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A rapid HPLC method for the quantification of 3,5,4′-trimethoxy-*trans*-stilbene (TMS) in rat plasma and its application in pharmacokinetic study

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ABSTRACT

A rapid HPLC-UV method had been developed and validated to quantify 3,5,4′-trimethoxy-*trans*-stilbene (TMS), a naturally occurring and pharmacologically active analog of resveratrol in rat plasma. The samples were mixed with three volumes of acetonitrile to precipitate protein. Chromatographic separation was achieved on a RP-HPLC column (Agilent ZORBAX Eclipse Plus C18: 250 mm × 4.6 mm i.d., 5 μm), which was protected by a guard column (Agilent ZORBAX Eclipse Plus C18: 12.5 mm × 4.6 mm i.d., 5 μm) through isocratic delivery of a mobile phase of acetonitrile: water (75:25, v/v) at a flow rate of 1.2 ml/min. The assay was executed at 30 °C and the UV absorbance at 320 nm was monitored. The retention time of TMS and *trans*-stilbene (internal standard) was 6.5 and 8.3 min, respectively. The calibration curve was linear within the range of 15–1000 ng/ml ($R^2 > 0.998$) and 15 ng/ml was the lower LOQ. The intra- and inter-day precisions were good and the RSD was all lower than 7.3%. The mean absolute recovery of TMS in plasma ranged from 99.2 to 104.1%. This HPLC method had been successfully applied to study the pharmacokinetics of TMS, which was fully dissolved with hydroxypropyl-β-cyclodextrin (HP-β-CyD). In comparison with resveratrol, TMS had greater plasma exposure, longer elimination half-life and lower clearance. As TMS had superior pharmacokinetic characteristics, its potential as a preventive or therapeutic agent in resveratrol-effective conditions or diseases should be considered.

1. Introduction

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene Fig. 1I) is a polyphenonic compound originally isolated from white hellebore [1]. Later, it is also observed in a variety of edible plants including grapevine, cranberry, blueberry, bilberry and peanut [2–5]. Since the report of the presence of resveratrol in wine in 1992, it was linked to some of the cardio-protective effects of red wine [2]. Resveratrol then attracted great interests and its health-promoting pharmacological effects have been extensively reported [2–7]. Besides cardio-protection, resveratrol is also associated to cancer chemoprevention/therapy, anti-oxidation, anti-diabetes, anti-obesity, anti-ageing and neuro-protection effects [2–5]. However, resveratrol does not appear to be a suitable candidate for further drug development due to its unfavorable pharmacokinetics such as short half-life [8,9], extensive metabolism (glucuronide or sulphate conjugation) [10,11] and low bioavailability [10,12]. Therefore, it is of great interest to identify new analogs of resveratrol with similar or even stronger biological activities but a better pharmacokinetic profile.

3,5,4′-trimethoxy-*trans*-stilbene (TMS, Fig. 1II) is a naturally occurring analog of resveratrol [13,14]. The anti-neoplastic, anti-allergic and anti-angiogenic activities of TMS have been reported recently [15–18]. From the viewpoint of metabolism, TMS may be more favorable than resveratrol because all of its hydroxyl groups, which are subjected to extensive glucuronide or sulphate conjugation in the metabolic pathways of resveratrol are protected by methoxylation. Moreover, methoxylation increases lipophilicity and may enhance cell membrane permeability. Hence, improved pharmacokinetic profile, i.e. longer elimination half-life and higher intestinal membrane permeation could be postulated. On the other hand, methoxylation decreases aqueous solubility and could hinder the oral bioavailability of TMS. Therefore, it is of interest to assess the pharmacokinetics of TMS in a pre-clinical model and compare its kinetic profile with resveratrol. A reliable method for the quantification of TMS in rat plasma would facilitate such pharmacokinetic study.

Recently, Ma et al. developed HPLC-UV methods to determine TMS in rat plasma and applied them to assess the pharmacokinetics after oral administration [19,20]. However, the intravenous pharmacokinetics was not attempted. Hence, many important kinetic parameters, including clearance, elimination half-life and absolute bioavailability remained unknown. Furthermore, in the reported methods, the elution time gap between the internal standards and

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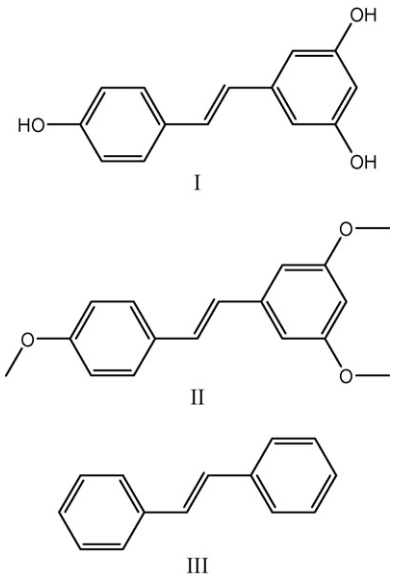

Fig. 1. Chemical structures of resveratrol (I), 3,5,4′-trimethoxy-*trans*-stilbene (II, TMS) and *trans*-stilbene (III, internal standard).

the huge background interferences from plasma was very narrow (1 or 1.5 min) [19,20]. Therefore, the risk for the polar metabolites of TMS to co-elute with internal standards cannot be neglected especially when these metabolites are at high concentration. In this study, a non-polar internal standard was used and the chromatographic condition was re-optimized. This HPLC-UV method was subsequently applied in a pharmacokinetic study to quantify TMS in plasma samples collected from Sprague–Dawley rats after single intravenous or oral administration. To the authors' knowledge, this is the first study to assess the intravenous pharmacokinetic profile and measure the absolute bioavailability of TMS. A comparison of the pharmacokinetic parameters between resveratrol and TMS was also carried out.

2. Experimental

2.1. Special precautions

All laboratory procedures involving the manipulations of TMS and *trans*-stilbene were executed in a dimly lit environment to prevent photo-isomerization.

2.2. Chemicals and reagents

3,5,4′-trimethoxy-*trans*-stilbene (TMS, purity ≥97%) and *trans*-stilbene (purity 96%) were purchased from Alexis Biochemicals (San Diego, CA 92121, USA) and Sigma–Aldrich (St. Louis, MO 63178, USA), respectively. Hydroxypropyl-β-cyclodextrin (HP-β-CyD) (degree of substitution: 0.6) was kindly donated by Roquette Freres S.A. (Lestrem, France). Analytical grade DMSO was from MP Biomedicals (Solon, OH 44139, USA). HPLC grade acetonitrile and methanol were from Fisher Scientific (Fair Lawn, NJ 07410, USA). Purified water (18.2 MΩ cm at 25 °C) was generated from a Millipore Direct-Q® ultra-pure water system (Billerica, MA 01821, USA) and used throughout this study.

2.3. Instrumentation

A Shimadzu (Kyoto, Japan) 2010A liquid chromatography, which comprises a quaternary gradient low-pressure mixing pump, an online degasser, an auto-sampler, a column oven, a dual-wavelength UV–Vis detector and a system controller was used to establish the HPLC analysis. The HPLC system was controlled by the software of Class-VP Version 6.12 SP1 (Shimadzu, Kyoto, Japan). Chromatographic data analysis was also executed with the same software.

2.4. Chromatographic conditions

The chromatographic separation was achieved on a RP-HPLC column (Agilent ZORBAX Eclipse Plus C18: 250 mm × 4.6 mm i.d., 5 μm), which was protected by a guard column (Agilent ZORBAX Eclipse Plus C18: 12.5 mm × 4.6 mm i.d., 5 μm) through isocratic delivery of a mobile phase of acetonitrile-water (75:25, v/v) at a flow rate of 1.2 ml/min at 30 °C. UV absorbance at both 320 and 303 nm was recorded but only the data acquired at 320 nm was used for the assay.

2.5. Sample preparation

Stock solutions of TMS and *trans*-stilbene (internal standard) were prepared in DMSO to obtain a final concentration of 1 mg/ml weekly. Such stock solutions were stored at room temperature (24 °C) and protected from light. The calibration standards for rat plasma were prepared through serial dilution of the TMS stock with pooled blank rat plasma. Three volumes of acetonitrile (internal standard concentration = 100 ng/ml) was added to one volume of rat plasma to precipitate proteins. After vigorous vortexing, the samples were centrifuged at 10,000 × *g* for 10 min at 4 °C. Finally, the supernatant was carefully transferred to a glass insert that was placed into an auto-sampler vial. 40 μl supernatant was injected into the HPLC during analyses. The minimal amount of plasma required for an analysis was 25 μl.

2.6. Method validation

This HPLC method was validated with regards its selectivity, linearity, precision (intra- and inter-day), accuracy, sensitivity and absolute recovery.

Selectivity was confirmed by comparing the chromatograms obtained from three types of plasma samples, namely drug-free samples collected from six individual rats, such samples spiked with TMS and internal standard, as well as samples obtained from the actual pharmacokinetic study.

The ratio between the peak area of TMS and that of *trans*-stilbene (internal standard) was adopted as the analytical response. Linear regression was executed using GraphPad Prism Version 5.01 (La Jolla, CA 92037, USA) via least sum-of-squares method, where x was the concentration of TMS, y was the analytical response, and $1/x^2$ was used as a weighting factor. The calibration standards of the following concentrations 15, 50, 100, 250, 500, 750, 1000 ng/ml were used to assess linearity. During the quantification of actual samples from pharmacokinetic study, the samples with TMS concentration >1000 ng/ml would be diluted with blank plasma till within the calibration range.

The sensitivity of this method was assessed by the determination of LOD and lower LOQ. A signal to noise ratio equal to 3 was defined as LOD while a signal to noise ratio equal to 10 was defined as lower LOQ.

To evaluate the intra- and inter-day precision, five replicates of calibration standards were analyzed on the same day and on 5 different days.

To evaluate the accuracy of this assay, the analytical recovery of the calibration standards (both intra- and inter-day) were calcu-

lated as:

$$\text{Analytical recovery}\,(\%) = \frac{\text{TMS detected}}{\text{TMS spiked}} \times 100\%$$

The analytical recovery of another set of independent reference standards with 45, 200 and 800 ng/ml TMS spiked was also calculated.

The absolute recovery of TMS was assessed at these concentrations: 15, 45, 200, 800 and 1000 ng/ml. The absolute recovery was calculated through this equation:

$$\text{Absolute recovery}\,(\%) = \frac{\text{Peak area of TMS in spiked plasma sample}}{\text{Peak area of TMS in acetonitrile}} \times 100\%$$

2.7. Preparation of dosing solution

The TMS-HP-β-CyD inclusion complex solution was prepared according to the following. 100 mg of TMS was suspended in 20 ml of 0.3 M HP-β-CyD solution prepared with isotonic saline. The resultant suspension was sonicated for 1 h and then shaken on a horizontal rotary shaker for 1 day. Finally, the suspension was filtered through a 0.22 μm syringe-driven filter (Millipore, Billerica, MA 01821, USA). The TMS-HP-β-CyD solution was diluted for 100 times and 10 μl was injected into the HPLC to determine the TMS concentration. The calibration range was 5–50 μg/ml. The TMS-HP-β-CyD inclusion complex solution was diluted to 2 mg/ml and used for the pharmacokinetic study. The procedure for the preparation of TMS-HP-β-CyD inclusion complex was modified from previous publications [21,22].

2.8. Animals

This pharmacokinetic study was performed according to the guidelines for the humane use of animals in scientific research. The animal experimental protocol was modified from a previous study [23] and approved by the Institutional Animal Care and Use Committee of the National University of Singapore (NUS). Adult male Sprague–Dawley rats (290–310 g) were obtained from the Laboratory Animal Center of NUS. The rats were maintained on a 12-h light/dark cycle. Food and water was supplied freely through out the study. On the day before the pharmacokinetic study, a polyethylene tube (i.d. 0.58 mm, o.d. 0.965 mm, Becton Dickinson, Sparks, MD 21152, USA) was placed into the right jugular vein under anesthesia. This catheter was used for intravenous drug administration as well as for blood sample collection. The rats were randomly divided into two groups. Group 1 ($n = 3$) received a single bolus intravenous administration of TMS at the dose of 4 mg/kg, serial blood samples were collected before dosing and at 5, 10, 20, 40, 60, 90, 120, 180, 240, 360, 480 and 720 min after intravenous injection. Group 2 received a single oral administration of TMS at the dose of 10 mg/kg through oral gavage, serial blood samples were collected before dosing and at 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 480, 600 and 720 min after oral administration. After each intravenous injection or blood sampling, 0.2 ml heparin-saline (10 I.U./ml) was used to flush the catheter. The blood samples were centrifuged at 1000 × *g* at 4 °C for 10 min, and the plasma was collected and stored at −80 °C until HPLC analysis. The stability of TMS in plasma under frozen condition has been confirmed [19].

2.9. Pharmacokinetic calculation

Both compartmental and non-compartmental parameters were calculated with the software of WinNonlin standard version 1.0 (Scientific Consulting Inc., Apex, NC 27502, USA). The area under the plasma concentration versus time curve ($AUC_{0\rightarrow 12\,h}$) in rats that received oral administration (Group 2) was calculated via the trapezoidal rule with the time points from 0 to 720 min, whereas the AUC in rats that received intravenous dosing (Group 1) was calculated through the same rule with the exception of using the logarithmic scale. Clearance (Cl), mean transit time (MTT) and terminal elimination half-life ($t_{1/2\lambda z}$) was also calculated by the non-compartmental method. The absolute bioavailability (F) of TMS was calculated as:

$$F\,(\%) = \frac{\left[AUC_{0\rightarrow 12\,h}(\text{Group 2})/10\ \text{mg/kg}\right]}{\left[AUC_{0\rightarrow 12\,h}(\text{Group 1})/4\ \text{mg/kg}\right]} \times 100\%.$$

Since a rapid distribution phase followed by a prolonged terminal elimination phase was observed after intravenous dosing in all of the tested rats, the plasma TMS concentration–time data was fitted into the classical two-compartment first-order open model ($C = A{\cdot}e^{-\alpha\cdot t} + B{\cdot}e^{-\beta\cdot t}$) using non-linear least squares curve fitting with a weighting factor of $1/y^2$ as described previously [23,24].

3. Results and discussion

3.1. Selection of chromatographic condition

The aim of this study was to establish a rapid method to quantify TMS in rat plasma. Therefore, the first priority was to achieve a complete separation of TMS and *trans*-stilbene from endogenous interference with a reasonable elution time. Different from resveratrol, TMS does not have any ionizable group. To minimize the complexity of the mobile phase, buffer solution or pH adjustment was not applied. Three types of RP-HPLC columns, namely Waters xBridge (250 mm × 4.6 mm i.d., 5 μm), Agilent ODS Hypersil (250 mm × 4 mm i.d., 5 μm) and Agilent ZORBAX Eclipse Plus C18 (250 × 4.6 mm i.d., 5 μm) were tried. Based on the separation efficacy, ZORBAX Eclipse Plus C18 column was selected to establish this HPLC assay. The maximal injection volume was found to be 40 μl. Any injection volume of TMS (1000 ng/ml) greater than 40 μl would lead to the appearance of unsymmetrical peaks. To increase the assay sensitivity, 40 μl supernatant was injected into the HPLC system during each assay.

3.2. Method validation

The specificity of this simple HPLC method was confirmed. Under the above-mentioned chromatographic conditions, the retention time for TMS and internal standard was 6.5 and 8.3 min, respectively. As evident from the typical chromatograms of a blank rat plasma sample, a blank rat plasma spiked with TMS (100 ng/ml) and internal standard (300 ng/ml), a plasma sample collected 2 h after intravenous injection of TMS (4 mg/kg), and a plasma sample collected 3 h after oral administration of TMS (10 mg/kg), TMS and internal standard were well separated and no significant endogenous interference from rat plasma was observed (Fig. 2). Polar metabolites (Fig. 2 peaks 3 and 4) were also observed but not identified.

Both intra- and inter-day calibration curves (15–1000 ng/ml) were linear with a correlation coefficient (R^2) larger than 0.998. The LOD (signal to noise ratio = 3/1) was about 5 ng/ml while the lower LOQ (signal to noise ratio = 10/1) was 15 ng/ml. The precision and the reproducibility of this assay were also confirmed. The inter- and intra-day RSD of the analytical response of the calibration standards at all concentrations was less than 7.3% (Tables 1 and 2). This method appeared to be fairly accurate. The analytical recovery of the all calibration standards ranged from 97.6 ± 2.7 to 103.3 ± 1.2% while the analytical recovery of the independent reference standards ranged from 95.5 ± 1.7 to 101.8 ± 1.5% (Tables 1–3). The

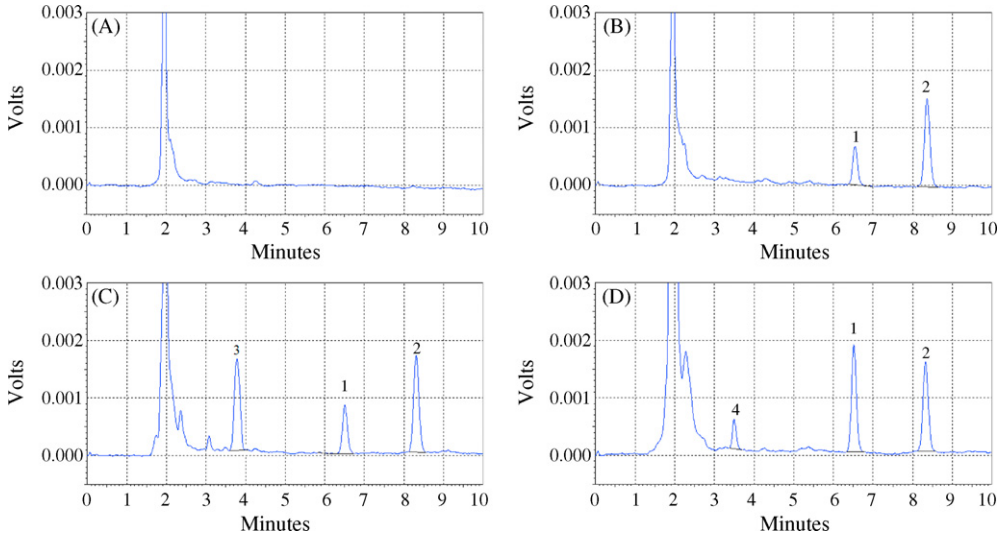

Fig. 2. Chromatograms of (A) a blank plasma sample, (B) a blank plasma sample spiked with TMS (100 ng/ml) and *trans*-stilbene (internal standard) (300 ng/ml), (C) a plasma sample from a rat 2 h after receiving an intravenous dose of TMS (4 mg/kg), (D) a plasma sample from a rat 3 h after receiving an oral dose of TMS (10 mg/kg). Peak 1, TMS; peak 2, internal standard; peak 3 and 4, unidentified metabolites of TMS.

Table 1
Intra-day precision and accuracy.

TMS concentration (ng/ml)	Analytical response	RSD (%)	Analytical recovery (%)
15	0.0464 ± 0.0032	6.9	100.7 ± 5.4
50	0.1782 ± 0.0103	5.8	96.4 ± 5.2
100	0.3849 ± 0.0113	2.9	100.0 ± 2.8
250	1.0082 ± 0.0252	2.5	102.6 ± 2.5
500	1.9498 ± 0.0493	2.5	98.5 ± 2.5
750	2.9546 ± 0.0581	2.0	99.3 ± 1.9
1000	4.1038 ± 0.0488	1.2	103.3 ± 1.2

Results were presented as mean ± SD ($n = 5$). Equation: $y = 0.003995x - 0.01414$, $R^2 = 0.9988$.

absolute recovery of TMS at 15 and 1000 ng/ml was 99.0 ± 6.1 and 102.1 ± 0.8%, respectively. Similar recovery was also observed with the independent reference standards (Table 3).

Ma et al. also reported HPLC-UV methods for the measurement of TMS in rat plasma recently [19,20]. The sensitivity of the current method (LOQ = 15 ng/ml) was slightly better than the reported method (LOQ = 25 ng/ml) [19] while the precision, accuracy, absolute recovery and analytical time were similar. However, in those methods, either chlorzoxazone or diethylstilbestrol was used as internal standards [19,20]. These compounds did not appear to be ideal internal standard for TMS. Structurally, chlorzoxazone is not related with TMS. Furthermore, both chlorzoxazone and diethylstilbestrol eluted from the RP-HPLC system before TMS and the elution time gap between these internal standards and the huge background interferences from plasma was very narrow (1 or 1.5 min) [19,20]. As observed in this study, the polar metabolites of TMS were present in the plasma at ultra-high levels within 1 h after intravenous administration. These metabolites eluted from the RP-HPLC system fairly early. Therefore, the risk for these metabolites of TMS to co-elute with chlorzoxazone or diethylstilbestrol cannot be neglected especially when these metabolites are at high concentration. The application of *trans*-stilbene as internal standard appeared to be a better choice as it is structurally related with TMS but less polar. So, under RP-HPLC system, the retention time of *trans*-stilbene would be longer than that of TMS. This minimizes the chance of co-elution with the polar metabolites.

In summary, a rapid HPLC method to quantify TMS in rat plasma was developed. As this HPLC protocol had good linearity, sensitivity, precision, reproducibility and accuracy, it appeared to be suitable for pharmacokinetic study of TMS.

Table 2
Inter-day reproducibility and accuracy.

TMS concentration (ng/ml)	Analytical response	RSD (%)	Analytical recovery (%)
15	0.0471 ± 0.0035	7.3	100.2 ± 5.7
50	0.1855 ± 0.0109	5.9	99.1 ± 5.4
100	0.3860 ± 0.0148	3.8	99.5 ± 3.7
250	1.0002 ± 0.0208	2.1	101.1 ± 2.1
500	1.9434 ± 0.0538	2.8	97.6 ± 2.7
750	2.9683 ± 0.0596	2.0	99.1 ± 2.0
1000	4.0469 ± 0.0731	1.8	101.2 ± 1.8

Results were presented as mean ± SD ($n = 5$). Equation: $y = 0.003994x - 0.01291$, $R^2 = 0.9995$.

3.3. Pharmacokinetics

This HPLC method was used to quantify the plasma concentration of TMS in rats after single intravenous or oral administration. The pharmacokinetic profiles are shown in Fig. 3 and the major pharmacokinetic parameters are listed in Table 4. The pharmacokinetics of TMS upon intravenous administration had not been studied before. This was probably due to the lack of a suitable dosage form as TMS is insoluble in water. Although DMSO or ethanol can fully dissolve TMS, these solvents are inappropriate dosing vehicles. When TMS solutions prepared with DMSO or ethanol were diluted with isotonic buffer, TMS immediately precipitated (the authors' observation). To overcome the solubility barrier, HP-β-CyD, a parenterally safe excipient was applied to dissolve

Table 3
Absolute recovery and analytical recovery of TMS.

TMS concentration (ng/ml)	Absolute recovery (%)	Analytical recovery (%)
45	101.1 ± 2.7	95.5 ± 1.7
300	104.2 ± 1.8	101.8 ± 1.5
800	99.2 ± 0.8	99.9 ± 1.2

Results were presented as mean ± SD ($n = 5$).

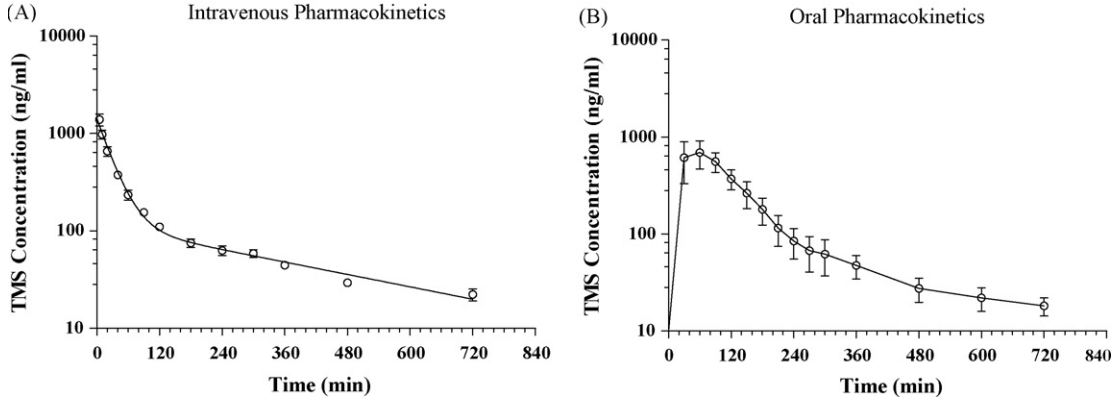

Fig. 3. Plasma pharmacokinetic profiles of TMS in Sprague–Dawley rats (A) after an intravenous administration (4 mg/kg), (B) after an oral administration (10 mg/kg). Symbols represent mean values and error bars represent SD ($n = 3$).

TMS and worked as a dosing vehicle for both intravenous and oral administrations.

After intravenous administration, TMS appeared to be eliminated from the body through a bi-exponential process. Therefore, the plasma TMS concentration versus time data of individual rat was fitted into the classical two-compartment first-order elimination model. The correlation of the fitting of individual rat was excellent ($R = 0.9874$, 0.9906 and 0.9957, respectively), indicating that an appropriate model had been applied. To the authors' knowledge, this is the first time in which the pharmacokinetic profiles of TMS were studied after intravenous administration. TMS appeared to have a fairly long terminal elimination half-life ($t_{1/2\lambda z} = 366 \pm 104$ min) and it still remained at measurable level (>15 ng/ml) 12 h after dosing. Similarly, the clearance was limited (46.5 ± 6.2 ml/min/kg).

After oral administration, TMS fully dissolved with 0.3 M HP-β-CyD was rapidly absorbed and the time to maximal plasma concentration (t_{max}) was not greater than 90 min post dosing. After reaching maximal plasma concentration (C_{max}), plasma TMS concentration declined gradually with a $t_{1/2\lambda z}$ (349 ± 79 min) similar to that observed after intravenous dosing. Furthermore, the MTT of TMS after oral administration was very similar to that after intravenous dosing, also pointing out a short absorption time and a prolonged elimination time. The absolute bioavailability (F) was $54.9 \pm 28.1\%$, indicating TMS was an orally available stilbenoid. Again, it is the first time in which the absolute bioavailability of TMS is measured.

The oral pharmacokinetics of TMS had been attempted in one previous study where Ma et al. administered TMS in 1% carboxymethyl cellulose suspension to rats at an ultra-high dose of 86 mg/kg [19]. However, the kinetic profile of TMS reported by Ma et al. was very different from what was observed in the present study. After oral administration of TMS in suspension form at 86 mg/kg, a delayed absorption was observed with a t_{max} achieved at about 2.5 h. Furthermore, the C_{max} normalized by the dose (C_{max}/Dose) reported by Ma et al. was only about 16 mg/ml; while such value in the present study was 72 ± 37 mg/ml. The differences in pharmacokinetic parameters between these two studies could be explained by the poor aqueous solubility of TMS. The oral absorption of a drug is determined by many different biological and physicochemical factors. Lipophilicity and solubility are the most important physicochemical factors that affect both the extent and the rate of absorption [25]. As the membranes of the gastrointestinal epithelial cells are composed of tightly packed phospholipids interspersed with proteins, the transcellular passage of drugs depends on their permeability characteristics to penetrate the lipid bi-layer of the epithelial cell membrane, which in turn depends on the lipophilicity of the drugs [25]. The lipophilicity required for good intestinal absorption is not a problem in TMS, because it is a member of the stilbenoid family with a cLogP value of 4.6 [26]. However, according to the authors' measurement, the intrinsic solubility of TMS in pure water was less than 15 ng/ml. Therefore, when given in a suspension formulation, most of the dosed TMS was not dissolved and could not be absorbed. The delayed absorption observed by Ma et al. was probably due to the slow dissolution of TMS in intestine, where bile secretion may enhance the solubility of TMS. In the present study, when a solution form of TMS was administered, the solubility was no longer an issue. This resulted in a rapid and complete absorption. It could be further postulated that food could enhance the oral bioavailability of TMS as bile secretion is stimulated by ingestion. However, such a hypothesis needs to be proven in future studies.

Table 4
Pharmacokinetic parameters of TMS.

Parameters	Intravenous	Oral
Dose (mg/kg)	4	10
$AUC_{0\rightarrow 12\,h}$ (min ng/ml)	74707 ± 10063	102498 ± 52414
V_c (ml/kg)	2971 ± 667	–
Cl (ml/min/kg)	46.5 ± 62	–
$t_{1/2\lambda z}$ (min)	366 ± 104	349 ± 79
C_{max} (ng/ml)	–	721 ± 376
t_{max} (min)	–	30 or 90
MTT (min)	144 ± 12	147 ± 9
F (%)	–	54.9 ± 28.1

Results were presented as mean ± SD ($n = 3$).

The pharmacokinetics of resveratrol had been assessed in same animal model recently, allowing a pharmacokinetic comparison to be made between resveratrol and TMS [27]. After intravenous administration of either resveratrol or TMS formulated with HP-β-CyD, a rapid distribution phase was observed. The apparent volume of distribution of the central compartment (V_c) was comparable (resveratrol (5 mg/kg): 2.49 ± 0.72 l/kg; TMS (4 mg/kg): 2.97 ± 0.67 l/kg). However, resveratrol has a very short half-life and 1 h after intravenous injection of resveratrol (5 mg/kg), the plasma resveratrol level dropped to an immeasurable level (<5 ng/ml) [27]. The plasma resveratrol exposure normalized by dose (AUC/Dose) was only 2833 ± 422 min mg/ml with a rapid clearance of 1296 ± 216 ml/min/kg. The elimination of TMS appeared to be much slower and even 12 h after dosing (4 mg/kg), the plasma level was still higher than LOQ (15 ng/ml). Similarly, the AUC/Dose was 19859 ± 1936 min mg/ml with a slow clearance of 46 ± 6 ml/min/kg. When fully solublized randomly methylated-β-cyclodextrin (RM-β-CyD), resveratrol also had an ultra-fast absorption rate (t_{max}: 5–15 min)

[27]. Both resveratrol and TMS had comparable bioavailability in this animal model [27]. After oral administration of resveratrol at 15 mg/kg, the C_{max}/Dose and AUC/Dose were 47 ± 3 mg/ml and 1406 ± 301 min mg/ml, respectively. However, such values were smaller than those values observed with TMS (C_{max}/Dose = 72 ± 37 mg/ml, AUC/Dose = 10250 ± 5241 min mg/ml). In summary of the pharmacokinetic comparisons between resveratrol and TMS, TMS had greater plasma exposure, longer half-life and lower clearance than resveratrol. The pharmacokinetic characteristics of TMS were much more favorable than that of resveratrol.

It had been well documented that enterohepatic circulation plays an important role in the pharmacokinetics of resveratrol and secondary peak usually appears in the plasma resveratrol versus time curve [2,9,27]. However, such phenomenon was not observed with TMS although the impact of enterohepatic circulation remained unclear. Since aqueous solubility played an important role in the oral pharmacokinetics of TMS, future pharmacokinetic studies of TMS should also examine the impact of bile secretion on TMS absorption.

4. Conclusions

A rapid HPLC method for the quantification of TMS in rat plasma had been developed and validated. This reliable method had been successfully applied to assess the pharmacokinetics of TMS formulated with 0.3 M HP-β-CyD. TMS had good oral bioavailability with a fairly long terminal elimination half-life. Since aqueous solubility appeared to be one of the crucial factors that determine the oral absorption of TMS, HP-β-CyD or other CyD that can enhance the solubility of TMS may be suitable excipients to deliver TMS. From the angle of pharmacokinetics, TMS appeared to be a superior analog of resveratrol as it was orally available and had greater plasma exposure, longer elimination half-life and lower clearance. As TMS had superior pharmacokinetic characteristics, its potentials as a preventive or therapeutic agent in resveratrol-effective conditions or diseases should be considered.

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References

[1] M. Takaoka, Nippon Kagaku Kaishi 60 (1939) 1090–1100.
[2] J.A. Baur, D.A. Sinclair, Nat. Rev. Drug Discov. 5 (2006) 493–506.
[3] S. Pervaiz, FASEB J. 17 (2003) 1975–1985.
[4] P. Saiko, A. Szakmary, W. Jaeger, T. Szekeres, Mutat. Res. 658 (2008) 68–94.
[5] G.J. Soleas, E.P. Diamandis, D.M. Goldberg, Clin. Biochem. 30 (1997) 91–113.
[6] P. Kopp, Eur. J. Endocrinol. 138 (1998) 619–620.
[7] M. Siemann, L.L. Creasy, Am. J. Enol. Vitic. 43 (1992) 49–52.
[8] M. Asensi, I. Medina, A. Ortega, J. Carretero, et al., Free Radic. Biol. Med. 33 (2002) 387–398.
[9] J.F. Marier, P. Vachon, A. Gritsas, J. Zhang, et al., J. Pharmacol. Exp. Ther. 302 (2002) 369–373.
[10] T. Walle, F. Hsieh, M.H. DeLegge, J.E. Oatis Jr., U.K. Walle, Drug Metab. Dispos. 32 (2004) 1377–1382.
[11] D.J. Boocock, G.E. Faust, K.R. Patel, A.M. Schinas, et al., Cancer Epidemiol. Biomarkers Prev. 16 (2007) 1246–1252.
[12] E. Wenzel, V. Somoza, Mol. Nutr. Food Res. 49 (2005) 472–481.
[13] G.E. Blair, J.M. Cassady, J.E. Robbers, V.E. Tyler, R.F. Raffauf, Phytochemistry 8 (1969) 497–500.
[14] W.D. MacRae, G.H.N. Towers, Phytochemistry 24 (1985) 561–566.
[15] Y. Bader, S. Madlener, S. Strasser, S. Maier, et al., Oncol. Rep. 19 (2008) 801–810.
[16] M. Belleri, D. Ribatti, S. Nicoli, F. Cotelli, et al., Mol. Pharmacol. 67 (2005) 1451–1459.
[17] H. Matsuda, S. Tewtrakul, T. Morikawa, M. Yoshikawa, Bioorg. Med. Chem. 12 (2004) 4871–4876.
[18] M.H. Pan, J.H. Gao, C.S. Lai, Y.J. Wang, et al., Mol. Carcinog. 47 (2008) 184–196.
[19] N. Ma, W.Y. Liu, H.D. Li, X.Y. Jiang, et al., Methods Find. Exp. Clin. Pharmacol. 30 (2008) 215–220.
[20] N. Ma, W.Y. Liu, H.D. Li, X.Y. Jiang, et al., Yao Xue Xue Bao 42 (2007) 1183–1188.
[21] H.S. Lin, S.Y. Chan, K.S. Low, M.L. Shoon, P.C. Ho, J. Pharm. Sci. 89 (2000) 260–267.
[22] H.S. Lin, C.S. Chean, Y.Y. Ng, S.Y. Chan, P.C. Ho, J. Clin. Pharm. Ther. 25 (2000) 265–269.
[23] H.S. Lin, W.W. Leong, J.A. Yang, P. Lee, et al., Int. J. Pharm. 341 (2007) 238–245.
[24] H.S. Lin, A.B. Barua, J.A. Olson, K.S. Low, et al., J. Pharm. Sci. 90 (2001) 2023–2031.
[25] J.H. Lin, A.Y. Lu, Pharmacol. Rev. 49 (1997) 403–449.
[26] SciFinder Scholar, Version 2007, Registry Number: 22255-22-7 (Assessed online on 4 August 2008).
[27] S. Das, H.S. Lin, P.C. Ho, K.Y. Ng, Pharm. Res. 25 (2008) 2593–2600.