.

- Is the evaluation of formulations and bioavailability, based on RSV levels, a valid strategy?
- Are there scientific data that reveal therapeutic activity of RSV metabolites, so their contribution should be considered when assessing bioavailability of formulations?

The study of RSV pharmacokinetics in humans concluded that high doses of RSV might be insufficient to achieve therapeutic concentrations required for the systemic prevention of a disease. Therefore, to achieve an optimal response of RSV, new strategies are required to enhance its bioavailability and reduce perceived toxicity. Furthermore, new delivery systems can increase drug solubility and stability, may allow systemic drug administration, and can enhance drug bioavailability and offer control over the absorption and distribution profiles.

Declaration of interest

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