Phase I Dose Escalation Pharmacokinetic Study in Healthy Volunteers of Resveratrol, a Potential Cancer Chemopreventive Agent

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Abstract

The red grape constituent resveratrol possesses cancer chemopreventive properties in rodents. The hypothesis was tested that, in healthy humans, p.o. administration of resveratrol is safe and results in measurable plasma levels of resveratrol. A phase I study of oral resveratrol (single doses of 0.5, 1, 2.5, or 5 g) was conducted in 10 healthy volunteers per dose level. Resveratrol and its metabolites were identified in plasma and urine by high-performance liquid chromatography-tandem mass spectrometry and quantitated by high-performance liquid chromatography-UV. Consumption of resveratrol did not cause serious adverse events. Resveratrol and six metabolites were recovered from plasma and urine. Peak plasma levels of resveratrol at the highest dose were 539 ± 384 ng/mL (2.4 μmol/L, mean ± SD; $n = 10$), which occurred 1.5 h post-dose. Peak levels of two monoglucuronides and resveratrol-3-sulfate were 3- to 8-fold higher. The area under the plasma concentration curve (AUC) values for resveratrol-3-sulfate and resveratrol monoglucuronides were up to 23 times greater than those of resveratrol. Urinary excretion of resveratrol and its metabolites was rapid, with 77% of all urinary agent-derived species excreted within 4 h after the lowest dose. Cancer chemopreventive effects of resveratrol in cells *in vitro* require levels of at least 5 μmol/L. The results presented here intimate that consumption of high-dose resveratrol might be insufficient to elicit systemic levels commensurate with cancer chemopreventive efficacy. However, the high systemic levels of resveratrol conjugate metabolites suggest that their cancer chemopreventive properties warrant investigation. (Cancer Epidemiol Biomarkers Prev 2007;16(6):1246–52)

Introduction

There is intense interest in the role of nutrition and specific diet constituents in the etiology and prevention of cancer (1). Experiments in animals suggest that dietary phytochemicals like selenium, folate, and the phytoalexin resveratrol (*trans*-3,5,4′-trihydroxy-stilbene; for structure, see Fig. 1), which occurs in grapes, peanuts, and various berries, can prevent certain malignancies or delay their onset. As a constituent of red wine, resveratrol is under scrutiny not only as a putative cancer chemopreventive agent but also because it might account for the ''French Paradox,'' the reduced risk of cardiovascular disease in Southern France, despite high intake of saturated fats, with moderate wine consumption (2). Very recently, resveratrol was shown to protect mice against the detrimental health effects associated with a high-calorie diet (3, 4), and the implications of the results for humans elicited considerable media attention.

Resveratrol interferes with all three stages of carcinogenesis initiation, promotion, and progression (5). Experiments in cells and isolated subcellular systems *in vitro* implicate a multitude of mechanisms in the pharmacologic activity of resveratrol (reviewed in ref. 6). These mechanisms include inhibition of the transcription factor NF-κB (7), cytochrome *P*450 isoenzyme 1A1 (8), androgenic actions (9) and expression and activity of cyclooxygenase enzymes (10), and activation of SIRT1, an enzyme regulating longevity, apoptosis, and DNA repair (11). Resveratrol has been shown to induce Fas/Fas ligand–mediated apoptosis (12), p53 (13), and cyclin A, cyclin B1, and cyclin-dependent kinases 1 and 2 (14). Furthermore, it possesses antioxidant (15, 16) and antiangiogenic properties (17, 18). Resveratrol prevented or delayed the development of esophageal (19) and mammary malignancies (7, 9), colonic aberrant crypt foci (20), and intestinal adenomas in the Apc^{Min} mouse model (21), although the latter effect was subsequently shown to be very weak (10, 22). Resveratrol also suppressed solid hepatoma growth and metastasis in rats, which harbored s.c. implanted hepatoma cells (23).

The resveratrol content of red wine is between 0.2 and 5.8 mg/L (24). As a nutraceutical, resveratrol is commercially available in the United States and Europe at between 50 μg and 60 mg per dosage form. Mechanistic experiments *in vitro* suggest tentatively that a concentration of at least 5 μmol/L resveratrol is required to elicit pharmacologic effects relevant to chemoprevention (5-18). P.o. administration of resveratrol in animals furnished resveratrol levels, which were considerably below this figure, with much higher concentrations of its metabolites, mainly resveratrol 3-sulfate and monoglucuronide conjugates (25). Ingestion by humans of 25 mg resveratrol with white wine or nonalcoholic beverages yielded systemic levels of 7.5 to 40 nmol/L (26, 27). Doses of 25 mg p.o. or 0.2 mg i.v. led to the detection of five metabolites, two resveratrol monoglucuronides, two monosulfates, and two conjugates of a product of aliphatic chain double bond reduction of

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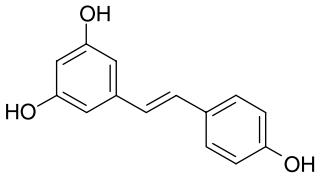

Figure 1. Structure of resveratrol.

resveratrol, and unchanged resveratrol was virtually undetectable (28). In the light of these reports, we wished to explore the feasibility of advancing resveratrol into clinical evaluation as a cancer chemopreventive agent and test the hypothesis that consumption of up to 5 g, much higher doses than those previously given to humans, is safe and furnishes systemic levels of phytochemical commensurate with cancer chemopreventive activity.

Materials and Methods

Resveratrol Formulation. Volunteers received uncoated immediate-release caplets containing 500 mg resveratrol. Caplets were manufactured by Royalmount Pharma using material synthesized under good manufacturing practice. Resveratrol in these caplets was stable under normal conditions (60% relative humidity, 25°C) for the period of time covering the duration of the trial and under conditions of accelerated decomposition (75% relative humidity, 40°C) for at least 180 days.

Study Participants and Inclusion/Exclusion Criteria. Forty healthy were recruited into the study at either the Universities of Leicester (United Kingdom) or Michigan (United States). Volunteer eligibility criteria included ages 18 to 80 years, ability to give written informed consent, willingness to abstain from ingestion of large quantities of resveratrol-containing foods, normal urine analysis, WHO performance status of 0 or 1, and adequate hematologic, hepatic, and renal functions, as characterized by the following criteria measured in blood: neutrophil count, >1,500/mm^3 (Michigan) or >10^9/L (Leicester); platelet count, 120,000 to 450,000/mm^3; hemoglobin concentration, 11.5 to 19.0 g/dL for men, 10.5 to 17.5 g/dL for women; bilirubin level, 0.05 to 1.2 mg/dL (Michigan) or 2.0 to 19.0 μmol/L (Leicester); transaminases (alanine aminotransferase, aspartate aminotransferase), <80 IU/L; creatinine, 0.7 to 1.3 mg/dL (Michigan) or 50 to 140 μmol/L (Leicester). Volunteers remained local while on the study. Exclusion criteria included pregnant or lactating women and women contemplating pregnancy for the duration of the protocol; any chronic medications except for oral or depot contraceptives and hormone replacement therapy; excessive alcohol intake (>210 or 140 mL of pure alcohol per week for men or women, respectively); any cancer diagnosis that was under treatment, clinically detectable, or had been treated within the past 5 years (except basal cell or squamous cell skin carcinomas); concurrent participation in other experimental studies or such participation within the past 6 months. Volunteers had to refrain from consumption of resveratrol-containing foodstuffs from 5 days before administration of resveratrol until they came off study. They were not allowed to take vitamin supplements from 2 weeks before resveratrol until they came off study. Written informed consent was obtained according to the respective state and local institutional guidelines. The study was reviewed and approved by the Leicestershire, Northamptonshire & Rutland Research Ethics Committee (United Kingdom) and the University of Michigan Institutional Review Board (IRBMED, USA). It was conducted in accordance with the applicable Guidelines on Good Clinical Practice.

Clinical Trial Design. Ten subjects were entered at each dose level beginning at 1 g and then escalating sequentially to 2.5 and 5 g. After pharmacokinetic data were completed for the 5 g dose level, 10 subjects were studied at a dose level of 0.5 g. Recruitment to the next dose level occurred only when lack of unacceptable toxicity of the previous dose had been established with a 14-day waiting period after the final subject received the intervention. On the day of administration, participants swallowed resveratrol caplets and remained within a supervised investigational unit for the subsequent 12-h period for pharmacokinetic study and toxicity monitoring. Participants were then discharged home and returned to clinic to allow collection of the 24-h blood sample. Individuals were followed up by telephone for 1 week after dosing to record potential concomitant medications or adverse events. Routine biochemical (renal, liver, and bone profiles) and hematologic analyses (complete blood count and coagulation) were done before and at 24 h after dosing. Toxicity, which was monitored via telephone interview or in personal interview with subjects, was graded using National Cancer Institute Common Toxicity Criteria (version 2.0).

Sample Collection and Management. Blood samples were collected via a sited cannula into heparinized tubes before and after resveratrol administration at the following time intervals: 0.17, 0.33, 0.50, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h. Samples were kept on ice and centrifuged (4°C, 2,900 × *g*, 15 min). Plasma was aspirated and transferred to a polypropylene tube. Urine was collected over four time intervals (0-4, 4-8, 8-12, and 12-24 h) post-administration. Subjects provided four fecal samples, one pre-dose and three consecutive ones post-dose. Samples of plasma, urine, and feces were frozen (−80°C) until analysis. Samples from the University of Michigan were shipped to Leicester on dry ice via 2-day express delivery service.

Sample Preparation. After defrosting, plasma and urine samples were extracted with acidified methanol [5% methanol with 2% acetic acid (pH 4), equal volume for plasma, twice the volume for urine]. The extractant was centrifuged (9, 000 × *g*, 15 min) and the supernatant was analyzed. Fecal matter was homogenized (5-mL water containing 2% phosphoric acid) and the homogenate was centrifuged (4°C, 2,900 × *g*, 15 min). Analyte was isolated from the supernatant using solid-phase extraction (Oasis HLB, Waters; volume, 3 mL; packing weight, 30 mg). Solid-phase cartridges were primed (1-mL acidified methanol followed by 1-mL water), washed (1 -mL acidified methanol), and analyte was eluted with methanol. While urine extractant and column eluents obtained from fecal extracts were injected onto the high-performance liquid chromatography column directly, plasma extractant was dried under nitrogen and reconstituted in mobile phase before high-performance liquid chromatography analysis. Resveratrol content in stool was related to dry feces weight, which was obtained by drying (100°C) stool to weight constancy (~24 h).

High-Performance Liquid Chromatography Analysis. Resveratrol and its metabolites were extracted and separated using a gradient UV-high-performance liquid chromatography system (Waters Breeze) as described before (29). Separation was achieved on a Waters Atlantis C18 column (4.6 × 150 mm, 3 μm; Waters) in combination with a Waters Atlantic C18 guard column (4.6 × 20 mm, 5 μm). Column oven temperature was 35°C (flow rate, 1 mL/min). The gradient elution system (A: 5 mmol/L ammonium acetate; B: 98% methanol, both with 2% propan-2-ol) was as follows with respect to B: 0 to 7 min 20%, 7 to 16 min 50%, 16 to 18 min 55%, 18 to 23 min 95%, then reequilibration to 0% B for 6 min before the next injection. Injection volume was 100 μL. The retention time of resveratrol was 18.6 min; its lower limit of detection was 5 ng/mL. This

method has been validated for resveratrol in terms of interday and intraday variability, recovery, accuracy, and precision (29). As resveratrol metabolites were not available in sufficient quantities for method development, their quantities were calculated based on the assumption that recovery characteristics and relationship between peak area ratios and concentrations were the same as those for resveratrol. Metabolite concentrations are therefore described as ''resveratrol equivalents.'' Authentic resveratrol 3-sulfate was provided by Royalmount Pharma. The identity of resveratrol and its metabolites was confirmed using an Agilent 1100 series high-performance liquid chromatography with in-line Applied Biosystems/MDS SCIEX API 2000 ion spray triple quadrupole mass spectrometer (Applied Biosystems) using the same chromatographic conditions as described above.

Data Analysis. The following pharmacokinetic variables were calculated for resveratrol and its three major metabolites using a noncompartmental pharmacokinetic approach and the ''PhAST'' (Phoenix Automated Statistics and Tabulation) validated proprietary software (MDS Pharma Services, 1999): area under the plasma concentration versus time curve (AUC; by the trapezoidal method), maximal plasma concentration (C_{max}) and time of maximal plasma concentration (T_{max}), average concentration over the total collection period (C_{av} = $AUC_{0\text{-}24}/24$ h), apparent elimination half-life ($\ln 2/k_{el}$, where k_{el} is apparent elimination rate constant), apparent total clearance (CL/F = dose/AUC_{inf}), apparent renal clearance of resveratrol (CL_R; approximated by $Ae_{0\text{-}24}/AUC_{0\text{-}24}$, where $Ae_{0\text{-}24}$ is estimated total amount excreted in urine over the total collection period), and apparent volume of distribution of resveratrol [V/F = dose/($k_{el} \times AUC_{inf}$)]. For a few subjects, pre-dose samples contained peaks coeluting with resveratrol or its metabolites. In these cases, AUC and C_{max} values were adjusted assuming these peaks constituted pre-dose concentrations (C_0) of resveratrol or resveratrol metabolite. These adjustments were made using the formulas $AUC_{infcorr} = AUC_{infobs} - C_0/k_{el}$ and $C_{maxcorr} = C_{maxobs} - (C_0 \times e^{-k_{el}} \times T_{max})$.

Results

Safety of Resveratrol. Forty volunteers (22 female, 18 male; 30 Caucasians, 2 African Americans, 7 Asians, 1 mixed race), of age range 19 to 61 years (mean, 32.5 years), ingested a single dose of resveratrol at 0.5, 1.0, 2.5, or 5.0 g. Blood and urine samples were collected over 24 h for analysis. Resveratrol was well tolerated with follow-up of these individuals failing to reveal any serious adverse reaction either clinically or by biochemical and hematologic analyses. Twenty-three of the 40 (57.5%) volunteers across all four dose levels presented with one or more minor adverse events (Table 1). Overall, there were 51 such events, which resolved within 2 to 4 days post-dosing. In 2 (5%) individuals, both of whom were on the 1.0 g dose, events potentially related to resveratrol administration were observed. One of these volunteers experienced an increase in blood bilirubin to 24 μmol/L (upper normal limit, 17 μmol/L), the other an increase in alanine aminotransferase to 55 IU/L (upper normal limit, 53 IU/L). Both events occurred on day 4 post-administration and resolved within the subsequent week.

Identification of Resveratrol Metabolites. Samples of plasma and urine were analyzed by high-performance liquid chromatography-tandem mass spectrometry, which furnished resveratrol, identified by its molecular ion m/z 227 ($[M + H]^+$), and six conjugated metabolites. The metabolites were characterized as two monosulfates, one disulfate, two monoglucuronides, and one glucuronide-sulfate by the following multiple reaction monitoring transitions: 307→227 (loss of a sulfate), 387→227 (loss of two sulfates), 403→227 (loss of a glucuronic acid), and 483→227 (loss of two glucuronic acids), respectively. One monosulfate, the most abundant resveratrol metabolite (Fig. 1), was confirmed as resveratrol 3-sulfate by coelution with authentic standard.

Plasma Pharmacokinetics of Resveratrol and Its Metabolites. Figure 2 shows the mean plasma concentration versus time curves for resveratrol and three most abundant metabolites, two resveratrol monoglucuronides and resveratrol 3-sulfate. Pharmacokinetic variables derived from these plots are summarized in Table 2. Resveratrol seemed to be rapidly absorbed, yielding peak concentrations (C_{max}) at between 0.83 and 1.5 h post-dose. The mean average (C_{av}) and peak plasma concentrations (C_{max}) of the parent molecule across the four dose levels ranged from 8.4 to 52 ng/mL (0.04-0.23 μmol/L) and from 73 to 539 ng/mL (0.3-2.4 μmol/L), respectively. The corresponding concentrations of the three resveratrol conjugates exceeded those of their progenitor molecule by up to ~20-fold. Of the metabolites, resveratrol 3-sulfate displayed the highest C_{av} and C_{max} concentrations with ranges of 172 to 1,089 ng/mL (0.56-3.5 μmol/L) and 1,135 to 4,294 ng/mL (3.7-14 μmol/L), respectively. The equivalent values determined for the two glucuronides are 51 to 344 ng/mL (0.13-0.85 μmol/L) and 370 to 1,735 ng/mL (0.92-4.3 μmol/L), respectively. The values for the mean area under the plasma concentration versus time curve to time infinity (AUC_{inf}) for resveratrol ranged from 224 ng h/mL at the lowest dose to 1,319 ng h/mL at the highest dose. The corresponding AUC_{inf} for resveratrol 3-sulfate was 18- to 23-fold higher than that determined for resveratrol. The AUC_{inf} values for the

Table 1. Adverse events in healthy volunteers after a single dose of resveratrol

Nature of event*	No. individuals			
	Dose level (g)			
	0.5	1.0	2.5	5.0
Unlikely to be drug related				
Raised urea	1	0	1	0
Raised creatinine	1	0	0	0
Low total protein	1	0	0	0
Raised albumin	0	0	0	1
Raised phosphate	2	0	1	2
Low phosphate	0	1†	0	0
Raised bilirubin	0	0	1	0
Raised calcium	1	0	0	0
Raised glucose	1	3	1	1
Low CO_2	0	0	2‡	2
Raised lactate dehydrogenase	1	0	0	1
Low lactate dehydrogenase	0	0	0	1
Raised cholesterol	0	2	0	0
Low WBC count	0	0	1	0
Raised neutrophil count	0	1	0	0
Raised lymphocyte count	0	1	0	0
Raised eosinophil count	0	0	0	1
Low basophil count	0	0	1	0
Raised platelet count	1	1	0	0
Low hematocrit	2	0	0	0
Raised chloride	1	1	0	1
Cellulitic foot infection	0	1	0	0
Flatulence	0	1	0	0
Nausea	0	1	0	0
Loose stool	0	2	0	0
Panic attack	0	1	0	0
Headache	0	1	1	0
Possibly drug related				
Raised bilirubin	0	2‡	0	0
Raised alanine aminotransferase	0	1	0	0

*All (except where indicated) National Cancer Institute Common Toxicity Criteria grade 1.
† National Cancer Institute Common Toxicity Criteria grade 2.
‡ In one of these two individuals, event was National Cancer Institute Common Toxicity Criteria grade 2.

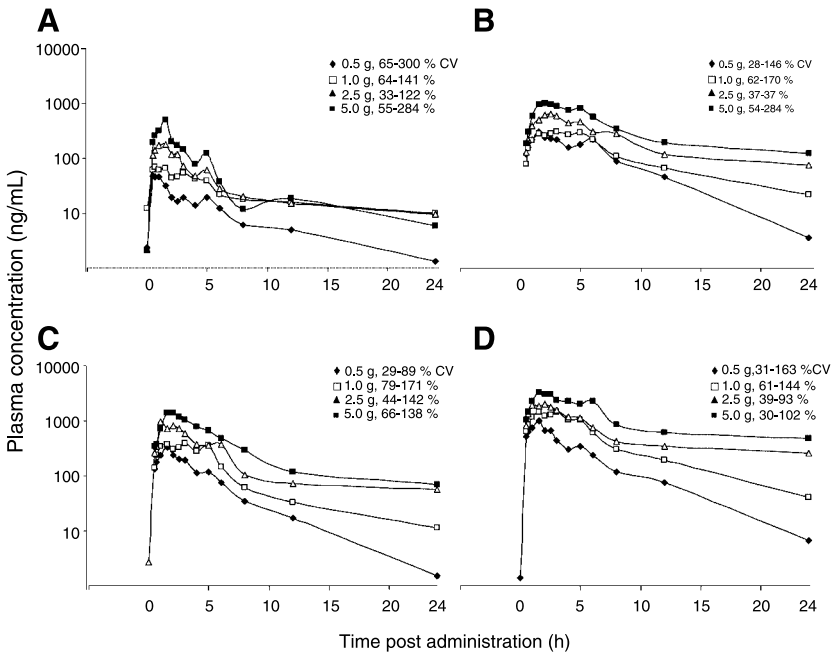

Figure 2. Mean plasma concentrations of resveratrol (**A**), two resveratrol monoglucuronides (**B** and **C**), and resveratrol-3-sulfate (**D**) versus time in healthy volunteers who received a single dose of resveratrol at 0.5 (♦), 1 (□), 2.5 (△), or 5 g (■). *Points,* mean of 10 volunteers per dose level. *Insets,* coefficients of variation.

glucuronides exceeded those of the progenitor molecule by factors of 4 to 6. When plotted versus dose, mean AUC and C_{max} values for resveratrol and its metabolites increased with dose, but in a slightly less than dose-proportional manner (Fig. 3). The plasma half lives of the three resveratrol conjugates, 3.2 to 11.5 h for the sulfate and 2.9 to 10.6 h for the glucuronides, were similar to those of parent resveratrol (2.9-8.9 h). The elimination phase for resveratrol was not well characterized because of the observed concentration increase in the terminal portion of the profiles, possibly due to enterohepatic recirculation. The magnitude of the mean apparent whole body clearance (2,235-4,930 L/h) and mean

Table 2. Pharmacokinetics of resveratrol and three metabolites in the plasma of volunteers after a single oral dose

Variable	Dose level (g)			
	0.5	1.0	2.5	5.0
Resveratrol				
AUC_{inf} (ng h/mL)	223.7*	544.8 (57.2)	786.5 (36.2)	1,319 (59.1)
C_{max} (ng/mL)	72.6 (48.9)	117.0 (73.1)	268.0 (55.3)	538.8 (72.5)
T_{max} (h)	0.833 (0.5-1.5)	0.759 (0.5-4.0)	1.375 (0.5-4.0)	1.500 (0.67-5.0)
C_{av} (ng/mL)	8.36 (57.8)	18.04 (51.6)	32.25 (43.0)	51.90 (80.7)
Half-life (h)	2.85*	8.87 (91.1)	4.22 (51.6)	8.52 (95.8)
CL/F (L/h)	2,235*	2,593 (65.1)	3,471 (29.9)	4,930 (50.0)
CL_R (L/h)	1.177 (102.5)	0.696 (71.5)	0.656 (53.1)	1.443 (139.2)
V/F (liters)	9,198*	19,298 (54.3)	22,226 (67.3)	66,991 (112)
Glucuronide 1				
AUC_{inf} (ng h/mL)	1,919 (33.6)	3,059 (60.9)	5,664 (27.7)	9,923 (40.9)
C_{max} (ng/mL)	404.6 (35.3)	473.6 (76.8)	874.4 (37.5)	1,285 (55.4)
T_{max} (h)	2.00 (1.0-6.0)	2.250 (1.0-6.0)	2.375 (1.0-8.0)	2.00 (1.5-5.0)
C_{av} (ng/mL)	76.9 (37.2)	110.3 (56.1)	215.5 (43.5)	344.1 (51.5)
Half-life (h)	2.85 (48.6)	7.27 (93.9)	10.6 (92.9)	7.90 (39.1)
CL/F (L/h)	282.7 (27.3)	493.5 (74.7)	469.5 (25.7)	590.6 (45.2)
Glucuronide 2				
AUC_{inf} (ng h/mL)	1,287 (21.7)	2,589 (66.4)	4,320 (32.9)	8,546 (62.3)
C_{max} (ng/mL)	369.5 (39.6)	672.6 (81.1)	1,626 (71.5)	1,735 (66.4)
T_{max} (h)	1.500 (1.0-5.0)	1.750 (1.0-5.1)	2.000 (1.0-6.0)	2.520 (1.5-8.0)
C_{av} (ng/mL)	51.0 (27.6)	99.9 (66.2)	193.8 (39.3)	317.8 (65.6)
Half-life (h)	3.09 (69.8)	6.64 (92.1)	8.42 (88.9)	5.83 (51.2)
CL/F (L/h)	408.8 (26.7)	642.5 (83.0)	636.9 (32.6)	1,017 (94.6)
3-Sulfate				
AUC_{inf} (ng h/mL)	4,049 (26.6)	10,053 (73.2)	16,984 (41.7)	30,898 (46.1)
C_{max} (ng/mL)	1,135 (25.7)	2,102 (81.3)	2,786 (27.2)	4,294 (48.0)
T_{max} (h)	1.500 (1.0-5.0)	2.000 (1.0-5.0)	2.000 (1.0-5.2)	2.050 (1.0-6.0)
C_{av} (ng/mL)	172.0 (23.2)	402.6 (70.5)	597.0 (27.0)	1,089 (49.4)
Half-life (h)	3.21 (56.6)	4.51 (82.8)	11.5 (95.5)	7.71 (42.3)
CL/F (L/h)	131.2 (25.8)	151.8 (62.7)	171.2 (40.0)	207.8 (63.9)

NOTE: Values are the mean of $n = 10$ with coefficient of variation (in percent) or range in brackets.
Abbreviations: AUC_{inf}, area under the concentration versus time curve to time infinity; C_{max}, maximal plasma concentration; T_{max}, median time of maximal plasma concentration; C_{av}, average plasma concentration; CL/F, apparent total body clearance (calculated as dose/AUC_{inf}); CL_R, apparent renal clearance approximated by amount excreted with urine within 24 h over $AUC_{0\text{-}24}$; V/F, apparent volume of distribution.
*$n = 1$, value for AUC_{inf} at the lowest dose could be established in only one participant.

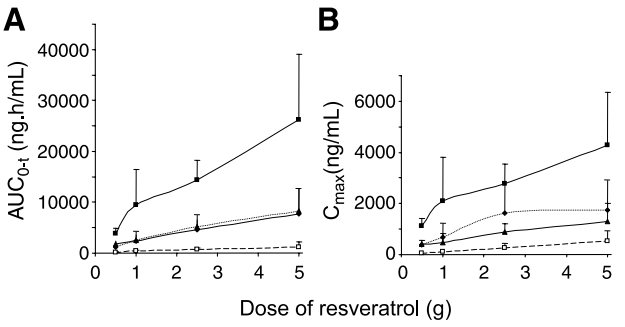

Figure 3. Relationship between dose of resveratrol and $AUC_{0\text{-}t}$ (**A**) or C_{max} (**B**) for resveratrol (□), two resveratrol monoglucuronides (◆, ▲), and resveratrol-3-sulfate (■) in healthy volunteers, who received a single dose of resveratrol at either 0.5, 1, 2.5, or 5 g. *Points,* mean of 10 volunteers per dose level; *bars,* SD.

volume of distribution (9,198-22,226 liters) of parent resveratrol is consistent with low bioavailability.

Levels of Resveratrol and Its Metabolites in Urine and Feces. Whereas the amount of unchanged resveratrol excreted in the urine within 24 h post-administration was below 0.04% of the dose (Fig. 4A), urinary excretion of the three resveratrol conjugates ranged from 0.51% (one of the glucuronides at the 0.5 g dose level) to 11.4% of the dose (resveratrol 3-sulfate at the 0.5 g dose level). Excretion rates were highest during the initial 4-h post-dose collection period (Fig. 4B), and 77% of all urinary species derived from the low dose resveratrol was passed during this time period. Individual chromatographic peak areas of resveratrol and its metabolites in feces were measured in stool samples, but total fecal mass was not recorded. Whereas the concentration of resveratrol ranged from 0 to 23 μg/g dry weight of feces, fecal concentrations of resveratrol metabolites, where measurable, were less than 1% of those determined for resveratrol, consistent with enterohepatic recirculation.

Discussion

The aim of this study was to determine whether single high oral doses of resveratrol are safe and yield systemic concentrations associated with chemopreventive activity in cells *in vitro* (5-18). Serious adverse events were not observed. The low toxicity prevalence and the limited number of subjects studied do not permit a statistically valid conclusion about safety. Assuming that the reproducibility of adverse events found in this trial can be generalized to a large, randomized clinical trial, resveratrol might be considered safe for chemopreventive intervention in healthy individuals. Nevertheless, it is important to be mindful of the possibility that, on chronic administration, resveratrol may generate unanticipated adverse effects.

The pharmacokinetic analysis suggests that ingestion of resveratrol equivalent to the amount contained in several hundred bottles of red wine produces C_{max} concentrations of between 0.3 and 2.4 μmol/L, markedly below the resveratrol concentrations required in *in vitro* experiments to elicit pharmacologic effects associated with cancer chemoprevention (>5 μmol/L). One might argue that concentrations required to elicit pharmacologic effects in experiments *in vitro* are only a crude estimate of concentrations needed for efficacy *in vivo*. Nevertheless, the low systemic availability of parent agent after consumption of high-dose resveratrol needs to be taken into consideration in the mechanistic interpretation of results of *in vivo* experiments using this agent. In contrast to the low systemic levels of parent resveratrol, its most abundant metabolite conjugate, resveratrol 3-sulfate, was present in the plasma at the 4 to 14 μmol/L C_{max} concentration range, and the more abundant of the two monoglucuronides was found at C_{max} concentrations of 0.9 to 4.3 μmol/L. The poor bioavailability of resveratrol, as reflected by its clearance, apparent volume of distribution, and urinary excretion, is reminiscent of comparable data obtained in humans for other polyphenolic phytochemicals, exemplified by the green tea constituent epigallocatchin gallate (30). The low bioavailability of resveratrol across mice, rats, and humans has previously been reported (28, 31-33). In these studies, resveratrol was administered at doses which were considerably lower than those used here. The pharmacokinetic observations described here in humans are essentially consistent with data from experiments in rodents. In rodents, maximal resveratrol concentration was attained rapidly, 10 min post-dose or earlier (34), and parent compound and metabolic conjugates tended to be undetectable beyond 1 h post-dosing (25). The presence of a second peak of resveratrol in the plasma drug concentration-versus-time profile observed here (Fig. 2) and the predominant amount of resveratrol compared with its metabolites in the feces are consistent with the hypothesis that resveratrol

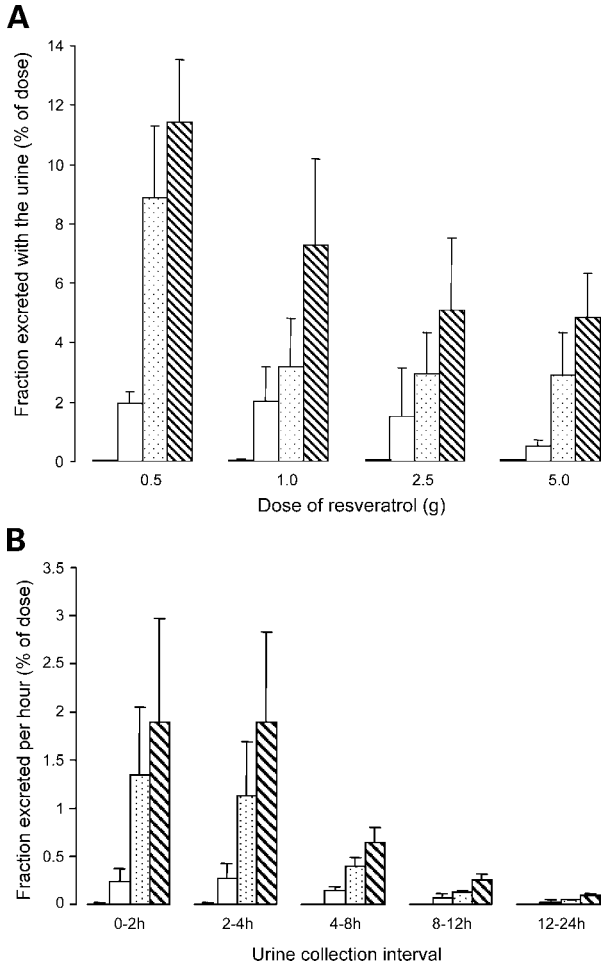

Figure 4. Excretion of resveratrol (*closed columns*), two resveratrol monoglucuronides (*open* and *dotted columns*), and resveratrol-3-sulfate (*striped columns*) in the urine of healthy volunteers who received resveratrol. **A.** Cumulative excretion over 24 h after ingestion of resveratrol at 0.5, 1, 2.5, or 5 g. **B.** Rate of excretion (per hour) during five collection intervals within 24 h post-administration of 0.5 g resveratrol. *Columns,* mean of 10 individuals, expressed as percentage of dose; *bars,* SD.

undergoes enterohepatic recirculation. This interpretation is congruent with results obtained in rats after oral resveratrol (35) and in human volunteers who received a low oral dose (25 mg) of ^{14}C-labeled resveratrol (28).

In the light of the amply documented antioncogenic properties of resveratrol in cells *in vitro* (5-18), its chemopreventive efficacy in rodent models (7, 9, 19-22) is thought to be mediated via the parent compound. The extensive sulfation and glucuronidation of resveratrol with consequent poor parent compound bioavailability, as described here and previously (28, 35), calls into question the role of parent resveratrol in the mediation of cancer chemopreventive efficacy, a notion which has been speculated on before (36). Whereas the pharmacologic properties of resveratrol conjugates are unknown, conjugated metabolites of naturally occurring flavonoids, polyphenols chemically resembling resveratrol, have been suggested to be responsible for, or contribute to, the pharmacologic activity of the parent molecule. For example, in vascular smooth muscle cells, quercetin 3-*O*-glucuronide inhibited both activity of c-Jun NH_2-terminal kinase and binding of transcription factor activator protein-1 to DNA, as potently as its metabolic progenitor (37). Quercetin conjugates also seem to retain, at least in part, the antioxidant properties of the parent molecule (38, 39). The glucuronide of the flavonoid luteolin underwent β-glucuronidase–catalyzed deconjugation at sites of inflammation producing parent aglycon (40). The work described here suggests that systemic concentrations of total resveratrol conjugates achievable in the human biophase after oral resveratrol can reach the range of 1×10^{-5} to 2×10^{-5} mol/L, grossly estimated on the basis of the sum of concentrations of resveratrol 3-sulfate and resveratrol glucuronides, as described above, and of resveratrol disulfate and resveratrol glucuronide sulfate, which were detected but not quantitated. It seems conceivable that such concentrations engage biochemical mechanisms germane to cancer chemoprevention in tissues targeted for chemoprevention of malignancies either directly or via generation of resveratrol by deconjugation. These possibilities clearly warrant experimental verification. Furthermore, the potential role needs to be explored which metabolites may play in the recently described effects of resveratrol on energy homeostasis and aging in mice *in vivo*, effects probably mediated in part via activation of the senescence regulator SIRT1 (3, 4).

In conclusion, the results presented here suggest that resveratrol undergoes avid metabolism in humans, which limits the availability of the parent molecule at organs remote from the site of absorption targeted for chemoprevention. It remains to be determined if repeated dosing schedules can achieve higher systemic concentrations of resveratrol than those observed here after a single dose, or whether sulfate and glucuronide metabolites, which are generated abundantly in the human biophase after resveratrol ingestion, possess efficacy in and of themselves.

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References

1. Taylor PR, Greenwald P. Nutritional interventions in cancer prevention. J Clin Oncol 2005;23:333–45.
2. Renaud S, de Lorgeril M, Rylander R. The French paradox: dietary factors and cigarette smoking-related health risks. Ann N Y Acad Sci 1993;686: 299–309.
3. Baur JA, Pearson KJ, Price NL, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006;444:337–46.
4. Lagouge M, Argmann C, Gerhart-Hines Z, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. Cell 2006;127:1–14.
5. Jang M, Cai L, Udeani GO, et al. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997;275: 218–20.
6. Gescher AJ, Steward WP. Relationship between mechanisms, bioavailability, and preclinical chemopreventive efficacy of resveratrol: a conundrum. Cancer Epidemiol Biomarkers Prev 2003;12:953–7.
7. Banerjee S, Bueso-Ramos C, Aggarwal BB. Suppression of 7,12-dimethylbenz(*a*)anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-κB, cyclooxygenase 2, and matrix metalloprotease 9. Cancer Res 2002;62:4945–54.
8. Ciolino HP, Yeh GC. Inhibition of aryl hydrocarbon-induced cytochrome *P*-450 1A1 enzyme activity and CYP1A1 expression by resveratrol. Mol Pharmacol 1999;56:760–7.
9. Bhat KPL, Lantvit D, Christov K, Mehta RG, Moon RC, Pezzuto JM. Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. Cancer Res 2001;61:7456–63.
10. Sale S, Tunstall RG, Ruparelia KC, et al. Comparison of the effects of the chemopreventive agent resveratrol and its synthetic analogue *trans*-3,4,5,4′-tetramethoxystilbene (DMU-212) on adenoma development in the Apc(Min+) mouse and cyclooxygenase-2 in human-derived colon cancer cells. Int J Cancer 2005;115:194–201.
11. Howitz KT, Bitterman KJ, Cohen HY, et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* life span. Nature 2003;425:191–6.
12. Clement MV, Hirpara JL, Chawdhury SH, Pervaiz S. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. Blood 1998;92: 996–1002.
13. Soleas GJ, Goldberg DM, Grass L, Levesque M, Diamandis EP. Do wine polyphenols modulate p53 gene expression in human cancer cell lines? Clin Biochem 2001;34:415–20.
14. Delmas D, Passilly-Degrace P, Jannin B, Malki MC, Latruffe N. Resveratrol, a chemopreventive agent, disrupts the cell cycle control of human SW480 colorectal tumor cells. Int J Mol Med 2002;10:193–9.
15. Fremont L, Belguendouz L, Delpal S. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. Life Sci 1999;64:2511–21.
16. Lee SK, Mbwambo ZH, Chung H, et al. Evaluation of the antioxidant potential of natural products. Comb Chem High Throughput Screen 1998;1: 35–46.
17. Cao Y, Fu ZD, Wang F, et al. Anti-angiogenic activity of resveratrol, a natural compound from medicinal plants. J Asian Nat Prod Res 2005;7: 205–13.
18. Tseng SH, Lin SM, Chen JC, et al. Resveratrol suppresses the angiogenesis and tumor growth of gliomas in rats. Clin Cancer Res 2004;10: 2190–202.
19. Li ZG, Hong T, Shimada Y, et al. Suppression of *N*-nitrosomethylbenzylamine (NMBA)-induced esophageal tumorigenesis in F344 rats by resveratrol. Carcinogenesis 2002;23:1531–6.
20. Tessitore L, Davit A, Sarotto I, Caderni G. Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21(CIP) expression. Carcinogenesis 2000;21:1619–22.
21. Schneider Y, Duranton B, Gosse F, Schleiffer R, Seiler N, Raul F. Resveratrol inhibits intestinal tumorigenesis and modulates host-defense-related gene expression in an animal model of human familial adenomatous polyposis. Nutr Cancer 2001;39:102–7.
22. Ziegler CC, Rainwater L, Whelan J, McEntee MF. Dietary resveratrol does not affect intestinal tumorigenesis in Apc(Min/+) mice. J Nutr 2004;134: 5–10.
23. Miura D, Miura Y, Yagasaki K. Hypolipidemic action of dietary resveratrol, a phytoalexin in grapes and red wine, in hepatoma-bearing rats. Life Sci 2003;73:1393–400.
24. Gu X, Creasy L, Kester A, Zeece M. Capillary electrophoretic determination of resveratrol in wines. J Agric Food Chem 1999;47:3223–7.
25. Yu CW, Shin YG, Chow A, et al. Human, rat, and mouse metabolism of resveratrol. Pharm Res 2002;19:1907–14.
26. Soleas GJ, Yan J, Goldberg DM. Measurement of *trans*-resveratrol, (+)-catechin, and quercetin in rat and human blood and urine by gas chromatography with mass selective detection. Methods Enzymol 2001; 335:130–45.
27. Goldberg DM, Yan J, Soleas GJ. Absorption of three wine-related polyphenols in three different matrices by healthy subjects. Clin Biochem 2003;36:79–87.
28. Walle T, Hsieh F, DeLegge MH, Oatis JE, Walle UK. High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab Dispos 2004;32:1377–82.
29. Boocock D, Patel KR, Faust GES, et al. Quantitation of *trans*-resveratrol and detection of its metabolites in human plasma and urine by high performance liquid chromatography. J Chromatogr B 2007;848:182–7.
30. Chow HHS, Cai Y, Alberts DS, et al. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatchin gallate and polyphenon E. Cancer Epidemiol Biomarkers Prev 2001;10:53–8.
31. Andlauer W, Kolb J, Siebert K, Furst P. Assessment of resveratrol bioavailability in the perfused small intestine of the rat. Drugs Exp Clin Res 2000;26:47–55.
32. Kuhnle G, Spencer JPE, Chowrimootoo G, et al. Resveratrol is absorbed in the small intestine as resveratrol glucuronide. Biochem Biophys Res Commun 2000;272:212–7.

33. Meng XF, Maliakal P, Lu H, Lee MJ, Yang CS. Urinary and plasma levels of resveratrol and quercetin in humans, mice, and rats after ingestion of pure compounds and grape juice. J Agric Food Chem 2004;52:935–42.
34. Asensi M, Medina I, Ortega A, et al. Inhibition of cancer growth by resveratrol is related to its low bioavailability. Free Radic Biol Med 2002;33: 387–98.
35. Marier JF, Vachon P, Gritsas A, Zhang J, Moreau JP, Ducharme MP. Metabolism and disposition of resveratrol in rats: extent of absorption, glucuronidation, and enterohepatic recirculation evidenced by a linked-rat model. J Pharmacol Exp Ther 2002;302:369–73.
36. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the *in vivo* evidence. Nat Rev Drug Discov 2006;5:493–506.
37. Yoshizumi M, Tsuchiya K, Suzaki Y, et al. Quercetin glucuronide prevents VSMC hypertrophy by angiotensin II via the inhibition of JNK and AP-1 signaling pathway. Biochem Biophys Res Commun 2002;293:1458–65.
38. Morand C, Crespy V, Manach C, Besson C, Demigne C, Remesy C. Plasma metabolites of quercetin and their antioxidant properties. Am J Physiol 1998; 275:R212–9.
39. Manach C, Regerat F, Texier O, Agullo G, Demigne C, Remesy C. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutr Res 1996;16:517–44.
40. Shimoi K, Saka N, Nozawa R et al. Deglucuronidation of a flavonoid, luteolin monoglucuronide, during inflammation. Drug Metab Dispos 2001; 29:1521–4.