BIOAVAILABILITY, MULTIPLE-DOSE PHARMACOKINETICS, AND BIOTRANSFORMATION OF THE ALDOSE REDUCTASE INHIBITOR ZOPOLRESTAT IN DOGS

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ABSTRACT:

Zopolrestat (Alond) is a new drug that is being evaluated as an aldose reductase inhibitor for the treatment of diabetic complications. The bioavailability in dogs of a 2 mg/kg oral dose of zopolrestat was 97.2%. In a 1-year, multiple-dose, pharmacokinetic study, systemic exposure increased with increasing dose (50, 100, and 200 mg/kg/day), and there were no consistent changes in exposure with multiple dosing. Renal clearance at 1 year appeared to be higher in males. The magnitude of the potential gender difference in exposure was relatively small and was unlikely to have had a meaningful impact on the pharmacokinetics of zopolrestat in dogs. In studies with bile duct-cannulated dogs, radioactivity from [14C]zopolrestat was primarily eliminated as unchanged drug and acyl glucuronide in the bile and feces (77.3% of the dose) and in urine (18.3% of the dose). The concentrations of acyl glucuronide in urine and feces were approximately 50% of the zopolrestat concentrations. Minor metabolites (each accounting for <1% of the dose) included those resulting from hydroxylation of the phthalazine ring and glutathione conjugation of the benzothiazole ring.

Inhibition of the polyol pathway in diabetic patients may ameliorate the complications of diabetes (Raskin and Rosenstock, 1987; Kinosita and Nishimura, 1988). A novel, carboxylic acid, aldose reductase inhibitor (Mylarl et al., 1991), zopolrestat (Alond, CP-73,850) (fig. 1), has been shown to normalize sorbitol, fructose, and myo-inositol levels in sciatic nerve, retina, and kidney in diabetic rats (Beyer et al., 1990) and to normalize renal plasma flow in galactosemic rats (Oates and Ellery, 1990). The pharmacokinetics, tissue distribution, and biotransformation of zopolrestat in rats (Inskeep et al., 1991; Schneider et al., 1998) have been reported. In addition, the pharmacokinetics of zopolrestat have been reported for normal (Inskeep et al., 1994) and diabetic (Inskeep et al., 1995) human volunteers. In this report, we describe the pharmacokinetics and biotransformation of zopolrestat in dogs after oral dosing.

Materials and Methods

Bioavailability. Three beagle dogs (12.4 ± 0.8 kg of body weight) were dosed iv with an aqueous solution (20 mg/ml) of the sodium salt of zopolrestat (2 mg/kg) via the cephalic vein. Blood from each dog was drawn into heparin-containing tubes just before dosing and at 0.25, 0.5, 1, 3, 6, 12, and 24 hr after dosing. After a suitable wash-out period, the same three dogs were fasted and dosed orally, by gastric gavage, with an aqueous suspension (1.0 mg/ml) of the free acid of zopolrestat (2 mg/kg); blood was collected at the same times as described for the iv dose. Plasma from these samples was stored frozen until assayed for drug.

Multiple-Dose Pharmacokinetics. In a 1-year study, four groups of four dogs/gender (10.2 ± 0.9 kg of body weight for males and 9.0 ± 0.8 kg of body weight for females) were assigned to control, 50 mg/kg/day, 100 mg/kg/day, or 200 mg/kg/day dosing groups. Dosing formulations (50 mg/ml) were prepared in 0.5% methylcellulose and were administered orally by gavage. No dosing occurred on the day after the first dose, so that single-dose pharmacokinetics could be examined. Daily dosing was resumed 48 hr after the first dose. Plasma samples were obtained from each treated dog in the study at 0.25, 0.5, 1, 2, 4, 8, 16, 24, 30, and 48 hr after the first dose (day 1) and at the end of the study (1 year). In addition, plasma samples were obtained (by jugular venipuncture) from each treated dog at 0.25, 0.5, 1, 2, 4, 8, 16, and 24 hr after dosing at 3 and 6 months. In control animals, only the 1-hr postdose time point was assayed on all sampling days. At the end of the study, urine samples were collected from each treated dog in a 24-hr period during the 1 week before blood collection. Plasma and urine samples were stored frozen until assayed.

Biotransformation. The bile ducts of two male and two female beagle dogs were cannulated and exteriorized so that continuous collections of bile could be obtained. Because the bile duct cannulae prevented flow of bile into the gastrointestinal tract, the dogs received 25-ml portions of control dog bile with each meal before and throughout the course of the study. The dogs were dosed with 10 ml of dosing solution/kg of body weight, with a 5 mg/ml solution of [14C]zopolrestat (specific activity, 1 μCi/mg).

The dogs were housed in metabolism cages. Urine, bile, and feces samples were collected at 0–8 and 8–24 hr on day 1 and in 24-hr periods throughout the next 4 days. Urine collection vessels contained 1.0 ml of 4.0 M phosphoric acid. Blood samples were collected from the jugular vein at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 30, 36, 48, 72, 96, 120, 198, 266, 343, 434, 530, 603, 675, and 843 hr after dosing.

Assay of Zopolrestat in Plasma and Urine. All reagents used were obtained from J.T. Baker, American Burdick and Jackson, Mallinckrodt, or Fisher and were HPLC grade or the equivalent. Zopolrestat concentrations in plasma were determined by reverse-phase HPLC assays similar to one described earlier (Inskeep et al., 1991). The lower limit of quantification was 0.5 μg/ml for the bioavailability study and 0.2 μg/ml for the pharmacokinetic study. Concentrations of zopolrestat and an acyl glucuronide of zopolrestat in urine were determined by similar reverse-phase HPLC assays, after dilution of sample aliquots with ACN.1 The lower limit of quantification of zopolrestat in urine was 5.0 μg/ml. Because analytical standards for the zopolrestat acyl glucuronide were not available, concentrations for the conjugate were estimated using the zopolrestat standard curves; the concentrations of zopolrestat acyl glucuronide are reported as microgram-equivalents of parent compound.

Quantification of Radioactivity in Biological Samples. Radioactivity in plasma, bile, and urine was determined by LSA after dilution in Packard...
deliver the Ecolite cocktail to the liquid scintillation flow cell. The connecting tubing inner diameter and flow cell volume (cell tubing i.d., 0.028 inch; total cell volume, 100 μi.d. fused silica (to the β-RAM). With this configuration, the HPLC flow rate of 75 μl/min was split, such that the mass spectrometer received ~15 μl/min and the β-RAM received ~60 μl/min of HPLC effluent. After chromatographic resolution, the β-RAM was used to quantify the radioactivity associated with each urinary metabolite. The β-RAM provided peak representation, an integrated printout of dpm/peak, and the percentage of radiolabeled material in each peak, relative to the total amount of activity detected. The β-RAM was operated in the homogeneous liquid scintillation counting mode, with the addition of scintillation cocktail to the HPLC effluent at 240 μl/min. Total flow to the β-RAM was ~300 μl/min, resulting in a cell residence time of 20 sec. Recovery of urinary radioactivity from the HPLC column was determined in a separate experiment. After injection of 5 μl of urine extract, the HPLC effluent was collected and counted by LSA. The recovery of radioactivity was approximately 87%.

**Bile Profiling.** Radioactivity profiles for bile samples were determined for each animal. Bile samples were concentrated by an ACN precipitation method. With this procedure, recoveries of radioactivity ranged from 91 to 96%. To quantify biliary radioactivity, 5-μl injections of bile extract containing ~5000–8000 dpm were made. The HPLC, β-RAM, and MS conditions were identical to those used for urine profiling.

Recovery of biliary radioactivity from the HPLC column was determined in a separate experiment. After injection of 5 μl of bile extract, the HPLC effluent was collected and counted by LSA. The recovery of radioactivity was approximately 93%.

**Preparation of Biological Samples for MS Analysis.** Aliquots (0.2 ml) of pooled dog plasma (2–6 hr after dosing) were denatured with 0.8 ml of ACN and vigorously vortex-mixed. After centrifugation, the supernatant was transferred to a clean tube and washed with 2 ml of n-hexanes. The mixture was centrifuged, and the hexane was aspirated to waste. The remaining ACN layer was evaporated under a nitrogen stream for ~20 min. Residues were reconstituted in 20 μl of 1:1 ACN/NH₄OAc (20 mM) (pH 4), and 5-μl injections were made into the microbore HPLC system.

Aliquots (0.5 ml) of dog urine were centrifuged, and the supernatant was transferred to another tube. A 4-ml volume of ACN was added to the urine, and the sample was vigorously vortex-mixed for 0.5–1 min. After centrifugation, the ACN was transferred to a clean tube and evaporated under a nitrogen stream for ~20 min. The residues were reconstituted in 200 μl of the HPLC mobile phase. Aliquots (5 μl) were injected into the microbore HPLC system. Pellets of dog bile precipitate (30 mg) were taken from each dog sample, and 10 ml of ACN was added. The pellets were ground with a steel rod, vortex-mixed, sonicated, and centrifuged. The ACN supernatant was transferred to a clean tube and evaporated under a nitrogen stream for ~20 min. The residues were reconstituted in 200 μl of the HPLC mobile phase. Aliquots (5 μl) were injected into the microbore HPLC system.

Pellets of dog bile precipitate (30 mg) were taken from each dog sample, and 10 ml of ACN was added. The pellets were ground with a steel rod, vortex-mixed, sonicated, and centrifuged. The ACN supernatant was transferred to a clean tube. The grinding, vortex-mixing, and sonication process was repeated twice. The ACN supernatants were combined and evaporated to ~2-ml volume. Aliquots of ACN (25 μl) were evaporated to dryness under a nitrogen stream and reconstituted to the original volume with 1:1 ACN/NH₄OAc (20 mM) (pH 4). Injections (5 μl) were made into the microbore HPLC system.

**HPLC Analysis of Metabolites in Plasma, Urine, and Bile.** HPLC analysis used a Keystone BDS Hypersil C₈ column (1 × 250 mm). An ABI 140A dual-syringe pump provided a constant flow of 75 μl/min. The mobile phase 100-μm i.d. fused silica to the ion-spray interface and the β-RAM was a combination of 50-μm i.d. fused silica (to the ion-spray interface) and

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1 Abbreviations used are: ACN, acetonitrile; LSA, liquid scintillation analysis; MRM, multiple reaction monitoring; β-RAM, β-radioactivity monitor; NH₄OAc, ammonium acetate; kₑᵣ, elimination rate constant; tₘᵢₓ, time of the first occurrence of the maximal plasma concentration; Cₘₐₓ, maximal plasma concentration.
and gradient conditions were as follows: solvent A, 1:9 ACN/20 mM NH₄OAc (pH 4); solvent B, 9:1 ACN/20 mM NH₄OAc (pH 4); at 0 min, A = 90%; at 30 min, A = 70%; at 35 min, A = 35%; at 45 min, A = 35%; at 50 min, A = 90%. The column was equilibrated for an additional 15 min before the next injection. Under these conditions, zopolrestat eluted at approximately 37 min.

Simultaneous MS and β-RAM Detection. A Sciex API III+ biomolecular mass analyzer was used for metabolite identification. The ion-spray interface was operated in the positive- or negative-ion mode, at a voltage of ±5000 V. The atmospheric pressure chemical ionization interface was operated in the positive- or negative-ion mode, with a corona discharge current of ±4 μA. Collision-induced dissociation studies were performed with argon as the collision gas and with collision gas thickness values of 2.0–2.40 torr.

Calculation of Pharmacokinetics. For the bioavailability study in dogs, the sampling intervals were not adequate for estimation of $k_{\text{el}}$, half-life, clearance, or volume of distribution. Oral bioavailability, however, was estimated by comparing the AUC₀₋₁₂ value after the 2 mg/kg dose with the AUC₀₋₁₂ value after the 2 mg/kg iv dose. $T_{\text{max}}$ and $C_{\text{max}}$ were determined as described below.

Noncompartmental pharmacokinetics were assessed using RS/1 (release 4.3.1; Bolt Beranek and Newman, Cambridge, MA). The AUC values were calculated by linear trapezoidal approximation, using 0 as the time 0 concentrations for day 1 and the 24-hr concentration as an approximation of the 0-hr concentration for all other sampling days. The $k_{\text{el}}$ value was determined as the slope of the least-squares regression of the natural logarithm of the concentration-time curve over the indicated intervals that defined the logarithmic-linear portion of the curves for each dog. The half-lives on day 1 and at 1 year were calculated as $\ln(2)/k_{\text{el}}$, where $k_{\text{el}}$ is the time of the last quantifiable plasma concentration and $C_{\text{el}}$ is the calculated concentration at $T_{\text{el}}$ from the aforementioned regression. AUC₀₋₁₂ on day 1 theoretically should be equivalent to AUC₀₋₁₂ at 3 months, 6 months, and 1 year. Thus, AUC₀₋₁₂ and not AUC₀₋₂₄ on day 1 was used in comparisons with AUC₀₋₂₄ on other days of the study. Renal clearance was estimated by dividing the total amount of zopolrestat excreted in the 24-hr urine sample by the plasma AUC₀₋₂₄ for the 1-year samplings.

**Results**

Bioavailability. After iv administration of zopolrestat at 2 mg/kg to three dogs, plasma concentrations decreased in a biphasic manner and were near or below the lower limit of quantification (0.5 μg/ml) of the assay at 24 hr (fig. 2). After oral administration at the same dose level, $T_{\text{max}}$ was observed at 0.5 hr, with a corresponding mean $C_{\text{max}}$ of 3.25 ± 0.52 μg/ml. The mean oral bioavailability, based on AUC₀₋₁₂, was 97.2 ± 5.6%.

Multiple-Dose Pharmacokinetics. On all four sampling days, zopolrestat concentrations in plasma exceeded 0.2 μg/ml for all zopolrestat-treated dogs at all time points except at 48 hr. Zopolrestat could not be quantified in any of the control dog plasma samples collected at 1 hr after dosing with vehicle (data not tabulated). $C_{\text{max}}$ (fig. 3) and AUC (fig. 4) values for male and female dogs increased with increasing dose on all four sampling days. The increase in AUC was approximately linear over the entire dose range, whereas the increase in $C_{\text{max}}$ did not appear to be linear beyond the 100 mg/kg/day dose. $C_{\text{max}}$ and AUC for males and females varied significantly.

### Table 1

**Summary of pharmacokinetics of zopolrestat in dogs dosed orally at 50, 100, or 200 mg/kg/day for 1 year**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Gender</th>
<th>N</th>
<th>Day 1</th>
<th>Year 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AUC₀₋₁₂</td>
<td>AUC₀₋₂₄</td>
</tr>
<tr>
<td>mg/kg/day</td>
<td></td>
<td></td>
<td>μg/hr/ml</td>
<td>μg/hr/ml</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>4</td>
<td>673 ± 234</td>
<td>323 ± 214</td>
</tr>
<tr>
<td>50</td>
<td>F</td>
<td>4</td>
<td>653 ± 268</td>
<td>892 ± 543</td>
</tr>
<tr>
<td>100</td>
<td>M</td>
<td>4</td>
<td>653 ± 234</td>
<td>608 ± 488</td>
</tr>
<tr>
<td>100</td>
<td>F</td>
<td>4</td>
<td>2059 ± 199</td>
<td>1806 ± 697</td>
</tr>
<tr>
<td>100</td>
<td>M &amp; F</td>
<td>8</td>
<td>1930 ± 307</td>
<td>1672 ± 171</td>
</tr>
<tr>
<td>200</td>
<td>M</td>
<td>4</td>
<td>1994 ± 249</td>
<td>1739 ± 475</td>
</tr>
<tr>
<td>200</td>
<td>M &amp; F</td>
<td>8</td>
<td>3070 ± 774</td>
<td>2736 ± 540</td>
</tr>
<tr>
<td>200</td>
<td>F</td>
<td>4</td>
<td>3641 ± 1236</td>
<td>2452 ± 975</td>
</tr>
<tr>
<td>200</td>
<td>M &amp; F</td>
<td>8</td>
<td>3355 ± 1003</td>
<td>2594 ± 745</td>
</tr>
</tbody>
</table>

Values are reported as mean ± SD.

* $t_{\frac{1}{2}} = 0.693/\text{mean } k_{\text{el}}$
Throughout the 1-year duration of the study, but there was no consistent increase or decrease in exposure with time (figs. 3 and 4). Mean AUC appeared to be slightly higher in females than in males throughout the study (fig. 4). However, AUC was quite variable (SD values were typically approximately 20–50% of mean AUC values), and exposure appeared higher in males in some cases (e.g. 1 year at 100 and 200 mg/kg/day).

Mean half-lives ranged from 5.25 to 6.99 hr on day 1 and from 6.13 to 10.82 hr at 1 year (table 1). There were no consistent differences in half-lives between male and females. Renal clearance and the percentage of the dose of zopolrestat excreted into the urine decreased with increasing dose (table 2). Urinary elimination of zopolrestat appeared to be higher in males than in females.

**Biotransformation.** Recovery of Administered Dose of Radioactivity. Most of the radioactivity administered to the dogs in this study was recovered in urine, bile, and feces within 2 days after dosing. Means ± SD of recoveries of administered radioactivity for the four dogs during the 5 days after dosing were 18.3 ± 9.0% in urine, 66.1 ± 27.0% in bile, and 11.2 ± 12.1% in feces. Mean ± SD of total recovery of the administered dose was 95.6 ± 12.3%.

Mean bile flow for 5 days after dosing for the four bile duct-cannulated dogs was 5.04 ± 0.57 ml/hr. However, the bile duct cannulae for two of the dogs were blocked for some of the 0–8-hr interval. The one dog with the greatest amount of radioactivity in the feces (29.2% of the dose) was one with interrupted bile flow during the first 8 hr after dosing.

**Plasma Profiles of Drug and Total Radioactivity.** Cmax for radioactivity and zopolrestat occurred at approximately 1 hr after dosing (fig. 5). At early time points (0.25–2 hr after dosing), mean zopolrestat concentrations ranged from 73 to 92% of total radioactivity. However, zopolrestat concentrations decreased more rapidly than did radioactivity, so that mean zopolrestat concentrations represented only 16% of total radioactivity at 24 hr after dosing. The mean AUC0–24 for zopolrestat was only 55% of the AUC0–24 for total radioactivity.

The mean half-life of zopolrestat was 10.7 hr. Although the amount of radioactivity in plasma decreased rapidly during the first 24 hr after dosing.
dosing, radioactivity decreased with a mean half-life of 255 hr thereafter (fig. 6).

Identification of Major Metabolites in Plasma, Urine, and Bile. All of the metabolites observed in this study with dogs were observed in another study with rats, using similar methods (Schneider et al., 1998). Because detailed information on the identification of all metabolites is included in that study, only summaries of the MS data for each metabolite in dogs are provided here.

Within 24 hr after dosing, only one major metabolite, in addition to unchanged zopolrestat, was observed in plasma. Nonmetabolized zopolrestat eluted at a retention time identical to that of an authentic standard and had the expected MRM response for the molecular ion at m/z 420 to product ions at m/z 374 or 216. An intense signal was detected for these MRM sequences in all dogs. The protonated molecular ion of the major metabolite (designated metabolite 18) was m/z 596, with a product ion at m/z 420. Numerous chromatographic peaks were detected at retention times of approximately 23–28 min. These results were indicative of acyl glucuronide conjugates of zopolrestat. An intense signal was detected for this MRM sequence in all dogs. The protonated molecular ion of the major metabolite (designated metabolite 18) was m/z 596, with a product ion at m/z 420. Numerous chromatographic peaks were detected at retention times of approximately 23–28 min. These results were indicative of acyl glucuronide conjugates of zopolrestat. The next most abundant form of radioactivity was unchanged zopolrestat (36%). The urinary radioactivity associated with other metabolites identified by MS was below the quantifiable limits of the β-RAM detector.

Urine samples from the four dogs were analyzed by simultaneous MS and β-RAM detection. The amount of radioactivity associated with each metabolite or drug peak was quantified. A representative radiochromatogram is shown in fig. 7, and table 3 provides a summary profile of radioactivity percentages for the urine samples. In male and female dogs, the radioactivity profiles were comparable, but the relative unchanged drug/metabolite ratios were variable. The majority of urinary radioactivity (64%) was present as the acyl glucuronide conjugates of zopolrestat (metabolite 18). The next most abundant form of radioactivity was unchanged zopolrestat (36%). The urinary radioactivity associated with other metabolites identified by MS was below the quantifiable limits of the β-RAM detector.

Bile samples from the four dogs were analyzed by simultaneous MS and β-RAM detection. The amount of radioactivity associated with each metabolite or drug peak was quantified. Representative radiochromatograms are shown in fig. 8, and table 4 provides a summary profile of radioactivity percentages for each bile sample. As in the urine profiles of male and female dogs, the radioactivity profiles were comparable, but the relative unchanged drug/metabolite ratios were variable. Unchanged drug accounted for most (71%) of the radioactivity, and acyl glucuronides of zopolrestat (metabolite 18) were the next most abundant form of radioactivity (29%). The biliary radioactivity associated with all other metabolites was below the quantifiable limits of the β-RAM detector.

Identification of Minor Metabolites in Plasma, Urine, and Bile. The structures of all identified plasma, urinary, and biliary metabolites are shown in fig. 9 and listed in table 5. A series of small chromatographic peaks with retention times ranging from 25 to 33 min, designated metabolites 15, 16, and 17, were observed in all three matrices. The protonated molecular ion of these metabolites was m/z 436. The MRM response for the molecular ion at m/z 436 to the product ion at m/z 216 is indicative of a single oxidation of the phthalazine ring system.

In addition to the metabolites observed in all three matrices, one

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**TABLE 3**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Gender</th>
<th>Sample Radioactivity</th>
<th>Acyl glucuronides</th>
<th>Zopolrestat</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>35014</td>
<td>M</td>
<td>57</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35016</td>
<td>M</td>
<td>73</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>04710</td>
<td>F</td>
<td>&lt;20&lt;sup&gt;®&lt;/sup&gt;</td>
<td>&gt;80&lt;sup&gt;®&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05020</td>
<td>F</td>
<td>61</td>
<td>39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD 64 ± 7 36 ± 7

<sup>®</sup> Only drug was detected by the β-RAM. The presence of glucuronide was confirmed by MS data. This data point was not included in the mean/SD calculations.

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**TABLE 4**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Gender</th>
<th>Sample Radioactivity</th>
<th>Acyl glucuronides</th>
<th>Zopolrestat</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>35014</td>
<td>M</td>
<td>38.7</td>
<td>61.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35016&lt;sup&gt;®&lt;/sup&gt;</td>
<td>M</td>
<td>3.8</td>
<td>96.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>04710</td>
<td>F</td>
<td>46.9</td>
<td>53.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05020</td>
<td>F</td>
<td>28.0</td>
<td>71.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD 29.4 ± 16.2 70.6 ± 16.2

<sup>®</sup> All samples were processed as described in Materials and Methods. An exception was dog 35016, for which 100 μl of the ACN extract was evaporated and residues were reconstituted in 25 μl of mobile phase. This provided a 4-fold concentration for the estimation of the radioactivity associated with the acyl glucuronides.
metabolite (metabolite 5) was observed in urine and bile but not plasma. The protonated molecular ion of this metabolite was $m/z$ 539. The HPLC retention time was approximately 17.5 min. Trace amounts of this metabolite were detected with the MRM sequence of $m/z$ 539 to the product ion at $m/z$ 406. These results were consistent with those previously seen for a cysteine conjugate with the benzothiazole moiety of zopolrestat.

One metabolite (metabolite 11) was observed only in bile. The protonated molecular ion of this metabolite was $m/z$ 612. The HPLC retention time was approximately 26 min. Trace amounts of this metabolite were detected with the MRM sequence of $m/z$ 612 to the product ion at $m/z$ 436. These results were consistent with those previously seen for an oxidized zopolrestat plus a glucuronic acid conjugate (neutral loss of 176 amu). The product ion at $m/z$ 436 represents the oxidized drug molecule. Without full-scan product-ion spectra, it was not possible to determine the site of oxidation with this MRM experiment. Glucuronide conjugation may occur at either the site of oxidation or the carboxylic acid functional group.

**Discussion**

Systemic exposure increased with increasing dose, and there were no consistent changes in exposure with multiple dosing. There was a trend for higher exposure in females than in males. Renal clearance at 1 year did appear to be higher in males, and this would be consistent with slightly higher exposure in females. Overall, however, the magnitude of the potential gender difference in exposure was relatively

![Structures of metabolites of zopolrestat observed in plasma, urine, or bile from dogs dosed orally at 50 mg/kg with [14C]zopolrestat.](image)

**TABLE 5**

<table>
<thead>
<tr>
<th>Metabolite No.</th>
<th>HPLC Retention Time (min)</th>
<th>Molecular Ion ($m/z$)</th>
<th>Elimination Route</th>
<th>Description of Metabolite Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>17.5</td>
<td>539</td>
<td>Plasma, Bile, Urine</td>
<td>Benzothiazole cysteine conjugate</td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>612</td>
<td>Y</td>
<td>Phthalazine oxidation product and glucuronic acid conjugate</td>
</tr>
<tr>
<td>15</td>
<td>25–33</td>
<td>436</td>
<td>Y</td>
<td>Single oxidation of phthalazine moiety product</td>
</tr>
<tr>
<td>16</td>
<td>25–33</td>
<td>436</td>
<td>Y</td>
<td>Single oxidation of phthalazine moiety product</td>
</tr>
<tr>
<td>17</td>
<td>25–33</td>
<td>436</td>
<td>Y</td>
<td>Single oxidation of phthalazine moiety product</td>
</tr>
<tr>
<td>18</td>
<td>23–28</td>
<td>596</td>
<td>Y</td>
<td>Acyl glucuronide conjugate</td>
</tr>
</tbody>
</table>

Y, presence of metabolite in the matrix noted.
small and, with the high degree of variability in $C_{\text{max}}$ and AUC observed in this study, was unlikely to have had a meaningful impact on the pharmacokinetics of zopolrestat in dogs. Renal clearance also appeared to decrease with increasing dose. In humans, zopolrestat is a free acid that is actively secreted in the kidney (Inskeep et al., 1994), a process that is saturable. Thus, saturation of active renal secretion in dogs may decrease renal clearance as the dose increases. An additional contributing factor could be urinary pH. In humans, renal clearance of zopolrestat decreases as urinary pH decreases (Inskeep et al., 1996). Assuming a similar relationship in dogs, if urinary pH were decreased with higher doses of zopolrestat, renal clearance would also decrease.

Recovery of radioactivity exceeded 90% of the administered dose for three of the four dogs, with a mean recovery of 95.6%. Most of the radioactivity was recovered in the bile, indicating that the major route of elimination of zopolrestat and/or its metabolites in dogs is biliary. Urinary elimination accounted for an average of 18% of the dose. Six percent or less of the dose was recovered in feces from three of the four dogs, indicating that zopolrestat was well absorbed. It is not clear why absorption appeared to be lower (<73% of the orally administered dose) in the fourth dog.

Zopolrestat concentrations in plasma during the first 2 hr after dosing accounted for >80% of total radioactivity in plasma. However, by 6 hr after dosing, circulating metabolites accounted for approximately 50% of total radioactivity in plasma. By 96 hr (4 days) after dosing, little or no zopolrestat remained in plasma and the amount of radioactivity ranged from 0.8 to 3.3% of the $C_{\text{max}}$ for $^{14}$C. However, from that point on, radioactivity persisted in plasma, decreasing with a mean half-life of 255 hr.

Other than acyl glucuronidation of the carboxylic acid moiety of zopolrestat, very little biotransformation of zopolrestat was observed in dogs. Most (66.1%) of the administered dose was recovered in bile, where the predominant species were unchanged drug (70.6% of radioactivity) and acyl glucuronide (29.4% of radioactivity). In urine, where 18.3% of the administered dose was recovered, the acyl glucuronide accounted for 64% of the radioactivity and unchanged drug accounted for 36% (in three of the four dogs). Only unchanged drug, and no glucuronide, was observed in the urine of one of the dogs. It is possible that the acid added to urine collections from this dog were inadequate to prevent hydrolysis of the acyl glucuronide. Although several additional oxidative and/or conjugative metabolites of zopolrestat were identified by MS, all of them accounted for <1% of the total administered dose and thus represent minor constituents of zopolrestat disposition in dogs.

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**References**


