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Resveratrol-Associated Renal Toxicity

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Resveratrol, (3,5,4′-trihydoxystilbene) a compound found in grapes, mulberries, and peanuts, has antimycotic, antiviral, and beneficial cardiovascular and cancer preventive activities. It is being developed for several clinical indications. To evaluate the potential toxicity of resveratrol, rats were administered by gavage 0, 300, 1000, and 3000 mg *trans*-resveratrol per kilogram body weight per day for 4 weeks. Most of the adverse events occurred in the rats administered 3000 mg per kilogram body weight per day. These included increased clinical signs of toxicity; reduced final body weights and food consumption; elevated BUN, creatinine, alkaline phosphatase, alanine aminotransferase, total bilirubin, and albumin; reduced hemoglobin, hematocrit, and red cell counts; and increased white cell counts. Increases in kidney weights and clinically significant renal lesions, including an increased incidence and severity of nephropathy, were observed. Diffuse epithelial hyperplasia in the bladder was considered, equivocal and of limited biological significance. No histological effects on the liver were observed, despite the clinical chemistry changes and increased liver weights in the females. Effects seen in the group administered 1000 mg resveratrol per kilogram body weight per day included reduced body weight gain (females only) and elevated white blood cell count (males only). Plasma resveratrol concentrations in blood collected 1 h after dose administration during week 4 were dose related but were relatively low given the high dosage levels; conjugates were not measured. Under the conditions of this study, the no observed adverse effect level was 300 mg resveratrol per kilogram body weight per day in rats.

Key Words: **resveratrol; cancer chemoprevention; kidney.**

Resveratrol, (3,5,4′-trihydoxystilbene), a compound found in grapes, mulberries, and peanuts, is a phytoalexin, used by the plant to defend itself against fungal and other attacks (Savouret and Quesne, 2002). It is one of the ingredients in the traditional Asian medicine Ko-jo-kon for treatment of fungal, inflammatory, hypertensive, allergic, and lipid diseases (Nonomura *et al*., 1963). The publication of Jang *et al*. in 1997 attributing cancer preventive activity to resveratrol led to an increase in research and publications related to resveratrol. Favorable cancer preventive attributes include, for example, activities as an antioxidant, anti-inflammatory, antiproliferative, antimutagen, and pro-apoptotic (Banerjee *et al*., 2002; Bhat *et al*., 2001a,b; Gusman *et al*., 2001; Joe *et al*., 2002; Schneider *et al*., 2001). Mechanisms of action have been reported to include inhibition of NfkappB and AP-1 (Manna *et al*., 2000; She *et al*., 2002) and modulation of cyclo-oxygenase, lipoxygenase, nitric oxide synthetase, and protein kinases (Adhami *et al*., 2001; Martinez and Moreno, 2000; Surh *et al*., 2001) associated with the process of carcinogenesis. Resveratrol is also a phytoestrogen and has some structural similarity to diethylstilbesterol (DES) (Gehm *et al*., 1997). However, resveratrol has a higher affinity for the estrogen receptor β (ER β) than α and transcriptionally activates ER β at low concentrations (Ramsey *et al*., 2004). ER α and ER β are distinct gene products with nonoverlapping functions (Gustafsson, 2003), and ER β ligands may have important cancer-preventive properties (Paruthiyil *et al*., 2004). Additionally, potentially beneficial cardiac properties have been ascribed to resveratrol (Fremont, 2000), and it has shown efficacy as a topically applied antiviral against herpes simplex (Docherty *et al*., 2003). For these reasons, resveratrol is an attractive pharmaceutical candidate.

To date, few studies have evaluated the toxicity of resveratrol in animals. Juan *et al*. (2002) administered 20 mg resveratrol orally per kilogram body weight to rats for 28 days and reported no treatment-related effects except mild changes in serum liver enzymes. A single dose of 2000 mg resveratrol per kilogram body weight did not cause any detectable, toxicologically significant changes in the rats. Other published experiments in rats tend to use dose levels less than 20 mg resveratrol per kilogram body weight and for durations that are shorter than 4 weeks (Turner *et al*., 1999). Given the minimal toxicity data in rats and the lack of data for systemic toxicity in other species, the toxicity and target organs of resveratrol were unknown.

Here we report the toxicological effects in rats of 300, 1000, and 3000 mg resveratrol per kilogram body weight (kg bwt) administered by gavage for 4 weeks. These data identify the kidney as a target organ for toxicity caused by the highest dose of resveratrol and provide data that will

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prove useful in supporting the safety evaluation of resveratrol for clinical use.

MATERIALS AND METHODS

Test Article. Resveratrol (lot no. 09672) was provided by the National Cancer Institute in collaboration with Royalmount Pharmaceuticals, Montreal, Quebec Canada. It was stored at 2–8°C, ambient humidity, and protected from light. Compound identity was confirmed by GC-MS, and the purity was determined by HPLC to be 99.67 ± 0.03%.

Animals. Male and female CD® Virus Antibody Free (VAF) rats (Charles River Breeding Laboratories, Kingston, NY) were housed in an AAALAC Intl. accredited facility according to the *Guide for the Care and Use of Laboratory Animal*s (National Research Council, 1996). The study was conducted in compliance with Good Laboratory Practices. Animals were singly housed in polycarbonate cages with Anderson bed-o'cobs® bedding (Heinold, Kankakee, IL) at 64–79°F, 30–70% room humidity, and 14/10 light/dark cycle. The animals were approximately 6 to 7 weeks old and weighed 173–223 g (males) and 143–187 g (females) at dosing initiation. Certified Rodent Chow No. 5002 (PMI Feeds Inc., St. Louis, MO) and tap water were provided *ad libitum*. Animals were assigned randomly to treatment groups based on body weight.

Dosing formulations. The vehicle was 0.5% methylcellulose/0.2% Tween 80. Each dosing suspension was prepared individually by mixing resveratrol with the vehicle in a homogenizer for at least 2 min. Dosing formulations were stored at 2–8°C, homogenized daily prior to dosing for at least two min, allowed to warm to room temperature before administration, and stirred continuously while the dosing procedure was ongoing. The concentrations and stability of the dosage formulations were confirmed by HPLC prior to the beginning and during the course of the study; all concentrations were within 10% of theoretical.

Study design. The dosages in the 28-day study were based on a 14-day range finding study in which 0, 50, 150, 500, and 1500 mg/kg bwt/day were administered to five rats per sex; no toxicological effects were observed in body weights, food consumption, hematology and clinical chemistry, organ weights, and gross and histologic (control and high dose groups) pathology. In the present study, 20 animals/sex/group were dosed once daily with 0 (vehicle only), 300, 1000, or 3000 mg/kg bwt/day resveratrol for 28 days by gavage (10 ml/kg bwt/day). The amount administered was based on the most recently measured body weight. Body weight measurements, food consumption calculations, and physical exams were conducted weekly. All animals were observed daily for clinical signs of toxicity.

In week 4, blood samples were collected for hematology and clinical chemistry measurements from 10 animals/sex/group (anesthetic: CO_2:O_2, 70%:30%) from the orbital sinus. Hematology parameters were measured using a Sysmex K1000 Hematology Analyzer and included erythrocyte count, hematocrit, hemoglobin, leukocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, red blood cell morphology, and reticulocyte count. Clinical chemistry parameters were measured using a Boehringer Mannheim/Hitachi 704 and included alanine aminotransferase, aspartate aminotransferase, albumin, alkaline phosphatase, albumin/globulin ratio, total bilirubin, BUN/creatinine ratio, calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, total protein, triglycerides, and urea nitrogen. Blood samples collected at scheduled terminal necropsy from the vena cava from 10 animals/sex/group were used to measure coagulation parameters using a MLA, Inc. Electra 700 Automatic Coagulation Timer (activated partial thromboplastin time, prothrombin time, and fibrinogen). Urinalysis was performed using Boehringer Mannheim Chemstrip 9 Reagent Strips.

In week 4, blood samples were collected 1 h post dosing for resveratrol plasma exposure verification from 10 animals/sex/group (anesthetic: CO_2:O_2, 70%:30%) from the orbital sinus. The blood samples were collected into tubes containing EDTA and centrifuged at 1500 × *g* for 10 min to isolate plasma. Plasma was collected, stored at −80°C and analyzed for resveratrol concentration by HPLC using a Waters Associates system with a LiChosorb RP8 Column, mobile phase of acetonitrile:0.025 M sodium monophosphate buffer, pH 4.2, 30:70 v/v, and detection at 310 nm. The standard curve was linear over the range of 25–4000 ng/ml. Carbamazapine was used as the internal standard.

Animals found dead or sacrificed moribund were necropsied. All other animals were euthanized by CO_2 asphyxiation and necropsied one day after the last dose. The necropsy procedure was a thorough and systematic examination and dissection of the viscera and carcass, and collection, weighing (organs marked with*), and fixation of the following tissues/organs: adrenal glands, aorta, brain*, cecum, colon, duodenum, epididymides, esophagus, eyes, femur with marrow, gross lesions, heart*, ileum, jejunum, kidneys*, liver*, lungs/bronchi*, mesenteric lymph node, mammary gland, ovaries/fallopian tubes, pancreas, pituitary, prostate, mandibular salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum with marrow, stomach, testes*, thymus, thyroid/parathyroid*, tissue mass, trachea, urinary bladder, uterus (corpus and cervix), and vagina. All tissues and organs collected at necropsy were examined microscopically in the vehicle control and high-dose groups. The kidneys and urinary bladders were subsequently examined microscopically in the low- and mid-dose groups. Where applicable, all tissue changes received a severity grade where: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked. Mean group severity scores for each change were determined by dividing the sum of the severity scores by the number of tissues examined in that group.

Statistical analyses. For each sex, analysis of variance tests were conducted on body weight, food consumption, hematology, clinical chemistry, coagulation, organ/brain weight ratios, and plasma resveratrol level data. If a significant F ratio was obtained ($p < 0.05$), Dunnett's t-test was used for pair-wise comparisons to the control group.

RESULTS

In-Life Results

In the 3000 mg/kg bwt/day dose group, two males were found dead (days 10 and 24) and one male was sacrificed moribund (day 24). Renal tubule dilatation, papillary necrosis, acute pelvic inflammation, and increased incidence and severity of nephropathy were seen in the animals sacrificed at day 24 and were interpreted as treatment related. The animal found dead at day 10 had cardiac inflammation and a thoracic mass surrounding the heart, and the death was not attributed to treatment. One female treated with 300 mg/kg bwt/day died of gavage trauma on day 11. The predominant treatment-related clinical signs of toxicity in this dose group were dehydration in seven males and ten females, piloerection in seven males and ten females, and red material in the urine/cage in five males. The frequencies varied from single to multiple occurrences. Occult blood tests on the urine failed to identify treatment-related blood in the urine (data not shown). Additional clinical signs included labored breathing in one female, hunched posture in two males and one female, decreased activity in two males, and rough coat in two males and three females. In the 1000 mg/kg bwt/day dose group, labored breathing and dehydration were noted in one female on day 27–28. One male in the 0 mg/kg bwt/day dose group appeared dehydrated on day 6–7. No other clinical signs were observed in the animals treated with 0, 300, and 1000 mg/kg bwt/day.

In the 3000 mg/kg bwt/day dose group, body weight gain and final body weight were significantly decreased ($p < 0.05$, $n = 20$/sex/group minus early deaths). The mean (standard deviation) of the total weight gain and final body weights in the males were 138 g (32) and 330 g (36.9) versus 181 (22.3) and 375 g (29.1) in the control group, respectively. In the females the weight gain and final weights were 67 g (20) and 228 g (22.5) versus 81 (9.1) and 224 g (15.6) in the control group, respectively. In the 1000 mg/kg bwt/day females, there was also a significant reduction ($p < 0.05$) in weight gain, 70 g (11.5) versus 81 g (9.1) in the controls. Food consumption was significantly decreased ($p < 0.05$) in the 3000 mg/kg bwt/day dose group only during weeks 1 and 4 and additionally during week 3 in the females.

Clinical Pathology

In the 3000 mg/kg bwt/day dose group, females had significantly ($p < 0.05$, $n = 10$/sex/group in this and all clinical pathology measurements) elevated blood urea nitrogen (BUN) and creatinine levels. The mean (standard deviation) values were 20.3 mg/dl (3.17) and 0.50 mg/dl (0.13) versus 15.8 mg/dl (0.82) and 0.37 mg/dl (0.03) in the controls, respectively. These were nonsignificantly elevated in the males. These changes are consistent with the identification of kidney toxicity based on organ weights and histology, described below. The BUN/creatinine ratios were not statistically different from the controls. An increase in the BUN/creatinine ratio in males in the 1000 mg/kg/day dose group was sporadic and not considered treatment-related because there was no concurrent increase in high-dose group animals. Animals administered 3000 mg/kg bwt/day had significantly higher ($p < 0.05$) serum alanine aminotransferase (ALT) and alkaline phosphatase (ALKP) levels. The mean (standard deviation) values in the males were 62 IU/l (16.2) and 362 IU/l (84.2) in the males versus 48.5 IU/l (8.5) and 288 IU/l (50.2) in the controls, respectively. In the females the values were 76 IU/l (21.5) and 276 IU/l (126) versus 47 IU/l (8.8) and 177 IU/l (21.1) in the controls, respectively. Total bilirubin was significantly increased ($p < 0.05$) in the females [0.22 mg/dl (0.04) vs. 0.16 mg/dl (0.04) in the controls] and nonsignificantly increased in males. These data suggest effects of treatment on the liver at this dose level, but no histopathological changes were observed. Males in the 3000 mg/kg bwt/day dose group had a significant increase ($p < 0.05$) in albumin levels and a concurrent increase in albumin/globulin ratio levels [4.7 g/dl (0.14) and 2.43 g/dl (0.37) vs. 4.2 g/dl (0.23) and 1.95 g/dl (0.27) in the controls, respectively] while there was a slight nonsignificant increase in these parameters in females in the same dose group. These increases may have been associated with the clinical observations of dehydration.

Animals in the 3000 mg/kg bwt/day dose group had a significant reduction ($p < 0.05$) in hemoglobin concentration [14.9 g/dl (0.65) in the males and 13.5 g/dl (1.16) in the females vs. 15.7 g/dl (0.50) and 15.1 g/dl (0.43) in the male and female controls, respectively]. Red blood cell counts were significantly and nonsignificantly decreased in females and males, respectively, in this dose group. Females in this dose group also had a reduction ($p < 0.05$) in hematocrit and mean corpuscular volume [37.2% (2.43) and 58.4 fl (1.66) vs. 40.4% (1.42) and 56.0 fl (1.62) in the controls, respectively], but this was not seen in males. These data are suggestive of anemia in the 3000 mg/kg bwt/day dose group animals, and the females appeared more sensitive than the males. The anemia may have been related to renal injury resulting in reduced erythropoietin synthesis in the kidney, which would subsequently cause anemia. White blood cell counts (1000/μl) were significantly increased ($p < 0.05$) in both sexes in the 3000 mg/kg bwt/day dose group [15.9 (2.64) in the males and 18.6 (6.93) in the females vs. 12.9 (2.46) and 13.6 (2.56) in the respective controls] and also in the males in the 1000 mg/kg bwt/day dose group [16.1 (3.24) vs. 12.9 (2.46) in the controls]. In the differential counts there were slight increases in the mature neutrophil and lymphocyte counts in the affected groups. There were no treatment-related changes in coagulation parameters.

Gross and Microscopic Pathology

At necropsy, kidney weights were significantly increased ($p < 0.05$, $n = 20$/sex/group minus early deaths) above controls in males and females treated with 3000 mg/kg bwt/day [mean and standard deviation were 164 g (22) and 121 g (20) vs. 148 g (13) and 108 g (8) in the male and female controls, respectively]. In males, heart [58 g (11)] and lungs/bronchi [80 g (10)] weights were significantly decreased ($p < 0.05$) in the 3000 mg/kg bwt/day dose group versus controls, 68 g (10) and 90 g (14), respectively. In females, adrenal glands [3.1 g (0.4) vs. 3.8 g (0.4) in the controls], liver [663 g (112) vs. 561 g (53)], spleen [36 g (13) vs. 29 g (3)], and thyroid/parathyroid weights [1.2 g (0.2) vs. 1.0 g (0.2)] were significantly increased ($p < 0.05$) in the 3000 mg/kg bwt/day dose group. Adrenal gland weights were also significantly decreased in the 1000 mg/kg bwt/day dose group females [3.3 g (0.5)]. Thyroid/parathyroid gland weights were significantly lowered in males in the 300 mg/kg bwt/day dose group, but this was not interpreted as test article related, because this effect was not seen in the higher dose groups.

Focal renal lesions and/or nodules were observed in two males and three females in the 3000 mg/kg bwt/day dose group during the scheduled gross necropsy. These observations generally correlated to a microscopic finding of tubule dilatation or nephropathy. All other gross observations were considered incidental and typical of normal rats.

Kidneys of animals in the 3000 mg/kg bwt/day dose group had the following microscopic lesions (Table 1): renal tubule dilatation, papillary necrosis, ulceration of pelvic epithelium, acute inflammation of the pelvis, acute inflammation of pelvic adventitia, glomerular necrosis, papillary fibrosis, hyperplasia of pelvic epithelium, and increased incidence of nephropathy. The incidence and severity of nephropathy in animals treated with

TABLE 1
Histologic Changes in the Kidneys of Rats Administered Resveratrol Orally for 4 Weeks

	0		300		1000		3000	
Dose mg/kg bwt/day	M	F	M	F	M	F	M	F
Kidney lesion								
Tubule dilatation	0/20	0/20	0/20	0/19	0/20	0/20	8/17 (1.24)[a]	9/20 (1.25)
Papillary necrosis	0/20	0/20	0/20	0/19	0/20	0/20	2/17 (0.12)	5/20 (0.45)
Ulceration, pelvic epithelium	0/20	0/20	0/20	0/19	0/20	0/20	1/17 (0.18)	1/20 (0.15)
Inflammation, acute pelvic	0/20	0/20	0/20	0/19	0/20	0/20	1/17 (0.06)	3/20 (0.30)
Inflammation, acute pelvic adventitia	0/20	0/20	0/20	0/19	0/20	0/20	2/17 (0.29)	2/20 (0.15)
Glomerular necrosis	0/20	0/20	0/20	0/19	0/20	0/20	2/17 (0.18)	3/20 (0.25)
Papillary fibrosis	0/20	0/20	0/20	0/19	0/20	0/20	2/17 (0.18)	3/20 (0.20)
Hyperplasia, pelvic epithelium	0/20	0/20	2/20 (0.20)	0/19	1/20 (0.10)	0/20	12/17 (2.00)	10/20(1.05)
Nephropathy	11/20 (0.65)	8/20 (0.50)	12/20 (0.60)	6/19 (0.32)	12/20 (0.65)	7/20 (0.35)	16/17 (1.82)	15/20(1.70)

[a]Values represent incidence (mean group severity score).

300 and 1000 mg/kg bwt/day was similar to controls. Therefore, nephropathy in these animals was not considered related to treatment. The low incidence of renal pelvic epithelium hyperplasia in males treated with 300 and 1000 mg/kg bwt/day was not interpreted as biologically relevant in the absence of the other findings present in the males treated with 3000 mg/kg bwt/day.

Diffuse epithelial hyperplasia in the urinary bladder was observed in 7 of 17 males (severity 0.65) and 4 of 20 females (severity 0.35) treated with 3000 mg/kg bwt/day. This was also observed in 2 of 20 males and 2 of 20 females (both severities 0.10) and in 1 of 19 females (severity 0.05) treated with 1000 and 300 mg/kg bwt/day, respectively.

On study day 24 one male being treated with 3000 mg/kg bwt/day was found dead, and a second male was sacrified due to its moribund condition. Gross lesions in the male that was found dead included multiple pale nodules in kidney, which correlated to a microscopic finding of infarction. The gross lesions in the animal that was sacrificed moribund included dark pigmentation changes and multiple pale nodules in the kidneys that correlated to microscopic findings of papillary necrosis and/or dilatation of tubules. These renal lesions were interpreted as the probable cause of death or moribund condition in these two males.

Plasma Resveratrol Concentrations

Resveratrol concentrations were measured in plasma collected in week 4, 1 h after dose administration, from 10 animals per sex per group. The concentrations [mean and (standard deviation)] in the males treated with 0, 300, 1000, and 3000 mg/kg bwt/day were, respectively, 0 (0), 576 (178), 991 (250), and 2728 ng/ml (961). In the females similarly treated the respective concentrations were 0 (0), 333 (250), 704 (460), and 1137 ng/ml (674). The molecular weight of resveratrol is 228.247, and a plasma concentration of approximately 1.1 μg/ml is approximately 5 μM, as discussed below.

DISCUSSION

Oral administration of 3000 mg resveratrol per kilogram body weight to rats for 28 days resulted in nephrotoxicity observed as elevated serum BUN and creatinine levels (statistically significant in the females), increased kidney weights, gross renal pathology changes, and an increased incidence and severity of histopathological changes in the kidneys. The seemingly high incidence of nephropathy in the control group (Table 1) is related to the identification of the kidney as a target organ and the diagnostic criteria [e.g., incidence of basophilic tubule(s) or eosinophilic cast(s)] that are then applied to resolve treatment effects (severity and/or number of focal lesions). In the two males that died early on day 24, microscopic evaluation of the kidneys identified lesions that were the probable cause of early death. One possible pathogenesis of the renal lesions could be increased concentration of the test article (or its metabolite) as a function of the renal osmotic concentration gradients, resulting in toxic levels in the renal pelvis. This would result in necrosis of the tissue, obstruction of selected renal tubules and thus dilatation of those tubules behind the obstructed region. Inflammation and pelvic epithelium hyperplasia are expected responses to the presence of necrotic tissue. The other histopathological observation of diffuse epithelial hyperplasia of the urinary bladder was interpreted as an equivocal finding of limited biological significance. The administration of 1000 or 300 mg resveratrol/kg bwt/day did not result in nephrotoxic findings.

The predominant clinical signs of toxicity in the 3000 mg/kg bwt/day dose group were dehydration, piloerection, and red material in the cage/urine. Dehydrated animals frequently appear piloerect. Dehydration was supported by reductions in body weights gains in the 3000 mg/kg bwt/day dose group and in females in the 1000 mg/kg bwt/day dose group. The reduction of body weight gains in the 3000 mg/kg bwt/day dose group may be related to decreased food consumption in this dose group, which did not occur in the 1000 mg/kg bwt/day dose group.

Dehydration in the 3000 mg/kg bwt/day dose group was further supported by hyperalbuminemia (statistically significant only in the females), typically seen in dehydrated animals. Occult blood tests on the urine failed to identify treatment-related blood in the urine

Anemia occurred in the females treated with 3000 mg/kg bwt/day dose group and possibly to some degree in the males. The anemia may have been related to the renal injury, as reduced erythropoietin synthesis in the kidneys would have occurred. White blood cell counts were significantly increased in animals in the 3000 mg/kg/bwt day dose group and in males in the 1000 mg/kg bwt/day dose group. The elevations may have been associated with the renal pelvic inflammation.

Clinical chemistry changes (i.e., increased ALT, ALKP, and total bilirubin in the 3000 mg/kg bwt/day dose group) suggest liver toxicity, but this was not supported histologically. Similarly, organ weights that were changed as a result of treatment did not show evidence of histological changes.

There was a dose-related increase in plasma resveratrol levels in each sex. Plasma levels were higher in males than in females. In the 3000 mg/kg bwt/day dose group the mean plasma resveratrol levels were approximately 2.7 and 1.1 μg per ml for males and females, respectively. In this study the samples were collected approximately 1 h post dose and were not additionally analyzed for glucuronide and sulfate conjugates of resveratrol. In a single-dose pharmacokinetic study in mice we have found that resveratrol reached a maximum concentration at 30 min and that the conjugates reached maximum concentrations at 1 h. Additionally, the maximum mean concentrations of resveratrol glucuronide and sulfate were approximately 18- and 13-fold higher, respectively, than resveratrol (unpublished data). Thus, the resveratrol measurements in this study confirmed dose-related exposure but were not intended to provide pharmacokinetic measurements. Resveratrol is known to have a short half-life, approximately 0.5 h, in rats (Bertelli *et al*., 1998) and to be metabolized rapidly (Andlauer *et al*., 2000; de Santi *et al*., 2000; Kuhnle *et al*., 2000) and similarly by rat and human liver microsomes (Yu *et al*., 2002). It circulates as conjugates at higher concentrations than the parent form (unpublished data). As with steroid hormones, the sulfated form may serve as a substrate for tissue sulfatases that could make higher local tissue concentrations. *In vitro* studies have suggested that 5–10 μM concentrations of resveratrol are needed to demonstrate cancer-preventive effects (Jang *et al*., 1997; Joe *et al*., 2002; Manna *et al*., 2000; She *et al*., 2002), and it has not been clear whether or not such concentrations could be achieved *in vivo*. In this study we have demonstrated that concentrations of resveratrol can be achieved *in vivo* that are toxic. However, the plasma measurements were not optimized to measure the maximum concentrations of resveratrol and its metabolites, and the latter probably reached significantly higher concentrations than 1–3 μg/ml. Thus, additional studies are needed to define the potential therapeutic index of resveratrol and its metabolites.

In a 4-week rat toxicity study Juan *et al*. (2002) reported that administration of 20 mg resveratrol/kg bwt resulted in mild changes in serum liver enzyme levels (AST), as well as increases in brain and testicular weights. These findings were not observed in the present study at the lowest dose tested, which was 15-fold higher than used by Juan. However, in the present study alterations in liver enzyme activities at the highest dose, without accompanying histological changes, were seen and testicular: brain ratios (data not shown) were nonsignificantly increased in all the males relative to the control group. Therefore, the findings in the present study are consistent with those of Juan. In the current study no adverse effects were observed in animals treated with 300 mg resveratrol/kg bwt for 28 days. In animals treated with 1000 mg reseveratrol/kg bwt the changes observed were dehydration and labored breathing in one female on day 27–28, a mild reduction in final body weight of approximately 5% and in weight gain of 14% ($p < 0.05$) in the females, slight but significant ($p < 0.05$) increases in WBC and lymphocytes (data not shown) in the males, and a decrease in adrenal weights in the females. No histological changes were observed. Therefore, under the conditions of this study the no observed adverse effect level was 300 mg reseveratrol/kg bwt, and the kidney was identified as the major target organ of toxicity in animals treated with 3000 mg/kg bwt.

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