ABSTRACT:
The excretion and biotransformation of carvedilol [1-[carbazolyl-(4-oxy)-3-[2-methoxyphenoxymethyl]amino]-2-propanol], a new, multiple-action, neurohormonal antagonist that exhibits the combined pharmacological activities of β-adrenoceptor antagonism, vasodilation, and antioxidation, were investigated in dogs, rats, and mice. Carvedilol was absorbed well, and biliary secretion was predominant in each species. Carvedilol was metabolized extensively in each species, and elimination of unchanged compound was minor in bile duct-catheterized rats and dogs. In dogs, glucuronidation of the parent compound and hydroxylation of the carbazolyl ring, with subsequent glucuronidation, were the major metabolic pathways. Rats showed the simplest metabolite profile; the primary metabolites were formed by hydroxylation of the carbazolyl ring, with subsequent glucuronidation. Mice displayed the most complicated metabolite profile; glucuronidation of the parent compound and hydroxylation of either the carbazolyl or phenyl ring, with subsequent glucuronidation, were the major metabolic routes. O-Dealkylation was a minor pathway in all species examined.

Carvedilol [1-[carbazolyl-(4-oxy)-3-[2-methoxyphenoxymethyl]amino]-2-propanol] is a new, multiple-action, neurohormonal antagonist that is used in the treatment of hypertension (Eggersen et al., 1987; Begogni, 1991), angina (Van der Does et al., 1991; Nahrendorf et al., 1992; Hauf-Zachariou et al., 1997), and congestive heart failure (Packer et al., 1996). It exhibits nonselective β-adrenoceptor antagonism, produces vasodilation via α1-adrenoceptor blockade (Sponer et al., 1987a,b, 1990), and acts as a potent antioxidant (Feuerstein et al., 1994, 1995). β-Adrenoceptor-blocking drugs have been extensively used clinically for the treatment of hypertension, and the metabolism and pharmacokinetics of many of these drugs have been described in the literature (Bourne, 1981). Common structural features of β-adrenoceptor blockers include either an arylethanolamine or an arylxyloisopropanolamine moiety. The compounds differ in the nature of the aryl group, as well as the group(s) linked to the amine moiety. Carvedilol contains an oxyisopropanolamine moiety with aromatic substituents linked to both the oxy and amine ends of the molecule, which provide its combined activities.

Carvedilol is metabolized extensively in animals and humans, and its pharmacokinetics were described previously for monkeys and humans (Neugebauer et al., 1990; Fujimaki et al., 1990). Fujimaki and Hakusui (1989, 1990) showed that, in rats, carvedilol metabolites were secreted primarily in bile, and those authors described the two major biliary metabolites, which were formed by aromatic ring hydroxylation and subsequent glucuronidation. Hydroxylation of carvedilol in rats occurred with some stereoselectivity (Fujimaki et al., 1991), and the radioabeled carvedilol metabolites were shown to undergo enterohepatic recycling (Fujimaki and Hakusui, 1989). Neugebauer and Neubert (1991) described the excretion of carvedilol from human subjects, as well as the characterization of several of the metabolites circulating in plasma and excreted in urine. Oldham and Clarke (1992) reported the human cytochrome P450 enzymes that catalyze the metabolic monoxygenation of carvedilol. In this report, we describe and compare the absorption, excretion, and biotransformation of carvedilol in rats, dogs, and mice.

Materials and Methods

Chemicals. Carvedilol (racemic, free base) was obtained from the Drug Substances and Products Department at SmithKline Beecham Pharmaceuticals. Racemic [14C]carvedilol (free base) was obtained from Boehringer-Mannheim and was purified before use by the Radiochemistry Department of SmithKline Beecham Pharmaceuticals (Upper Merion, PA). Authentic reference standards for des-carbazolyl-carvedilol (M18), des-methyl-carvedilol (M2), 4′-hydroxyphenyl-carvedilol (M4), 5′-hydroxyphenyl-carvedilol (M5), 1-hydroxy carbazolyl-carvedilol (M14), and 8-hydroxy carbazolyl-carvedilol (M16) were obtained from Boehringer-Mannheim. All other chemicals and reagents used for these studies were of reagent grade or better. Solvents used for chromatography were obtained from J. T. Baker, Inc. (Phillipsburg, NJ), and were HPLC grade. HPLC-grade water was obtained from a Millipore (Milford, MA) Milli-Q water system. Ready-Safe scintillation cocktail was purchased from Beckman Instruments (Palo Alto, CA). Carbo-Sorb II and Permafluor V, used with the Packard Tri-Carb sample oxidizer, were obtained from Packard Instruments Co. (Downers Grove, IL).

Animals, Dosing, and Sample Collection. Bile Duct Catheterization of Dogs. Two female beagle dogs were surgically prepared for collection of bile, in the Department of Laboratory Animal Science at SmithKline Beecham Pharmaceuticals. The animals were placed under light surgical anesthesia (with 5% sodium thiamylal iv, as much as needed, ~0.5 ml/kg) for endotracheal intubation, and then isoflurane/O2 gas was administered for maintenance of surgical anesthesia. A midline abdominal incision was made, and the gallbladder was removed to prevent storage of bile. The bile duct was transected...
between the cystic duct and the duodenum, and both ends of the duct were cannulated with polypropylene tubing. The free ends of the tubing were exteriorized through the hind flank; they could be joined externally in a junction to restore normal bile flow or separated to facilitate collection of bile.

Postoperative analgesics, i.e., Nubain (1 mg/kg sc, every 4 hr) and buprenorphine (0.1–0.2 mg/kg im or sc, every 8–12 hr), were administered as needed. Routine blood chemistry analyses (including measurement of liver enzyme activities) were performed during the recovery period. Normal activity levels were reached at 6–8 weeks after surgery. The metabolism experiment was not initiated until normal blood chemistry results were observed.

Dose Suspension for Dogs. A stock solution of [14C]carvedilol was prepared by dissolving [14C]carvedilol in absolute ethanol. The dosing suspension used for oral administration was prepared by adding nonradiolabeled carvedilol to the ethanolic [14C]carvedilol stock solution and diluting this mixture with 0.5% aqueous Methocel (Dow Corning Corp., Midland, MI). The final dosing solution contained 10.69 mg of [14C]carvedilol/ml of dose suspension (5% ethanol) and 5.99 μCi/ml [14C]carvedilol.

Dosing and Sample Collection for Dogs. Two female bile duct-catheterized and two additional intact (surgically unaltered) female beagle dogs (7–11 kg; Marshall Farms, North Rose, NY) were used in the study. The dogs were fasted overnight, and food was restored 1.5 hr after dosing. Free access to water was allowed throughout the study period. [14C]Carvedilol was administered at a target dose of 10 mg/kg (1 ml of dose solution/kg of body weight), using a syringe fitted with a plastic gavage tube. After administration of the dose solution, 5 ml of water was administered to rinse the gavage tube. The absolute amount of drug administered to each animal was determined gravimetrically from the difference in the weight of the dosing syringe before and after dose administration. The target dosage was 60 μCi/10 ml of [14C]carvedilol/kg.

Immediately after drug administration, the dogs were housed individually in metabolism cages equipped for the separation and collection of urine and feces. Urine was collected for 24 hr and over 24-hr periods thereafter. Total fecal samples were collected as voided for 24 hr and over 24-hr periods thereafter and were frozen. For collection of bile, the junction in the bile duct catheter described above was separated, and bile was collected in a 10-ml test tube that was secured in a pouch attached to the back of the dog. The tube was changed every 1–2 hr for 24 hr. Bile flow was approximately 5–6 ml/hr for each animal. After 24 hr, the bile duct catheter of one dog was reconnected to the duodenal catheter to restore normal bile flow to the intestine. Thereafter, bile was collected for 1 hr at 24-hr intervals, to monitor biliary elimination of radioactivity. Near the end of the first 24-hr period, the duodenal catheter of the other dog had become blocked internally (because of a sharp bend), and normal bile flow could not be restored. Consequently, total bile was collected for the 24–96-hr period, in 24-hr periods, in a polyethylene bag. Bile samples were quickly frozen on dry ice as collected. At the end of the collection period, each cage was rinsed with a 50% aqueous ethanol solution. Whole blood samples (~5 ml) were taken from a cephalic vein, using heparinized plastic syringes, at 1, 3, and 6 hr after dosing; plasma was separated by centrifugation and frozen on dry ice. All samples were assayed for radioactivity.

Intravenous Dose Solution for Rats. The dosing solution for iv administration was prepared by adding nonradiolabeled carvedilol to a stock solution of [14C]carvedilol in ethanol and diluting this mixture with an aqueous solution composed of 5.25% (w/v) glucose, 1% (w/v) N,N-dimethylformamide, and 0.1% (v/v) acetic acid. The final dose solution contained 10.0 mg of [14C]carvedilol/ml (20% ethanol) and 20.0 μCi/mg of [14C]carvedilol (target dose volume, 0.25 ml/kg).

Oral Dose Suspension for Rats. The dosing suspension used for oral administration contained 0.5% aqueous Methocel (with 12% ethanol), 15.0 mg of [14C]carvedilol/ml, and 6.66 μCi/mg of [14C]carvedilol (target dose volume, 2 ml/kg).

Dosing and Sample Collection for Rats. Rats (Sprague Dawley, 300–400 g, N = 5 or 6/gender/dose group; Charles River, Raleigh, NC) were bile duct-catheterized and treated as described previously (Schafer et al., 1992). The animals were dosed the next day (17–22 hr after surgery), either by injection into the tail vein with the iv [14C]carvedilol dosing solution (50 μCi/2.5 mg of [14C]carvedilol/kg) or by gavage with the oral [14C]carvedilol dosing suspension (200 μCi/30 mg of [14C]carvedilol/kg), and were placed in Bollman cages. The dextrose drink solution provided after surgery (Schafer et al., 1992) was replaced with physiological saline solution and rat chow approximately 1 hr after dosing.

Bile was collected for 1 hr before dosing. After administration of carvedilol, bile was collected over the periods of 0–2, 2–4, 4–6, 6–12, 12–24, and 24–48 hr, into tubes that were kept on ice and out of direct light. Urine and feces were collected on ice for the periods of 0–24 and 24–48 hr. Each sample was frozen at −80°C immediately after collection. At the end of the experiment (48 hr), the rats were euthanized with an overdose of methoxyflurane. The residual drug-related material retained in the animals was determined by liquid scintillation counting, after the rat carcasses were dissolved in a mixture containing 96 g of KOH, 670 ml of ethanol, and 100 ml of water.

Blood samples were obtained from a separate set of rats (N = 3/gender/time), by exsanguination via the vena cava after inhaled methoxyflurane anesthesia, at 1, 3, and 6 hr after dosing. Heparin was used as an anticoagulant.

Oral Dose Suspension for Mice. [14C]Carvedilol dissolved in ethanol (10 mg/ml) was admixed with unlabeled carvedilol. This solution was taken to dryness under a gentle stream of nitrogen and suspended in 25 ml of 0.5% Methocel. The final dose suspension contained 10.0 mg of [14C]carvedilol/ml and 5.0 μCi/mg of [14C]carvedilol (target dose volume, 10 ml/kg).

Dosing and Sample Collection for Mice. Mice (Crl:NMR BR, 20–25 g; N = 18/gender; Charles River Wiga) received the [14C]carvedilol dose suspension by gavage (500 μCi/100 mg of [14C]carvedilol/kg). Animals were housed in groups of three in metabolism cages, and urine and feces were collected from each cage at room temperature for 96 hr, in 24-hr periods. Because of the small samples produced, some mixing of urine with the fecal samples was unavoidable. Samples were frozen at −80°C immediately after collection. Mice were allowed free access to food and water throughout the course of the study. At the end of the experiment (96 hr), mice were euthanized using ether anesthesia, and the carcasses were dissolved in ethanolic KOH for determination of residual radioactivity.

Blood samples were collected from a separate group of mice (N = 3/gender/time), by exsanguination via the vena cava or cardiac puncture after rapid ether anesthesia, at 2 and 6 hr after dosing. Heparin was used as an anticoagulant.

Measurement of Radioactivity in Biological Samples. Radioactivity in triplicate aliquots of bile, urine, plasma, and cage washings was determined by liquid scintillation counting, using Ready-Safe scintillation cocktail (Beckman Instruments, Palo Alto, CA), with a Beckman model 5801 liquid scintillation counter. Without thawing, the frozen fecal specimens were lyophilized. Each fecal sample was ground, either in a Waring blender (for dogs) or with a mortar and pestle (for mice and rats), until a uniform texture was observed. Triplicate aliquots (150–500 μg) were analyzed using a Packard Tricarb sample oxidizer (model 306) with liquid scintillation counting. Combustion efficiency was determined using Spec Chec 14C-quality assurance standards (Packard Instruments) and was found to be routinely >95%.

Triplicate aliquots of heparinized whole blood (100–200 μg) were digested with 1 ml of tissue solubilizer (Protocol; ICN/ethanol (1/2, v/v)) for 2 hr at 55–60°C in an oven. After cooling, four 250-μl aliquots of 30% H2O2 were added. When the bleaching and foaming subsided, the vials were returned to the oven for 30 min (with loosened caps) to drive off excess O2. The alkaline solubilization mixture was neutralized with 0.5 ml of 0.5 N HCl and analyzed by liquid scintillation counting.

Samples were counted until a 2 σ value of 0.50 or a preset time of 10 min (whichever occurred first) was reached. The lower limit of detection was established as 2 times the background level.

Separation and Quantification of Metabolites. Carvedilol metabolites were separated and quantified by HPLC with liquid scintillation counting of collected fractions. The binary gradient HPLC system consisted of a Waters (Milford, MA) model 710B WISP autosampler, model 680 automated gradient controller, and model 510 pumps, a Beckman (Palo Alto, CA) model 166 variable-wavelength detector, and an Isco (Lincoln, NE) Foxy fraction collector. Fractions (0.5 min) were collected, and 14C-labeled metabolites were quantified by liquid scintillation counting. In each case, the recovery of radioactivity from the HPLC column was quantitative.

For HPLC method 1, bile and urine samples were diluted with water and injected directly into the HPLC system. Metabolites were separated on a Brownlee RP-300 column (7 × 250 mm), with an RP-300 guard column (4.6 × 30 mm), using the following linear gradient conditions: solvent A, 0.1 M ammonium acetate, pH 5.0; solvent B, acetonitrile/water (80/20, v/v); 0 min,
For HPLC method 2, the urinary metabolites, which eluted near the column void fraction and were not separated adequately using the HPLC conditions described above, were separated on a Beckman (Fullerton, CA) Ultrasphere C18 column (10 × 250 mm), with a Brownlee RP-300 guard column (4.6 × 30 mm), using the following linear gradient conditions (same mobile phases as for method 1): 0 min, 0% B; 45 min, 45% B; 55 min, 100% B; flow rate, 3.0 ml/min.

Fingerprinted fecal samples were extracted three times with acetonitrile/0.1 M ammonium acetate, pH 5.0 (3:1, v/v). The combined extract was dried in vacuo and redissolved in acetonitrile/0.1 M ammonium acetate, pH 5.0 (1:1, v/v). Particulates were removed by centrifugation, and metabolites were analyzed by HPLC using the conditions described above.

Plasma samples (1.5–2.4 ml) were subjected to solid-phase extraction using 12-ml Varian Analytichem (Harbor City, CA) C18 Meg-Elute columns. Each plasma sample was diluted with an equivalent volume of 0.1 M ammonium acetate, pH 5.0, and diluted with water to a total volume of 5 ml. After slow application of the sample with vacuum, the column was washed with 10 ml of water and eluted with 10 ml of acetonitrile/0.1 M ammonium acetate, pH 5.0 (3:1, v/v). The extract was dried in vacuo and redissolved in 0.5 ml of acetonitrile/0.1 M ammonium acetate, pH 5.0 (1:1, v/v). The sample was centrifuged to remove particulates, and 200 μl was analyzed by HPLC as described above. Recovery of radioactivity from the solid-phase extraction procedure was typically >90%. Alternatively, metabolite profiles for some plasma samples were determined by direct injection of plasma (100–400 μl) into the HPLC system, after centrifugation and dilution with 1 volume of water. HPLC method 1 (above) was used for all plasma samples. The HPLC guard column used with this system provided adequate phase protection from these crude samples for the 7-mm Brownlee RP-300 column.

**Identification of Metabolites.** MS, FAB mass spectra were obtained for isolated metabolites using a VG-7070-EHF mass spectrometer (Fisons Instruments, Danvers, MA) equipped with a VG continuous-flow interface and a saddle-field fast atom gun (operated at 6 kV). Solvent (10 mM ammonium acetate, pH 5/glycerol, 95:5, v/v) was pumped through a Rheodyne 1-channel injector into the source, at a flow rate of 4 μl/min, by a Brownlee 230 Micropump (Brownlee Labs, Santa Clara, CA). A solvent containing acetonitrile/10 mM ammonium acetate, pH 5/glycerol, 30:70:5 (v/v/v/v), was used for the analysis of some of the more hydrophobic metabolites. The source was heated to 40°C, and spectra were obtained by automatically switching between positive- and negative-ion modes. Some CID product-ion mass spectra were obtained with FAB ionization using a VG ZAB-SE 4F tandem, double-focusing, mass spectrometer equipped with a high-voltage (35-kV) cesium ion gun. The collision energy was 10 keV, and helium (adjusted for 75% beam attenuation) was used as the collision gas. Thioglycerol was used as the liquid matrix.

Rat and dog bile and dog and mouse fecal extracts were analyzed by LC/MS and LC/MS/MS using a Finnigan TSQ-70 mass spectrometer operated with thermospray ionization. LC/MS and LC/MS/MS spectra were obtained by automatically switching between positive- and negative-ion modes in alternating scans. MS/MS spectra were obtained with a collision energy of 15 eV. The HPLC gradients used for these analyses were the same as those used to generate the metabolite profiles. A Brownlee RP-300 column (4.6 × 250 mm), operated with a flow rate of 1 ml/min, was used with the following ion source conditions: block temperature, 244°C; vaporizer temperature, 126°C; repeller voltage, +50 V.

Mouse urine samples were analyzed by LC/MS and LC/MS/MS using a Sciex API III mass spectrometer with ion-spray ionization, operated in positive-ion mode (with Dr. Thomas Covey at Sciex, Inc., Thornhill, Toronto, Canada). The ion-spray potential was 5000 V; the orifice potential was set to 70 V for MS analyses and 60–65 V for MS/MS experiments. MS/MS experiments were conducted using argon as a collision gas, at a thickness setting of 6.90 × 10^12 atoms/cm², and a collision energy of 20 eV. A Brownlee RP-300 column (2.1 × 250 mm) was used with the gradient in HPLC method 1 (see above) and a flow rate of 0.2 ml/min. The effluent was split so that approximately 0.05 ml/min was introduced into the ion-spray source.

**NMR Spectroscopy.** Proton NMR spectra were obtained using either a Bruker WM-360 or AM-400 NMR spectrometer. Carvedilol metabolites were lyophilized three times from D₂O and dried before being dissolved in DMSO-d₆ for analysis. Protons were assigned based on their chemical shifts, relative to those of authentic carvedilol, as well as from results of decoupling and NOE experiments, as appropriate.

**Results**

**Elimination of [¹⁴C]Carvedilol-Related Material in Rats, Dogs, and Mice.** The elimination of radiolabel by rats, dogs, and mice is summarized in table 1. With intact animals, the majority of the radioactivity was recovered in feces, whereas only a small percentage of the dose was excreted in urine. In bile duct-catheterized animals, the majority of the dose was excreted in bile. Radioactivity was recovered quantitatively from bile duct-catheterized male (table 1) and female (data not shown) rats, with little variability among animals or between genders, and results were consistent with data from Fujimaki and Hakusui (1989, 1990). Recovery of radioactivity from the two bile duct-catheterized dogs was approximately 88%, slightly higher than that (82%) from intact dogs. Recovery of radioactivity was essentially quantitative from male (table 1) and female (data not shown) mice, and the relative amounts of the dose excreted in urine and feces were the same for both genders. Excretion and biotransformation data for bile duct-catheterized dogs and rats indicated that carvedilol was well absorbed in each species and biliary secretion of metabolites was predominant. Although bile duct-catheterized mice were not studied, the data from intact mice were consistent with good absorption and the predominance of biliary secretion of metabolites.

**Metabolite Identification. General Procedures.** Radioisotopically labeled carvedilol metabolites excreted from animals and circulating in plasma were characterized using a variety of techniques that were available during the course of these studies. Metabolites in dog and rat bile were characterized by MS and NMR. Metabolites in dog urine and feces were identified by MS. Metabolites in rat urine (which contained a very small percentage of the administered dose) were identified by comparing HPLC retention times with those of metabolites in rat bile. Mouse fecal metabolites of carvedilol were characterized by thermospray LC/MS/MS, and mouse urinary metabolites were identified by ion-spray LC/MS/MS. Carvedilol-related products circulating in plasma from all species were identified by comparing HPLC retention times with those of metabolites in other biological fluids. When possible, authentic compounds were used to aid in metabolite identification, particularly for hydroxylated metabolites. Proposed structures of the metabolic products of carvedilol are shown in fig. 1. Racemic carvedilol was used in all of these studies. The absolute stereochemistry of the metabolites, as well as the presence of mixtures of enantiomers or diastereomers in HPLC peaks, was not generally determined, unless diastereomers happened to be resolved with the HPLC conditions used and authentic standards were available. The metabolite identification numbers (e.g. M4) used in this report are the same as those used in the report by Neugebauer and Neubert (1991), as well as the regulatory documentation for carvedilol, but differ from those used in the report by Schaefer et al. (1992) and Schaefer (1992).

Product-ion MS/MS spectra provided valuable information to indicate which aromatic ring (carbazolyl vs. phenyl) of carvedilol had undergone oxidative metabolism and to indicate, in some cases, the positions of glucuronidation, as well as to confirm the structure of the conjugating moiety. As a reference, the product-ion mass spectrum for either ejection or dissociation, as appropriate.
unchanged carvedilol is shown in fig. 2a. Carvedilol displayed an intense [M+H]⁺ ion at m/z 407. The ions at m/z 283 and 222 resulted from dissociation of carvedilol with charge retention on the carbazolyl side of the molecule (i.e. elimination of the phenyl moiety), and the ions at m/z 224 and 180 were formed from elimination of the carbazolyl ring with charge retention on the phenyl side of the molecule. The ion at m/z 100 was formed from elimination of both hydroxycarbazole and methoxyphenol. These characteristic fragmentations were subsequently used to determine whether oxidized metabolites of carvedilol were formed by hydroxylation of the phenyl or carbazolyl ring. Product-ion mass spectra for metabolites formed from hydroxylation of the phenyl or carbazolyl ring. Subsequently, the product-ion mass spectrum of the [M+H]⁺ ion at m/z 583 displayed the aglycone at m/z 407, as well as the same product ions that were observed for unchanged carvedilol. Product ions that contained the glucuronide moiety, or a portion of the glucuronide moiety, were not observed.

Metabolite M25 was identified previously as carvedilol carbazolyl-N-glucuronide, based on MS and NMR data (Schaefer et al., 1992). Subsequently, the product-ion mass spectrum of the [M+H]⁺ ion at m/z 583 for M25 was obtained; it displayed ions at m/z 407, 283, 224, 222, 183, and 180, resulting from fragmentation of the carvedilol portion of the metabolite. Interestingly, an additional ion was observed in the spectrum at m/z 449, corresponding to protonated carvedilol (407 amu) plus C₆H₄O₂ (42 amu) from the glucuronol moiety. Similarly, ions observed at m/z 264 and 325 represented addition of C₆H₄O₂ (42 amu) to the ions at m/z 222 and 283, respectively. The ion at m/z 264 contained the 42-amu fragment from the glucuronol moiety and confirmed the linkage of the glucuronide to the carbazolyl nitrogen, because the carbazolyl nitrogen is the only functional group of this ion to which a glucuronide could be linked (this structural assignment was also proven by NMR data) (Schaefer et al., 1992). These fragments that resulted from a shift of 42 amu were not observed in the spectra for the carvedilol-O-glucuronides M1a and M1b or the phenolic glucuronides M17 and M15 and correlated with linkage of the glucuronide to the carbazolyl nitrogen. In addition, an intense fragment ion at m/z 224 (resulting from cleavage of carvedilol with charge retention on the phenyl-containing fragment) was observed in the thermospray LC/MS (MS1) mass spectrum for M25 and also appeared to correlate with the carbazolyl-N-glucuronide structure. A fragment resulting from this relatively facile cleavage was not

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**TABLE 1**

### Elimination of radiolabel from dogs, rats, and mice

<table>
<thead>
<tr>
<th></th>
<th>Dogs (Female)</th>
<th>Rats (Male)</th>
<th>Mice (Male)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose (mg/kg) (route)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 (po) (N = 2)</td>
<td>10 (po) (N = 2)</td>
<td>2.5 (iv) (N = 5)</td>
</tr>
<tr>
<td><strong>Recovery (% of dose)</strong></td>
<td>100 (N = 6)</td>
<td>100 (N = 6)</td>
<td>100 (N = 6)</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–24 hr</td>
<td>7.73, 8.11燨</td>
<td>0.42, 0.39</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>24–48 hr</td>
<td>0.08, 0.06</td>
<td>0.05, 0.03</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>72–96 hr</td>
<td>2.82, 8.59</td>
<td>6.54, 8.93</td>
<td>12.04 ± 11.81</td>
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<tr>
<td>Urine total</td>
<td>64.96, 65.53</td>
<td>8.00, 8.14</td>
<td>20.26 ± 11.05</td>
</tr>
<tr>
<td>Feces</td>
<td>0.00, 0.42</td>
<td>0.15 ± 0.06</td>
<td>5.98 ± 11.65</td>
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<tr>
<td>0–24 hr</td>
<td>6.09, 0.05</td>
<td>0.23, 0.13</td>
<td>1.41 ± 0.17</td>
</tr>
<tr>
<td>Feces total</td>
<td>73.86, 74.21</td>
<td>20.96, 44.23</td>
<td>26.24 ± 5.57</td>
</tr>
<tr>
<td>Bile</td>
<td></td>
<td></td>
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<tr>
<td>0–6 hr</td>
<td>50.03, 25.83</td>
<td>16.40 ± 12.70</td>
<td>47.52 ± 11.88</td>
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<tr>
<td>6–24 hr</td>
<td>10.14, 6.15</td>
<td>3.99 ± 2.58</td>
<td>19.97 ± 10.23</td>
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<tr>
<td>24–48 hr</td>
<td>0.74 ± 0.51</td>
<td>0.45 ± 0.16</td>
<td>19.99 ± 5.91</td>
</tr>
<tr>
<td>Bile total</td>
<td>60.17, 34.95</td>
<td>81.12 ± 11.44</td>
<td>67.94 ± 6.09</td>
</tr>
<tr>
<td>Carcass</td>
<td>NA</td>
<td>4.01 ± 4.59</td>
<td>0.54 ± 0.10</td>
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<tr>
<td>Cage rinses</td>
<td>0.07, 0.02</td>
<td>NA</td>
<td>0.56 ± 0.23</td>
</tr>
<tr>
<td>Total</td>
<td>82.01, 82.82</td>
<td>87.76, 88.14</td>
<td>98.19 ± 2.56</td>
</tr>
</tbody>
</table>

Values are percentages of the dose recovered.

a Dog (female) excreta were collected for 96 or 120 hr.

b Bile duct-catheterized rats (male) were used, and excreta were collected for 48 hr.

© Mouse (male) excreta were collected for 96 hr.

Individual values for duplicate animals.

Mean ± SD.

NA, not applicable.

* Included with determination of urinary radioactivity.
FIG. 1. Summary of the metabolites of carvedilol identified in rats, dogs, and mice.

Gluc, glucuronide conjugate; Sulfate, sulfate conjugate.
observed with this intensity for any carvedilol aliphatic or phenolic \(O\)-glucuronides.

A fourth carvedilol glucuronide (M27) also showed ions at \(m/z\) 583 and 407 for the \([M+H]^{+}\) and aglycone, respectively, in the positive-ion FAB mass spectrum, with a corresponding \([M-H]^{-}\) ion at \(m/z\) 581 in the negative-ion FAB mass spectrum. The thermospray mass spectrum of M27 showed only the aglycone at \(m/z\) 407. Treatment of M27 in dog plasma with \(\beta\)-glucuronidase from bovine liver (Sigma Chemical Co., St. Louis, MO) resulted in quantitative conversion of M27 to the parent compound carvedilol. This hydrolysis was blocked by D-saccharic acid-1,4-lactone, confirming that M27 was a glucuronide conjugate of carvedilol. M27 was resistant to treatment with sulfatase and was chemically stable under the conditions used for these incubations. Glucuronide conjugates of the aliphatic hydroxyl and the carbazolyl amine had been identified previously and had very different HPLC retention times. Thus, based on all available data, M27 was proposed to be formed from linkage of glucuronic acid to the aliphatic amine, the only other likely position for conjugation.

An additional conjugation product (M23) was identified as carvedilol carbamoyl glucuronide (\([M+H]^{+}\) at \(m/z\) 627) (Schaefer et al., 1992; Schaefer, 1992). Diastereomers formed from carbamoyl glucuronidation of racemic carvedilol could be resolved chromatographically (Schaefer et al., 1992; Schaefer, 1992), but the diastereomers were not quantified individually in these experiments.

**Hydroxylation Products of Carvedilol and Their Respective Conjugates.** Four monohydroxylated metabolites were identified. Metabolites M16 and M14 showed the same relative HPLC retention times and product-ion mass spectra (\([M+H]^{+}\) at \(m/z\) 423) as authentic 8-hydroxy-carbazolyl-carvedilol and 1-hydroxy-carbazolyl-carvedilol, respectively. Metabolites M4 and M5 represented phenyl hydroxylation products and showed the same relative HPLC retention times and product-ion mass spectra (\([M+H]^{+}\) at \(m/z\) 423) as authentic 4\(^{-}\)-hydroxy-phenyl-carvedilol and 5\(^{-}\)-hydroxyphenyl-carvedilol (Neugebauer and Neubert, 1991), respectively. Metabolites M17 and M15 were identified as the phenolic-\(O\)-glucuronides of 8-hydroxy-carbazolyl-carvedilol and 1-hydroxy-carbazolyl-carvedilol, respectively, based on NMR and MS/MS data. These metabolites were also described previously by Fujimaki and Hakusui (1990). Both metabolites showed an \([M+H]^{+}\) ion at \(m/z\) 599, and the product-ion mass spectra for these metabolites were essentially identical. CID of the \([M+H]^{+}\) ion at \(m/z\) 599 yielded the protonated aglycone at \(m/z\) 423, which dissociated further as described above (fig. 2b). Ions at \(m/z\) 199, 238, and 299 indicated that the metabolites were hydroxylated on the carbazolyl ring, whereas the ion at \(m/z\) 224 indicated that the phenyl ring was unchanged. Product ions that retained the glucuronide moiety were not readily discernible. M17 was isolated, purified, and characterized by proton NMR. DMSO-\(d_6\).

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**Table 2**

Summary of NMR data for carvedilol and metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Shifts of Aromatic Protons</th>
<th>Catechol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\delta_{ppm})</td>
<td></td>
</tr>
<tr>
<td>Carvedilol(^{a})</td>
<td>7.06 7.26 6.66 8.28 7.09 7.30 7.39</td>
<td>6.89, 6.84</td>
</tr>
<tr>
<td>M8(^{b})</td>
<td>7.19 7.28 6.67 7.93 6.98 7.09</td>
<td>6.97, 6.88</td>
</tr>
<tr>
<td>M17(^{b})</td>
<td>7.07 6.55 8.19 7.07 7.30 7.60</td>
<td>6.92, 6.84</td>
</tr>
<tr>
<td>M15(^{b})</td>
<td>7.09 6.90 8.22 7.11 7.32 7.62</td>
<td>6.90, 6.81</td>
</tr>
<tr>
<td>M4(^{b})</td>
<td>7.07 7.28 6.70 8.17 7.08 7.30 7.42</td>
<td>6.92, 6.83</td>
</tr>
<tr>
<td>M5(^{b})</td>
<td>7.06 7.30 6.68 8.22 7.07 7.30 7.40</td>
<td>6.94, 6.84</td>
</tr>
</tbody>
</table>

---

\(^{a}\) Spectra obtained in methanol-\(d_4\).

\(^{b}\) Spectra obtained in DMSO-\(d_6\).

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Fig. 2. Product-ion mass spectra of carvedilol (a), a carbazolyl hydroxylation product (M14) (b), and a phenyl hydroxylation product (M5) (c).
was selected as the solvent to preserve exchangeable protons, such as
the proton on the carbazolyl nitrogen. Proton assignments (aromatic
protons are shown in table 2) were made based on results from COSY
spectra and chemical shifts relative to carvedilol and other metabo-
lites. Metabolite M15 was also analyzed by NMR using DMSO-d6.
Proton assignments were made based on results from COSY spectra,
as well as NOE experiments to differentiate M15 from M21 (see below).
[NOE experiments to prove the assignment of M15 were not
reported by Fujimaki and Hakusui (1990).] Signals at 8.21, 7.09, 7.35,
and 7.55 ppm corresponded to H5, H6, H7, and H8 of the carbazolyl
moiety, respectively. However, only two protons, at 7.07 and 6.60
ppm, which were coupled (J2,3 = 9.1 Hz), were found on the other
carbazolyl ring, indicating it as the site of hydroxylation. The exact position of hydroxylation was not determined for this minor metabolite.

The thermospray mass spectrum of M28 displayed an [M + H]+ ion
at m/z 599, an aglycone ion at m/z 423, and an intense fragment ion at
m/z 224 (similar to M25; Schaefer et al., 1992). Thus, M28 was
identified as a glucuronide conjugate of hydroxylated carvedilol.
However, the CID mass spectrum of the [M + H]+ ion of M28 at m/z
599 also displayed a pattern of product ions that was similar to that of
M25. The aglycone appeared at m/z 423, and the ions at m/z 238 (222
+ 16 amu) and 199 (183 + 16 amu) confirmed that the carbazolyl
group was hydroxylated. Although carbazolyl hydroxylation products
typically showed ions at m/z 238, 299, and 423, other ions that were
42 amu higher (at m/z 280, 341, and 465, respectively) were also observed.
The ion at m/z 280 indicated that the glucuronide was linked to
the carbazolyl group at either the nitrogen or the phenolic oxygen.
The distinctive 42-amu shift of carbazolyl-related ions was not ob-
served in spectra from other hydroxylated carvedilol O-glucuronides
(M17 and M15) but was observed in the spectrum of M25, suggesting
that the glucuronide portion of M28 was linked to the carbazolyl
nitrogen. In addition, the HPLC retention time of M28 was similar to
that of M25 and was much shorter than that of other glucuronide
conjugates. The exact position of hydroxylation on the carbazolyl
group of M28 was not determined.

M32 was detected in trace amounts in mouse urine and was also
identified as a glucuronide conjugate of hydroxylated carvedilol
([M + H]+ at m/z 599). It showed a short HPLC retention time that was
similar to those of M28 and M25. Using ion-spray ionization, the
metabolite produced a weak, but interpretable, mass spectrum that
was similar to that of M28. CID analysis of the [M + H]+ ion at m/z
599 yielded ions at m/z 224 and 299, which indicated that hydroxy-
lation had occurred on the carbazolyl moiety. Although an ion at m/z
423, representing the aglycone, was not detected in this weak spec-
trum, an ion shifted by 42 amu (m/z 465) was observed, similar to
the spectrum for M28. This result suggested that the glucuronide portion
of M32 was also linked to the carbazolyl nitrogen. The exact position
of hydroxylation was not determined for this minor metabolite.

The thermospray and FAB mass spectra for M26 showed ions at
m/z 503, corresponding to the [M + H]+ ion for an hydroxylated
carvedilol sulfate, and m/z 423, resulting from elimination of SO3.
The ion-spray mass spectrum for the same metabolite from urine con-
firmed the [M + H]+ ion at m/z 503. CID analysis of the [M + H]+ ion
at m/z 503 for M26 revealed product ions at m/z 224, 238, 299, and
423, consistent with hydroxylation on the carbazolyl moiety. The exact position of hydroxylation was not determined.

The thermospray mass spectra for M67 showed an ion at m/z 423,
with no evidence of conjugation. However, the ion-spray mass spec-
trum for the same metabolite showed an [M + H]+ ion at m/z 503,
consistent with a sulfate conjugate of hydroxylated carvedilol. The
product-ion mass spectrum of the ion at m/z 423 for M67 displayed
ions at m/z 222, 240, 283, and 423, indicating that hydroxylation had
occurred on the phenyl ring. The exact position of hydroxylation of
M67 was not determined, although Neugebauer and Neubert (1991)
previously described the sulfate conjugates of 4′-hydroxyphenyl-
carvedilol (M6) and 5′-hydroxyphenyl-carvedilol (M7).

Oxidative Cleavage Products of Carvedilol and Their Respective
Conjugates. Several metabolites of carvedilol appeared to have been
formed by oxidative O-dealkylation reactions. Although carvedilol
contains several sites that could be susceptible to oxidative cleavage,
products resulting from oxidative cleavage at only two sites were
observed, and these metabolites were relatively minor.

Metabolite M8 was identified as des-carbazolyl-carvedilol. The

NMR spectrum for M8 indicated the absence of all carbazolyl-related signals, confirming the elimination of this group (table 2). An intense $[\text{M+H}]^+$ ion at $m/z$ 242 was observed in the thermospray and FAB mass spectra for M8. The product-ion mass spectrum of the $[\text{M+H}]^+$ ion at $m/z$ 242 displayed an ion at $m/z$ 180, resulting from elimination of ethylene glycol, and ions at $m/z$ 118 and 100, resulting from elimination of methoxyphenol and subsequent elimination of water, respectively. In addition, the HPLC retention time of M8 was identical to that of authentic des-carbazolyl-carvedilol (Neugebauer and Neubert, 1991).

Metabolites M9a and M9b were identified in dog urine and eluted from the HPLC column slightly before M8. Both metabolites showed an ion at $m/z$ 228 using thermospray ionization, which was consistent with the $[\text{M+H}]^+$ ion for des-methyl-des-carbazolyl-carvedilol (des-methyl-M8). CID of the ion at $m/z$ 228 produced identical daughter ion spectra for M9a and M9b, which were also similar to that of M8. An ion at $m/z$ 166 corresponded to elimination of ethylene glycol, and ions at $m/z$ 118 and 100 resulted from elimination of hydroxyphenol and subsequent elimination of water, respectively. Because these compounds yielded the same mass spectra but appeared at different retention times, either (or both) M9a or M9b was likely to be conjugated; however, an intact conjugated metabolite was not detected using thermospray ionization, probably because of decomposition in the ion source.

Metabolite M2 showed the same HPLC retention time as authentic des-methyl-carvedilol (Neugebauer and Neubert, 1991). This assignment was confirmed by the $[\text{M+H}]^+$ ion at $m/z$ 393 in the mass spectrum.

Metabolites M3a and M3b were identified as glucuronide conjugates of des-methyl-carvedilol. Both metabolites showed an $[\text{M+H}]^+$ ion at $m/z$ 569 and an aglycone ion at $m/z$ 393 using thermospray ionization. Metabolites M3a and M3b showed the same relative retention times as products formed in vitro from the incubation of des-methyl-carvedilol with dog liver microsomes fortified with UDPGA (data not shown). Based on the very similar retention times exhibited by these metabolites, they were probably diastereomers formed from glucuronidation of (R)- and (S)-des-methyl-carvedilol. Based on HPLC retention times, relative to those of glucuronide conjugates of other carvedilol metabolites, the glucuronide moiety of M3a and M3b was likely linked to the phenol. The microsomal incubation also yielded another pair of glucuronide conjugates of des-methyl-carvedilol (M38a and M38b, also identified in humans) (data not shown), which eluted slightly before the diastereomeric carvedilol-O-glucuronides M1a and M1b and were resolved chromatographically to the same extent as M1a and M1b. Thus, M38a and M38b were likely diastereomers with the glucuronide moiety linked to the aliphatic hydroxyl group of des-methyl-carvedilol.

Metabolite M22 showed an $[\text{M+H}]^+$ ion at $m/z$ 473 and an intense $[\text{M-H}]^-$ at $m/z$ 471 in the positive- and negative-ion FAB mass spectra, respectively. Ions at $m/z$ 393 and 391 in the positive- and negative-ion mass spectra, respectively, resulted from elimination of SO$_3$. Thus, M22 was identified as a sulfate conjugate of des-methyl-carvedilol.

Profile and Quantification of Urinary, Biliary, and Circulating (Plasma) Metabolites in Rats. The metabolite profiles observed in rats were less complicated than those observed in other species and were very similar for male and female animals (data not shown). The majority (67.94%) of the orally administered radiolabeled carvedilol was eliminated in bile by male rats. The biliary metabolite profile, shown in fig. 3a and summarized in table 3, showed two abundant metabolites, which were identified as 8-hydroxyderivatilol glucuronide (M17) and 1-hydroxyderivatilol glucuronide (M15) and accounted for 18.5 and 37.8% of the biliary radioactivity, respectively. The data for these two metabolites were consistent with the results of Fujimaki and Hakusui (1990). Other, less abundant, biliary metabolites included des-carbazolyl-carvedilol (M8), carvedilol carbazolyl-N-glucuronide (M25), diastereomeric carvedilol-O-glucuronides (M1a and M1b), hydroxycarvedilol sulfate (M26), and carvedilol carbamoyl glucuronide (M23). Each of these minor metabolites represented <9% of the biliary radioactivity. Parent carvedilol was observed in only trace amounts in rat bile. This unchanged carvedilol could have resulted, at least in part, from degradation of M23, which
was found to decompose completely in rat bile at room temperature overnight.

The profiles of metabolites in urine and plasma from rats were qualitatively similar to the biliary profiles, but the relative quantities of the metabolites were somewhat different. Metabolites M17, M15, and M1b were the most abundant products in urine. Unchanged carvedilol was a major product in rat plasma at 1, 3, and 6 hr after dosing.

**Profile and Quantification of Urinary, Biliary, Fecal, and Circulating (Plasma) Metabolites in Dogs.** Biliary secretion was also predominant in dogs (table 1), and the profile of biliary metabolites in fig. 3b showed a very complex mixture composed of conjugates of carvedilol and hydroxylated carvedilol. Quantification of the biliary metabolites is summarized in table 3. Unchanged carvedilol was present in trace quantities in dog bile. Similar to findings for rats, 8-hydroxycarvedilol glucuronide (M17) and 1-hydroxydextracarvedilol glucuronide (M15) were abundant metabolites. Diastereomeric carvedilol-O-glucuronides (M1a and M1b) were also formed in relatively large amounts. The remainder of the profile was composed of relatively minor metabolites, including des-carbazolyl-carvedilol (M8), carvedilol carbazolyl-N-glucuronide (M25), 3-hydroxydextracarvedilol glucuronide (M21), carvedilol-N-glucuronide (M27), des-methylcarvedilol glucuronides (M3a and M3b), hydroxycarvedilol sulfate (M26), des-methyl-carvedilol sulfate (M22), and carvedilol carbamoyl glucuronide (M23).

The carvedilol-related products observed in feces from intact dogs correlated well with the metabolites identified in bile. As expected, the majority of the fecal metabolites corresponded to products formed from hydrolytic cleavage of the conjugates observed in bile, with the exception of M25. The most abundant fecal products were the parent compound carvedilol, carvedilol carbazolyl-N-glucuronide (M25), 8-hydroxydextracarvedilol (M16), and 1-hydroxydextracarvedilol (M14). Lesser quantities of des-carbazolyl-carvedilol (M8), des-methyl-carvedilol (M2), and 4’-hydroxydextracarvedilol (M4) were also identified. Metabolite profiles were also obtained for feces from bile duct-catheterized dogs. Interestingly, the profiles were qualitatively similar to those observed in intact normal dogs, suggesting that the bile duct-catheterized dogs might have had collateral bile flow that bypassed the catheter and drained into the intestine. Alternatively, these fecal metabolites could have been formed in the gut (by gut microflora) or in the intestinal wall and excreted directly back into the lumen, or they could have been excreted from the blood directly into the lumen of the intestine (Mayer et al., 1996; Sparreboom et al., 1997).

The profile of metabolites in dog urine was quite different from that from bile. Only metabolites that were very polar were observed in dog urine. The major metabolites were identified as des-carbazolyl-
carvedilol (M8) and des-carbazolyl-des-methyl-carvedilol (M9a and M9b).

The profile of carvedilol-related products circulating in dog plasma was also quite different from the profiles from bile and urine. Unchanged carvedilol was a major product in dog plasma at 1, 3, and 6 hr after dosing. Carvedilol-N-glucuronide (M27) and des-carbazolyl-carvedilol (M8) were also identified as major circulating metabolites, and carvedilol-des-carbazolyl-N-glucuronide (M25) was identified as a minor circulating metabolite.

**Profile and Quantification of Fecal, Urinary, and Circulating (Plasma) Metabolites in Mice.** Mouse feces displayed a very complex profile of carvedilol-related products (fig. 3c). LC/MS analyses revealed numerous coeluting or closely eluting metabolites, indicating that the metabolite profile was even more complicated than the radiochromatogram had suggested. In several cases, different metabolites were not resolved chromatographically and could not be quantified individually (table 3). The most abundant fecal product was the parent compound carvedilol. This represented drug that was not absorbed, as well as carvedilol that was released upon gut hydrolysis of conjugates that had been secreted in bile. Considering the chemical characteristics of carvedilol and the lack of appreciable amounts of unchanged carvedilol excreted in dog and rat bile, significant biliary secretion of unchanged carvedilol in mice was unlikely. Hydroxylation represented a major metabolic pathway in mice. Interestingly, many glucuronide and sulfate conjugates of carvedilol were also present as major metabolites in mouse feces. In addition to the parent compound carvedilol, the most abundant metabolites in mouse feces (based on LC/MS data and the radiochromatograms) were 1-hydroxy-carvedilol (M14), 5'-hydroxyphenyl-carvedilol (M5), 4'-hydroxyphenyl-carvedilol (M4), 1-hydroxy-carvedilol glucuronide (M15), carvedilol hydroxycarbazolyl sulfate (M26), carvedilol carbaaryl-N-glucuronide (M25), carvedilol-Ö-glucuronides (M1a and M1b), and carvedilol carbamoyl glucuronide (M23).

Although only a small percentage of the dose of [14C]carvedilol was excreted by mice in urine (male, 3.18%; female, 10.27%), the urinary metabolites were characterized structurally to confirm the data obtained for fecal metabolites and to aid in the identification of metabolites circulating in plasma. The results indicated that the metabolites excreted in urine by the mice were qualitatively similar to those observed in feces, but the profile showed a predominance of the more polar metabolites. Unchanged carvedilol was a very minor product in urine. For male mice, the most abundant urinary metabolites included des-carbazolyl-carvedilol (M8), carvedilol carbaaryl-N-glucuronide (M25), hydroxycarbazolyl-carvedilol-N-glucuronide (M28), and 8-hydroxy-carvedilol glucuronide (M17). Urine samples from female mice showed a very similar profile and the same metabolites (data not shown), with the addition of minor amounts of 4'-hydroxyphenyl-carvedilol glucuronide (M29), an hydroxycarbazolyl-carvedilol glucuronide (M39), and 1-hydroxy-carvedilol glucuronide (M15).

The profiles of carvedilol-related components circulating in plasma also displayed a complex mixture of products; however, unchanged carvedilol was clearly the most abundant component in both male and female mice at 2 and 6 hr after dosing. The plasma metabolite profiles obtained 2 hr after dosing were not significantly different from those obtained 6 hr after dosing. Qualitatively, the plasma metabolite profiles for male and female mice were very similar. However, an obvious difference between the profiles for male and female mice was the difference in the quantities of circulating metabolites, relative to that of the parent compound carvedilol. Although the metabolites were present in similar relative proportions, male mice showed lower levels of metabolites circulating in plasma, compared with female mice. At 2 and 6 hr after dosing, unchanged carvedilol accounted for 56.2 and 64.5%, respectively, of the radioactivity in male mouse plasma, and each of the metabolites observed accounted, individually, for <7% of the circulating radioactivity at each time point. In female mice, unchanged carvedilol accounted for 12.8 and 19.2% of the plasma radioactivity at 2 and 6 hr, respectively, and most of the metabolites were present at higher relative levels than in male plasma.

**Discussion**

Carvedilol was absorbed well and was metabolized extensively, to numerous metabolic products, in rats, dogs, and mice. Metabolites of carvedilol were excreted primarily in the bile in rats and dogs (and likely in mice), consistent with the molecular weight and relatively hydrophobic nature of carvedilol. The majority of the dose was excreted in the first 24 hr. The principle metabolites of carvedilol that were excreted in the bile were formed primarily by hydroxylation and subsequent conjugation, as well as by direct conjugation of the parent drug. Because carvedilol was well absorbed, enterohepatic cycling of carvedilol and metabolites after hydrolysis of conjugates in the gastrointestinal tract was likely. Indeed, Fujimaki and Hakusui (1989) have characterized the extensive enterohepatic cycling of carvedilol and metabolites in rats. Enterohepatic recycling was also possible in dogs, although the recovery of radioactivity from the bile duct-catheterized dogs appeared to be only slightly greater than that from intact dogs. The incomplete recovery of radioactivity from normal intact dogs could be the result, at least in part, of a very long elimination half-life resulting from recycling of carvedilol and metabolites.

Species differences in the metabolism of carvedilol were clearly evident. Carvedilol metabolite profiles for mice were significantly more complicated than those for rats and dogs. Rats showed oxidation as the principle initial metabolic step, but mice and dogs (and humans) (Neugebauer and Neubert, 1991) showed glucuronidation of carvedilol, as well as oxidation. In rats and dogs, hydroxylation of the carbaaryl ring was predominant; however, mice showed hydroxylation of the carbazolyl and phenyl rings. Humans displayed phenyl ring hydroxylation (Neugebauer and Neubert, 1991), as well as carbazolyl ring hydroxylation (Schafer W, unpublished data).

The chemical structure of carvedilol revealed many potential sites for biotransformation via both oxidation and conjugation pathways. Several sites on carvedilol that could be susceptible to conjugation with glucuronic acid were apparent. In fact, glucuronide conjugates of unchanged carvedilol were identified for each of the potential conjugation sites, although all of these products were not identified in every species. The aliphatic secondary hydroxyl group at the chiral center was readily conjugated with glucuronic acid to form diastereomers (M1a and M1b); this represented a major biotransformation pathway for carvedilol in dogs and mice, as well as humans (Neugebauer and Neubert, 1991), and a relatively minor pathway in rats. Conjugation of glucuronic acid to the carbazolyl amine (M25) was also observed in each of the animal species examined. This glucuronide was observed in dog feces, as well as bile, indicating that it was resistant to hydrolysis by gut microflora. Other experiments demonstrated that the metabolite was also resistant to hydrolysis by bovine β-glucuronidase (Schafer et al., 1992). This product, however, was not observed in vitro using dog or rat liver microsomes fortified with UDPGA (Schafer, 1992). A third glucuronide conjugate of carvedilol, linked to the aliphatic amine (M27), was identified indirectly and was observed only in dog bile and dog plasma. Finally, the aliphatic secondary amine of carvedilol was also found to react with CO2, with subsequent glucuronidation to form a carbamoyl glucuronide conjugate (M23). This metabolite was identified in dogs, rats, and mice.
Characterization of this product and its formation in vitro were described previously (Schaefer, 1992; Schaefer et al., 1992). Carbamoyl glucuronide conjugates formed at the carbazolyl amine were not observed and might not be formed because of the diminished nucleophilicity of the aromatic amine. Other aliphatic primary and secondary amine-containing compounds, including tocanidene (Elvin et al., 1980; Kwok et al., 1990), SK&F 86466 (Straub et al., 1988), sertraline (Tremaine et al., 1989), rimantadine (Brown et al., 1990), and Org 3770 (Delbressine et al., 1990), have also been shown to form carbamoyl glucuronide conjugates.

Hydroxylation of the carbazolyl and/or phenyl rings of carvedilol represented important metabolic routes in all of the animal species examined, as well as humans (Neugebauer and Neubert, 1991). Positions of hydroxylation on the carbazolyl ring included the 1-, 3-, and 8-positions, and hydroxylation of the phenyl ring occurred at the 4′- and 5′-positions. Neugebauer and Neubert (1991) previously identified these phenyl hydroxylation products in human urine. In animals, the hydroxylation products were excreted in bile and/or urine primarily as glucuronides and, to a lesser degree, as sulfates conjugates. Aromatic ring hydroxylation is an important metabolic pathway for many other β-blockers. Of particular relevance is amosulalol, which is a combined β/α-adrenoceptor antagonist that contains an N-O-methoxyphenoxymethyl moiety, similar to carvedilol. Hydroxylation was observed at the 4′- and 5′-phenyl positions of this compound in rats, dogs, and monkeys (Sasaki et al., 1984). Exact positions of hydroxylation and conjugation for several minor conjugates of carbazolyl and phenyl hydroxylation products were not determined. Because carvedilol has several potential sites for conjugation, each of these minor metabolites might not represent unique sites of hydroxylation but, rather, might represent different sites of conjugation of only a few different hydroxylated products. In addition, glucuronide metabolites of hydroxylated metabolites that showed similar HPLC retention times and nearly identical mass spectra could represent diastereomers produced from racemic carvedilol.

β-Blockers are frequently metabolized by oxidative N- or O-dealkylation, although this has generally represented a relatively minor pathway (Bourne, 1981). Carvedilol has several sites that could be susceptible to oxidative dealkylation; however, products from only two of these metabolic routes were observed in animals, and these represented minor metabolic pathways. O-Demethylation to yield des-methyl-carvedilol (M2) was observed in dogs and mice, and was previously observed in humans (Neugebauer and Neubert, 1991), but was not detected in rats in this study. Des-methyl-carvedilol was conjugated with sulfuric acid (M22) before excretion in dogs and mice. Mice and dogs also formed a distinct pair of glucuronide conjugates of des-methyl-carvedilol (M3a and M3b), which were likely to be diastereomers formed from glucuronidation at the phenol. A glucuronide conjugate of des-methyl-carvedilol was also described in humans (Neugebauer and Neubert, 1991). A metabolite resulting from elimination of the carbazolyl ring (M8) was observed in all species examined (including humans) (Neugebauer and Neubert, 1991), although it represented a small percentage of the dose. This metabolite was likely formed by sequential hydroxylation at the carbon adjacent to the ether oxygen, followed by elimination of the resulting hemiacetal to yield hydroxy carbazolone and an aldehyde. The aldehyde was apparently reduced before excretion, because only the corresponding alcohol was observed in excreta. The corresponding carboxylic acid that could result from oxidation of the aldehyde was not detected in these studies. Additional metabolites (M9a and M9b) formed from both demethylation and elimination of the carbazolyl group were observed in dog and human urine (Neugebauer and Neubert, 1991), but not in samples from other species. Several additional, minor, dealkylation products were previously described in humans (Neugebauer and Neubert, 1991). The routes by which other compounds with β-blocking activity are metabolized and excreted have been studied extensively; they vary widely from compound to compound. Some of the smaller, more polar, β-blockers (such as atenolol, nadolol, practolol, and sotalol) are cleared primarily by the kidney, without significant contributions from biotransformation, whereas others are metabolized extensively by many different pathways, including aliphatic and/or aromatic hydroxylation, oxidative dealkylation, and conjugation of either the parent drug or metabolites (Bourne, 1981). In addition, significant differences in the metabolic routes have been observed among species. For example, glucuronidation and side chain oxidation of prananolol were predominant in dogs; however, ring oxidation represented the major metabolic pathway in rats and hamsters (Bargar et al., 1983). Many β-blockers [including bevantolol (Latts, 1986) and metoprolol, alprenolol, and timolol (Bourne, 1981)], although they are metabolized in the liver, are excreted primarily in the urine. For others [including labelol (Lalonde et al., 1990) and amosulalol (Sasaki et al., 1984; Kamimura et al., 1985)], biliary secretion of metabolites is significant. The extent of metabolism and biliary secretion of metabolites tends to correlate with the size and hydrophobicity of the parent drug.

Acknowledgments. We are grateful to Dr. Tom Goodwin and Jack Kissinger for surgically preparing and maintaining the bile duct-catheterized dogs, to Drs. Peter Neubert and J.-P. Höck for synthesizing and providing the authentic hydroxy metabolite standards, and to Dr. Tom Covey for providing valuable help with the Sciex LC/MS/MS analyses.

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