Comparative Pharmacokinetics of the Polyphenol, Pterostilbene, in Rhesus Macaques, Cynomolgus Macaques, and Common Marmosets

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Abstract
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STUDY OBJECTIVE

In consideration of the potential limitations that its bioavailability and metabolism may impose on the pharmacodynamic activity of resveratrol, congeners and analogs of resveratrol are attracting increasing attention as possible agents for cancer chemoprevention. The present study was performed to compare plasma drug levels of pterostilbene in three species of nonhuman primates receiving oral administration of the polyphenol.

INTRODUCTION

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene; Figure 1) is a naturally occurring plant polyphenol that is present in grapes, berries, peanuts, and other foodstuffs. Resveratrol is thought to act as a phytoalexin, protecting plants against pathogens. Since the suggestion in the early 1990's that the apparent cardioprotective effects of red wine ("the French Paradox") may be mediated by resveratrol, the compound has been studied for a variety of beneficial health effects. In 1997, Jang *et al.*, reported that resveratrol has a number of biological activities that are consistent with cancer chemoprevention, and that the agent can inhibit carcinogenesis in experimental animals. Since then, a broad range of desirable health effects have been ascribed to resveratrol. Beneficial health effects that have been attributed to resveratrol include:

- Cancer chemoprevention activity
- Cancer chemotherautic activity
- Anti-aging activity
- Neuroprotective (anti-Alzheimer's) activity
- Anti-viral activity
- Cardioprotective activity

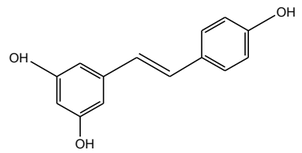


Figure 1. Structure of Resveratrol

The cancer chemopreventive and chemotherapeutic effects of resveratrol may be mediated by any of a large number of mechanisms. Possible mechanisms of resveratrol action include:

- Antioxidant activity
- Free radical scavenging
- Anti-inflammatory activity
- Modulation of the activity of carcinogen metabolizing enzymes
- Inhibition of cell proliferation
- Induction of apoptosis
- Inhibition of tumor angiogenesis
- Chemosensitization

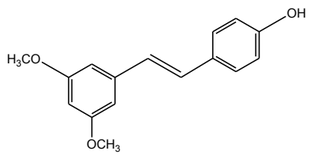


Figure 2. Structure of Pterostilbene

Although it demonstrates a large number of *in vitro* activities that are consistent with efficacy in disease prevention and therapy, the oral bioavailability of resveratrol is poor, and the compound undergoes rapid first pass metabolism. Plasma levels of resveratrol are very low after oral administration; the primary resveratrol species found in the plasma are glucuronide and sulfate conjugates. Pharmacokinetic studies in human volunteers have led to questions concerning whether even very high oral doses of resveratrol can generate plasma levels of parent drug that are necessary to achieve the chemopreventive and other desirable activities that have been reported in experimental models.

The poor oral bioavailability and rapid metabolism of resveratrol are common challenges to the use of polyphenols for disease prevention or therapy. However, some methylated polyphenols demonstrate much greater intestinal absorption than resveratrol does, and appear to have better hepatic stability. Pterostilbene (4-[(E)-2-(3,5-dimethoxyphenyl)ethenyl]phenol; Figure 2), is a naturally occurring dimethylether analog of resveratrol. Like resveratrol, pterostilbene is a phytoalexin that is produced by plants in response to microbial infestation or exposure to UV light. Pterostilbene has been reported to demonstrate cancer chemopreventive activity, and may also offer other resveratrol -like health benefits.

MATERIALS & METHODS

Animals

- **Marmosets:** Common marmosets (*Callithrix jacchus*) were obtained from Alpha Genesis, Yemassee, SC. Marmosets were approximately 1 to 1.5 years of age and weighed approximately 350 grams at the time of study initiation.
- **Cynomolgus macaques:** Cynomolgus macaques (*Macaca fascicularis*) were obtained from Worldwide Primates, Miami, FL. Cynomologus monkeys were approximately 3 years of age and weighed approximately 3 to 4 kg at the time of study initiation.
- **Rhesus macaques:** Rhesus monkeys (*Macaca mulatta*) were obtained from PrimGen, Hines, IL. Rhesus macaques were approximately 3 years old and weighed approximately 6 to 8 kg at the time of study initiation.

Animal Husbandry: Monkeys were housed in Class 3 metal squeeze-back primate cages. All monkeys were fed a diet of certified monkey chow (biscuits), fresh fruit, and primate treats that included a vitamin supplement and a mineral supplement. In addition, marmosets were provided mash (biscuits moistened with Ensure®), yogurt, marshmallows, sweet potatoes, sunflower seeds, and cashews. City of Chicago drinking water was supplied *ad libitum* by an automatic watering system.

Experimental Design: Using group sizes of 3 or 4 animals for each species, each monkey received a single oral (gavage) dose of pterostilbene at 100 mg/kg body weight (marmosets) or 50 mg/kg body weight (cynos and rhesus), using a dosing volume of 5 ml/kg. The 100 mg/kg dose of pterostilbene that was administered to marmosets was approximately equivalent to the 50 mg/kg dose of pterostilbene that was administered to the macaques (on an mg/m^2 basis). Dosing formulations were prepared as suspensions in a vehicle of 0.5% methycellulose with 0.2% Tween 80. All animals were fasted overnight prior to drug administration.

Table 1. Study Design

Group	Species	Number of Animals	Pterostilbene Dose (mg/kg)*	Route of Administration	Number of Doses
1	Marmoset	4	100	Oral	1
2	Cynomolgus	3	50	Oral	1
3	Rhesus	3	50	Oral	1

*Doses administered to all three species on an equivalent mg/m^2 basis.

In Vivo Evaluations

- **Mortality/Moribundity Observations:** Twice daily
- **Body Weight:** Prior to dosing
- **Clinical Observations**: Prior to dosing and at 0.5 and 4 hours post-dose
- **Blood sampling for Pharmacokinetics:** 0.5 ml of whole blood was collected from each monkey at six time points post-dosing (0.5, 1, 2, 4, 8, and 24 hours)

Plasma Drug Level and Pharmacokinetic Analyses

- **Sample Preparation:** Plasma samples were extracted with acetonitrile, vortex-mixed, and centrifuged at 4°C for 10 minutes to remove plasma proteins. The supernatant was decanted into a clean tube and evaporated under nitrogen. The resulting residue was reconstituted in methanol, sonicated for 5 minutes and diluted with water. The diluted residue samples were vortex-mixed, centrifuged, and decanted into an autosampler vial for instrumental analysis.
- **Quantitation of Plasma Pterostilbene Levels:** Samples were analyzed using a tandem mass spectrometer (API 3000) equipped with a high-performance liquid chromatograph (HPLC; Agilent 1200). Chromatographic separation of pterostilbene from matrix components was performed using a Luna C18(2), 3µ column, 30 × 2.0 mm (Phenomenex). The column temperature was maintained at 25°C, and a flow rate of 0.25 ml/min was used. Mobile phase consisted of Solvent A: 5 mM ammonium acetate in water/isopropanol (98:2, v/v), and Solvent B: methanol/isopropanol (98:2, v/v). Initial conditions were Solvent A at 90% 0.5 minutes, decreased to 5% in 3.5 minutes and held constant for 5 minutes, returning to initial conditions for another 3 minutes of re-equilibration time. Total run time was 12 minutes.

RESULTS AND CONCLUSIONS

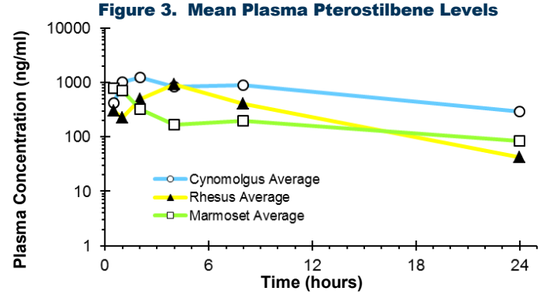
Mortality and Clinical Observations: No mortality or clinical signs of toxicity were seen in any animal as a result of pterostilbene administration.

Plasma Drug Levels: Plasma levels of pterostilbene were above the limit of quantitation in all animals at 0.5, 1, 2, 4, and 8 hours after dosing (Table 2). At 24 hours, pterostilbene was above the limit of quantitation in 8 of 10 monkeys, but was undetectable in the plasma of one rhesus macaque and one marmoset.

Table 2. Plasma Drug Levels in Individual Marmoset, Cynomolgus, and Rhesus Monkeys Receiving a Single Oral (Gavage) Dose of Pterostilbene

		Time Post-Dose (Hours)					
Species	Animal #	0.5	1	2	4	8	24
Marmoset	1	1108	518	206	356	468	8.00
	2	891	1135	640	79.1	30.7	15.1
	3	553	690	262	180	247	232
	4	614	505	200	62.0	37.0	BQL
Cynomolgus	1	531	2247	544	1508	1744	289
	2	66.8	146	2674	123	72.9	9.67
	3	656	603	482	856	835	589
Rhesus	1	7.75	263	167	69.3	105	BQL
	2	9.93	8.98	90.5	967	463	5.78
	3	880	399	1210	1727	655	80.0

Figure 3. Mean Plasma Pterostilbene Levels



- Peak plasma drug levels (C_{max}) were generally higher in cynomolgus monkeys than in the other two species; however, considerable interindividual variability was seen in all species.
- Pterostilbene was more rapidly absorbed after an oral dose in marmosets (T_{max} ranging from 0.5 or 1 hour) than in either species of macaque (T_{max} ranging from 1 to 4 h).
- Elimination half-life ($t_{½}$) was comparable in all three species; one cynomolgus monkey appeared to be an outlier.
- Total drug exposures (AUC) in rhesus and cynomolgus monkeys were approximately 2X and 4X that seen in marmosets.
- Several macaques (both rhesus and cynomolgus) demonstrated delayed absorption of pterostilbene, with substantial increases in plasma pterostilbene levels seen at 4 and 8 hours after dosing. These late increases were associated with prolonged terminal elimination half-life. This observation is consistent with results from similar studies with resveratrol in other species and is attributed to enterohepatic recirculation.
- Although marmosets are utilized much less commonly than are macaques in preclinical development studies, their smaller body size provides important advantages if drug supply is limited or if synthesis costs are high. These results demonstrate largely comparable plasma drug level data with a model compound in all three species of nonhuman primate, and suggest that the common marmoset may provide a useful nonhuman primate alternative for preclinical toxicology and pharmacology studies.