0090-9556/93/2103-0435$02.00/0
DRUG METABOLISM AND DISPOSITION
Vol. 21, No. 3
Printed in U.S.A.
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PRESYSTEMIC ELIMINATION OF THE β-BLOCKER PAFENOLOL IN THE RAT AFTER ORAL AND INTRAPERITONEAL ADMINISTRATION AND IDENTIFICATION OF A MAIN METABOLITE IN BOTH RATS AND HUMANS

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(Received July 14, 1992; accepted December 17, 1992)

ABSTRACT:

Pafenolol is a β_1-adrenoreceptor antagonist exhibiting some interesting oral absorption properties in both rat and humans. The blood concentration-time profile exhibits two peaks, and the bioavailability is low and dose-dependent due to an incomplete and nonlinear intestinal uptake. The origin of the presystemic metabolism was studied in rats after oral and intraperitoneal administration of tritium-labeled pafenolol with reference to the intravenous route by means of urinary excretion data of pafenolol and metabolites specifically assayed by HPLC and radioisotope detection. The oral bioavailability increased from 15.8 ± 4.1% (1.0 μmol/kg) to 33.3 ± 5.8% (25 μmol/kg, $p < 0.001$). This was primarily due to a change in the fraction of the absorbed dose (f_a) from 21.9 ± 4.6 to 39.5 ± 7.9% ($p < 0.01$). The bioavailability following an intraperitoneal dose was almost complete indicating that the presystemic metabolism was due to gut wall metabolism. Saturation of the presystemic metabolism contributed only by ~15–20% to the 2-fold increase of bioavailability. This clearly indicates that the underlying mechanism for the low and dose-dependent bioavailability was an incomplete and nonlinear intestinal uptake. The metabolic pattern showed that at least eight metabolites are formed in the rat. One of these is an α-OH pafenolol, identified as the main metabolite in human urine by mass spectrometry.

Pafenolol, (±)-*N*-isopropyl-*N*'-2-[-4-(2-hydroxy-3-isopropylaminopropoxy)-phenyl]ethylurea (fig. 1), is a highly selective β_1-adrenoceptor antagonist (1, 2). The drug is a weak base, with pK_a = 9.7, water solubility = 1.0 mg/ml, and K_D = 0.3 (*n*-octanol and phosphate buffer at pH 7.4 and 25°C) (3, 4). Pafenolol is discontinuously absorbed from the gastrointestinal tract in fasted subjects following administration of a solution of 150 ml. A first peak in the blood concentration-time profile is usually observed during the first hour postdose. A second more pronounced peak appears 3–4 hr after dosing. Bioavailability in humans is dose-dependent and increases from ~25% after an oral 25 mg dose to 45% when the dose is raised to 100 mg. Following iv[1] administration, ~55% and 25% of the given amount are recovered as unchanged drug in urine and feces, respectively. Another 10% are recovered as metabolites. The half-life is about 3.5 hr following iv administration and 6 hr when the drug is given as an oral solution. The longer half-life of the oral dose is due to prolonged absorption of pafenolol, probably from the ileocolonic region leading to absorption rate-limited kinetics (3, 4).

We have previously shown that following oral administration of a solution of pafenolol to fasted and fed rats the blood concentration-time profiles exhibit two peaks. More than 90% of the available dose is absorbed during the second absorption phase that appears ~4 hr postdose (5). This has been demonstrated to be due to site-specific intestinal uptake of pafenolol probably from the ileocolonic region (6). The oral bioavailability increases from ~15 to 30% when the dose is changed from 1 to 25 μmol/kg, primarily due to increased intestinal uptake. Total clearance in rat, which consists of renal excretion (50%), intestinal excretion (25–30%), and metabolism (20–25%), is constant when the iv dose is increased from 0.3 to 3.0 μmol/kg. The half-life is also absorption rate-limited in rat and estimated to about 2 and 3 hr after iv and oral administration, respectively. Because of the strong similarities between the pharmacokinetics of pafenolol in humans and rats, the rat is a suitable model for further investigations of the mechanisms mediating the absorption of pafenolol from the gastrointestinal tract (5).

The main purpose of this study was to investigate the cause of the presystemic metabolism after oral and ip administration of pafenolol to rats with reference to the iv route by means of urinary excretion data of pafenolol and metabolites. Metabolic profiling was conducted by HPLC. The pattern was compared with that obtained after administration of a radiolabeled dose to humans. Furthermore, the study aimed at identifying the major metabolite in human urine and to confirm its presence in rat urine.

Materials and Methods

Drug and Chemicals. Specifically tritium-labeled pafenolol (19.0 MBq/μmol) was used (fig. 1). The radiochemical purity, determined by HPLC and on-line detection by a radioactivity detector (Berthold Wildbad, Germany), was higher than 97%. The radioactive substance was stored as base in a 99.5% ethanol solution at −20°C. The doses were prepared by evaporating the ethanol under nitrogen, dissolving the residue in saline followed by adjustment of pH to 5.0 with 0.1 M HCl. Unlabeled pafenolol (molecular weight = 337.5) was added to achieve the required dose. The pH was raised to 6.8–7.0 by 0.1 M NaOH. The specific radioactivity of the different doses was determined by HPLC and UV detection followed by scintillation counting of collected fractions. Pafenolol, [^{3}H]pafenolol, and α-OHpafenolol were synthesized by the Depart-

[1] Abbreviations used are: iv, intravenous; ip, intraperitoneal; CI, chemical ionization; TMS, trimethylsilane.

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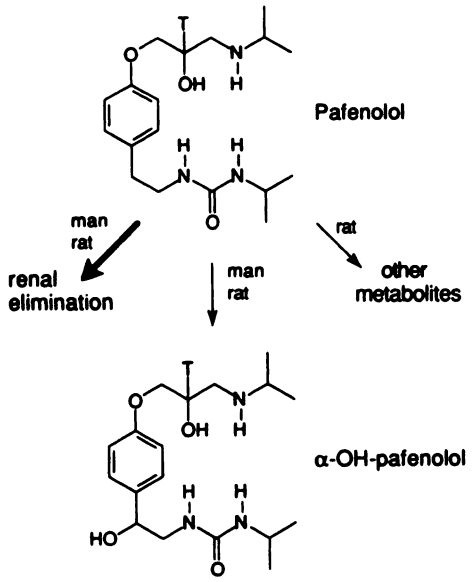

FIG. 1. *Chemical structure of pafenolol and the main metabolite in rat and human urine.*

T shows the position of the tritium labeling. In rats seven other not yet identified metabolites were recovered in urine.

ment of Organic Chemistry, Astra Hässle AB, Mölndal, Sweden. All other chemicals used were of analytical grade.

Animals. Male albino Sprague–Dawley rats (ALAB, Sollentuna, Sweden), weighing 220–270 g, were used in the study. The rats were housed under standard conditions in the animal unit of Biomedical Center, Uppsala University. All rats were deprived of food for 12–16 hr before drug administration. Food was provided 7 hr thereafter. Tap water was freely available. During the period when food was withheld the rats were kept in cages with wide-screen bottoms to prevent coprophagy. Surgery was performed the day before the experiment. The animals were anesthetized by ip injection of xylazine (15 mg/kg) and ketamine (80 mg/kg). An iv and ip catheter were inserted into the jugular vein and peritoneum, respectively, during anesthesia the day before the experiment. The catheters were passed under the skin and exteriorized at the back of the neck. The rats who received the oral doses by gavage were given the same dose of the anesthetics the day before the experiment to mimic the pretreatment procedure of the iv and ip experiments. The rats were not restrained or anesthetized at any time during the experiments.

Mass Balance. Each dose in this study was given to groups of five rats. The iv doses were 0.3 and 3.0 μmol/kg (1 μmol = 0.3 mg), and the ip and oral doses were 1.0 and 25 μmol/kg. The parenteral doses were given as bolus injections (0.3–0.5 ml iv and 0.5–0.7 ml ip) through a silastic catheter (o.d., 0.95 mm) placed in vena jugularis and in peritoneum, respectively. The oral dose was given as a bolus by intragastric intubation, and the volume was 0.5–0.7 ml. Each dose was administered as a saline solution (pH = 7.4). The amount of radioactivity was ~40 μCi/dose. The animals were individually housed in metabolic cages enabling separate collection of urine and feces. The excreta were collected quantitatively for 0–6, 6–12, 12–24, and 24–48 hr. At the end of each sampling interval, the cages were rinsed carefully with water. The recovery in the wash water was added to the urinary recovery.

Determinations of Total Radioactivity in Urine and Feces. Urine samples (0.1–1.0 ml) were weighed and diluted to 1.0 ml with water before measurements of radioactivity. Total radioactivity was determined by liquid scintillation counting after addition of 10 ml of Beckman Ready Safe®. Feces samples were weighed, and water equal to 4 times the amount of feces was added. The samples were homogenized in an Ultra Turrax® for 5 min. Aliquots of the homogenate (0.1–0.2 g) were treated at 80°C for 30 min with a mixture of 0.2 ml H_2O_2 and 0.2 ml of 70% $HClO_4$. After cooling to room temperature, 10 ml of scintillation fluid (Beckman EP®) was added, and the radioactivity was measured by liquid scintillation counting (Beckman instrument, model 244). Counting efficiency was determined by reference to external standards.

Determinations of Pafenolol in Urine and Feces. After extraction of urine and feces homogenate, pafenolol was assayed by a normal-phase liquid chromatographic method (7) with UV detection followed by liquid scintillation counting of collected fractions from the UV peak that contained unchanged pafenolol. The analytical equipment consisted of an HPLC pump (LDC Consta Metric III), a manual injector (Rheodyne 7125) fitted with a 250-μl sample loop, and a UV spectrophotometer (LDC Spectro Monitor III) set to 277 nm. The mobile phase consisted of methanol containing 25% of NH_3 solution and CH_2Cl_2 (17/83 v/v). The flow rate was 1.0 ml/min. The size of the separation column was 150 × 4.5 (i.d.) mm, and the packing material was Lichrosorb Si 60 (5 μm).

Unlabeled pafenolol was used as internal standard; 50 μl of a 3 mM saline solution was added to each sample. Samples ranging in weight from 0.05–0.5 g were diluted 10–1000 times with water. The pH was raised above 11.5 with 50 μl NaOH (1 M), and the samples were extracted with CH_2Cl_2 for 10 min. The volume ratio between the organic and aqueous phase was 2.5. After centrifugation (2250*g* for 10 min.) 100–200 μl of the organic phase was injected onto the column. The fraction corresponding to the UV peak of pafenolol was collected in scintillation vials, and the radioactivity was determined as described previously. The recovery varying between 95–102% was used to adjust the amount of radioactivity in each sample. The coefficient of variation of repeated determinations of 10 standards, was 3 and 6% for the highest and lowest radioactivity concentrations, respectively. The highest and lowest radioactivity injected on to the column was 80,000 and 1000 dpm/100 μl.

Determination of Metabolites in Rat Urine. The gradient liquid chromatographic system used to determine pafenolol and its metabolites in rat urine was identical to that for the identification of α-OH pafenolol (see below). The radioactivity of the eluate was measured by a HPLC Radioactivity Monitor LB 506 C-1 (Berthold, Wildbad, Germany), using a 1 ml flow cell and continuous addition of scintillator fluid (Ria Luma, 4 ml/min). The signals were stored in a PC-based evaluation system, and peak integration was performed. Pooled urine (0–6 hr) from each group of rats was analyzed by direct injection of 100 μl onto the HPLC column protected by a Brownlee New Guard RP-2 (15 × 3.2 mm) precolumn.

The potential formation of conjugates was evaluated by treating 250 μl aliquots of pooled urine from orally dosed rats in three different ways.

1. pH was adjusted to 4.5 by glacial acetic acid followed by addition of β-glucuronidase (EC 3.2.1.31, Sigma) from limpets to a final concentration of 1000 units/ml. The sample was incubated for 16 hr at 37°C.
2. Sulfatase (EC 3.1.6.1, Sigma) was added to a final concentration of 3 units/ml followed by incubation at 37°C for 16 hr.
3. HCl (3 M) was used to lower the pH to 1. The sample was heated at 80°C for 1 hr.

Treated samples were separated by gradient HPLC with on-line radioactivity detection as described above, and their patterns were compared with those of the untreated urine.

Identification of α-OH Pafenolol in Rat Urine. Pooled urine (50 ml) from rats (n = 3) over 0–24 hr, following an oral dose of 25 μmol/kg of pafenolol, was extracted 3 times at pH 12 with 200 ml of methylene chloride. The phases were separated, the organic solvent was evaporated under nitrogen, and the residue was reconstituted in 1 ml of H_2O containing 5% CH_3OH. After centrifugation at 1500*g* for 5 min the sample (100 μl) was injected on to a Nucleosil 120, 3 μm C_{18} column (12.0 cm × 4.5 i.d.). The gradient system consisted of an LKB pump (model 2150) connected to an LKB 2152 HPLC controller. The mobile phases A and B were 20 and 35% methanol, respectively, in 100 mM NH_4Ac (pH = 6.8). A linear gradient was formed from 0–100% phase B over a 15-min period at a flow rate of 1 ml/min. The HPLC effluent was monitored by a Finnigan TSQ 70 quadrupole mass spectrometer in a positive ion mode with a Finnigan thermospray interface. The jet and vaporizer temperatures were 250°C and 91°C, respectively. Daughter ion spectra were acquired with Argon at 1 mtorr and a collision energy of 10

eV. Computer-averaged and background-subtracted mass spectra were produced. Both pafenolol and α-OH pafenolol references were analyzed by the same LC/MS method.

Identification of α-OH Pafenolol in Human Urine. Pooled urine (0–48 hr, 100 ml) from a study in 10 healthy volunteers was extracted 3 times at pH 12 with 300 ml of CH_2Cl_2 (4). The phases were separated and the combined organic layers were evaporated in a Rotavapor®. The residue was reconstituted in 10 ml of H_2O, and the sample was injected repeatedly onto a preparative HPLC column (25.0 cm × 7.8 i.d.) packed with LiChroPrep RP8 (5–20 μm). The mobile phase was 8% methanol in 50 mM $HClO_4$ buffer, with a flow rate of 3.5 ml/min. The effluent was collected in fractions and aliquots were counted for radioactivity. The radioactive fractions were pooled and extracted as described. The residue was dissolved in 1 ml of an aqueous buffer (pH 5.5) and washed twice with CH_2Cl_2. The pH was adjusted to 12, and the metabolite was derivatized while extracting into 5 ml of CH_2Cl_2 containing 30 μl of 2 M phosgene in toluene (Fluka, Buchs, Switzerland). The mixture was shaken for 5 min and the organic phase was evaporated under nitrogen. The residue was dissolved in 50 μl of *N,O*-bis(trimethylsilyl)acetamide (Macherey-Nagel, Germany), and the sample was heated for 1 hr at 60°C. Mass spectra of derivatized metabolite were recorded in a Finnigan MAT 44 S instrument equipped with a direct probe inlet system and CI probe with methane as reagent gas. The temperature of the probe was increased linearly at a rate of 50°C/min up to 300°C. Data were evaluated by a Finnigan MAT SS 200 data system. The synthetic reference of the metabolite was derivatized and analyzed by the same procedure.

Pharmacokinetic Evaluation. The oral and ip bioavailability (F) was calculated from the dose-adjusted ratios of the cumulative amounts of unchanged pafenolol in urine after the extravascular (ev) and iv doses. The mean value of dose adjusted amounts in urine after the iv doses was used as a reference, because the dose levels used do not influence the elimination of pafenolol (5, 6). Assuming that metabolites possibly formed in the intestine or peritoneum were not absorbed or were excreted into the bile before reaching the systemic circulation, the fractions of the oral and ip doses absorbed were obtained from the following ratio:

$$f_a = \left(\frac{\text{Total radioactivity in urine}_{(ev)}/\text{dose}_{(ev)}}{\text{Total radioactivity in urine}_{(iv)}/\text{dose}_{(iv)}}\right) \times 100. \quad (1)$$

The degree of first-pass extraction of pafenolol in the gut wall and/or liver (E) of the fraction of pafenolol absorbed was calculated according to:

$$E = 1 - F/fa. \quad (2)$$

First-pass extraction (E) following the ip doses was assumed to be due to liver extraction only because drugs absorbed from the peritoneum enter directly into the splanchic blood vessels (8). The metabolized fraction of the given dose (f_m) was calculated from the differences between the fraction of the dose recovered as total radioactivity (f_{total}), in urine and feces, and the fraction of parent drug ($f_{unchanged}$) in the same excreta, as described in eq. 3:

$$f_m = f_{total} - f_{unchanged}. \quad (3)$$

The fraction of the absorbed dose that was metabolized systemically (f_{ms}) (*i.e.* after the drug has passed the gut, liver, and lung) was calculated from the equation:

$$f_{ms} = \frac{f_m}{fa} - E. \quad (4)$$

Data are presented as mean ± SD. Student's unpaired t test was used to determine the significant level of differences between observed parameters. The level of $p < 0.05$ was considered statistically significant.

Results

Mass Balance. The mean recovery of the six doses following the different administration routes varied between 86.0 ± 1.9 and 90.2 ± 1.7%. The excretion of the parent drug and metabolites was virtually completed by 12 hr postdose, because >90% of the total cumulative amount of pafenolol and metabolites in urine and feces was recovered.

Elimination of the IV Doses. The elimination of pafenolol was linear for the iv doses, and the results are presented as mean values of both doses. The drug was eliminated primarily by excretion of unchanged form in urine and feces, 45.1 ± 6.1 and 25.5 ± 2.0%, respectively (table 1). The corresponding recoveries of metabolites in the excreta were 12.1 ± 3.7 and 6.9 ± 1.9%, respectively. The mean of the metabolite fractions, f_{ms}, was 19.0 ± 3.6% (table 2). The ratio between metabolites recovered in urine and feces was 2.1 ± 1.3.

Absorption and Elimination of the IP Dose. Following ip administration, pafenolol was eliminated in a similar way as after iv administration (table 1). The absorption of pafenolol from peritoneum was complete and only a minor fraction was extracted during the first-pass through the liver. The dose level had no influence on the elimination of ip given drug (tables 1 and 2). The average excretion of unchanged pafenolol in urine and feces following the ip doses was 44.8 ± 4.6 and 19.4 ± 2.7%, respectively. The degree of systemic metabolism (f_{ms}) was 24.0 ± 4.6% (table 2).

Absorption and Elimination of the Oral Dose. The oral bioavailability administration increased from 15.8 ± 4.1 to 33.8 ± 5.8% ($p < 0.001$) when the dose was raised from 1.0 to 25 μmol/kg. In parallel, fa increased from 21.9 ± 4.6 to 39.5 ± 7.1% ($p < 0.01$) (table 1). The fraction of the dose recovered in the 0–6 hr urine was ~33% for the low and 55% for the high dose, indicating a more rapid absorption for the high oral dose. The same fraction excreted following the iv reference was ~80%. The degree of systemic metabolism of the low and high oral dose (f_{ms}) was 28.3 ± 8.8 and 31.3 ± 5.5%, respectively (table 2). The first-pass extraction (E) decreased from 28.6 ± 5.5 to 15.5 ± 5.4% ($p < 0.01$) when the oral dose was increased 25-fold.

Metabolic Profiling of Pooled Rat Urine. The HPLC chromatograms of detected radioactivity in untreated 0–6 hr urine following the low iv, ip, and oral doses are shown in fig. 2 and apparently several hydrophilic metabolites were formed. The major peak was, however, pafenolol. A quantitative evaluation of the radioactive peaks in the 0–6 and 6–12 hr urine collections are shown in table 3. Following the low and high oral doses, ~43 and 21% of the excreted amounts were recovered as metabolites in the 0–12 hr interval. The corresponding values for the iv and ip doses varied between 19 and 28% and showed no dose-dependency.

The quantitative formation of metabolites 1–3 seemed to be dependent on the route of administration (fig. 2). For the low oral dose these metabolites accounted for ~23% of the excreted radioactivity. This was 2.4 times higher than the fraction following the corresponding ip dose (table 3). Pretreatment of pooled urine from the low oral dose with β-glucuronidase, sulfatase, or strong acid did not affect the metabolite pattern as displayed in fig. 2 (lower panel). Consequently the contribution of the phase II reactions to the presystemic elimination of pafenolol in the rat seemed to be of minor importance. No further attempts were made to identify these metabolites.

Identification of a Main Metabolite in Rat Urine. The metabolite with the retention time of 6:50 min (fig. 2) was present in all experiments and corresponded to 2.9–6.5% of the excreted amount in urine (table 3). The metabolite was isolated from urine after oral administration of pafenolol by extraction with CH_2Cl_2 at pH 12. A single analysis by LC/MS was sufficient to

TABLE 1

Mean recoveries (±SD) of total radioactivity and pafenolol (% of dose) in urine and feces in unfed rats (N = 5) over a collection period of 48 hr and the calculated fraction absorbed and bioavailability

Dose	Total Radioactivity			Fraction Absorbed	Pafenolol		Bioavailability
	Urine	Feces	$u+f$		Urine	Feces	
μmol/kg	%	%	%	% fa	%	%	% F
0.3 iv	57.2 ± 2.8	31.6 ± 2.2	88.8 ± 3.2		43.4 ± 2.2	26.3 ± 1.5	
3.0 iv	*57.1 ± 5.5*	*32.8 ± 2.6*	*89.9 ± 3.9*		*46.7 ± 7.0*	*24.4 ± 2.2*	
Mean	57.1 ± 4.9	32.2 ± 2.4	89.4 ± 3.4		45.1 ± 6.1	25.5 ± 2.0	
1.0 ip	55.5 ± 6.6	32.5 ± 3.9	88.0 ± 4.9	97.0 ± 11.6	41.8 ± 3.3	20.6 ± 3.1	92.7 ± 7.4
25 ip	58.3 ± 3.3	27.7 ± 2.8	86.0 ± 1.9	101 ± 5.7	47.8 ± 3.7	18.4 ± 1.8	105 ± 8.2
1.0 po	12.5 ± 2.6[a]	77.7 ± 3.7	90.2 ± 1.7	21.9 ± 4.6[b]	7.1 ± 1.9[a]	71.8 ± 5.6	15.8 ± 4.1[a]
25 po	22.6 ± 4.0[a]	66.6 ± 2.8	89.2 ± 2.3	39.5 ± 7.1[b]	15.1 ± 2.6[a]	55.8 ± 3.8	33.3 ± 3.8[a]

[a] $p < 0.001$.
[b] $p < 0.01$.

TABLE 2

Mean values (±SD) of excreted pafenolol metabolites over a collection period of 48 hr following iv, ip, and oral administration of pafenolol to unfed rats (N = 5), first-pass extraction ratio (E) and metabolized fraction of systemically available dose

Dose	f_m (% of Dose)			f_m/f_a (% of Absorbed Dose)			E	f_{ms}[a]
	Urine	Feces	Total	Urine	Feces	Total		
μmol/kg							%	%
0.3 iv	13.8 ± 3.4	5.3 ± 1.9	19.2 ± 4.1	—	—	—	0[b]	19.2 ± 4.1
3.0 iv	*10.4 ± 3.5*	*8.4 ± 1.7*	*18.8 ± 4.5*	—	—	—	0[b]	*18.8 ± 4.5*
Mean	12.1 ± 3.7	6.9 ± 1.9	19.0 ± 3.6					19.0 ± 3.6
1.0 ip	13.7 ± 4.2	11.9 ± 1.1	25.6 ± 4.3	13.9 ± 3.0	12.6 ± 2.7	26.5 ± 2.1	3.0 ± 6.8	23.7 ± 6.7
25 ip	10.5 ± 1.8	9.4 ± 1.9	19.9 ± 3.0	10.4 ± 1.9	9.3 ± 2.2	19.7 ± 3.5	0 ± 2.7	24.2 ± 1.8
1.0 po	5.4 ± 0.8	6.5 ± 2.2	11.9 ± 1.9	25.0 ± 2.5	31.6 ± 6.3	56.3 ± 11.6	28.6 ± 5.5[c]	28.3 ± 8.8
25 po	7.5 ± 1.8	10.7 ± 1.6	18.3 ± 2.6	19.0 ± 2.9	27.7 ± 4.4	46.7 ± 4.8	15.5 ± 5.4[c]	31.3 ± 5.5

[a] Per cent of the available dose metabolized is calculated according to eq. 4. Presystemic metabolism is not included.
[b] First-pass extraction in the lung is assumed to be 0.
[c] $p < 0.001$.

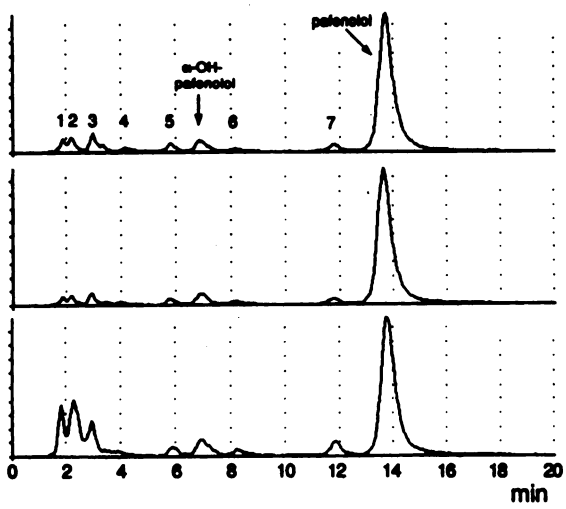

FIG. 2. *Determination of pafenolol and metabolites in pooled (N = 5)* rat urine (0–6 hr) by radioisotope detection.

The dosages (0.3, 1.0, and 1.0 μmol/kg), shown from top to bottom, were given iv, ip, and orally, respectively.

obtain a mass spectrum of the metabolite (fig. 3). By scanning the first quadrupol of the mass spectrometer, a protonated molecular ion at m/z 354, an increase of the molecular weight of pafenolol by 16 amu, was recorded at the retention time of the radioactive peak. Collision-induced decomposition resulted in daughter ions at m/z 336 and 251, originating from elimination of H_2O from MH^+ followed by loss of O═C═N—$CH(CH_3)_2$ and charge localization on the rest of the molecule. The synthetic reference of α-OH pafenolol exhibited the same LC and MS characteristics as this metabolite.

Identification of the Main Metabolite in Human Urine. Only one radioactive metabolite was found in human urine, representing ~5% of the total urinary recovery. Aqueous phase cyclization of the aminopropanol group with phosgene formed a derivative that was isolated by solvent extraction followed by silylation. The CI mass spectrum and the structure of the derivative are displayed in fig. 4. The protonated molecular ion at m/z 452 easily eliminated TMS-OH to give a base peak at m/z 362. Further loss of $NHCH(CH_3)_2$ and O═CH═N—$CH(CH_3)_2$ gave rise to ions at m/z 304 and 277, respectively. The interesting ion of low abundance at m/z 336 most likely originated from the cleavage of the carbon–carbon bond in the para substituent, being alpha to the aromatic ring with charge retention on the O-TMS containing moiety. Identical analytical data were obtained from reference standard α-OH pafenolol.

Discussion

The excretion data in table 1 show that systemically available pafenolol is eliminated by three separate routes, of which renal excretion of unchanged drug accounts for ~50% of the given iv dose. Based on a previously determined total clearance value of ~50 ml/min/kg (5, 6), the urinary excretion of pafenolol after the iv dose in this study indicates that the renal clearance was ~25 ml/min/kg. This suggests that both glomerular filtration and tubular secretion are involved in the renal elimination and

TABLE 3

Pafenolol and metabolites in pooled 0–12 hr rat urine (N = 5) after different doses (μmol/kg) of pafenolol

Data are given as per cent of excreted amount as determined by LC and radioisotope detection. Per cent of dose excreted in the same interval is added for comparison.

Pafenolol Metabolite No.	LC Retention Time	Dose and Administration Route					
		iv		ip		po	
		0.3 μmol/kg	3.0 μmol/kg	1.0 μmol/kg	25 μmol/kg	1.0 μmol/kg	25 μmol/kg
	min						
1	1:55	3.4	2.7	2.6	2.0	7.2	3.9
2	2:10	3.5	3.8	2.3	2.9	6.9	5.5
3	3:00	6.4	5.3	4.8	3.0	9.1	5.9
4	4:10	1.7	1.3	1.4	0.7	2.2	1.0
5	5:50	2.8	2.8	2.7	2.3	3.3	2.6
α-OH	6:50	4.9	4.5	5.1	2.9	6.5	4.6
6	8:10	1.2	1.2	1.5	0.7	2.0	1.4
7	11:45	2.8	2.8	2.8	1.9	4.4	3.0
Pafenolol	13:45	72.0	74.4	75.3	81.6	56.6	79.0
Urinary recovery 0–12 hr pool [mean ± SD (% of dose)]		53.8 ± 3.2	54.1 ± 6.3	52.1 ± 5.3	54.8 ± 2.6	10.8 ± 2.3	21.9 ± 6.6

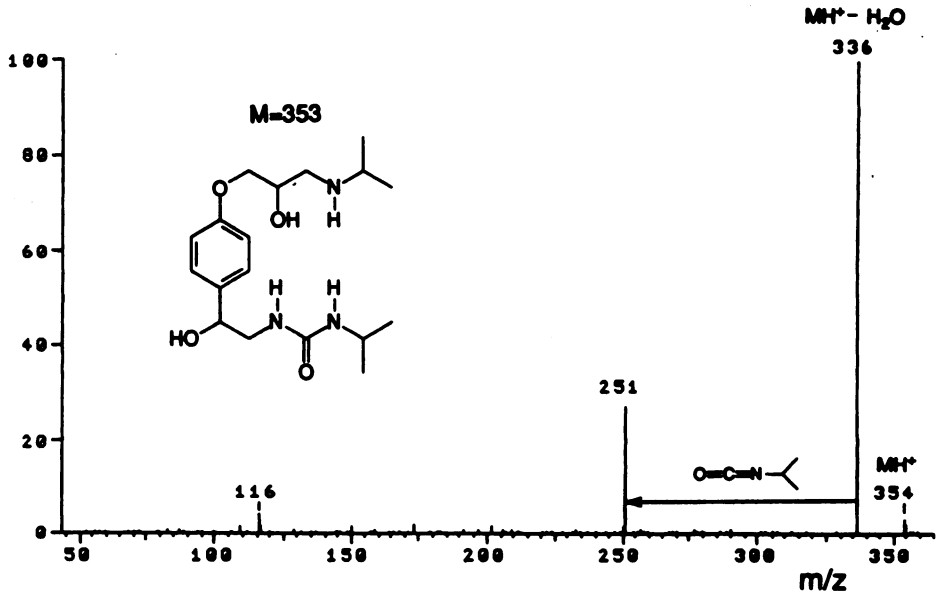

FIG. 3. *Mass spectrum of α-OH pafenolol isolated from rat urine and determined by LC and thermospray ionization with daughter ion scan of the protonated molecular ion.*

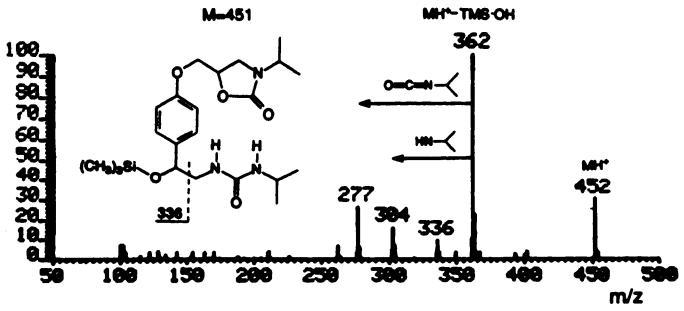

FIG. 4. *Mass spectrum of α-OH pafenolol isolated from human urine as derivative and analyzed by direct probe/chemical ionization (CH_4).*

that the drug has a high extraction over the kidney (9, 10). The second most important excretion route is *via* feces, which accounts for ~25% after an iv dose. Metabolism is the third process and accounts for ~20% of the systemically available dose. These results are in good agreement with previous observations following oral and iv administration of pafenolol to rat (11). In that study it was shown that intestinal excretion direct from blood to the lumen (exsorption) accounts for ~20% of the total elimination of pafenolol in rat (11).

The virtually complete bioavailability of pafenolol following intraperitoneal administration indicates that first-pass extraction in the liver is negligible. This agrees with estimates of bioavailability from blood concentrations (6). Furthermore, the elimination of systemically available pafenolol is unaffected of the ip dose range studied (*i.e.* 1.0–25 μmol/kg).

In contrast to the ip dose the bioavailability after oral administration was low, variable, and dose-dependent. This is due to a poor and variable intestinal uptake (f_a) that also is the main reason of the dose-dependency in F. The major part (90%) of the oral absorption of pafenolol in rat has been shown to be confined to uptake from the ileocolonic region, where also this dose-dependent uptake occurred (6). The mechanism underlying this nonlinear increase in fa and F might be a saturable binding

between the drug and bile acids in the gastrointestinal lumen that can form nonabsorbable complexes at low pafenolol concentrations (5, 6, 11). Such a nonabsorbable complex has earlier been demonstrated to be the underlying mechanism for the low oral absorption of nadolol, another β-blocker (12, 13).

In this study saturable first-pass metabolism has been shown to contribute to ~15% of the nonlinear increase in bioavailability of a high oral dose of pafenolol. In an earlier study we concluded that the first-pass metabolism is located in the gut and/or liver (11). Intraperitoneal administration of pafenolol proved that the presystemic metabolism is mainly located in the gut, probably in the intestinal epithelium. This conclusion is valid provided that rapid absorption of the ip dose does not cause saturation of the liver enzymes.

As orally administered pafenolol is mostly absorbed from the ileocolonic region (6), metabolism by the gut microflora might contribute to the presystemic elimination of pafenolol. However, metabolism mediated by microorganisms in the intestine are usually anaerobic processes leading to more lipophilic products (14). For pafenolol oral administration results in an increase of hydrophilic metabolites compared with iv and ip dosing according to the metabolic profiles of the urine (fig. 2). Larger fractions were found under peaks 1, 2, and 3 for the low oral dose. These peaks are associated with the most polar metabolites and consequently, the effect of the microflora on the presystemic elimination of pafenolol can probably be ignored. Approximately 15–25% more of these metabolites are found in urine after oral administration of the low dose compared with the lower doses given iv or ip. Since compared to the low dose these metabolites are less abundant in the HPLC chromatogram after the high oral dose; this presystemic formation at least to some extent is mediated by some saturable enzyme(s). The structures of the metabolites are not known but incubations with β-glucuronidase, sulfatase, or strong acid (3 M HCl) indicate that glucuronide and sulfate conjugates of pafenolol are not formed.

Before we had access to thermospray to couple LC and MS, direct probe mass spectrometry was used to get the molecular ion of α-OH pafenolol. The long side chain was cyclized to an oxazolidone ring according to a method described for the α-hydroxylated metabolites of metoprolol (15). The study on metoprolol implied silylation as well as the capillary column gas chromatography and electron impact ionization. Despite full derivatization of α-OH pafenolol and CI conditions extensive fragmentation occurred most likely due to heat transfer during sample evaporation. Interestingly, the minor ion at *m/z* 336 in the mass spectrum of α-OH pafenolol (fig. 4) is the base fragment induced by electron impact of α-OH metoprolol metabolites (15). By using a thermospray interface the pafenolol metabolite can be analyzed without derivatization (fig. 3) to get molecular weight information. The instability of the α-OH group during the analysis is apparent from the loss of H_2O from the MH^+ ion (fig. 3) and the abundant elimination of the TMS-OH group in the CI spectrum of the derivative (fig. 4). A highly diagnostic fragment was observed at *m/z* 251 (α-OH pafenolol) and at *m/z* 253 (pafenolol, data not shown) formed by the loss of 85, O=C=N—$CH(CH_3)_2$, from the MH^+-H_2O or MH^+ ion, respectively. Because fragment 85 is related to the *N*-isopropyl urea group of pafenolol, specific monitoring of metabolites by a neutral loss (16, 17) of 85 in a triple-stage quadrupol should be possible.

It is concluded from this study that pafenolol has a route-dependent metabolism probably located in the gut wall, and that phase II reactions are not involved in the presystemic elimination of pafenolol. Furthermore, the metabolite α-OH pafenolol is formed in both humans and rats. In humans, it is the main metabolite, whereas it is one of several metabolites formed by the rat.

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