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Research Paper

The Impact of Aqueous Solubility and Dose on the Pharmacokinetic Profiles of Resveratrol

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Purpose. This study aimed at the investigation of the impact of aqueous solubility and dose manipulation on the pharmacokinetics of resveratrol.
Methods. Water soluble intravenous and oral formulations of resveratrol were prepared with hydroxypropyl-β-cyclodextrin (HP-β-CD) and randomly methylated-β-cyclodextrin (RM-β-CD), respectively. Sodium salt and suspension of resveratrol in carboxymethyl cellulose (CMC) were used as the reference intravenous and oral formulations, respectively. The pharmacokinetics of resveratrol was assessed in Sprague–Dawley rats. Plasma resveratrol concentrations were measured by high performance liquid chromatography (HPLC).
Results. Both HP-β-CD and RM-β-CD enhanced the aqueous solubility of resveratrol. After intravenous administration, rapid elimination of resveratrol was observed at all tested doses (5, 10, and 25 mg kg^{-1}) regardless of formulation types; with non-linear elimination occurring at the dose of 25 mg kg^{-1}. RM-β-CD significantly increased the maximal plasma concentration of orally administered resveratrol, but, it did not increase the oral bioavailability in comparison with the CMC suspension. Furthermore, the oral bioavailability remained unchanged among all tested doses (15, 25, and 50 mg kg^{-1}).
Conclusions. Aqueous solubility barrier might affect the speed but not the extent of resveratrol absorption. Further, dose manipulation (up to 50 mg kg^{-1}) did not have a significant impact on the oral bioavailability of resveratrol.

KEY WORDS: aqueous solubility; bioavailability; dose manipulation; pharmacokinetics; resveratrol.

INTRODUCTION

Resveratrol (*trans*-3, 5, 4′-trihydroxystilbene, Fig. 1) is a polyphenolic phytoalexin produced by a variety of plants in response to stress (1). Resveratrol has wide pharmacological activities. It is well known for its anti-oxidant, anti-inflammatory, analgesic, cardio-protective, neuro-protective, chemo-preventive and anti-aging activities (1–3). Resveratrol has shown great promise in the treatment of cancers and two Phase I/II clinical trials are currently underway to determine if resveratrol is useful for the treatment of colorectal cancer (4). More recently, resveratrol is shown to improve general health and survival of mice on a high-caloric diet, pointing to new approaches for treating obesity-related disorders and diseases of aging (2).

As the pharmacology of resveratrol was subjected to extensive studies during the past decade, its pharmacokinetics has also been investigated in pre-clinical models as well as in humans (1, 5). Unfortunately, the pharmacokinetic properties of resveratrol are not as favorable when compared with its beneficial pharmacological activities in various disease models (5). Resveratrol has a very short initial half-life (~8–14 min) (1). Upon administration, it is metabolized quickly and extensively in the body (5). Further, its oral bioavailability in human is observed to be very low (6–8).

Oral bioavailability is generally believed to be dependent on the aqueous solubility, membrane permeability, and metabolic stability of the given drugs (9, 10). As a polyphenolic compound (log P>3.1), gastrointestinal permeability of resveratrol across the epithelial cells is not considered to be a concern. Indeed, two recent published studies had indicated that transport of resveratrol across biological barrier did not have difficulty (11, 12). However, the high lipophilicity of resveratrol also leads to low aqueous solubility (13), which may impair its oral bioavailability. Despite such proposition, it is interesting to note that no study to date has examined the direct role of aqueous solubility on oral bioavailability of resveratrol. Poor metabolic stability of resveratrol has been well documented for restricting the oral bioavailability of resveratrol (1, 7, 14). Resveratrol undergoes extensive phase

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ABBREVIATIONS: AUC, area under curve; C_{max}, maximum plasma concentration; CD, cyclodextrin; Cl, clearance; CMC, carboxy methyl cellulose; F, bioavailability; HP-β-CD, hydroxypropyl-β-cyclodextrin; HPLC, high performance liquid chromatography; LOQ, limit of quantitation; PBS, phosphate buffer solution; RM-β-CD, randomly methylated-β-cyclodextrin; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; T_{max}, time to reach C_{max}; $t_{1/2}$, half life; V, volume of distribution.

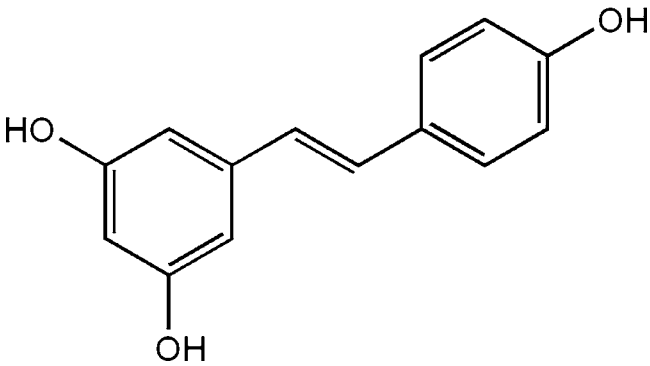

Fig. 1. Chemical structure of *trans*-3, 5, 4′-trihydroxystilbene.

II metabolism to from glucuronide and sulfate conjugates (1, 11, 12, 14–16). However, two recent studies have reported metabolic saturation of resveratrol in Caco-2 cells which led to increased transport of resveratrol across the Caco-2 monolayer (11,12). These results suggest that metabolism of resveratrol in gastrointestinal tract could be saturated at higher resveratrol concentration and dose escalation of resveratrol might be employed as a tool to enhance its oral bioavailability.

Cyclodextrins (CDs), a family of cyclic oligosaccharides derived from starch, are well known for their abilities to form inclusion complexes with a variety of guest molecules (17). They have traditionally been used to increase oral bioavailability by increasing the dissolution of a given drug. Inclusion of resveratrol into CDs led to an enhancement in its aqueous solubility (13). The objective of this work was to determine if oral bioavailability of resveratrol can be enhanced by manipulating the aqueous solubility and doses of resveratrol. To achieve this objective, the pharmacokinetics of resveratrol and those formulated in HP-β-CD, RM-β-CD and CMC were compared after intravenous and oral administration, respectively. Our data indicated that neither aqueous solubility nor dose manipulation exerted a significant impact on the oral bioavailability of resveratrol.

MATERIALS AND METHODS

Chemicals

Resveratrol (99.9%), sodium salt of CMC, carbamazepine, sodium chloride and sodium phosphate monobasic were purchased from Sigma (St Louis, MO). RM-β-CD (degree of substitution: ~1.8) and HP-β-CD (degree of substitution: ~0.6) were kindly donated by Wacker Chemie AG (Burghausen, Germany) and Roquette Freres S.A. (Lestrem, France), respectively. HPLC grade methanol and acetonitrile were obtained from Tedia Company (Fairfield, OH). Sodium hydroxide was received from Kanto Kagaku Singapore Private Limited. (Singapore). Purified water (18.2 M Ω cm at 25°C) was obtained from a Millipore Direct-Q® ultra-pure water system (Billerica, MA) and used throughout the study. Di-sodium hydrogen phosphate anhydrous was purchased from Fluka (Steinheim, Germany).

High Performance Liquid Chromatography (HPLC) Assay

A Shimadzu (Kyoto, Japan) 2010A HPLC system was used to quantitate resveratrol in both formulation and plasma samples. The chromatography was run with a reversed phase HPLC column (ODS Hypersil, 5 μm, 250 mm×4 mm, Agilent, Palo Alto, CA), which was protected with a guard column (Agilent). The assay was performed at 35°C through isocratic delivery of the mobile phase, consisting of acetonitrile and 30 mM phosphate buffer solution (PBS) pH 7.0 (30:70 *V/V*) at a flow rate of 1 ml min^{-1} and the ultraviolet detection wavelength was 320 nm.

Stock solution of resveratrol (1 mg ml^{-1}) and the internal standard, carbamazepine (1 mg ml^{-1}) were prepared in methanol, stored in refrigerator and protected from light. Standard working solutions of resveratrol (50 μg ml^{-1}) and carbamazepine (50 μg ml^{-1}) in water methanol mixture (1:1) were prepared freshly before experiments. The procedure for plasma clean up was modified from a previously reported method (18). Briefly, 5 μl of internal standard working solution (50 μg ml^{-1}) was first spiked into 100 μl plasma in a clean 2 ml centrifuge tube. The samples were mixed well and 40 μl of PBS (30 mM, pH 6) was added to the tube and the tube was mixed for another 15 s. Finally, ethyl acetate (300 μl) was added and the tube was mixed for 30 s. After ethyl acetate extraction, the tube was centrifuged at 5,500×*g* for 10 min and the upper organic layer was carefully transferred to a clean tube. The extraction procedure was repeated for another two times and the combined organic layers were evaporated to dryness under nitrogen gas. The residue was reconstituted with 75 μl mobile phase and centrifuged for 1 min at 5,500×*g* and 30 μl of the supernatant was injected to the HPLC system.

The calibration curve, obtained by spiking resveratrol into pooled rat plasma, was linear (R^2>0.99) within the range of 5–1,000 ng ml^{-1}. The intra-day and inter-day variation were less than 5%. The limit of quantitation (LOQ) of the assay was 5 ng ml^{-1}. The extraction recovery rate of resveratrol in plasma was 95±3.8%, 95±4.2%, and 97±4.4% for 500, 100, and 25 ng ml^{-1}, respectively. For the pharmacokinetic study, plasma samples with high resveratrol concentration were properly diluted with pooled plasma to the calibration range before clean up procedure.

For the concentrated samples from the formulation preparation, clean up procedure was not required. They were diluted with methanol-PBS, pH 7.4 (1:1) and only diluted samples (5 μl) were injected into the HPLC. The calibration range for the formulation samples was 1–100 μg ml^{-1}.

Phase-solubility Study

Resveratrol–cyclodextrin inclusion complexes were prepared with a method modified from a previous report (19). Briefly, an excess amount of resveratrol (40 mg ml^{-1}) was added to HP-β-CD or RM-β-CD solutions prepared at different concentrations (0, 0.01, 0.025, 0.05, 0.10, 0.15, 0.20, 0.30 M) with 10 mM PBS at pH 7.4. The suspensions were vortexed and sonicated for 1 h and kept on a horizontal rotary shaker (200 rpm) for 3 days. The suspension was filtered through a 0.22 μm membrane filter (Whatman Ltd., Kent, UK) to obtain a clear solution. All samples were prepared in triplicate. Final clear solutions were diluted properly with methanol-PBS, pH 7.4 (1:1) to the calibration range (1–100 μg ml^{-1}) and the concentrations of resveratrol in inclusion complex solutions were measured by HPLC. The apparent inclusion rate constant ($K_{1:1}$) was calculated with

the equation established by Higuchi and Connors (20): $K_{1:1} = \frac{Slope_1}{S_0 \cdot (1 - Slope_1)}$; where $Slope_1$ is slope of the phase-solubility curve and S_0 is the intrinsic solubility of resveratrol.

Solubility Study in Simulated Gastro-intestinal Fluid

Resveratrol solubility was determined in simulated gastro-intestinal fluid. Excess amount of resveratrol was added to simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluids (SIF, pH 6.8 and SIF, pH 7.5); vortexed and kept on a horizontal rotary shaker (200 rpm) for 3 days. The suspension was filtered through a 0.22 μm membrane filter (Whatman Ltd., Kent, UK) to obtain a clear solution. All samples were prepared in triplicate. The concentration of resveratrol in SGF and SIF was determined by HPLC assay (calibration range 0.1–10 μg ml^{-1}).

Preparation of Dosing Formulations

The parenteral safety of HP-β-CD has been well documented (17). Therefore, HP-β-CD (0.3 M) was used in the current study to prepare the intravenous injection formulation. The resveratrol concentration in HP-β-CD solution was measured with HPLC and subsequently diluted to 20 mg ml^{-1}. To find out whether the formation of inclusion complex with HP-β-CD affects the kinetic profile of resveratrol after intravenous administration, sodium salt solution of resveratrol was used as a control. It was prepared freshly by dissolving 20 mg of resveratrol in 1 ml of 0.9% NaCl–0.3% NaOH (*w*/*v*) solution. The final clear solution indicated complete dissolution of resveratrol. Such simple preparation method has been used to form intravenous formulations of retinoic acids in pre-clinical studies (21, 22). Since RM-β-CD usually has superior solubility and bioavailability enhancing ability, it was used to form a water soluble oral formulation of resveratrol. The preparation procedure has been described in the phase-solubility study. The resveratrol concentration was measured with HPLC and subsequently diluted to 25 mg ml^{-1}. The resveratrol suspension for oral dosing was prepared by suspending 25 mg resveratrol into 1 ml 0.5% CMC (pH 7.4). The suspension was prepared freshly and shaken vigorously before oral gavage.

Animals

The study design and the animal handling protocol of this pharmacokinetic study were modified from several previous studies (21–23) and approved by the Institutional Animal Care and Use Committee of the National University of Singapore. Adult male Sprague–Dawley rats (250–300 g) were supplied by the Laboratory Animal Center of the National University of Singapore. The rats were housed in Animal Holding Unit of the university under temperature- (22±1°C) and humidity- (60–70%) controlled environment. A 12-h light/dark cycle was maintained and the rats were given free access to food and water before surgery. A polyethylene tube (i.d. 0.58 mm, o.d. 0.965 mm, Becton Dickinson, Sparks, MD) was placed into the right jugular vein through surgical implant under anesthesia on the day before the pharmacokinetic study. This cannula was used for intravenous drug administration as well as blood sample collection. The rats were randomly divided into ten groups (n=4 per group); four groups received intravenous administration of resveratrol while six groups received oral dosing through gavage. Because oral absorption may be influenced by different dietary regimens and the inherent bile salt solubilization capacity in the intestine, the rats were kept in fasting condition overnight prior to the oral gavage and during blood collection but free assess to water was allowed. Such restriction was not applied to the rats that received intravenous administration. Resveratrol–HP-β-CD inclusion complex solution was intravenously administrated to the rats in group 1, 2, and 3 at the dose of 25, 10 and 5 mg kg^{-1}, respectively. Rats in group 4 received a single bolus dose intravenous injection of resveratrol sodium salt solution at a dose of 10 mg kg^{-1}. Ethical considerations (primarily a limitation of the high pH imposed by the sodium salt of resveratrol) prevented the use of higher dose of sodium resveratrol in this parallel study. Rats in groups 5, 6 and 7 were administered single dose of resveratrol–RM-β-CD inclusion complex solution by oral gavage at the dose of 50, 25, and 15 mg kg^{-1}, respectively. Similarly, groups 8, 9 and 10 were given single oral doses of resveratrol suspension in 0.5% CMC (pH 7.4) at the dose of 50, 25, and 15 mg kg^{-1}, respectively. Serial blood samples (200 μl) were collected from each animal through the catheter at 5, 15, 30, and 45 min, and 1, 1.5, 2, 3, 4 and 5 h after intravenous administration and at 5, 15, and 30 min, and 1, 1.5, 2, 3, 4, 6 and 8 h after oral gavage. The cannula was flushed and blood was replaced by an equivalent volume of heparin–saline (5 U ml^{-1} heparin in normal saline) after each draw of blood sample. After centrifugation at 5,500×*g* for 10 min, plasma samples were collected and stored at −80°C until HPLC analysis.

Pharmacokinetic Analysis

Most of the pharmacokinetic parameters were calculated by non-compartmental model using the software WinNonlin standard Version 1.0 (Scientific Consulting Inc., Apex, NC). The area under the plasma concentration *versus* time curve ($AUC_{0\to t}$) in rats that received oral administration (Groups 5 to 10) was calculated by the linear trapezoidal rule with the time point from 0 to the last detectable time point, whereas the $AUC_{0\to t}$ in rats that received intravenous dosing (Groups 1–4) was calculated through the same rule except the logarithmic scale was taken (22). Clearance (Cl) values in Groups 1–4 were calculated using non-compartmental methods $\left(Cl = \frac{Dose}{AUC_{0\to t}}\right)$. Bioavailability ($F$) of resveratrol after oral administration in Group 5–10 was calculated using following formula:

$$F(\%) = \frac{\frac{AUC_{0\to t}(\text{Groups } 5, 6, 7, 8, 9 \text{ or } 10)}{\text{Dose (Groups } 5, 6, 7, 8, 9, \text{ or } 10)}}{\frac{AUC_{0\to t}(\text{Group } 2)}{10\ mg\ kg^{-1}}} \times 100\%$$

The apparent volume of distribution (V) after intravenous administration were calculated by assuming first order kinetics with the first 3 data points (up to 0.5 h) in group 3; with the first 4 data points (up to 0.75 h) in group 2 and 4, and with the first 5 data points (up to 1 h) in group 1. Such plasma resveratrol concentration–time data was fitted into one com-

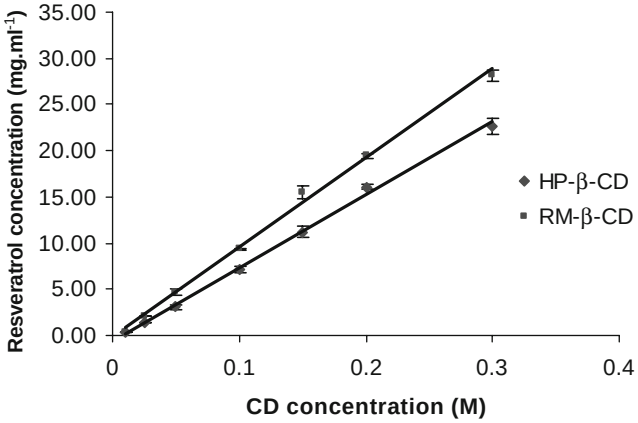

Fig. 2. Phase solubility diagrams for resveratrol with HP-β-CD and RM-β-CD at 25°C. Data represent the mean values (n=3) of the groups ± SD.

partment open model $\left(C = C_0 \cdot e^{-k \cdot t}\right)$ (fit with nonlinear least squares regression with a weighting factor of $1/Y^2$), where V was calculated as: $V = \frac{\text{Dose}}{C_0}$.

Statistics

Statistical analyses were performed using the software Graph-Pad Prism Version 2.00 (San Diego, CA). All experimental data were expressed as mean ± standard deviation (SD) except for the point pertaining to time to maximal concentration (T_{max}) after oral administration, as it was a non-continuous data due to the pre-decided sampling schedule. Statistical comparisons of the pharmacokinetic parameters among the different doses with the same formulations (resveratrol–HP-β-CD inclusion complex or resveratrol–RM-β-CD inclusion complex or resveratrol in 0.5% CMC) were performed by using one-way ANOVA with the *post hoc* Tukey test. Two-tail independent sample t test was used to compare the pharmacokinetic parameters between the two different formulations at the same dose (resveratrol sodium salt *versus* resveratrol–HP-β-CD, resveratrol suspended in 0.5% CMC *versus* resveratrol–RM-β-CD). Statistical comparisons of the T_{max} values among Groups 5, 6 and 7 were performed by using Kruskal–Wallis test with the *post hoc* Dunn test. Similar test was performed to compare T_{max} values among Groups 8, 9 and 10. Again two-tail Mann–Whitney test were used to compare the T_{max} of different formulations with same dose (Group 5 *vs.* 8, Group 6 *vs.* 9 and Group 7 *vs.* 10). Statistical significance was set at $p<0.05$.

RESULTS

Phase Solubility Study

Figure 2 shows the phase solubility profiles of resveratrol in HP-β-CD and RM-β-CD solutions. Both CDs produced a concentration-dependent increase in the solubility of resveratrol, with RM-β-CD produced a more enhanced effect than HP-β-CD on a per molar concentration basis (28.13 mg ml^{-1} for RM-β-CD *vs.* 22.61 mg ml^{-1} for HP-β-CD at 0.3 M CD concentration). This difference in enhanced solubility is probably due to the extended hydrophobic surface of the inner cavity of RM-β-CD molecules (24). Phase solubility plots were A_L^- type and linear (R^2>0.99) from 0.01 to 0.3 M CD concentration for both CDs. These results suggest that within the concentration range studied, the inclusion stoichiometry of resveratrol with CD was mostly 1:1, and is consistent with previous observation obtained at lower CD concentrations (13). Inclusion rate constants (K_{11}) for HP-β-CD and RM-β-CD solutions were calculated from the slope of Fig. 2 and using S_0 (0.0017 mM) obtained from phase solubility study. The calculated values of K_{11} (3.17×10^5 M^{-1} for HP-β-CD and 4.41×10^5 M^{-1} for RM-β-CD) indicate that the complexes formed between resveratrol and HP and RM-β-CDs are moderately stable.

Solubility Study in Simulated Gastro-intestinal Fluid

Resveratrol solubility in simulated gastro-intestinal fluid is pH dependant. Solubility of resveratrol in SIF, pH 6.8 and SIF, pH 7.5 was 0.11±0.01 μg ml^{-1} and 0.42±0.03 μg ml^{-1}, respectively; whereas, resveratrol was almost insoluble in SGF, pH 1.2. Such low solubility of resveratrol in buffers at different pH was also reported in a recently published study by Hung *et al.* (25).

Pharmacokinetics After Intravenous Administration

Figure 3 shows the pharmacokinetic profiles of resveratrol following single intravenous bolus dose of either sodium salt of resveratrol (10 mg kg^{-1}) or resveratrol–HP-β-CD complexes (5, 10 and 15 mg kg^{-1}). In all cases, plasma resveratrol concentration declined rapidly over the first hour that was followed by the appearance of a second peak at 2 h. The reappearance of a second peak after the initial decline of resveratrol is most likely due to enterohepatic recirculation, which is a well-documented phenomenon (14).

The pharmacokinetic parameters describing resveratrol in the plasma are shown in Table I. At 10 mg kg^{-1} of resveratrol, there are no significant differences (p>0.05) in the V and Cl values between sodium salt of resveratrol (Group 4) and resveratrol–HP-β-CD inclusion complex solution (Group 2). These results suggest that HP-β-CD did not exert

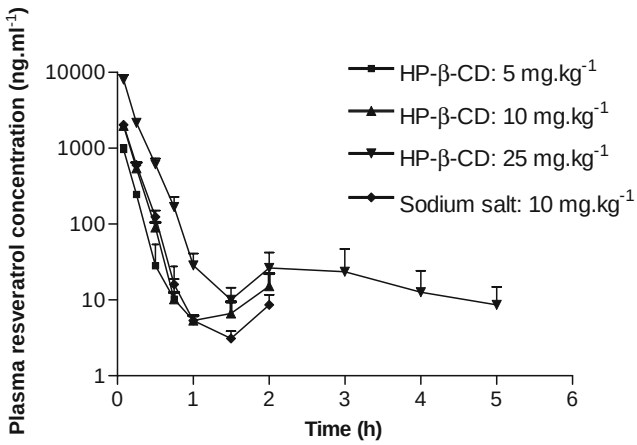

Fig. 3. Pharmacokinetic profiles of resveratrol after a single intravenous administration. Data represent the mean values (n=4) of the groups ± SD. Concentration of resveratrol was below the LOQ of HPLC assay after 45 min and 2 h at 5 and 10 mg kg^{-1} doses, respectively.

Table I. Pharmacokinetic Parameters of Resveratrol After Intravenous Administration

	Group 1	Group 2	Group 3	Group 4
Formulation	HP-β-CD	HP-β-CD	HP-β-CD	Sodium salt
Dose (mg kg^{-1})	25	10	5	10
V (L kg^{-1})	2.24±0.15	2.60±0.37	2.49±0.72	2.69±0.12
Cl (L h kg^{-1})	11.6±1.6*, **	20.0±2.6	21.6±3.6	18.9±1.8
AUC (ng h ml^{-1})	2,196.2±343.3	505.9±68.1	236.1±35.2	532.9±55.0

Data is presented as Mean ± SD, N=4.
*p<0.05 between Group 1 and 2; **p<0.05 between Group 1 and 3

a significant impact on the intravenous pharmacokinetic profile of resveratrol even though HP-β-CD dramatically enhanced the aqueous solubility of resveratrol. After intravenous administration of resveratrol–HP-β-CD inclusion complex solution, there were no significant difference in the V value between Groups 1 through 3 (p>0.05) and the Cl values remained unchanged for Groups 2 and 3 (p>0.05). In contrast, Cl value was significantly lower (11.6±1.6 L h kg^{-1}) in Group 1 than in Groups 2 and 3 (p<0.05). Based on these observations, it can be hypothesized that at higher dose (Group 1: 25 mg kg^{-1}), it is probable that elimination kinetics may become saturated and non-linear, which can invariably lead to higher resveratrol concentration in plasma. For this reason, AUC (Group 2) was chosen to determine the bioavailability of orally administered resveratrol.

Pharmacokinetics After Oral Administration

The plasma pharmacokinetic profiles and the pharmacokinetic parameters following single oral dosing of resveratrol–RM-β-CD complexes (15, 25 and 50 mg kg^{-1}) and resveratrol suspensions (15, 25 and 50 mg kg^{-1}) are shown in Fig. 4 and Table II, respectively. In all cases, formulating resveratrol in RM-β-CD resulted in a significant (p<0.05) higher maximal concentration (C_{max}) than the suspension formulations. However, there were no significant differences (p>0.05) in bioavailability (F value) between the suspension and solution formulations at all doses (Table II). Within each formulation, a significantly different C_{max} was observed between those of 50 and 25 mg kg^{-1} doses, 50 and 15 mg kg^{-1} doses (p<0.05), but not between 25 and 15 mg kg^{-1} doses (p>0.05) (Table II). T_{max} was not significantly different between solution and suspension formulations (p>0.05) at 15 and 25 mg kg^{-1} doses, but at 50 mg kg^{-1} dose, T_{max} was significantly different (p< 0.05) between solution and suspension formulations (Table II). The higher T_{max} seen in Group 8 (between 60 and 90 min) implies a delayed absorption of resveratrol. These findings indicated that the dose and the dosage form of resveratrol can affect the rate, but not the extent of its oral absorption.

DISCUSSION

In this study, we described the preparation of CD complexes with resveratrol and evaluated the pharmacokinetics and bioavailability of resveratrol in Sprague–Dawley rats following intravenous and oral administration of resveratrol. Our objective was to determine whether increased solubility of resveratrol due to complexation with CDs, and dose escalation, which may theoretically reduce

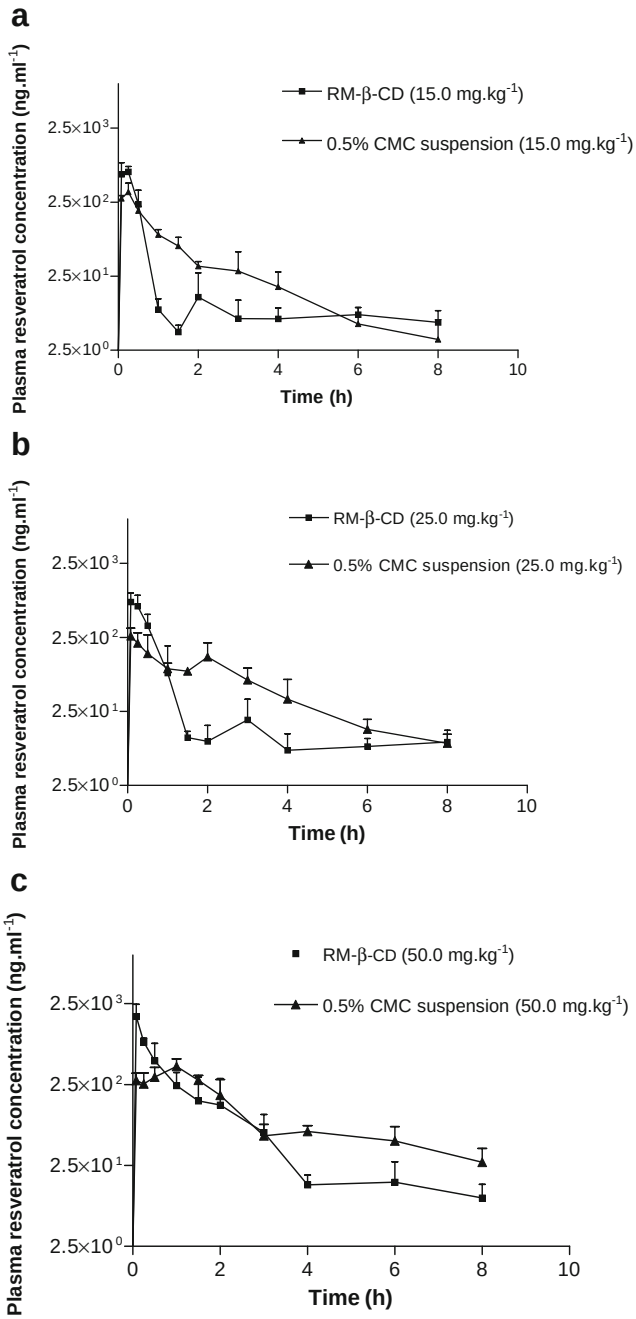

Fig. 4. Pharmacokinetics of resveratrol after a single oral dose. **a** 15 mg kg^{-1}; **b** 25 mg kg^{-1}; **c** 50 mg kg^{-1}. The *line* represents the predicted values. *Symbols* represent the mean observed values (n=4) ± SD.

metabolism of resveratrol, could lead to increased bioavailability of resveratrol.

Formulation with HP-β-CD significantly increased the aqueous solubility of resveratrol (~59,500 fold increase). Despite such increase, no statistical differences in pharmacokinetic parameters between sodium resveratrol (Group 4) and HP-β-CD–resveratrol (Group 2) after intravenous administration were found (Table I). These observations are consistent with previous findings with retinoic acids (21, 22) and miconazole (26) that HP-β-CD did not exert significant impact on intravenous pharmacokinetic of poorly soluble drugs. After intravenous administration, the major driving force for dissociation of weakly to moderately bound drugs from the cyclodextrin inclusion complex is simple dilution and the drug release is rapid (in fraction of seconds) and quantitative in most cases (27). From the phase solubility study, a moderate inclusion stability constant (3.17×10^5 M^{-1}) was found between resveratrol and HP-β-CD. This value is close to the reported binding constant between resveratrol and human serum albumin (1.76×10^5 L M^{-1}) (28). Hence, upon administration it is expected that resveratrol would rapidly dissociate from HP-β-CD and bind to the plasma proteins, and explain why HP-β-CD would increase the aqueous solubility of resveratrol but had no effect on its intravenous pharmacokinetic profiles (*V*, Cl, and AUC, Group 2 *vs*. Group 4, Table I).

Although many studies have looked at various aspects of resveratrol pharmacokinetics, our study is the first to demonstrate dose-dependent elimination of resveratrol *in vivo*. At higher intravenous resveratrol dose (Group 1, 25 mg kg^{-1}), a significant lower Cl and higher AUC were observed in animals compared to those given lower dose of resveratrol (Groups 2 and 3) (Table I). It is plausible at high intravenous dose, elimination process for resveratrol may be saturated, which implies that dose escalation beyond its metabolic threshold may increase the plasma levels of resveratrol. It is noteworthy to mention irrespective of intravenous doses and dosage forms, a second peak appeared at 2–4 h after the rapid initial decline of the plasma concentration of resveratrol. The appearance of a secondary peak is consistent with the findings of an earlier study (14) and is probably due to enterohepatic recirculation of resveratrol. As expected, the extra dissolution step causes the suspension formulations to display a more sustained plasma resveratrol concentration profile than solution formulations, which decreased rapidly upon administration. Hence, suspension formulation can be a useful mean to maintain resveratrol plasma concentration above minimum effective concentration (MEC) for longer time than solution formulation.

Oral bioavailability of small molecular drugs is generally believed to be determined by the aqueous solubility, membrane permeability, and metabolic stability of the given drugs (29). Previous works imply that poor oral bioavailability of resveratrol may not be a membrane permeability issue (7, 11, 12). Our present results did not suggest poor aqueous solubility was responsible for poor oral bioavailability of resveratrol as we did not find any significant differences in bioavailability of all dosages between resveratrol–RM-β-CD solutions and resveratrol suspension in 0.5% CMC (Table I). However, initial plasma resveratrol concentration and C_{max} values of RM-β-CD formulations were much higher than the corresponding doses of suspension formulations (4.1, 3.2 and 2 times higher in RM-β-CD formulations than suspension formulations at 50, 25 and 15 mg kg^{-1} dosages, respectively). These results indicated that aqueous solubility might play a crucial role on the oral absorption rate of resveratrol but it might not affect its extent of absorption. The present findings raise a concern that resveratrol might be crashing out of the complexes following immediate dilution in the GI tract, thus explaining why the enhanced solubility *via* cyclodextrin complexation did not result in increased oral bioavailability of resveratrol. While seem logical, the poor solubility of resveratrol in aqueous environment coupled with its rapid occurrence in the plasma will refute such a possibility (Fig. 4). Alternatively, it is possible that solubility of resveratrol is pH-dependent; hence its absorption from the GI tract will no longer be just an issue of solubility but a pH concern as well. To address this concern, we conducted solubility experiments utilizing simulated gastric and intestinal fluids (SGF and SIF). Although our data clearly indicated a pH-dependent effect (soluble in neutral SIF but almost insoluble in acidic SGF), the results are not enough to explain why bioavailability of resveratrol did not increase with enhanced solubility. This is because regardless of doses, all dosage forms face the same GI environment, and, therefore, same release and solubility conditions. While the mechanism responsible for our peculiar observation remains to be elucidated, we speculate a rapid and extensive metabolism of resveratrol (both at the intestinal and hepatic levels) (6–8, 11, 12, 14, 15, 30–32) at the studied doses may underscore the present results.

Alexandra *et al.* recently demonstrated a concentration-dependent biotransformation of resveratrol to its metabolites (mono-glucuronides and mono-sulfate) in Caco-2 cells (12). At high dose (200 μM), biotransformation was either inhibited

Table II. Pharmacokinetic Parameters of Resveratrol After Oral Administration

	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Formulation	RM-β-CD	RM-β-CD	RM-β-CD	CMC suspension	CMC suspension	CMC suspension
Dose (mg kg^{-1})	50	25	15	50	25	15
T_{max} (min)	5–15	5–15	5–15	60–90*	5–15	5–15
C_{max} (ng L^{-1})	1.75±0.72**, ***, ****	0.86±0.19#	0.71±0.05##	0.43±0.09###	0.27±0.06	0.36±0.08
AUC (ng h ml^{-1})	1,009.0±186.6	480.1±24.2	351.4±75.2	981.0±49.5	485.3±114.1	352.0±59.1
F (%)	39.9±7.38	38.0±1.91	46.3±9.92	38.8±1.96	38.4±9.02	46.4±7.78

Data is presented as Mean ± SD, $N=4$.
*$p<0.05$ between Group 5 and 8; **$p<0.05$ between Group 5 and 6; ***$p<0.05$ between Group 5 and 7; ****$p<0.05$ between Group 5 and 8; #$p<0.05$ between Group 6 and 9; ##$p<0.05$ between Group 7 and 10; ###$p<0.05$ between Group 8 and 9

or saturated, which resulted in an increase in the total amount of resveratrol transported across the Caco-2 monolayers (12). In the present study, we did not find any evidence to suggest that inhibition or saturation of biotransformation of resveratrol had occurred as there was no significant difference seen in bioavailability among all oral Groups even when the doses of resveratrol were increased (15–50 mg kg^{-1}). One interpretation of the data presented here is that the concentration (200 μM: 45.6×10^3 ng ml^{-1}) that led to metabolic saturation in Caco-2 cells is unlikely to be achievable at *in vivo* level. Assuming one-compartmental elimination kinetics, the concentration at time zero (C_0) or the maximal concentration after receiving intravenous resveratrol (10 mg kg^{-1}) can be calculated as:

$$C_0 = \frac{\text{Dose}}{V} \approx 3.8 \times 10^3 \text{ ng ml}^{-1}$$

In the present study, saturation in elimination did not appear until the intravenous dose was increased from 10 to 25 mg kg^{-1}. The results suggest that the dose that led to metabolic saturation must be greater than 10 mg kg^{-1} and imply that the least concentration that causes saturation of systemic elimination in rats have to be higher than 3.8×10^3 ng ml^{-1}. Unfortunately, such level was not achievable even after oral administration of 50 mg kg^{-1} resveratrol. It is interesting to comment although the local concentration of resveratrol in the intestinal tissue may be higher than that in the peripheral vein, it is still difficult to achieve a local concentration as high as 45.6×10^3 ng ml^{-1}; a concentration which is 25 fold higher than the C_{max} after oral dosing of 50 mg kg^{-1} resveratrol in RM-β-CD formulation. Taken together, the results suggest that dose manipulation may not be a viable and feasible approach to enhance the oral bioavailability of resveratrol.

CONCLUSION

In this study, we have shown that (1) HP-β-CD and RM-β-CD could increase resveratrol solubility to a great extent and HP-β-CD did not change the pharmacokinetic profile of resveratrol after intravenous administration, (2) oral bioavailability of resveratrol was not influenced by the formulation types (solution and suspension), although the suspended dosage form (CMC suspension) showed a lower C_{max} and a plasma concentration that is more sustained than the solution dosage form (RM-β-CD), (3) dose escalation (15–50 mg kg^{-1}) did not influence the oral bioavailability of resveratrol, although non-linear elimination was observed at 25 mg kg^{-1} intravenous dose. In spite of extensive metabolism of resveratrol in body, several studies in rodents and human indicated *in vivo* pharmacological activity of resveratrol. This situation, along with the findings of the present study, put forward one important question to answer in that it is unclear whether metabolites of resveratrol are also active.

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