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 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 1990s that the apparent cardioprotective effects of red wine (the French Paradox) may be mediated by pound 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 chemotherapeutic activity
- Anti-aging activity
- Neuroprotective (anti-Alzheimer’s) activity
- Anti-viral activity
- Cardiovascular activity

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-(3-5,3,5,4’,-tetramethoxyphenethyl)phenol; Figure 2), is a naturally occurring dimethoxy analog of resveratrol. Like resveratrol, pterostilbene is a phytoalexin that is produced by plants in response to microbial infection or exposure to UV light. Pterostilbene has been reported to demonstrate cancer chemopreventive activity, and may also offer other resveratrol-like health benefits.

**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 2 of 10 monkeys, but undetectable in the plasma of one rhesus monkey and one marmoset.

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

<table>
<thead>
<tr>
<th>Animal</th>
<th>Time Post-Dose (Hours)</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marmoset</td>
<td>1</td>
<td>1108</td>
<td>518</td>
<td>316</td>
<td>356</td>
<td>368</td>
<td>488</td>
</tr>
<tr>
<td>Marmoset</td>
<td>2</td>
<td>891</td>
<td>1136</td>
<td>640</td>
<td>751</td>
<td>307</td>
<td>15.1</td>
</tr>
<tr>
<td>Marmoset</td>
<td>3</td>
<td>914</td>
<td>505</td>
<td>262</td>
<td>185</td>
<td>247</td>
<td>333</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>1</td>
<td>531</td>
<td>234</td>
<td>544</td>
<td>1508</td>
<td>1744</td>
<td>289</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>2</td>
<td>68.8</td>
<td>146</td>
<td>2674</td>
<td>132.2</td>
<td>72.9</td>
<td>9.67</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>3</td>
<td>696</td>
<td>603</td>
<td>482</td>
<td>835</td>
<td>589</td>
<td></td>
</tr>
<tr>
<td>Rhesus</td>
<td>1</td>
<td>9.93</td>
<td>9.48</td>
<td>90.5</td>
<td>396</td>
<td>463</td>
<td>5.78</td>
</tr>
<tr>
<td>Rhesus</td>
<td>2</td>
<td>989</td>
<td>529</td>
<td>1270</td>
<td>1727</td>
<td>650</td>
<td>80.0</td>
</tr>
</tbody>
</table>

**In Vivo Evaluations**

- Mortality/Morbidity 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. 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 chromatography (HPLC; Agilent 1100). Chromatographic separation of pterostilbene from matrix components was performed using a Luna C18 column, 30 × 2.0 mm (Phenomenex). The column temperature was maintained at 25°C, and a flow rate of 0.25 μl/min was used. Mobile phase consisted of Solvent A: 5 mM ammonium acetate in water/methanol (80:20, v/v) and Solvent B: methanol/propanol (82:18, v/v). Initial conditions were Solvent A at 90% for 0.5 minutes, decreasing to 5% in 3.5 minutes and held constant for 5.5 minutes, returning to initial conditions for another 3 minutes of re-equilibration time. Total run time was 12 minutes.

**Figure 3. Mean Plasma Pterostilbene Levels**

Peak plasma drug levels (Cmax) 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 (T½ ranging from 0.5 or 1 hour) than in either species of macaque (T½ ranging from 1 to 4 h).

Elimination half-life (t½) 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 primates, and suggest that the common marmoset may provide a useful nonhuman primate alternative for preclinical toxicology and pharmacology studies.