Research Article

Pharmacokinetic and safety profile of *trans*-resveratrol in a rising multiple-dose study in healthy volunteers

Luis Almeida[1, 2], Manuel Vaz-da-Silva[1], Amílcar Falcão[3], Eva Soares[1], Raquel Costa[1], Ana I. Loureiro[1], Carlos Fernandes-Lopes[1], José-Francisco Rocha[1], Teresa Nunes[1], Lyndon Wright[1] and Patrício Soares-da-Silva[1, 2]

[1] Department of Research and Development, BIAL – Portela & Co SA, S Mamede do Coronado, Portugal
[2] Institute of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, Portugal
[3] 4Health Consulting, Cantanhede, Portugal

This was a double-blind, randomised, placebo-controlled study to investigate the pharmacokinetics and safety of *trans*-resveratrol. In four groups of ten healthy adult subjects (five males and five females), two subjects were randomized to receive placebo and eight subjects to receive *trans*-resveratrol 25, 50, 100 or 150 mg, six times/day, for thirteen doses. Peak plasma concentrations of *trans*-resveratrol were reached at 0.8 – 1.5 h postdose. Following the 13th dose of *trans*-resveratrol 25, 50, 100 and 150 mg, mean peak plasma concentration (C_{max}) was 3.89, 7.39, 23.1 and 63.8 ng/mL and mean area under the plasma concentration – time curve ($AUC_{0-\tau}$) was 3.1, 11.2, 33.0 and 78.9 ng · h/mL. Interindividual variability was high, with coefficients of variation >40%. *Trans*-resveratrol half-life was 1 – 3 h following single-doses and 2 – 5 h following repeated dosing. Trough (C_{min}) concentrations were ≤1 ng/mL following 25 and 50 mg, 3 ng/mL following 100 mg and <10 ng/mL following 150 mg. *Trans*-resveratrol pharmacokinetics showed circadian variation. Adverse events were mild in severity and similar between all groups. In conclusion, repeated administration was well-tolerated but produced relatively low plasma concentrations of *trans*-resveratrol, despite the high doses and short dosing interval used. Bioavailability was higher after morning administration.

Keywords: Bioavailability / Healthy subjects / Pharmacokinetics / Safety / *Trans*-resveratrol

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1 Introduction

Trans-resveratrol (*trans*-3,4′,5-trihydroxystilbene, CAS name 5-((1*E*)-2-(4-hydroxyphenyl)ethenyl)-1,3-benzenediol) is a nonflavonoid polyphenolic compound found in several plant species, a number of which are part of the human diet such as mulberries, peanuts, grapes and red wines [1, 2]. Especially in the last decade, *trans*-resveratrol has been the focus of a number of studies investigating its beneficial effects on health. Evidence has accumulated that *trans*-resveratrol acts as a free radical scavenger and a potent antioxidant, promotes nitric oxide production, increases HDL cholesterol and inhibits platelet aggregation and the oxidation of low-density lipoproteins [2, 3]. These biological effects may be cardioprotective and contribute to the phenomenon known as the 'French paradox': a decreased incidence of cardiovascular diseases in moderate consumers of red wines despite an intake of a high-fat diet [4, 5].

A recent focus of attention has been the potential chemopreventive and therapeutic effects of *trans*-resveratrol against cancer [6]. It has been demonstrated that *trans*-resveratrol is active against all three major stages of carcinogenesis (initiation, promotion and progression) [7]. In addition to its use in cancer chemoprevention, it has been suggested to use *trans*-resveratrol in combination with chemotherapeutic or cytotoxic drugs [8, 9]. Neuroprotective properties of *trans*-resveratrol have also been demonstrated [5], and some data suggest a modulating role in multiple mechanisms of Alzheimer's disease pathology [10] and a potential beneficial effect in diabetic neuropathy [11]. Finally, *trans*-resveratrol has been shown to extend the life-

Correspondence: Professor Patrício Soares-da-Silva, Department of Research & Development, BIAL, À Av. da Siderurgia Nacional, 4745-457 S. Mamede do Coronado, Portugal
E-mail: psoares.silva@bial.com
Fax: +351-22-9866192

Abbreviations: AUC, area under the plasma concentration – time curve; **ECG,** electrocardiogram; LLOQ, lower limit of quantification

span of several species, evidencing its potential as an anti-ageing agent [1].

Trans-resveratrol is an ingredient of some foods and of dietary supplements and herbal medicines with large consumption worldwide. However, despite its actual use as dietary supplement and its potential therapeutic interest, few formal clinical trials have been performed and only limited data on the kinetics of *trans*-resveratrol have been published [1] and were obtained mainly in single dose studies [12 – 15]. Therefore, further investigation into the pharmacokinetics, tolerability and safety of *trans*-resveratrol in multiple-doses in man was warranted.

In a previous study by Boocock *et al*. [13], the authors found *trans*-resveratrol bioavailability following oral administration of single doses of 500 mg, 1 g, 2.5 g and 5 g. However, remains to be determined if repeated dosing can achieve higher systemic availability of *trans*-resveratrol. In the present work we describe the results of a study aiming to evaluate the pharmacokinetics and safety of multiple dose regimens of *trans*-resveratrol 150, 300, 600 and 900 mg/day in healthy volunteers.

2 Methods

2.1 Study design and ethics compliance

This was a single-centre, phase I, double-blind, randomised, placebo-controlled study investigating four multiple rising oral doses of *trans*-resveratrol in four groups of ten young adult volunteers (five males and five females). Within each group, two subjects (one male and one female) were randomised to receive placebo and the remaining eight subjects to receive *trans*-resveratrol. The groups were studied sequentially in ascending order of dose and a timely review and evaluation of preliminary safety and pharmacokinetic data was performed before proceeding to a higher dose level.

Subjects received multiple doses of *trans*-resveratrol or matched placebo in the form of oral capsules. The dosing regimen consisted of *trans*-resveratrol 25 mg/placebo in group 1, 50 mg/placebo in group 2, 100 mg/placebo in group 3 and 150 mg/placebo in group 4, administered at 4 h intervals (6 times/day), for 48 h (13 doses, in total). The first dose was administered at approximately 8 h, in the morning of the first day of treatment. The products were given with 240 mL water for doses 1 and 13, and at least 150 mL of water for doses 2 – 12. First dose (dose 1) and last dose (dose 13) were administered after a fast of at least 8 h and subjects remained fasted until 2.5 h postdose. Meals were not served within 1 h before and 1 h after investigational product administration at doses 2 – 12. Drinking of water was allowed as desired except for 1 h before and after doses 1 and 13.

Trans-resveratrol was produced by Abatra Technology (Hi-Tech Development Zone, Xi'an, China) with a purity above 99%. The investigational products were manufactured by BIAL – Portela & Co SA (S. Mamede do Coronado, Portugal) in accordance with good manufacturing practice and were stored in a controlled temperature and humidity environment, protected from light, until the time of administration. The investigational products consisted of opaque hard capsules containing *trans*-resveratrol 25, 50, 100 and 150 mg, and corresponding placebo.

The study was conducted according to the principles of the Declaration of Helsinki and the International Conference on Harmonisation good clinical practice guidelines. An Independent Ethics Committee (Comissão de Ética Independente da UFH, S. Mamede do Coronado, Portugal) reviewed and approved the study protocol and the subject information. Written informed consent was obtained for each subject prior to enrolment in the study.

2.2 Subjects

Healthy male and female volunteers 18 – 45 years of age, nonsmokers or who smoke $\leq$10 cigarettes/day, and with a BMI ranging from 19 to 30 kg/m^2 were eligible for the study. Volunteers were considered to be healthy on the basis of medical history, physical examination, electrocardiogram (ECG), and clinical laboratory safety tests (haematology, plasma biochemistry, urinalysis and hepatitis B, hepatitis C and HIV serology) performed at screening. Subjects were screened between 28 and 7 days of admission. Tests for drugs of abuse and alcohol in urine were performed at screening and admission. Female subjects of childbearing potential had to have a negative pregnancy test at screening and admission and were required to use intrauterine device or double-barrier contraception methods until the poststudy follow-up visit. Subjects who had taken any medication within 2 wk prior to admission were excluded from participation. No concomitant medication was allowed during the study. Volunteers were admitted to the research unit approximately 24 h prior to receiving the first dose of study medication and remained resident in the unit under clinical supervision for at least 24 h after receiving the final dose, after which the subjects were discharged. Similar standard meals were served to all subjects. Meals were served at approximately the middle of the dosing interval because the impact of the presence of food on the *trans*-resveratrol pharmacokinetics was unknown at the time of the study. No foods rich in resveratrol (*e.g.* grapes, peanuts and their products) were served from admission until discharge. Xanthine, alcohol and grapefruit-containing food and beverages were prohibited. From the screening day until the follow-up visit, subjects were requested to abstain from performing strenuous physical exercise.

2.3 Assay of *trans*-resveratrol concentrations in plasma

Blood samples (7 mL) for the assay of plasma *trans*-resveratrol were taken by means of an intravenous catheter into

EDTA K3 Vacutainers at the following times: at predose and ¼, ½, ¾, 1, 1½, 2 and 3 h after the first dose; before doses 2, 3, 4, 5, 7, 8, 9, 10 and 11; at predose and ¼, ½, ¾, 1, 1½, 2, 3, 4, 6, 8, 12, 16 and 24 h after the last dose. After collection, the blood samples were centrifuged at approximately 1500 × *g* for 10 min at 4°C and the resulting plasma was stored at ≤ −70 °C until required for analysis.

Plasma concentrations of *trans*-resveratrol were determined by the Laboratory of Chemistry, BIAL, using an SPE process and an HPLC coupled with mass spectrometric detection (HPLC-MSD). The lower limit of quantification (LLOQ) was 0.5 ng/mL. The method involved the preparation of aliquots of 800 µL of plasma specimens that were placed in a glass tube and vortex-mixed. To each tube, 200 µL of 1 µg/mL of internal standard ((±)-naringenin, chemical name 4′,5,7-trihydroxylflavanone) working solution prepared in ACN were added and vortex-mixed. The samples were then placed on the automatic liquid handler (ASPEC-XL4) for SPE. The SPE cartridges (Oasis, HLB, 30 mg, 1 mL Waters) were conditioned with 1 mL of methanol and then washed twice with 1 mL of Milli-Q water. Specimens (800 µL) were loaded onto the cartridges and the cartridges were washed with 1 mL of Milli-Q water with 5% methanol and 1 mL of Milli-Q water. After the second wash, the cartridges were flushed with air push of 10 mL at 6 mL/min. The samples were eluted twice with 200 µL of methanol/THF (50:50 v/v) and with an air push of 2 mL at 6 mL/min. To the eluted sample, 100 µL of Milli-Q water was added and mixed twice with aspiring/dispensing cycles. The eluted samples (400 µL) were then transferred to glass vials and 5 µL injected into HPLC-MSD. The analysis of plasma samples extracts was performed using HPLC-MSD (Agilent, AP-ESI, 1100 Series, Agilent Technologies) with negative ion detection. Separation was performed on a Zorbax SB-C_{18}, 3.5 µm, 30 × 4.6 mm^2, column (Agilent) using a mobile phase A: water and B: methanol, with gradient conditions of 70% of A and 30% of B at 0 min; 50% of A: 50% of B at 5 min and 20% of A: 80% of B at 8 min. SIM with the detection of each compound of interest was used for quantification of *m*/*z* 227 (*trans*-resveratrol) and *m*/*z* 271 (internal standard). The method was validated in accordance with FDA guidance for industry [16]. For maximal sensitivity, the fragment energy was set to 120 V and further settings were 3500 eV for the capillary voltage, 350 °C nebuliser gas temperature and 40 psi nebuliser pressure. The selectivity, carryover, matrix effect on ionisation, accuracy, precision, linearity, stability and recovery of the method were evaluated. The method was found to be selective and no significant carryover and matrix effect on ionisation was observed. Standard curves were linear over the concentration range of 0.5–100 ng/mL, the intra- and interday CV (precision) was below 10.8% and the relative error (accuracy) ranged from −11.5 to 8.2%. The response factors ranged from 23 353^{-5} to 31 746^{-5} for *trans*-resveratrol and from 0.93 262^{-5} to 12 281^{-5} for the internal standard. *Trans*-resveratrol was found to be stable in human plasma after three freeze/thaw cycles of specimens stored at approximately −80 °C. The recovery was 76.8% for *trans*-resveratrol and 73.0% for the internal standard. Calibration curves over the nominal concentration range 0.5–100 ng/mL and a set of quality control (QC) samples (duplicates over three concentration levels) were analysed with each batch of study samples. Aliquots of QC spiking solutions (20 µL) were dispensed in blank plasma to assess the accuracy of the assay. *Trans*-resveratrol was synthesised in the Laboratory of Chemistry, BIAL, with purity >99.9%. The internal standard ((±)-naringenin) was supplied by Sigma–Aldrich (St. Louis, MO).

2.4 Pharmacokinetic analysis

The following *trans*-resveratrol pharmacokinetic parameters were derived from the individual plasma drug concentration–time profiles using a noncompartmental model and the WinNonlin software (version 4.0, Pharsight, Mountain View, CA): maximum observed plasma *trans*-resveratrol concentration (C_{max}); time of occurrence of C_{max} (t_{max}); area under the plasma concentration–time curve (AUC) from time zero to the last sampling time at which concentrations were at or above the limit of quantification ($AUC_{0\text{-}t}$) and AUC over the dosing interval (4 h) ($AUC_{0\text{-}\tau}$), both calculated by the linear trapezoidal rule; AUC from time zero to infinity ($AUC_{0\text{-}\infty}$), calculated from $AUC_{0\text{-}t} + (C_{last}/\lambda_z)$, where C_{last} is the last quantifiable concentration and λ_z the apparent terminal rate constant; apparent terminal half-life ($t_{1/2}$), calculated from $\ln 2/\lambda_z$; observed degree of accumulation ratio (R_O), calculated from the ratio $AUC_{0\text{-}\tau}$ (dose 13)/$AUC_{0\text{-}\tau}$ (dose 1); and theoretical degree of accumulation (R_T), calculated from $1/(1 - \exp^{-\lambda}z^{t})$, where *t* is the dosing interval. The steady-state was calculated by observation of the evolution of the predose plasma concentration.

Actual sampling times were used for the pharmacokinetic analysis. Special consideration was given to the estimation of λ_z and corresponding $t_{½}$ values. Values of λ_z were calculated from a minimum of three data points. Where an AUC was extrapolated to infinity, the percentage of the extrapolated area to the total area was assessed; if greater than 20%, the AUC value was flagged as unreliable. Plasma concentrations below the LLOQ were taken as zero for all calculations. Summary statistics of all data for each treatment and scheduled sampling time were reported, as appropriate, using the geometric mean, arithmetic mean, SD, CV, SEM, median, minimum and maximum. The statistical package SAS (Version 8.2, SAS Institute, Cary, USA) was used.

2.5 Safety assessments

Safety assessments included medical history, physical examination, vital signs, clinical laboratory analysis, 12-

Table 1. Summary of demographic data ($n = 8$ in each dose group)

Dose group	Variable	Placebo	*Trans*-resveratrol			
			25 mg	50 mg	100 mg	150 mg
Age (years)	Mean ± SD	25.1 ± 3.1	24.3 ± 1.1	23.9 ± 4.8	27.6 ± 8.4	23.5 ± 2.7
	Median (range)	25 (21–30)	24 (22–26)	22 (20–35)	26 (19–43)	23 (20–29)
Gender	Male	4 (50%)	4 (50%)	4 (50%)	4 (50%)	4 (50%)
	Female	4 (50%)	4 (50%)	4 (50%)	4 (50%)	4 (50%)
Race	Caucasian	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
BMI (kg/m^2)	Mean	23.8 ± 2.9	22.4 ± 1.7	23.0 ± 3.4	24.1 ± 3.9	23.5 ± 2.0
	Median (range)	24 (20–28)	22 (21–25)	22 (19–29)	24 (20–32)	23 (21–26)

lead ECG, continuous lead-II ECG and neurological examination.

At admission to the unit, physical examination and medical history were updated. During the study, blood pressure and pulse rate were measured and a brief neurological examination and 12-lead ECG recordings were made at frequent intervals. Continuous lead-II ECG monitoring was performed at 0–4 h after the last dose. Clinical laboratory tests (haematology and plasma biochemistry) were performed at admission and discharge. Seven to 10 days after discharge, a follow-up visit occurred during which subjects had a medical history update and clinical laboratory tests were performed. Haematology tests included haemoglobin, haematocrit, and red blood cell, white blood cell and platelet counts. Plasma biochemistry tests included aspartate amino transferase (AST), alanine amino transferase (ALT), γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine phosphokinase (CPK), creatinine, urea, sodium, potassium, calcium, phosphate, chloride, glucose, albumin, total protein, uric acid and bilirubin.

All clinical adverse events (AEs) were monitored throughout the study period. Their severity (intensity) was categorised according a three-point scale (mild, moderate and severe) and the causality (potential relationship to drug) was assessed by the investigator before breaking the blind. Laboratory test abnormalities considered clinically relevant were reported as AEs.

The biochemistry, haematology, coagulation and urinalysis parameters as well as heart rate, blood pressure and 12-lead ECG parameters were presented in tabular form with mean, median, SD, minimum and maximum. For the clinical laboratory data out of range values were flagged in the data listings. Clinically significant abnormalities regarding vital signs, 12-lead ECG, continuous lead II ECG monitoring, clinical laboratory tests, brief neurological examination and medical history and physical examination updates were reported as AEs. AEs were tabulated and summarised according to the MedDRA and classified by system organ class (SOC).

3 Results

Forty healthy volunteers (20 males and 20 females) participated in the study. The treatment groups were well matched in terms of age, height, weight and BMI (Table 1). All subjects tested negative for drugs and alcohol at screening and on admission. There were no clinically significant abnormalities at screening or at admission concerning laboratory parameters, vital signs and 12-lead ECG measurements. All subjects completed the study through to the final follow-up visit. The randomisation code was not broken before study completion.

3.1 Pharmacokinetics

Mean *trans*-resveratrol plasma concentration–time profiles following first dose (dose 1) and last dose (dose 13) are displayed in Figs. 1A–C. Mean *trans*-resveratrol trough (predose, C_{min}) plasma concentrations from dose 1–13 are shown in Fig. 1D.

Mean pharmacokinetic parameters of *trans*-resveratrol are listed in Table 2. Following the first and last oral doses of *trans*-resveratrol, maximum plasma concentrations (t_{max}) of *trans*-resveratrol were reached within 0.8–1.5 h postdose. Mean apparent terminal half-life ranged from 1 to 3 h following *trans*-resveratrol single-dose and from 2 to 5 h following repeated dosing. The systemic exposure to *trans*-resveratrol following repeated administration of 25, 50, 100 or 150 mg six times/day was assessed by estimating the R_O. Following repeated dosing, the mean R_O ranged from 1.93 (100 mg) to 5.15 (50 mg) (Table 2).

There was an increase in systemic exposure to *trans*-resveratrol with increasing doses of 25–150 mg on both dose 1 (first dose) and dose 13 (last dose). With dose 1, for a dose level increase in the ratio 1.0:2.0:2.0:1.5, $AUC_{0\text{-}\tau}$ values increased in the proportion 1.0:5.3:4.6:1.6 and C_{max} increased in the proportion 1.0:4.5:3.3:1.2 (Table 3). Over the dose range of 25–150 mg, there was a more than dose-proportional increase in $AUC_{0\text{-}\tau}$ and C_{max}: the dose proportionality factor (ratio of x-fold increase in the pharmacoki-

Table 2. Pharmacokinetic parameters of *trans*-resveratrol following the first dose (dose 1) and the final dose (dose 13) of repeated administration of *trans*-resveratrol 25, 50, 100 or 150 mg six times *per* day

Trans-resveratrol pharmacokinetic parameters	Dose regimen of *trans*-resveratrol							
	25 mg, 6 × /day		50 mg, 6 × /day		100 mg, 6 × /day		150 mg, 6 × /day	
	Dose 1	Dose 13	Dose 1	Dose 13	Dose 1	Dose 13	Dose 1	Dose 13
C_{max} (ng/mL) (%CV)	1.48 (40.3)	3.89 (66.4)	6.59 (87.5)	7.39 (62.7)	21.4 (113)	23.1 (74.2)	24.8 (79.4)	63.8 (50.0)
t_{max} (h) (range)	1.0 (0.3 – 4.0)	1.5 (0.8 – 3.0)	0.9 (0.3 – 3.0)	0.8 (0.5 – 3.0)	1.3 (0.5 – 3.0)	1.1 (0.3 – 3.0)	1.3 (0.5 – 4.0)	0.8 (0.5 – 3.0)
$AUC_{0-\tau}$ (ng · h/mL) (%CV)	0.814 (55.7)	3.10 (70.8)	4.27 (65.6)	11.2 (69.9)	19.5 (86.4)	33.0 (60.4)	32.0 (61.2)	78.9 (46.8)
AUC_{0-t} (ng · h/mL) (%CV)	NA	NA	NA	16.6 (112)	NA	50.7 (59.6)	NA	136.0 (43.4)
$AUC_{0-\infty}$ (ng · h/mL) (%CV)	NA	NA	NA	23.2 (85.6)[a]	NA	53.5 (58.2)	NA	141.0 (41.4)
$t_{½}$ (h) (%CV)	2.0 (104)	NA	1.8 (149)[b]	3.2 (51.0)[a]	1.1 (44.8)[a]	2.4 (42.6)	1.9 (72.9)[c]	4.8 (78.9)
R_0 (%CV)	–	5.15 (80.2)[a]	–	3.68 (107)	–	1.93 (49.5)	–	3.50 (77.4)

$n = 8$ *per* dose group, unless otherwise noted. For t_{max}, values are medians with range in parenthesis; for the other pharmacokinetic parameters, values are arithmetic means and coefficients of variation (%CV) in parenthesis. NA = Not assessable.
a) $n = 7$.
b) $n = 4$.
c) $n = 6$.

Table 3. Relationship between the extent of systemic exposure to *trans*-resveratrol with increasing doses of *trans*-resveratrol, after the first (dose 1) and last (dose 13) of repeated oral administration of *trans*-resveratrol 25, 50, 100 or 150 mg six times *per* day ($n = 8$ *per* dose group)

Dose (mg)	Fold increase in dose[a]	C_{max} (ng/mL)	Fold increase in C_{max}[a]	$AUC_{0-\tau}$ (ng · h/mL)	Fold increase in $AUC_{0-\tau}$
Dose 1					
25	1.0	1.48	1.0	0.814	1.0
50	2.0	6.59	4.5	4.27	5.3
100	2.0	21.4	3.3	19.5	4.6
150	1.5	24.8	1.2	32.0	1.6
Overall[b]	6.0		16.8		39.3
DPF[c]	1.0		2.8		6.6
Dose 13					
25	1.0	3.89	1.0	3.10	1.0
50	2.0	7.39	1.9	11.2	3.6
100	2.0	23.1	3.1	33.0	2.9
150	1.5	63.8	2.8	78.9	2.4
Overall[b]	6.0		16.4		25.5
DPF[+]	1.0		2.7		4.3

a) Fold increase in dose or parameters between adjacent doses.
b) Fold increase in dose or parameter over the dose range 25 – 150 mg *trans*-resveratrol.
c) Dose proportionality factor = ratio of fold increase in parameter divided by fold increase in dose.

netic parameter divided by *x*-fold increase in dose) was 2.8 for C_{max} and 6.6 for $AUC_{0-\tau}$ following the first dose, and 2.7 for C_{max} and 4.3 for $AUC_{0-\tau}$ following the last dose (Table 3). Predose plasma *trans*-resveratrol concentrations across doses 1 – 13 suggested that steady state had been reached in all dose groups, at least by dose 7, and are suggestive of diurnal variation in *trans*-resveratrol pharmacokinetics (Fig. 1D and Table 4).

3.2 Safety

A total of 18 treatment-emergent AEs were reported (Table 5). Among them, nine AEs were considered as having a possible relation with treatment: four AEs reported by two subjects (25%) in *trans*-resveratrol 25 mg group, one AE reported by one subject (12.5%) in *trans*-resveratrol 50 mg, one AE reported by one subject (12.5%) in *trans*-resveratrol 100 mg group, and three AEs reported by two subjects (25%) in *trans*-resveratrol 150 mg group. No case of a possibly related AE was reported with placebo. Among the AEs considered possibly related to treatment, the most frequent was frontal headache ($n = 3$): one in the 25 mg, one in the 50 mg and one case in the 150 mg group. The other AEs occurred just once: headache, myalgia of lower extremities and somnolence in the 25 mg group; epididymitis in the 100 mg group and dizziness and occipital headache in the

Table 4. Mean 'trough' concentrations (C_{min}) of *trans*-resveratrol following oral administration of *trans*-resveratrol 25, 50, 100 or 150 mg six times *per* day ($n = 8$ *per* dose group)

Dose group (mg)	C_{min} (ng/mL) Dose (time of administration)										
	1 (8 am)	2 (12 am)	3 (4 pm)	4 (8 pm)	5 (12 pm)	7 (8 am)	8 (12 pm)	9 (4 pm)	10 (8 pm)	11 (12 pm)	13 (8 am)
25	– a)	– a)	– a)	– a)	– a)	1.51	0.613	0.567	– a)	– a)	– a)
50	– a)	– a)	– a)	– a)	1.08	1.39	2.01	1.10	0.876	0.781	0.996
100	– a)	2.22	1.27	3.04	3.69	2.26	5.35	3.64	1.61	2.51	3.19
150	0.588	6.92	2.69	5.19	3.57	9.24	9.63	8.12	8.30	5.84	8.88

a) Below the LLOQ (0.5 ng/mL).

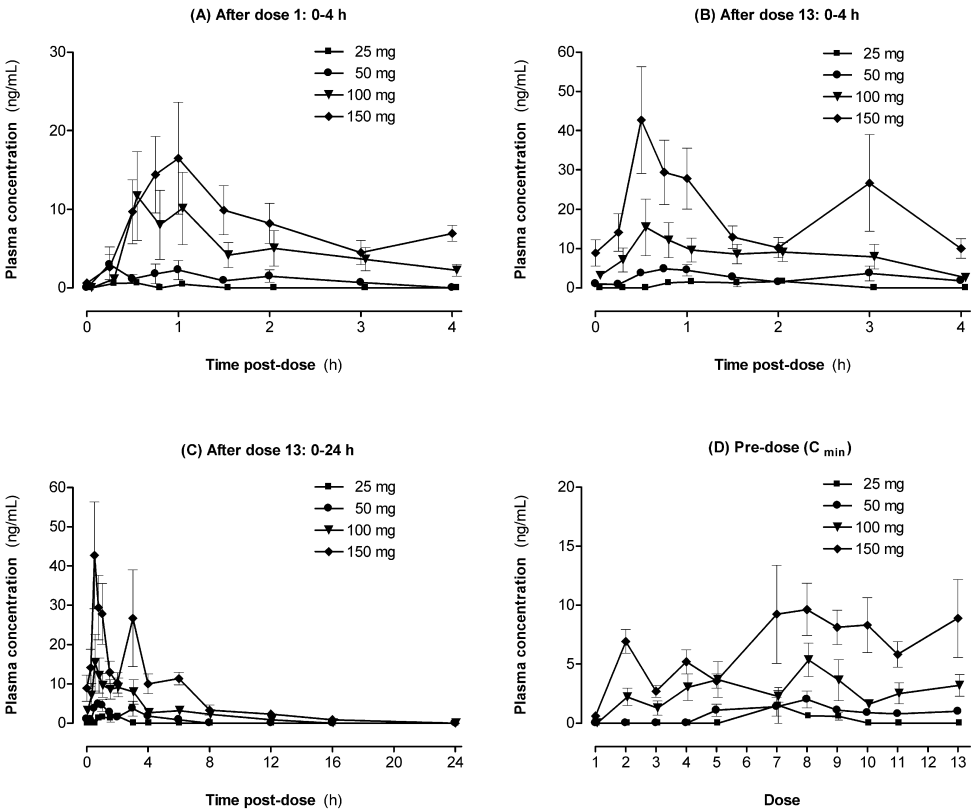

Figure 1. *Trans*-resveratrol plasma concentration-time profiles (mean ± SEM) following oral administration of *trans*-resveratrol 25, 50, 100 or 150 mg at 4 h intervals (six times *per* day): (A) 0 – 4 h profile after the first dose; (B) 0 – 4 h profile after the last dose (dose 13); (C) 0 – 24 h profile after the last dose (dose 13); (D) trough (i. e., predose) concentrations from first to last dose ($n = 8$ *per* dose group).

150 mg group. All clinical AEs were considered to be mild. All *trans*-resveratrol tested doses were well tolerated and no dose-related differences were apparent. No deaths, serious AEs or discontinuations due to AEs were reported.

Except for one case of increased creatinine phosphokinase reported in the follow-up visit and considered not related to treatment, no clinically significant abnormalities were found in the laboratory parameters. No clinically significant abnormalities were reported in the vital signs, neurological examinations or ECG recordings.

4 Discussion

The objectives of this study were to investigate the pharmacokinetics and safety of four multiple-dose oral regimens of *trans*-resveratrol (25, 50, 100 and 150 mg administered six times *per* day) in healthy volunteers. *Trans*-resveratrol was subjectively and objectively well-tolerated by the subjects in this study. All reported AEs were mild in severity and no drug-related abnormalities were found in the clinical laboratory tests, 12-lead ECG parameters, vital signs or neuro-

Table 5. Treatment-emergent AEs in healthy subjects following administration of placebo or *trans*-resveratrol 25, 50, 100 or 150 mg at 4 h intervals

AE	Placebo ($n = 8$)	*Trans*-resveratrol, six times *per* day			
		25 mg ($n = 8$)	50 mg ($n = 8$)	100 mg ($n = 8$)	150 mg ($n = 8$)
Headache	1	2	1	0	2
Dysmenorrhea	1	0	1	0	0
Nasopharyngitis	0	1	0	0	0
Dyspepsia	0	0	1	0	0
Precordial pain	0	0	0	1	0
Lower urinary tract infection	0	1	0	0	0
Creatine phosphokinase increased	0	1	0	0	0
Dorsal pain	1	0	0	0	0
Epididymitis	0	0	0	1	0
Myalgia of lower extremities	0	1	0	0	0
Dizziness	0	0	0	0	1
Somnolence	0	1	0	0	0

logical examination. There were no serious AEs or discontinuations due to AEs. However, it should be taken into account that the treatment was of short duration and therefore the results present low predictive value in regards to adverse effects that can be induced by *trans*-resveratrol on chronic administration.

Oral absorption of *trans*-resveratrol is high but bioavailability is very low [17, 18]. *Trans*-resveratrol is rapidly and extensively biotransformed by first pass metabolism. Identified metabolic pathways include sulphate and glucuronic acid conjugation of the phenol groups, but an extremely rapid sulphate conjugation by the intestine/liver appears to be the rate-limiting step in resveratrol bioavailability [17]. *Trans*-resveratrol sulphates are the dominant conjugates in plasma and urine [14].

Previous studies reported in the literature [13, 17, 19, 20] found poor *trans*-resveratrol bioavailability following single-doses of *trans*-resveratrol ranging from 25 to 5 mg. Walle *et al*. [17] found virtually undetectable resveratrol levels following administration of an oral single dose of 25 mg. Vitaglione *et al*. [20] found that the bioavailability of *trans*-resveratrol following moderate consumption of red wine in fasting or associated to a standard meal high or low in lipids was independent from the meal or its lipid content. In a recent study by our group [19], we have found that a high-fat and high-calorie standard meal significantly delays the rate of absorption of *trans*-resveratrol following administration of an oral 400 mg single dose of *trans*-resveratrol in the form of capsules. Mean ±SD maximum plasma concentrations (C_{max}) were 42.2 ± 36.6 ng/mL in fed and 47.3 ± 30.0 ng/mL in fasting conditions. Median time to C_{max} (t_{max}) was 2.0 h in fed and 0.5 h in fasting ($p < 0.0001$) [19]. However, the extent of absorption, as reflected by $AUC_{0-\infty}$ was not significantly affected (128 ng · h/mL in fasting and 131 ng · h/mL in fed conditions) and therefore it was concluded that *trans*-resveratrol can be administered without regard to meals [19]. In a recent phase I single-dose escalation study by Boocock *et al*. [13], single doses of 0.5, 1, 2.5 and 5 g were tested in groups of ten healthy subjects per dose level. C_{max} of resveratrol at the highest dose was 539 ± 384 ng/mL (2.4 µmol/L) and occurred 1.5 h post-dose. C_{max} of two glucuronides and a sulphate conjugate were three- to eight-fold higher than *trans*-resveratrol C_{max} and their AUC was up to 23 times higher than AUC of *trans*-resveratrol. Urinary excretion was rapid, with 77% of all urinary drug moieties recovered in urine within 4 h after the 500 mg dose.

In our knowledge, the current study was the first evaluation of the pharmacokinetics of *trans*-resveratrol in a multiple-dose regimen in healthy volunteers. Following repeated dosing of *trans*-resveratrol 25, 50, 100 or 150 mg administered at a very short interval (4 h) interval, the degree of observed accumulation (R_O) was only moderate, with mean accumulation ratios ranging from 1.9 (*trans*-resveratrol 100 mg) to 5.2 (*trans*-resveratrol 50 mg). This accumulation may be due to the short dosing interval (4 h) in relation to the $t_{½}$, but should be interpreted with caution because, especially with the lowest doses, the concentrations are close to the LLOQ of *trans*-resveratrol and, consequently, R_O may be overestimated. Systemic exposure to *trans*-resveratrol was low and $t_{½}$ relatively short. Between-subject variability in the pharmacokinetic parameters was relatively high, with average coefficients of variation around 60%. This high interindividual variability is in line with that reported by Boocock *et al*. [13] following single-doses of trans-resveratrol, and is probably due to individual differences in metabolism.

Despite the relatively short interval (4 h, only), C_{min} values were below the LLOQ following most 25 mg doses, and approximately 1 ng/mL following 50 mg, 3 ng/mL following 100 mg and < 10 ng/mL following 150 mg. The profile of C_{min} concentrations is highly suggestive of diurnal variation in the *trans*-resveratrol pharmacokinetics. Circadian variations in absorption, distribution, metabolism or elimi-

nation of drugs may be due to diurnal variations in gastric motility and emptying time, intestinal and hepatic blood flow, drug protein binding, enzyme activity, and renal function [21]. *Trans*-resveratrol C_{min} levels were highest in the morning and tended to decrease along the day, being lowest in the night. The *trans*-resveratrol chronopharmacokinetics can be the result of several factors. *Trans*-resveratrol and its metabolites suffer enterohepatic circulation [22] and enterohepatic circulation is susceptible to circadian variation [23]. *Trans*-resveratrol undergoes significant glucuronidation [14] and it has been reported that glucuronidation activity may vary along the day [24]; significant circadian pharmacokinetic variation has also been reported with other drugs that undergo marked glucuronidation [25]. A diurnal variation at the absorption level can also be speculated.

There was an increase in plasma concentrations of *trans*-resveratrol with increasing doses of 25 – 150 mg on both first dose and last dose. With first dose, for a dose level increase in the ratio 1.0:2.0:2.0:1.5, $AUC_{0\text{-}4}$ and C_{max} values increased in the ratio 1.0:5.3:4.6:1.6 and 1.0:4.5:3.3:1.2, respectively. Over the dose range of 25 – 150 mg, there was an increase more than dose-proportional in $AUC_{0\text{-}4}$ and C_{max} values following the first and last doses: the dose proportionality factor (ratio of fold increase in the pharmacokinetic parameter divided by fold increase in dosage) was 2.8 for C_{max} and 6.6 in $AUC_{0\text{-}4}$ following the first dose and 2.7 for C_{max} and 4.3 in $AUC_{0\text{-}4}$ following the last dose. Therefore, it can be concluded that the extent of systemic exposure to *trans*-resveratrol increased in a more than dose-proportional manner with increasing doses of *trans*-resveratrol. However, in all cases the plasma concentrations of *trans*-resveratrol were markedly below the *in vitro* concentrations required to show pharmacologic effects (>5 μmol/L) [1] and, therefore, even relatively high doses of *trans*-resveratrol administered several times *per* day might be insufficient to produce benefit. It has been hypothesised, however, that although the systemic bioavailability of *trans*-resveratrol is very low, accumulation of resveratrol in epithelial cells along the digestive tract and potentially active resveratrol metabolites may still produce cancer preventive and other effects [13, 17]. Other authors also suggested that, despite the fact that the *trans*-resveratrol glucuronides have been shown to be inactive *in vitro*, they may be active *in vivo*, because the ubiquitously existing human β-glucuronidase could convert such metabolites back to *trans*-resveratrol locally or systemically [26].

In conclusion, repeated oral administration of high daily doses of *trans*-resveratrol was well-tolerated but produced relatively low plasma concentrations of *trans*-resveratrol, despite the relatively high doses and frequent dosing regimen used. Bioavailability was higher when *trans*-resveratrol was administered in the morning.

Conflict of interest statement: Several authors are employees of BIAL – Portela & Co, SA, the sponsor of the study.

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