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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

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Abstract

Resveratrol, a naturally occurring polyphenol, has cancer chemopreventive properties in preclinical models. It has been shown to downregulate levels of insulin-like growth factor-1 (IGF-1) in rodents. The purpose of the study was to assess its safety, pharmacokinetics and effects on circulating levels of IGF-1 and insulin-like growth factor binding protein-3 (IGFBP-3) after repeated dosing. Forty healthy volunteers ingested resveratrol at 0.5, 1.0, 2.5 or 5.0g daily for 29 days. Levels of resveratrol and its metabolites were measured by HPLC-UV in plasma obtained before and up to 24h after a dose between days 21 and 28. IGF-1 and IGFBP-3 were measured by enzyme-linked immunosorbent assay in plasma taken pre-dosing and on day 29. Resveratrol was safe, but the 2.5 and 5g 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 (AUC) for the metabolites dramatically exceeded those for resveratrol, in the case of the AUC by up to 20.3-fold. Ingestion of resveratrol caused a decrease in circulating IGF-1 and IGFBP-3 ($P<0.04$ for both), respectively, compared to pre-dosing values, in all volunteers. At the 2.5g dose level the decrease was most marked. 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-1 and IGFBP-3 may contribute to cancer chemopreventive activity.

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Keywords

Resveratrol; chemoprevention; pharmacokinetics; pharmacodynamics

Introduction

Resveratrol, a polyphenol which occurs in red grapes and red wine, has been demonstrated 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 anticarcinogenesis (6) contrasts with a scarcity of studies in humans. Published human studies typically employed single doses of up to 25mg resveratrol, mostly as a constituent of wine, grape fruit 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 per day administered over two or three days (10,11) suggest that it is safe under the tested conditions. Pharmacodynamic properties of resveratrol after repeated dosing in humans have hitherto not been described.

The insulin-like growth factor (IGF) signalling system, which consists of IGFs, IGF binding proteins (IGFBPs) 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 levels of IGF-1, and an inverse relationship between 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-1, have an elevated risk of colorectal cancer (17). IGF-1 has also been suggested to contribute to the development of adenomatous polyps (18). The anti-carcinogenic activity of dietary restriction in preclinical models of carcinogenicity is thought to be mediated, at least in part, *via* reduction of circulating IGF-1 (19). Modulation of the IGF system has been proposed as a mechanism by which certain agents, for example 9-*cis*-retinoic acid, may prevent cancer (20). Resveratrol lowered circulating IGF-1 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 signalling pathway, in humans are virtually unknown. We conducted a trial of repeat high dose resveratrol in healthy volunteers with the aim to explore its safety, the pharmacokinetics of parent agent and its major metabolites and the effect of resveratrol on circulating levels of IGF-1 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 (UK) or Michigan (USA) 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 (website address: http://www.clinicaltrials.gov) as NCT 00098969. It was reviewed and approved by the Leicestershire, Northamptonshire & Rutland Research Ethics Committee (UK) and the University of Michigan Institutional Review Board (IRBMED, US) and conducted in accordance with the applicable guidelines on Good Clinical Practice. At the pre-dosing 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 4 different dosing groups were as follows: Age (in years): 0.5g: 35 (20-49), 1.0g: 36 (20-58), 2.5g: 37 (24-51), 5.0g: 42 (21-73); body mass index (in kg/m^2): 0.5g: 27.5 (20.0-42.4), 1.0g: 25.2 (18.6-39.4), 2.5g: 26.3 (19.3-39.0), 5.0g: 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 GMP standards by Pharmascience Inc, Montreal, Quebec (Canada). Caplets contained 500mg chemically synthesized resveratrol. The stability of the formulation was tested according to GMP stipulations. Participants were recruited sequentially to 4 dose levels of resveratrol (0.5, 1.0, 2.5 and 5.0g) and instructed to ingest the appropriate dose between 7 and 9 am daily for 29 days. Participants completed a form after each dose and were evaluated for adverse events and compliance with dosing on a weekly basis. 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 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 HPLC analysis

Blood samples for PK analysis of resveratrol and its metabolites were collected prior to resveratrol administration (pre-dose) on day 1 and at pre-dose and 0.25, 1.0, 1.5, 5, 12 and 24h post-dose 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 extraction of acidified plasma with methanol, and 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 (4.6mm×150mm 3μm, Waters, Elstree, UK) in combination with a Waters Atlantic C_{18} guard column (4.6mm×20mm, 3μm). Quantitation of resveratrol using a gradient HPLC-UV system (Waters Alliance 2695; Waters Corp., Manchester, UK) was performed as described before, and the method has been validated in terms of inter- and intra-day variability, recovery, accuracy and precision (22). The retention time of resveratrol was 18.6min, its lower limit of detection 5ng/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 parent resveratrol. Authentic resveratrol-3-*O*-sulfate (also provided by Pharmascience, Montreal), 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 co-chromatography. Metabolite

identity was confirmed by liquid chromatography-tandem mass spectrometry (LC/MS/MS) with selected reaction monitoring (SRM), 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, Warrington, UK) under chromatographic conditions described previously (9,22). Definitive isomer identification was not possible for resveratrol disulfate and resveratrol sulfate glucuronide.

Determination of IGF-1 and IGFBP-3

Blood samples to assay IGF-1 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 on days 30 or 31, who ingested resveratrol for the additional day or two. IGF-1 and IGFBP-3 concentrations in serum were determined using enzyme-linked immunosorbent assay (ELISA) kits DG100 and BAF675, respectively (R&D Systems, Oxon, UK), with pre- and post-dosing samples from each person analysed 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-1 assay includes a step which releases IGF-1 from binding proteins. Assays were performed according to the manufacturer's instructions and blinded with respect to subject. Samples were stable over the period elapsing between collection of pre and post dose blood when stored at −80°C, as illustrated by comparing samples analyzed fresh and after 3 months storage, which showed a variation of <5%.

Pharmacokinetic parameters

The following pharmacokinetic parameters were calculated for resveratrol and its metabolites using a non-compartmental 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}; $T_{½}$= 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 *vs* 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/(λ_z×AUC), respectively.

Statistical analysis

Descriptive statistics (mean, SD, coefficient of variation [CV], median) were calculated for plasma concentrations of resveratrol and its metabolites. Geometric mean and CV 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, Murray Hill, NJ) 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 0.5g dose, as many of the plasma concentration values at this dose were near or below the limit of quantitation.

Differences between pre- and post-intervention IGF protein levels in study volunteers (pre-minus post-intervention level) were subjected to statistical evaluation using Student's paired t test with the "Statistical Package for the Social Sciences" (SPSS) software. The test compares the mean of the differences between pre- and post-intervention values with zero; P values below 0.05 indicate that the difference between pre- and post-intervention values 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 5.0g. Resveratrol was safe, as borne out by the lack of serious adverse reactions detected by clinical, biochemical or hematological analyses during the study and study follow-up. Of the in total 44 volunteers who received resveratrol, including those who terminated the intervention prematurely, 28 reported one or more adverse events whilst on study. Seven of these individuals were on the 0.5g, four on the 1.0g, eight on the 2.5g and nine on the 5.0g doses. 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 5.0g) were gastrointestinal symptoms, including nausea, flatulence, abdominal discomfort and diarrhoea. Most of these events were mild (severity grade 1, NCI CTCAE v.4.0), although 4 participants on the 2.5g and 5.0g doses presented with nausea and/or diarrhoea of moderate severity (grade 2). The gastrointestinal side effects commenced after 2-4 days of the intervention and occurred half to one 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 presence of parent agent and metabolites. As reported before 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 co-chromatography with authentic reference material and/or HPLC-tandem mass spectrometry in volunteers' plasma (result 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 1h post-dose. The mean average plasma concentration (C_{av}) and C_{max} values of parent resveratrol across the four dose levels ranged from 0.04 to 0.55nmol/mL and 0.19 to 4.24nmol/mL, respectively. 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 6.1nmol/mL and from 2.5 to 18.3nmol/mL, respectively, across the 4 doses. The plasma elimination half-lives varied between 4.77 and 9.70h for resveratrol and between 3.09 and 8.14h for the major metabolites. The mean values for the areas under the plasma concentration *versus* time curve (AUC_{last}) for resveratrol were 175ng×h/mL at the lowest, and 4097ng×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-1 and IGFBP-3

IGF-1 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-1 and IGFBP-3 levels weakly, albeit significantly, when results from all trial participants were combined. Mean differences between pre- and post-intervention levels, 95% 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-1 and 109 (10-208), P=0.04 for IGFBP-3. Fig. 3 shows the effect of resveratrol on circulating levels of IGF-1 and IGFBP-3 in the individual volunteers. In those on the 2.5g dose, levels of IGF-1 were most prominently and consistently reduced (Fig. 3), with a difference between pre- and post-intervention IGF-1 levels (in ng/mL) of 29.6 (95% confidence interval 21.5-37.8, P<0.001). IGF-1 levels were not significantly affected in volunteers on the 0.5 or 1.0g doses. Mean IGFBP-3 concentrations in individuals on 1.0 or 2.5g resveratrol were also significantly reduced by resveratrol. The differences between pre- and post intervention IGFBP-3 levels with 95% confidence intervals (both in ng/mL) and P values were 279 (62–496, P=0.03) for the 1.0g dose and 210 (49–372, P=0.03) for the 2.5g dose (Fig. 3). The ratio IGF-1/IGFBP-3 for the 2.5g dose cohort was also strongly reduced (not shown). At 5g, resveratrol failed to affect the IGF system significantly.

Discussion

In this report we describe the pharmacokinetics of resveratrol after repeated oral administration of high doses and define potential pharmacodynamic endpoints pertinent to optimise future long-term intervention studies of resveratrol. Doses of up to 5g given daily for 29 days were safe, although the two highest doses used here (2.5 and 5g) 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 1.0g. The highest dose generated circulating peak levels of parent agent which approached concentrations reported to cause pharmacological 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 18.3μM. These results are important in the light of the suspicion that resveratrol metabolites may contribute to the pharmacological activity of the parent agent (2). Experimental evidence to support this notion is scarce, but recent publications suggests that resveratrol sulfate conjugates can 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). Whilst 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 0.5g 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 5g 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 5g 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 (result 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 3-5 half-lives, and the half-life of resveratrol administered once daily was 4.8-9.7h, approximately 15h 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 1.0g of the same resveratrol formulation used here daily for 7 days, resveratrol was still present at concentrations of between 8.3 and 674nmol/g tissue in surgically resected colon tissue beyond ~6 h after the last dose (29). This means that for 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-1 and IGFBP-3. These observations render IGF proteins potential biomarkers of pharmacological activity of resveratrol in humans. The reduction of IGF-1 and IGFBP-3 by resveratrol did not follow a linear dose-response relationship at the dose range tested here. Whilst the effect of resveratrol was not significant at the lowest (0.5g) or highest (5.0g) doses, significant decreases were observed at the medium dose of 2.5g. Unconventional dose–pharmacological response relationships have been described for resveratrol before. For example, in a murine model of aorta repair *in vivo*, resveratrol at 10mg/kg increased both endothelial nitric oxide synthase expression in injured arteries and the number of endothelial progenitor cells in the circulation, whilst 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 can 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 signalling. 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-1 have been causally associated with risk of several cancers (16), so that the ability to decrease IGF-1, which we have shown here may be achieved in humans by resveratrol, constitutes an anti-carcinogenic mechanism. Intervention with 9-*cis*-retinoic acid for 3 months decreased circulating IGF-1 in former smokers (20). Reduction in IGF-1 is often the corollary of elevation of IGFBP-3 concentrations, which sequester IGF-1 and decrease its bioavailability and thus its interaction with IGF receptors by which it engages mitogenic and anti-apoptotic 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-1, may constitute an anti-carcinogenic event.

Importantly, ingestion of resveratrol for 29 days did neither significantly affect circulating levels of prostaglandin E-2 (PGE-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). Effects of resveratrol on

levels of IGF-1, IGFBP-3, PGE-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 5g. There was a hint of pharmacodynamic activity in terms of effect on circulating IGF protein levels at the 2.5g dose level, which engendered a mean plasma peak level of 1.45μM. Future studies should establish whether resveratrol can modulate the IGF axis also when given for periods exceeding 29 days at doses below those eliciting gastrointestinal symptoms, and elucidate 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 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-1 and/or IGFBP-3 may serve as potential markers of chemopreventive efficacy when these dietary agents are eventually evaluated in definitive clinical chemoprevention studies.

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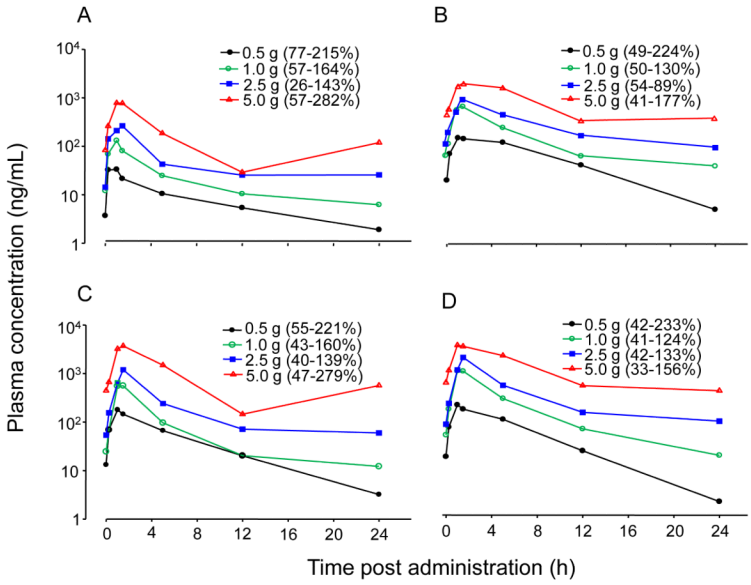

Figure 1.

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 0.5g (black, closed circles), 1g (green, open circles), 2.5g (blue, squares) or 5g (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 brackets.

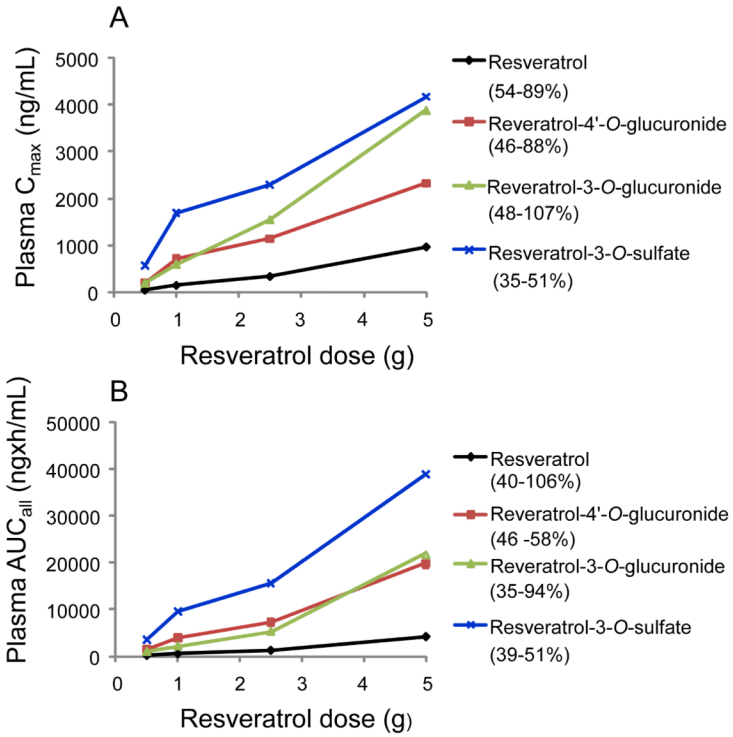

Figure 2.

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 5g ingested on between day 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 brackets.

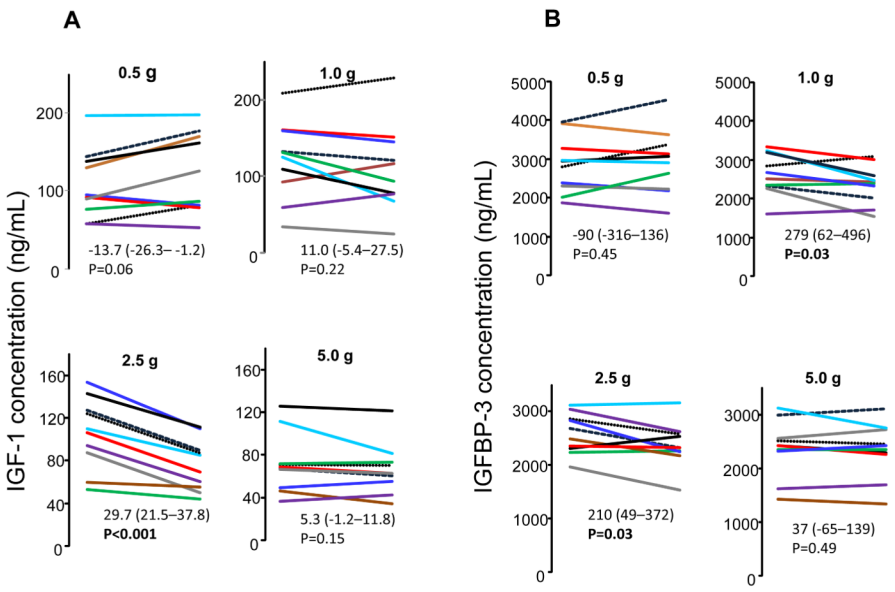

Figure 3.

Circulating levels of IGF-1 (**A**) and IGFBP-3 (**B**) in individual healthy volunteers before and after consumption of resveratrol at 0.5, 1.0, 2.5 or 5.0g daily for 28 days. Results of the statistical analysis by paired t-test, i.e. mean differences between pre- and post-intervention levels (pre minus post values in ng/mL), 95% confidence intervals (in brackets) and P values, are shown for each group of 10 individuals below the graphs. Negative values signify an increase rather than decrease in levels. Blood samples were taken just prior to the first dose of resveratrol and on day 29.

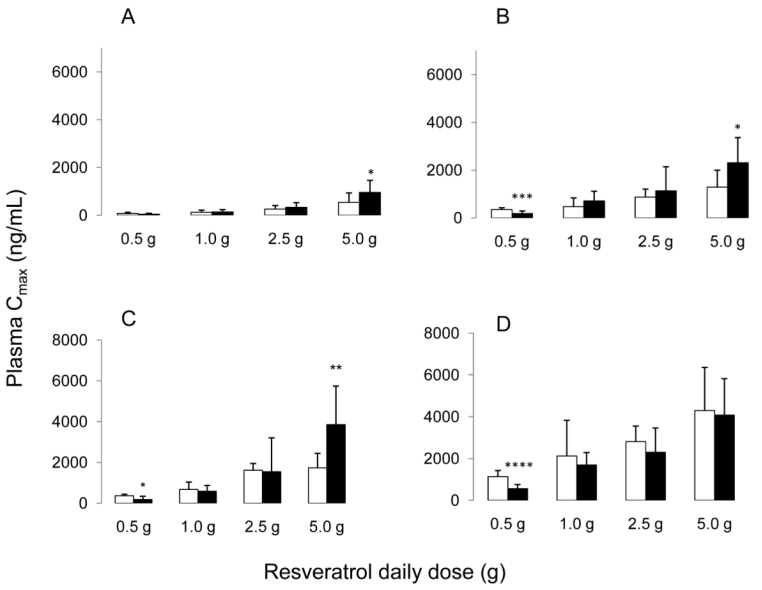

Figure 4.

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 bars) or between 21 and 28 daily doses (closed bars) 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. Asterisks indicate *P<0.05,**P=0.01, ***P=0.001 and ****P<0.0005.

Table 1

Number of healthy volunteers who experienced adverse events deemed intervention-related after daily ingestion of resveratrol for 29 days. Overall number of volunteers per dose was 10-12.

Symptom	Number of volunteers			
	Dose (g)			
	0.5	1.0	2.5	5.0
Raised blood bilirubin:				
Total	1	1		
Conjugated		2		
Unconjugated		2		
Skin discoloration		1		
Cystitis		1		
Abdominal pain			4	3
Acne			1	
Cramp			1	
Diarrhoea			2 (*1)	7 (*2, **1)
Discomfort on passing faeces			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

Severity grading (NCI CTCAE v.3.0): no star=1,

* =2,

** =3

Cancer Res. Author manuscript; available in PMC 2011 May 15.

Table 2

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

	Parameter				
	C_{max}*↑ (ng/mL)	**C_{av}*↑ (ng/mL)**	**T_{max}† (h)**	**$T_{½}$↑ (h)**	**AUC_{last}↑ (ng×h/mL)**
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)	1250 (40.0)
5.0	967 (53.5) [4.24]	126 (55.8) [0.55]	1.08 (0.5-1.5)	7.85 (25.1)	4097 (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)	1331 (56.4)
1.0	710 (57.9) [3.12]	178 (64.8) [0.78]	1.50 (0.83-5.0)	5.77 (44.6)	3774 (53.2)
2.5	1137 (88.0) [4.99]	323 (66.9) [1.42]	1.50 (0.25-5.0)	8.14 (38.6)	7245 (54.4)
5.0	2323 (45.5) [10.2]	667 (61.3) [2.93]	1.50 (1.0-5.0)	7.55 (21.2)	19984 (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)	2087 (37.7)
2.5	1546 (107) [6.78]	204 (64.0) [0.89]	1.42 (1.0-1.5)	6.43 (40.8)	5300 (47.1)
5.0	3886 (48.6) [17.1]	649 (34.8) [2.85]	1.50 (0.5-1.6)	5.19 (52.7)	22084 (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)	3558 (51.6)
1.0	1694 (35.2) [7.43]	377 (48.7) [1.65]	1.50 (0.83-5.0)	7.37 (51.4)	9464 (42.3)
2.5	2292 (50.7) [10.1]	604 (43.8) [2.65]	1.33 (1.0-1.5)	6.84 (40.6)	15638 (39.0)
5.0	4172 (40.3) [18.3]	1384 (42.3) [6.07]	1.25 (0.25-5.0)	7.98 (29.6)	38900 (49.5)

C_{max}=maximal plasma concentration. C_{av}=average plasma concentration. T_{max}=time of maximal plasma concentration (C_{max}). $T_{½}$=apparent first-order elimination half-live. 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).

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 days (n=10 per dose level).

Dose (g)	Apparent total body clearance (L/h)	Apparent volume of distribution (L)
0.5	2771 (58.9)*	16071 (56.5)
1.0	2366 (63.9)	42673 (86.1)
2.5	2219 (42.5)	32322 (44.5)
5.0	2548 (100)	38023 (133)

* Mean values with CV% in brackets