Clinical Studies

Repeat Dose Study of the Cancer Chemopreventive Agent Resveratrol in Healthy Volunteers: Safety, Pharmacokinetics, and Effect on the Insulin-like Growth Factor Axis

Victoria A. Brown[1], Ketan R. Patel[1], Maria Viskaduraki[1], James A. Crowell[2], Marjorie Perloff[2], Tristan D. Booth[3], Grygoriy Vasilinin[4], Ananda Sen[5], Anna Maria Schinas[4], Gianfranca Piccirilli[3], Karen Brown[1], William P. Steward[1], Andreas J. Gescher[1], and Dean E. Brenner[6]

Abstract

Resveratrol, a naturally occurring polyphenol, has cancer chemopreventive properties in preclinical models. It has been shown to downregulate the levels of insulin-like growth factor-1 (IGF-I) in rodents. The purpose of the study was to assess its safety, pharmacokinetics, and effects on circulating levels of IGF-I and IGF-binding protein-3 (IGFBP-3) after repeated dosing. Forty healthy volunteers ingested resveratrol at 0.5, 1.0, 2.5, or 5.0 g daily for 29 days. Levels of resveratrol and its metabolites were measured by high performance liquid chromatography-UV in plasma obtained before and up to 24 hours after a dose between days 21 and 28. IGF-I and IGFBP-3 were measured by ELISA in plasma taken predosing and on day 29. Resveratrol was safe, but the 2.5 and 5 g doses caused mild to moderate gastrointestinal symptoms. Resveratrol-3-*O*-sulfate, resveratrol-4′-*O*-glucuronide, and resveratrol-3-*O*-glucuronide were major plasma metabolites. Maximal plasma levels and areas under the concentration versus time curve for the metabolites dramatically exceeded those for resveratrol, in the case of areas under the concentration versus time curve, by up to 20.3-fold. Compared with predosing values, the ingestion of resveratrol caused a decrease in circulating IGF-I and IGFBP-3 ($P < 0.04$ for both), respectively, in all volunteers. The decrease was most marked at the 2.5 g dose level. The results suggest that repeated administration of high doses of resveratrol generates micromolar concentrations of parent and much higher levels of glucuronide and sulfate conjugates in the plasma. The observed decrease in circulating IGF-I and IGFBP-3 might contribute to cancer chemopreventive activity. *Cancer Res; 70(22); OF1–9. ©2010 AACR.*

Introduction

Resveratrol, a polyphenol which occurs in red grapes and red wine, has been shown to prevent cancer, or delay its onset, in a variety of rodent models of carcinogenesis (1, 2). Resveratrol can also retard parameters linked to aging and acts as a calorie-restriction mimetic in mice (3, 4), important findings in the light of emerging evidence of a possible association between calorie restriction and anticarcinogenesis (5). The abundance of information on biochemical effects of resveratrol in cultured cells potentially linked to anticarci-

nogenesis (6) contrasts with a scarcity of studies in humans. Published human studies typically employed single doses of up to 25 mg of resveratrol, mostly as a constituent of wine, grapefruit juice, or grape extract (7). Reports on trials of resveratrol in humans after single (8, 9) or multiple daily doses of up to 600 mg/d administered over 2 or 3 days (10, 11) suggest that it is safe under the tested conditions. The pharmacodynamic properties of resveratrol after repeated dosing in humans have hitherto not been described.

The insulin-like growth factor (IGF) signaling system, which consists of IGFs, IGF-binding proteins (IGFBP), and IGF receptors, crucially influences malignant development. IGFs possess potent antiapoptotic and mitogenic properties (12, 13) and affect cell differentiation, neoplastic transformation, and metastasis (13–15). The IGF system is regulated by IGFBPs, prominently IGFBP-3, which bind IGFs in the extracellular milieu with high affinity and specificity, thus reducing circulating levels of IGFs. Several studies suggest a direct relationship between the levels of IGF-I, and an inverse relationship between the levels of IGFBP-3, and risk of colorectal, prostate, breast, or lung cancer (16). Individuals suffering from acromegaly, a somatic disease associated with increased IGF-I, have an elevated risk of colorectal cancer (17). IGF-I has also been suggested to contribute to the development of adenomatous polyps (18). The anticarcinogenic

Authors' Affiliations: [1]Cancer Biomarkers and Prevention Group, Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester, United Kingdom; [2]Chemoprevention Agent Development Research Group, National Cancer Institute, Bethesda, Maryland; [3]Pharmascience, Inc.; [4]MDS Pharma Services, Montreal, Canada; [5]Department of Family Practice, University of Michigan Medical School; and [6]Departments of Internal Medicine and Pharmacology, University of Michigan Medical School and VA Medical Center, Ann Arbor, Michigan

Corresponding Author: Andreas J. Gescher, Department of Cancer Studies and Molecular Medicine, University of Leicester, LRI, RKCSB, Leicester LE2 7LX, United Kingdom. Phone: 44-116223-1856; Fax: 44-116223-1855; E-mail: ag15@le.ac.uk.

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activity of dietary restriction in preclinical models of carcinogenicity is thought to be mediated, at least in part, via reduction of circulating IGF-I (19). Modulation of the IGF system has been proposed as a mechanism by which certain agents, for example 9-*cis*-retinoic acid, might prevent cancer (20). Resveratrol lowered circulating IGF-I in diabetic mice on a high-calorie diet (3) and in prostate tumor tissue of TRAMP mice (21), a genetic model of prostate carcinogenesis. Information on the effect of resveratrol on IGFBP-3 has not been provided in these two studies.

The potential of resveratrol as a cancer chemopreventive agent and/or calorie-restriction mimetic in humans is a topic of considerable interest (2, 5), but potential biomarkers of its efficacy, such as levels of components of the IGF signaling pathway, in humans are virtually unknown. We conducted a trial of repeat high-dose resveratrol in healthy volunteers with the aim of exploring its safety, the pharmacokinetics of parent agent and its major metabolites, and the effect of resveratrol on circulating levels of IGF-I and IGFBP-3.

Materials and Methods

Volunteers

Healthy volunteers (55% male, 65% Caucasian, 15% Asian, 12.5% Afro-Caribbean or biracial, 7.5% Hispanic) were recruited into the study at either the Universities of Leicester (United Kingdom) or Michigan (United States) and gave written informed consent. Eligibility criteria included willingness to abstain from ingestion of large quantities of resveratrol-containing foods. Exclusion criteria included chronic medications including vitamins (except for oral or depot contraceptives and hormone replacement therapy). The study is registered at ClinicalTrials.gov (web site address: http://www.clinicaltrials.gov) as NCT 00098969. It was reviewed and approved by the Leicestershire, Northamptonshire and Rutland Research Ethics Committee (Nottingham, United Kingdom), and the University of Michigan Institutional Review Board (IRBMED, Ann Arbor, MI) and conducted in accordance with the applicable guidelines on Good Clinical Practice. At the predosing screening visit, the volunteer's medical history was recorded including regular/occasional use of medication and vitamins. Four subjects, who terminated intervention prematurely, were replaced, so that overall 40 individuals, 10 per dose level, completed the intervention. Mean and range (in brackets) of ages and body mass indices for the four different dosing groups were as follows: age (in years)—0.5 g, 35 (20–49); 1.0 g, 36 (20–58); 2.5 g, 37 (24–51); 5.0 g, 42 (21–73); body mass index (in kg/m^2)—0.5 g, 27.5 (20.0–42.4); 1.0 g, 25.2 (18.6–39.4); 2.5 g, 26.3 (19.3–39.0); and 5.0 g, 25.4 (19.2–32.8). The values did not differ significantly between dose groups.

Study design and resveratrol dose

Study participants ingested uncoated immediate-release caplets manufactured to good manufacturing practice standards by Pharmascience, Inc. Caplets contained 500 mg of chemically synthesized resveratrol. The stability of the formulation was tested according to good manufacturing practice stipulations. Participants were recruited sequentially to four dose levels of resveratrol (0.5, 1.0, 2.5, and 5.0 g) and instructed to ingest the appropriate dose between 7:00 and 9:00 a.m. daily for 29 days. Participants completed a form after each dose and were evaluated on a weekly basis for adverse events and compliance with dosing. An adverse event according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 was attributed to resveratrol by the site study team on the basis of detailed description of symptoms, their duration and other pertinent factors (medication, food intake). Volunteers were recruited to the next dose level when the absence of unacceptable toxicity in the final participant on the previous dose level had been established within a 14-day waiting period.

Sample preparation and high performance liquid chromatography analysis

Blood samples for pharmacokinetic analysis of resveratrol and its metabolites were collected prior to resveratrol administration (predose) on day 1, and at predose and 0.25, 1.0, 1.5, 5, 12, and 24 hours postdose on a day between the 21st and the 28th day of dosing. Blood samples were collected into lithium-heparinized tubes from which plasma was obtained and stored at −80°C until analysis.

Sample preparation, which entailed the extraction of acidified plasma with methanol, and high performance liquid chromatography (HPLC)-UV analysis for quantification of resveratrol and its metabolites were performed as described previously (9, 22). Separation was achieved on a Waters Atlantis C_{18} column (4.6 mm × 150 mm 3 μm; Waters) in combination with a Waters Atlantic C_{18} guard column (4.6 mm × 20 mm, 3 μm). Quantitation of resveratrol using a gradient HPLC-UV system (Waters Alliance 2695; Waters, Corp.) was performed as described before, and the method has been validated in terms of interday and intraday variability, recovery, accuracy, and precision (22). The retention time of resveratrol was 18.6 minutes, its lower limit of detection was 5 ng/mL. As authentic resveratrol metabolites were not available in sufficient quantities as reference materials, metabolite amounts were calculated as "resveratrol equivalents," on the assumption that recovery characteristics and relationship between peak area ratio and concentration were the same as for the parent resveratrol. Authentic resveratrol-3-*O*-sulfate (also provided by Pharmascience), resveratrol-4'-*O*-sulfate, resveratrol-3-*O*-glucuronide, and resveratrol-4'-*O*-glucuronide became available during the course of the study by in-house synthesis, permitting HPLC peak identification, so that resveratrol metabolites could be identified by cochromatography. Metabolite identity was confirmed by liquid chromatography-tandem mass spectrometry with selected reaction monitoring, operated in negative ion mode using an Agilent 1100 series HPLC with in-line Applied Biosystems/MDS SCIEX API 2000 ion spray triple quadrupole mass spectrometer (Applied Biosystems) under chromatographic conditions described previously (9, 22). Definitive isomer identification was not possible for resveratrol disulfate and resveratrol sulfate glucuronide.

Determination of IGF-I and IGFBP-3

Blood samples to assay IGF-I and IGFBP-3 were obtained, following overnight fasting, before the first dose and before the last dose of resveratrol on day 29, or in the case of three individuals who ingested resveratrol for an additional day or two, on days 30 or 31. IGF-I and IGFBP-3 concentrations in serum were determined using ELISA kits DG100 and BAF675, respectively (R&D Systems), with predosing and postdosing samples from each person analyzed on the same 96-well plate. Assays for both species were performed in parallel, and serum samples were analyzed in triplicate, standards in duplicate. The IGF-I assay includes a step which releases IGF-I from binding proteins. Assays were performed according to the instructions of the manufacturer and blinded with respect to subject. Samples were stable over the period elapsing between collection of predose and postdose blood when stored at −80°C, as illustrated by comparing samples analyzed fresh and after 3 months of storage, which showed a variation of <5%.

Pharmacokinetic parameters

The following pharmacokinetic parameters were calculated for resveratrol and its metabolites using a noncompartmental approach: C_{max} = maximal plasma concentration over the collection period; C_{av} = average plasma concentration over the collection period; T_{max} = time of C_{max}; $T_{1/2}$ = apparent first-order elimination half-life calculated as $\ln(2) / \lambda_z$ (λ_z = apparent first-order elimination constant calculated from semi-log plot of plasma concentration versus time curve); AUC_{last} = area under the plasma concentration versus time curve from time 0 to the last measurable concentration above the limit of quantitation, as calculated by the linear trapezoidal method. Apparent total body clearance (CL/F) and apparent volume of distribution (V/F) for resveratrol were calculated as dose/AUC and dose / ($\lambda_z \times$ AUC), respectively.

Statistical analysis

Descriptive statistics (mean, SD, coefficient of variation, median) were calculated for plasma concentrations of resveratrol and its metabolites. Geometric mean and coefficient of variation values were calculated for concentrations and derived pharmacokinetic parameters using R v.2.9.2 (open source implementation of S statistical programming language; Bell Laboratories) on MS Windows and Linux. The proportionality between C_{max} or AUC and dose for resveratrol was evaluated using the power model and confidence interval approaches as described by Chow and Liu (23) omitting results for the 0.5 g dose, as many of the plasma concentration values at this dose were near or below the limit of quantitation.

Differences between preintervention and postintervention IGF protein levels in study volunteers (preintervention minus postintervention levels) were subjected to statistical evaluation using Student's paired t test with the "Statistical Package for the Social Sciences" software. The test compares the mean of the differences between preintervention and postintervention values with zero; $P < 0.05$ values indicate that the difference between preintervention and postintervention was significantly different from zero.

Results

Safety of resveratrol

Healthy volunteers received resveratrol daily for 29 days at daily doses of 0.5, 1.0, 2.5, or 5.0 g. Resveratrol was safe, as borne out by the lack of serious adverse reactions detected by clinical, biochemical, or hematologic analyses during the study and study follow-up. Of the total 44 volunteers who received resveratrol, including those who terminated the intervention prematurely, 28 reported one or more adverse events while on the study. Seven of these individuals were on the 0.5 g dose, four on the 1.0 g dose, eight on the 2.5 g dose, and nine on the 5.0 g dose. Table 1 describes the nature of only those adverse events deemed to be possibly or probably associated with resveratrol intake. The majority of events reported by the volunteers on the two highest dose levels (2.5 and 5.0 g) were gastrointestinal symptoms, including nausea, flatulence, abdominal discomfort, and diarrhea. Most of these events were mild (severity grade 1, NCI CTCAE v.4.0), although four participants on the 2.5 and 5.0 g doses presented with nausea and/or diarrhea of moderate severity (grade 2). The gastrointestinal side effects commenced after

Table 1. Number of healthy volunteers who experienced adverse events deemed intervention-related after daily ingestion of resveratrol for 29 d

Symptom	No. of volunteers			
	Dose (g)			
	0.5	1.0	2.5	5.0
Increased blood bilirubin				
Total	1	1		
Conjugated		2		
Unconjugated		2		
Skin discoloration		1		
Cystitis		1		
Abdominal pain			4	3
Acne			1	
Cramp			1	
Diarrhea			2 (1*)	7 (2*, 1**)
Discomfort on passing feces			1	
Fatigue			1	
Flatulence			1	2
Nausea			2	3
Pruritis			1	
Chest pain				1
Dizziness				1
Dry mouth				1
Red/itchy eyes				1
Urine color change				1

NOTE: Overall number of volunteers per dose was 10 to 12.
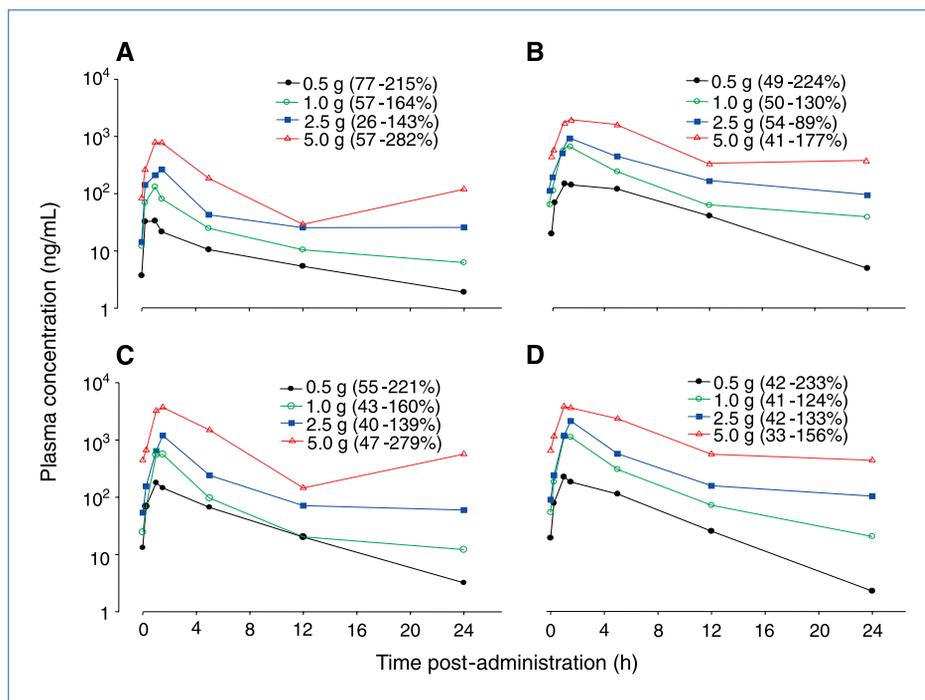Severity grading (NCI CTCAE v.4.0): no asterisk = 1; * = 2; ** = 3.

Figure 1. Mean plasma concentrations of resveratrol (A), resveratrol-4′-O-glucuronide (B), resveratrol-3-O-glucuronide (C), and resveratrol-3-O-sulfate (D) versus time in healthy volunteers after the last of between 21 and 28 daily doses of resveratrol at either 0.5 g (black, closed circles), 1 g (green, open circles), 2.5 g (blue, squares), or 5 g (red, triangles). Values are the mean ± SD of 10 volunteers per dose level. The range of coefficients of variation (as % of the mean) for the individual data points is shown in parentheses.

2 to 4 days of the intervention and occurred 30 minutes to 1 hour after resveratrol ingestion. Symptoms, which tended to improve throughout the day and return after the following dose, resolved within 2 days of completing the 29-day course. There was no weight loss in any participant, and all volunteers maintained normal performance status throughout the study period.

Pharmacokinetics of resveratrol and its metabolites

Plasma from 40 volunteers was collected at multiple time points after resveratrol ingestion on a day between the 21st and the 28th day of intervention, and analyzed for the presence of parent agent and metabolites. As reported previously in volunteers after a single dose of resveratrol (9), parent resveratrol, and six metabolic conjugates, resveratrol-3-O-sulfate, resveratrol-4′-O-sulfate, resveratrol disulfate, resveratrol-3-O-glucuronide, resveratrol-4′-O-glucuronide, and a resveratrol sulfate glucuronide, were identified by HPLC-UV cochromatography with authentic reference material and/or HPLC-tandem mass spectrometry in volunteers' plasma (results not shown). The most abundant circulating resveratrol metabolite was resveratrol-3-O-sulfate.

Plasma concentrations of resveratrol and its three major metabolites, resveratrol-3-O-sulfate, resveratrol-4′-O-glucuronide, and resveratrol-3-O-glucuronide, were measured by HPLC-UV, and plasma concentration versus time curves are shown in Fig. 1. Pharmacokinetic parameters derived from these plots are summarized in Table 2. Resveratrol was rapidly absorbed yielding peak concentrations (C_{max}) at 1 hour postdose. The mean average plasma concentration (C_{av}) and C_{max} values of parent resveratrol across the four dose levels ranged from 0.04 to 0.55 nmol/mL and 0.19 to 4.24 nmol/mL, respec-

tively. The corresponding concentrations of the major resveratrol conjugates exceeded those of their parent molecule by factors of between 3.8 and 16.5 for C_{av} and between 2.4 and 12.9 for C_{max}. Of the metabolites, resveratrol-3-O-sulfate displayed the greatest C_{av} and C_{max} values, ranging from 0.5 to 6.1 nmol/mL and from 2.5 to 18.3 nmol/mL, respectively, across the four doses. The plasma elimination half-lives varied between 4.77 and 9.70 hours for resveratrol and between 3.09 and 8.14 hours for the major metabolites. The mean values for the AUC_{last} for resveratrol were 175 ng × h/mL at the lowest, and 4,097 ng × h/mL at the highest dose. The respective AUC_{last} values at these doses for resveratrol-3-O-sulfate were 20.3- and 9.49-fold higher, those for resveratrol-4′-O-glucuronide 7.61- and 4.88-fold higher, and those for resveratrol-3-O-glucuronide 5.00- and 5.39-fold higher, than the AUCs for resveratrol (Table 2). The apparent total body clearances and apparent volumes of distribution for resveratrol are consistent with its rapid metabolism and low bioavailability (Table 3). When plotted versus dose, mean C_{max} and AUC values for resveratrol and its metabolites increased with dose in a manner grossly proportional to dose (Fig. 2). This relationship was analyzed statistically (23) for parent resveratrol, and the analysis supported dose proportionality for C_{max} and AUC at the 1.0 to 5.0 g dose levels.

Effect of resveratrol on circulating IGF-I and IGFBP-3

IGF-I and IGFBP-3 levels in plasma samples obtained on day 29 were compared with those taken just prior to the first dose of resveratrol. Consumption of resveratrol reduced IGF-I and IGFBP-3 levels weakly, albeit significantly, when results from all trial participants were combined. Mean differences between preintervention and postintervention levels, 95%

Table 2. Pharmacokinetic parameters of resveratrol and its three major metabolites in the plasma of healthy volunteers who received daily oral resveratrol for between 21 and 28 d (n = 10 per dose level)

	C_{max} (ng/mL)*,†	C_{av} (ng/mL)*,†	T_{max} (h)‡	$T_{1/2}$ (h)†	AUC_{last} (ng × h/mL)†
			Parameter		
Resveratrol, dose (g)					
0.5	43.8 (89.4) [0.19]	9.93 (69.7) [0.04]	1.00 (0.25–5.0)	4.77 (62.1)	175 (83.7)
1.0	141 (68.9) [0.62]	22.8 (68.4) [0.10]	1.00 (0.25–1.82)	9.70 (37.5)	503 (79.3)
2.5	331 (59.2) [1.45]	48.1 (46.5) [0.21]	1.00 (0.23–4.97)	9.17 (42.0)	1,250 (40.0)
5.0	967 (53.5) [4.24]	126 (55.8) [0.55]	1.08 (0.5–1.5)	7.85 (25.1)	4,097 (107)
Resveratrol-4′-O-glucuronide, dose (g)					
0.5	186 (56.7) [0.82]	50.2 (51.1) [0.22]	1.27 (1.0–5.0)	3.78 (42.2)	1,331 (56.4)
1.0	710 (57.9) [3.12]	178 (64.8) [0.78]	1.50 (0.83–5.0)	5.77 (44.6)	3,774 (53.2)
2.5	1,137 (88.0) [4.99]	323 (66.9) [1.42]	1.50 (0.25–5.0)	8.14 (38.6)	7,245 (54.4)
5.0	2,323 (45.5) [10.2]	667 (61.3) [2.93]	1.50 (1.0–5.0)	7.55 (21.2)	19,984 (59.0)
Resveratrol-3-O-glucuronide, dose (g)					
0.5	184 (86.3) [0.81]	37.7 (56.2) [0.17]	1.27 (1.0–5.0)	4.98 (41.6)	875 (51.4)
1.0	588 (48.4) [2.45]	94.4 (28.9) [0.42]	1.50 (0.83–5.0)	5.50 (24.5)	2,087 (37.7)
2.5	1,546 (107) [6.78]	204 (64.0) [0.89]	1.42 (1.0–1.5)	6.43 (40.8)	5,300 (47.1)
5.0	3,886 (48.6) [17.1]	649 (34.8) [2.85]	1.50 (0.5–1.6)	5.19 (52.7)	22,084 (93.9)
Resveratrol-3-O-sulfate, dose (g)					
0.5	563 (35.1) [2.47]	118 (26.9) [0.52]	1.04 (1.0–5.0)	3.09 (15.3)	3,558 (51.6)
1.0	1,694 (35.2) [7.43]	377 (48.7) [1.65]	1.50 (0.83–5.0)	7.37 (51.4)	9,464 (42.3)
2.5	2,292 (50.7) [10.1]	604 (43.8) [2.65]	1.33 (1.0–1.5)	6.84 (40.6)	15,638 (39.0)
5.0	4,172 (40.3) [18.3]	1,384 (42.3) [6.07]	1.25 (0.25–5.0)	7.98 (29.6)	38,900 (49.5)

Abbreviations: C_{max}, maximal plasma concentration; C_{av}, average plasma concentration; T_{max}, time of maximal plasma concentration (C_{max}); $T_{1/2}$, apparent first-order elimination half-life; AUC_{last}, area under the plasma concentration versus time curve from time 0 to the last sampling blood draw collected.
*Mean value in ng/mL.
†CV% (round brackets), mean value in nmol/mL [square brackets].
‡Median (range).

confidence intervals (both in ng/mL), and P values emanating from the paired t test were 8.1 (0.7–15.4; P = 0.04) for IGF-I and 109 (10–208; P = 0.04) for IGFBP-3. Figure 3 shows the effect of resveratrol on circulating levels of IGF-I and IGFBP-3 in the individual volunteers. In those on the 2.5 g dose, levels of IGF-I were most prominently and consistently reduced (Fig. 3), with a difference between preintervention and postintervention IGF-I levels (in ng/mL) of 29.6 (95% confidence interval, 21.5–37.8; P < 0.001). IGF-I levels were not significantly affected in volunteers on the 0.5 or 1.0 g doses. Mean IGFBP-3 concentrations in individuals on the 1.0 or 2.5 g doses of resveratrol were also significantly reduced by resveratrol. The differences between preintervention and postintervention IGFBP-3 levels with 95% confidence intervals (both in ng/mL) and P values were 279 (62–496; P = 0.03) for the 1.0 g dose and 210 (49–372; P = 0.03) for the 2.5 g dose (Fig. 3). The ratio IGF-I/IGFBP-3 for the 2.5 g dose cohort was also strongly reduced (data not shown). At 5 g, resveratrol failed to affect the IGF system significantly.

Table 3. Apparent total body clearance and volume of distribution for resveratrol in healthy volunteers who received daily oral resveratrol for between 21 and 28 d (n = 10 per dose level)

Dose (g)	Apparent total body clearance (L/h)	Apparent volume of distribution (L)
0.5	2,771 (58.9)*	16,071 (56.5)
1.0	2,366 (63.9)	42,673 (86.1)
2.5	2,219 (42.5)	32,322 (44.5)
5.0	2,548 (100)	38,023 (133)

*Mean values with CV% in brackets.

Discussion

In this report, we describe the pharmacokinetics of resveratrol after repeated oral administration of high doses and define potential pharmacodynamic end points pertinent to
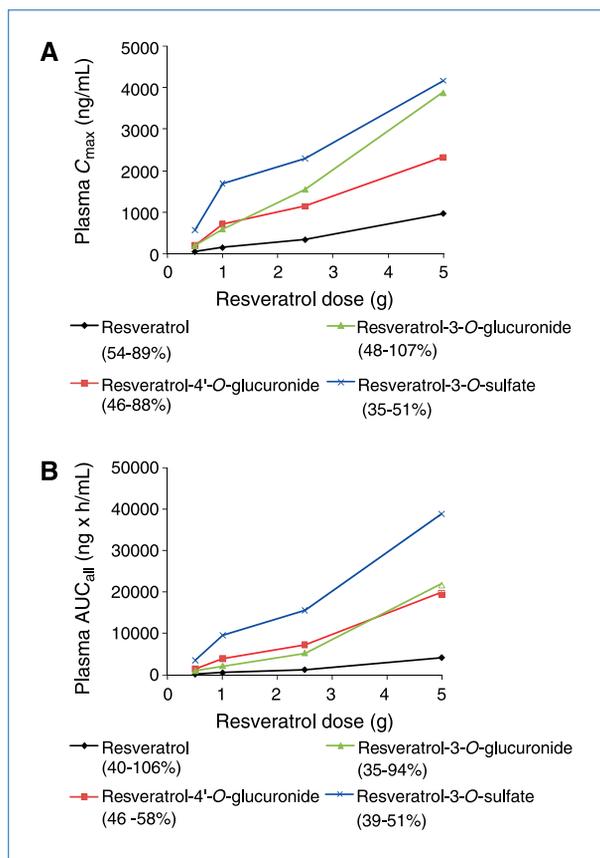
Figure 2. Relationship between dose of resveratrol and maximal plasma concentration (C_{max}; A) or area under the plasma concentration versus time curve (AUC_{last}; B) for resveratrol (black, rhombi), resveratrol-4′-O-glucuronide (red, squares), resveratrol-3-O-glucuronide (green, triangles), and resveratrol-3-O-sulfate (blue, crosses) in healthy volunteers, after a dose of resveratrol at either 0.5, 1, 2.5, or 5 g ingested between days 21 and 28 of daily dosing. Values are the mean of 10 volunteers for each dose level. The range of coefficients of variation (as % of the mean) for individual data points is shown in parentheses.

optimize future long-term intervention studies of resveratrol. Doses of up to 5 g given daily for 29 days were safe, although the two highest doses used here (2.5 and 5 g) caused gastrointestinal symptoms of mild to moderate severity. On the basis of these findings, we would tentatively recommend that in future intervention studies of resveratrol the daily dose should perhaps not exceed 1.0 g. The highest dose generated circulating peak levels of the parent agent which approached concentrations reported to cause pharmacologic activity in cells *in vitro* (24). Circulating levels of its major metabolites, resveratrol-3-O-sulfate, resveratrol-4′-O-glucuronide, and resveratrol-3-O-glucuronide, were much higher, in the case of the sulfate, the highest dose yielded a mean C_{max} of 18.3 µmol/L. These results are important in light of the suspicion that resveratrol metabolites may contribute to the pharmacologic activity of the parent agent (2). Experimental evidence to support this notion is scarce, but recent publications suggests that resveratrol sulfate conjugates could induce quinone reductase and inhibit cyclooxygenase enzymes, nitric oxide production,

and NFκB induction in cells *in vitro* (25, 26). It is not known whether resveratrol metabolites can engage estrogenic effects, a property which the parent agent is suspected to possess (27), although this notion has been disputed (28). Although the C_{max} and AUC values described here for resveratrol and its metabolites after multiple resveratrol doses are similar to those reported previously after single dose resveratrol at levels identical to those used here (9), there are subtle differences (Fig. 4). In the case of the 0.5 g dose, the C_{max} values for resveratrol-3-O-sulfate and the two monoglucuronides after repeat resveratrol were 50% to 60% of those after a single dose, consistent with multiple administration of resveratrol at this dose causing inhibition of its metabolic conjugation or augmentation of metabolite elimination. In contrast, after the 5 g dose, the C_{max} values for parent resveratrol and the two resveratrol glucuronides after multiple dosing were approximately double those after single dose resveratrol, indicative of accumulation of parent and glucuronides. After multiple administration of the 5 g dose, the AUC values for resveratrol and its metabolites were higher than those observed after single dosing; however, these differences did not reach significance levels (results not shown). Although the design of the study does not allow delineation of steady state, it is conceivable that steady state was achieved. Given that the time to steady state is three to five half-lives, and the half-life of resveratrol administered once daily was 4.8 to 9.7 hours, approximately 15 hours to 2 days would be required to attain steady-state. It needs to be stressed that the dosing regime was not optimized in this study, and a shorter dosing interval might have been used to increase the steady state concentrations and maintain levels within a narrower range. Likewise, sustained release formulations of resveratrol might possess pharmacokinetic properties superior to those of the caplet formulation used in this study. However, it is pertinent to point out that in a recent phase 1 study in colorectal cancer patients who ingested 0.5 or 1.0 g of the same resveratrol formulation used here daily for 7 days, resveratrol was still present at concentrations of between 8.3 and 674 nmol/g tissue in surgically resected colon tissue beyond ∼6 hours after the last dose (29). This means that for the prevention of colorectal malignancies by resveratrol, the once daily dosing schedule used in the present investigation might well be sufficient. The results suggest that repeated consumption of resveratrol may decrease circulating levels of IGF-I and IGFBP-3. These observations render IGF proteins potential biomarkers of pharmacologic activity of resveratrol in humans. The reduction of IGF-I and IGFBP-3 by resveratrol did not follow a linear dose-response relationship at the dose range tested here. Although the effect of resveratrol was not significant at the lowest (0.5 g) or highest (5.0 g) doses, significant decreases were observed at the medium dose of 2.5 g. Unconventional dose-pharmacologic response relationships have been described for resveratrol before. For example, in a murine model of aorta repair *in vivo*, resveratrol at 10 mg/kg increased both endothelial nitric oxide synthase expression in injured arteries and the number of endothelial progenitor cells in the circulation, although such responses were not elicited by 50 mg/kg (30). It needs to be emphasized that the current study only hints at the possibility that high-dose resveratrol

could depress circulating levels of IGF proteins, and these observations need to be corroborated in long-term studies with larger numbers of participants. If IGF protein modulation is indeed found to be a genuine property of resveratrol, its consumption for years—rather than weeks—may profoundly affect IGF axis signaling. The findings reported here for resveratrol have to be interpreted in the light of the importance of the IGF system for the development of malignancies. High levels of IGF-I have been causally associated with risk of several cancers (16), so that the ability to decrease IGF-I, which we have shown here may be achieved in humans by resveratrol, constitutes an anticarcinogenic mechanism. Intervention with 9-*cis*-retinoic acid for 3 months decreased circulating IGF-I in former smokers (20). Reduction in IGF-I is often the corollary of elevation of IGFBP-3 concentrations, which sequester IGF-I and decrease its bioavailability and thus its interaction with IGF receptors by which it engages mitogenic and antiapoptotic actions. The results presented here show that exposure to resveratrol did not elevate IGFBP-3 levels in humans, rather there was some reduction. It is difficult to interpret this finding in terms of contribution to the mechanisms by which resveratrol may exert chemoprevention. Circulating levels of IGFBP-3 are now thought to be directly associated with an increased risk of common cancers, albeit associations are modest and vary between sites

(31, 32). On the basis of these insights, one may argue that the resveratrol-induced decrease in circulating IGFBP-3, like the decrease in IGF-I, may constitute an anticarcinogenic event.

Importantly, the ingestion of resveratrol for 29 days neither significantly affected circulating levels of prostaglandin E_2, reflecting perturbation of the arachidonic acid cascade, nor influence leukocyte levels of the malondialdehyde-DNA adduct M_1dG, reflecting DNA oxidation, in a plausible and consistent fashion (results not shown). The effects of resveratrol on the levels of IGF-I, IGFBP-3, prostaglandin E_2, or M_1dG in individuals were not correlated with any of the pharmacokinetic parameters.

In summary, resveratrol has been shown here to be safe after 29 daily doses of 0.5 to 5 g. There was a hint of pharmacodynamic activity in terms of effect on circulating IGF protein levels at the 2.5 g dose level, which engendered a mean plasma peak level of 1.45 µmol/L. Future studies should establish whether resveratrol could also modulate the IGF axis when given for periods exceeding 29 days at doses below those eliciting gastrointestinal symptoms, and elucidate the mechanisms involved. Resveratrol is representative of a group of diet-derived putative cancer chemopreventive agents encompassing, among others, curcumin, tea polyphenols, and apigenin, which have attracted a lot of interest in
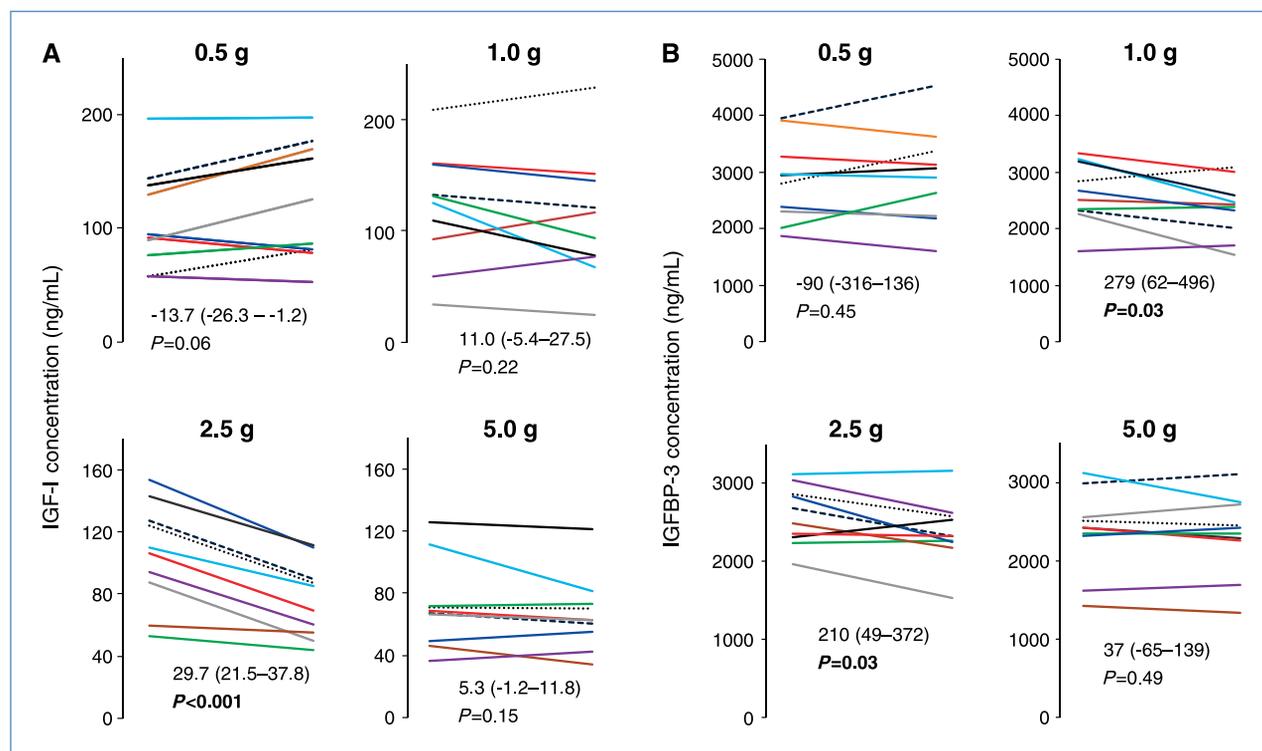

Figure 3. Circulating levels of IGF-I (A) and IGFBP-3 (B) in individual healthy volunteers before and after consumption of resveratrol at 0.5, 1.0, 2.5, or 5.0 g daily for 28 d. Results of the statistical analysis by paired *t* test, i.e., mean differences between preintervention and postintervention levels (preintervention minus postintervention values in ng/mL), 95% confidence intervals (in parentheses) and *P* values, are shown for each group of 10 individuals below the graphs. Negative values signify an increase rather than a decrease in levels. Blood samples were taken just prior to the first dose of resveratrol and on day 29.
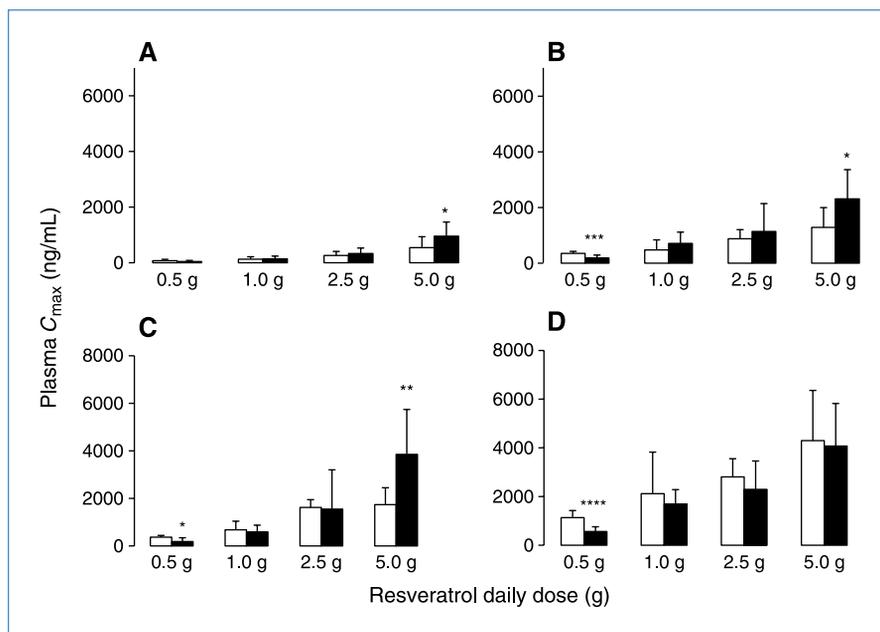
Figure 4. Maximal plasma concentrations (C_{max}) of resveratrol (A), resveratrol-4′-O-glucuronide (B), resveratrol-3-O-glucuronide (C), and resveratrol-3-O-sulfate (D) in healthy volunteers who received either a single dose (open columns) or between 21 and 28 daily doses (closed columns) of resveratrol at either 0.5, 1, 2.5, or 5 g. Single dose results have been published previously (9). Values are the mean ± SD of 10 volunteers at each dose level (*, $P < 0.05$; **, $P = 0.01$; ***, $P = 0.001$; and ****, $P < 0.0005$).

the cancer chemoprevention community. The interest stems from the fact that these agents can engage a plethora of intriguing anticarcinogenic mechanisms in cellular studies *in vitro*, although hardly any of these processes have hitherto been explored as potential efficacy biomarkers in humans. The indication described here that resveratrol affects the IGF axis hints at the possibility that IGF-I and/or IGFBP-3 may serve as potential markers of chemopreventive efficacy when these dietary agents are eventually evaluated in definitive clinical chemoprevention studies.

Disclosure of Potential Conflicts of Interest

T.D. Booth and G. Piccirilli are employees of Pharmascience, Inc. All other authors declared no potential conflicts of interest.

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Repeat Dose Study of the Cancer Chemopreventive Agent Resveratrol in Healthy Volunteers: Safety, Pharmacokinetics, and Effect on the Insulin-like Growth Factor Axis

Victoria A. Brown, Ketan R. Patel, Maria Viskaduraki, et al.

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