

## DISPOSITION OF RADIOLABELED IFETROBAN IN RATS, DOGS, MONKEYS, AND HUMANS

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### ABSTRACT:

Ifetroban is a potent and selective thromboxane receptor antagonist. This study was conducted to characterize the pharmacokinetics, absolute bioavailability, and disposition of ifetroban after i.v. and oral administrations of [<sup>14</sup>C]ifetroban or [<sup>3</sup>H]ifetroban in rats (3 mg/kg), dogs (1 mg/kg), monkeys (1 mg/kg), and humans (50 mg). The drug was rapidly absorbed after oral administration, with peak plasma concentrations occurring between 5 and 20 min across species. Plasma terminal elimination half-life was ~8 h in rats, ~20 h in dogs, ~27 h in monkeys, and ~22 h in humans. Based on the steady-state volume of distribution, the drug was extensively distributed in tissues. Absolute bioavailability was 25, 35, 23, and 48% in rats, dogs, monkeys, and humans, respectively. Renal

excretion was a minor route of elimination in all species, with the majority of the dose being excreted into the feces. After a single oral dose, urinary excretion accounted for 3% of the administered dose in rats and dogs, 14% in monkeys, and 27% in humans, with the remainder excreted in the feces. Extensive biliary excretion was observed in rats with the hydroxylated metabolite at the C-14 position being the major metabolite observed in rat bile. Ifetroban was extensively metabolized after oral administration. Approximately 40 to 50% of the radioactivity in rat and dog plasma was accounted for by parent drug whereas, in humans, approximately 60% of the plasma radioactivity was accounted for by ifetroban acylglucuronide.

Ifetroban (BMS-189201, [1*S*-(1 $\alpha$ , 2 $\alpha$ , 3 $\alpha$ , 4 $\alpha$ )]-2-[[3-[4-[(pentylamino)carbonyl]-2-oxazolyl]-7-oxa-bicyclo[2.2.1]hept-2-yl]methyl]benzenepropanoic acid, monosodium salt) is a potent and selective thromboxane receptor antagonist. It inhibits the platelet shape change reaction and demonstrates antiaggregation activity in animal and human platelet-rich plasma (Misra et al., 1993; Ogletree et al., 1993). It significantly reduces thrombus formation in a variety of animal models, including some that are insensitive to aspirin (Schumacher et al., 1993; Gomoll and Ogletree, 1994). The antiplatelet and antithrombotic activity occurs at doses that do not prolong bleeding. Ifetroban also competitively antagonizes the coronary vasoconstrictor response to U-46619, a synthetic thromboxane A<sub>2</sub> receptor agonist, and 8-epi PFG<sub>2 $\alpha$</sub> , a naturally occurring prostenoid produced by a mechanism independent of cyclooxygenase activity (Ogletree, 1992). Ifetroban is devoid of agonist activity in *in vitro* and *in vivo* animal models.

The objectives of this study were to: 1) assess the pharmacokinetics, absolute bioavailability, and disposition of ifetroban; 2) determine its routes, extent of excretion, and predominant metabolites; and 3)

compare its disposition in rats, dogs, monkeys, and humans after oral and *i.v.*<sup>1</sup> administrations of radiolabeled ifetroban.

### Experimental Procedures

#### Chemicals

Radiolabeled [<sup>14</sup>C]ifetroban, labeled in the oxazole ring (Fig. 1), had a radiochemical purity of 98% and a specific activity of 16.8  $\mu$ Ci/mg. This was diluted with nonradiolabeled ifetroban to a specific activity of 2  $\mu$ Ci/mg for the human study. [<sup>14</sup>C]ifetroban was synthesized at Bristol-Myers Squibb (BMS) Pharmaceutical Research Institute (Syracuse, NY). Radiolabeled [<sup>3</sup>H]ifetroban, labeled in the aromatic portion of the interphenylene moiety (Fig. 1, for rat study) or labeled in the pentyl side chain (Fig. 1, for monkey study), each had a radiochemical purity of 98% and specific activity of 2.3 mCi/mg. [<sup>3</sup>H]ifetroban was obtained from ChemSyn Science Laboratories (Lenexa, KS). Unlabeled ifetroban was obtained from BMS Pharmaceutical Research Institute (Princeton, NJ).

#### Rat Studies

Male Sprague-Dawley (SD) rats (~220–400 g) obtained from Harlan Sprague-Dawley, Inc. (Frederick, MD) or from Hilltop Lab Animals, Inc. (Scottsdale, PA) were used in these studies after approval of the BMS Animal Care and Use Committee. The animals were acclimated for several days before use in the study and fed a standardized rodent diet. After dosing, the animals were housed in individual stainless steel or plastic metabolism cages. Food was withdrawn ~18 h before dosing, but the animals had free access to water at all times. Food was provided again at either 4 or 8 h post dosing. Rats used for serial blood collection had catheters implanted in the jugular vein. Bile duct-cannulated rats were used for bile collection. The bile duct was cannulated approximately 48 h before dosing. Bile was recirculated into the duodenum until collection, and donor bile was infused into the duodenum at a rate of 1 ml/h during bile collection.

<sup>1</sup> Abbreviations used are: SD, Sprague-Dawley; BMS, Bristol-Myers Squibb; GI, gastrointestinal; MS, mass spectrometry; QC, quality control; TLC, thin-layer chromatography (chromatographic); ODS, octadecasilane; MS/MS, tandem mass spectroscopy; AUC<sub>(INF)</sub>, area under the plasma concentration-time curve from 0 to infinity; C<sub>max</sub>, maximum plasma concentration; CL<sub>T</sub>, total clearance; VD<sub>SS</sub>, steady-state volume of distribution.

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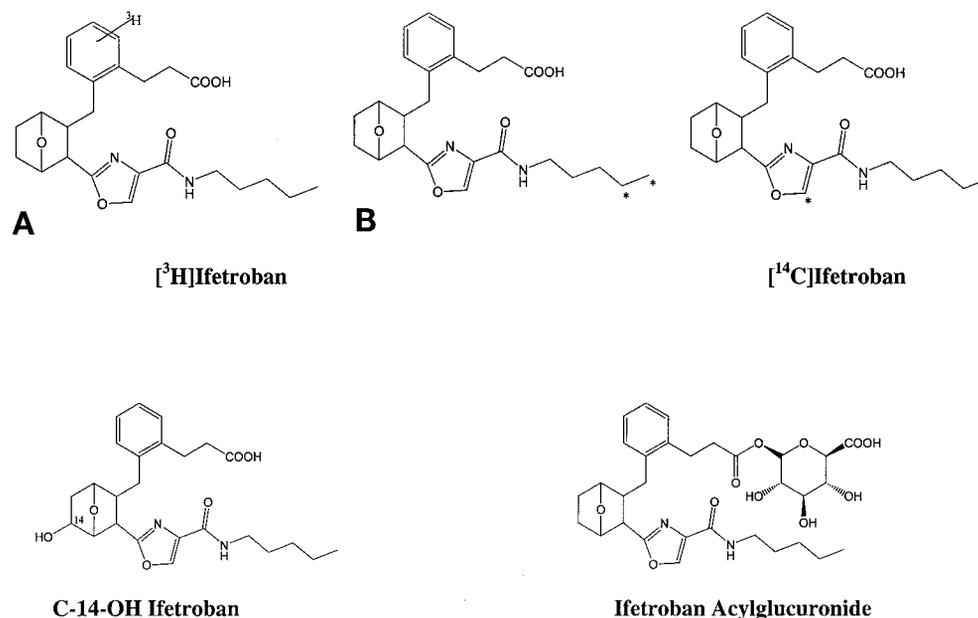


FIG. 1. Chemical structures of ifetroban and related compounds.

The asterisk (\*) on ifetroban indicates the position of <sup>3</sup>H or <sup>14</sup>C. [<sup>3</sup>H]ifetroban (A) was used in the rat study and [<sup>3</sup>H]ifetroban (B) was used in the monkey study.

In the i.v./oral disposition study, two groups of 25 SD rats were administered 5 mg/kg (85  $\mu$ Ci/kg) of [<sup>14</sup>C]ifetroban, prepared in a 5% aqueous sodium bicarbonate solution, either orally (2.5 ml/kg) or by i.v. injection (1 ml/kg) in the dorsal penis vein while under light Metofane (methoxyflurane; Schering-Plough Animal Health, Union, NJ) anesthesia. Five SD rats from each group were sacrificed by methoxyflurane overdose at 5 min and 2, 8, 24, and 168 h after administration of the dose. Blood was collected into tubes containing EDTA. In addition to blood and plasma, the following tissues and fluids were collected from each rat: adrenals, bone marrow, brain, cerebrospinal fluid, eyes, heart, kidneys, liver, large intestine and contents, lungs, muscle, skin, small intestine and contents, spleen, stomach and contents, and testes. All samples were analyzed for radioactivity by liquid scintillation counting.

In the i.v./oral pharmacokinetic study, two groups of five SD rats were administered 3 mg/kg ( $\sim$ 1 mCi/rat) [<sup>3</sup>H]ifetroban, prepared in a 0.2% aqueous sodium carbonate solution containing 10% ethanol, either orally (2 ml/kg) or by i.v. injection (1 ml/kg) in the jugular vein. Blood samples (0.6 ml) were collected at the following times postdose: at 2 (i.v. only), 5, 10, 20, 30, and 45 min, and at 1, 1.5 (oral only), 2, 4, 8, 12, 24, 36, 48, 72, and 96 h. Urine (collected over ice) and feces were collected before dosing and then daily for up to 96 h, at which time the rats were sacrificed. All samples were stored frozen at or below  $-20^{\circ}\text{C}$  until analysis. All samples of plasma, urine, feces, and carcass were analyzed for radioactivity by liquid scintillation counting. In addition, plasma samples were analyzed for intact ifetroban with a specific radiometric thin-layer chromatographic (TLC) assay.

For the absorption study in bile duct-cannulated rats, three SD rats were administered 3 mg/kg ( $\sim$ 160  $\mu$ Ci/rat) [<sup>3</sup>H]ifetroban by oral gavage. Bile, urine (collected over ice), and feces were quantitatively collected for 12 h after dosing, at which time the rats were sacrificed by methoxyflurane overdose. The gastrointestinal (GI) tract was then removed from the carcass and combined with the 0–12 h feces. The samples were analyzed for radioactivity by liquid scintillation counting.

The mass balance of radioactivity was determined in eight male SD rats after each received a single 5-mg/kg dose of [<sup>14</sup>C]ifetroban. Four rats received the dose i.v. as an injection into the dorsal penis vein, and four rats received the dose orally by gavage. All rats were housed individually in plastic metabolic cages for 168 h. Urine (collected over ice) and feces were collected during 0 to 12 and 12 to 24 h, and during every 24 h thereafter until the rats were sacrificed at 168 h. The carcasses were retained. All samples were stored frozen at or below  $-20^{\circ}\text{C}$  until analysis. All samples of urine, feces, and carcass were analyzed for radioactivity by liquid scintillation counting.

Pooled urine from the first collection interval was hydrolyzed by incubating

with glucuslase (1125 U/ml sample) at  $37^{\circ}\text{C}$ , pH 5.0, for 18 h according to the procedure outlined by Jajoo et al. (1990). Ifetroban was stable under the glucuslase hydrolysis conditions used. The activity of the enzyme was monitored by the incubation of additional tubes containing the standards phenolphthalein glucuronic acid, phenolphthalein disulfate, and *p*-nitrophenyl sulfate.

#### Dog Studies

Male beagle dogs ( $\sim$ 10–13 kg) were used in this study after approval of the BMS Animal Care and Use Committee. The animals were acclimated for several days before use in the study, and were fed a standardized canine diet. All treatments were administered after an overnight fast in a 5% aqueous sodium bicarbonate solution. The dogs were fed 8 h after dosing. Water was available ad libitum.

The mass balance study was conducted as a randomized, single dose, crossover design in four dogs. Each dog received 1 mg/kg (17  $\mu$ Ci/kg) i.v. and oral doses of [<sup>14</sup>C]ifetroban with a 2-week washout between doses. Blood samples (5 ml) were collected just before each dose and at the following times after each dose: 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 h. Blood was collected via the cephalic vein into K<sub>3</sub>EDTA tubes. Urine (collected over ice) and feces were collected in toto after each radiolabeled dose at 24-h intervals for 96 h. Plasma, urine, and feces were stored at or below  $-20^{\circ}\text{C}$  until analyzed.

#### Monkey Study

Male African green monkeys (5–7 kg) with indwelling vascular catheters, for blood sample collection, were used in this study after approval of the BMS Animal Care and Use Committee. The animals were acclimated for several days and fed a standardized diet. The monkeys were fasted overnight and for 4 h after dosing, chaired for 12 h after dosing, and then housed individually in metabolic cages for the remainder of the study. The oral dosing solution, prepared in a 0.2% aqueous sodium carbonate solution containing 10% ethanol, was administered by gavage, and the i.v. solution was administered as a bolus. The study was conducted as a randomized, single dose, crossover design in three monkeys. Each monkey received 1.0 mg/kg i.v. and oral doses of [<sup>3</sup>H]ifetroban solution. At least 3 weeks elapsed between doses. Blood samples (1 ml) were collected before dosing and at selected times up to 96 h after dosing. Blood was collected via the indwelling femoral vein catheters into heparinized tubes. Urine and feces were collected quantitatively before dosing and then daily for up to 4 days after dosing; the collection time for excreta was extended to 15 days for the second leg of the study when preliminary data from

the first leg suggested that 4 days was insufficient. Plasma, urine, and feces were stored at or below  $-20^{\circ}\text{C}$  until analyzed.

### Human Study

The study was conducted as a randomized, single dose, crossover design in 12 healthy male subjects. Each subject received 50 mg (100  $\mu\text{Ci}$ ) of [ $^{14}\text{C}$ ]ifetroban as a solution in normal saline infused for 30 min i.v. or administered orally. The treatments were separated by at least a 2-week washout. Each treatment was administered after a 10-h fast; the subjects continued to fast until 4 h after dosing.

The study protocol was approved by the Institutional Review Board and Radiation Safety Committee at the investigational site. All subjects gave consent to participate in the study by signing and dating an informed consent form after the study was completely explained to each person.

All subjects were in good health based on medical history, prestudy physical examinations, and clinical laboratory testing. The mean  $\pm$  S.D. age of all subjects who entered the study was  $29 \pm 7$  years, with a range of 20 to 44 years.

Serial blood samples were collected at predose, 10, 20, 30, 45, 50 (i.v. only) min and at 1, 1.25 (i.v. only), 1.5 (i.v. only) 2, 4, 5, 6, 7, 8, 12, 18 (p.o. only), 24, 36, 48, 60, 72, 84, 96, and 120 h post oral dose and from the start of the i.v. infusion. Within 1 h of collection, a 1-ml portion of blood was separated and stored frozen for total radioactivity measurement. The remainder of the blood sample was centrifuged to obtain plasma for total radioactivity measurement and for analysis of intact ifetroban. Blood and plasma were stored at or below  $-70^{\circ}\text{C}$  until analyzed. Urine was collected predose, 0 to 4, 4 to 8, 8 to 12, and 12 to 24 h, and over daily intervals for 7 days after oral and i.v. dosing. Feces were collected predose and over daily intervals for 7 days. All urine and feces samples were stored at or below  $-20^{\circ}\text{C}$  until analyzed.

### Analytical Methods

**Ifetroban.** Dog plasma, human plasma, and urine using gas chromatography/mass spectrometry (MS). This method involved the addition of internal standard (SQ 34,510) to 0.5 ml of plasma. After vortexing, the samples were loaded onto conditioned 200-mg cyclohexyl solid-phase extraction columns (Varian Sample Preparation Sample Products, Harbor City, CA). Ifetroban and internal standard were eluted with 1.5 ml of methanol after rinsing with 5 ml of distilled water followed by 5 ml of heptane, yielding 96 to 100% recovery of ifetroban. The extract was dried under nitrogen. The analyte as well as the internal standard were then derivatized by incubating with 50  $\mu\text{l}$  of a pentafluorobenzyl bromide solution in dry acetone and 10  $\mu\text{l}$  of diisopropylethylamine for 20 min at approximately  $40^{\circ}\text{C}$ . The derivatizing reagents were evaporated and the derivative was then reconstituted in 100  $\mu\text{l}$  of tetradecane, and 2  $\mu\text{l}$  was injected into a gas chromatograph/mass spectrometer.

For urine, no extraction was necessary as the 50- $\mu\text{l}$  sample was simply dried under nitrogen and derivatized in the same way as the plasma. Separation was achieved using helium as the carrier gas at a flow rate of 50 ml/min, with an injection port temperature of  $280^{\circ}\text{C}$ . The oven temperature started at  $220^{\circ}\text{C}$  and was programmed to increase at a rate of  $30^{\circ}\text{C}/\text{min}$  until a temperature of  $325^{\circ}\text{C}$  was reached. Ionization of analytes was accomplished with electron capture chemical ionization using ammonia as a buffer to thermalize electrons. The instrument was tuned using fragment ions ( $m/z$  414, 452, and 633) produced by pentafluorobenzyl bromide after electron capture. Spiked quality control (QC) samples were prepared before the initiation of the study in control plasma and urine using a reference standard for ifetroban, and were stored with the study samples. QC samples were analyzed with study samples to establish stability, assay accuracy, and precision.

The gas chromatograph used for both plasma and urine analyses was HP5890 equipped with 7673A autosampler (Hewlett-Packard, Avondale, PA). The mass spectrometer was a VG Trio-2 obtained from Fisons (Danvers, MA). A DB-1 fused silica capillary column (15 m, 0.25 mm i.d., 0.25- $\mu\text{m}$  film thickness) obtained from J&W Sci. (Folsom, CA) was used. The 5-mm HP splitless injection port liners used were obtained from Restek (Bellefonte, PA).

The standard curves were linear and met a priori acceptance criteria of  $\leq 20\%$  deviation of the back-calculated standard curve concentrations and the predicted QC concentrations from the respective nominal concentrations. The plasma standard curve range was 1 to 200 ng/ml in dog plasma (0.5-ml

extraction volume) and 0.5 to 500 ng/ml in human plasma (0.5-ml extraction volume). The human urine standard curve range was 5 to 5000 ng/ml (50- $\mu\text{l}$  extraction volume). During analysis of study samples, the mean observed concentrations of the QC samples were within 10% from nominal values. The between- and within-day variations were within 18%.

**Rat and monkey plasma using TLC.** The plasma samples from rats and monkeys dosed with [ $^3\text{H}$ ]ifetroban were analyzed for ifetroban by a TLC assay. Briefly, the method involved mixing a 0.2-ml aliquot of each plasma sample with 2 ml of acetonitrile, shaking horizontally with a mechanical shaker for 1 h, and centrifuging. The supernatant was removed and kept. The pellet was resuspended with acetonitrile and the mixture was centrifuged for 30 s. The supernatants were combined and evaporated to dryness under nitrogen. The dried residue was reconstituted with 0.25 ml of methanol containing 1 mg/ml nonradioactive ifetroban (reference standard) and 0.2 ml was spotted onto a reversed phase TLC plate (KC $_{18}$ F, 0.2 mm thick; Whatman International Ltd, Maidstone, England). The plates were developed in acetonitrile/*n*-butanol/water/trifluoroacetic acid (60:5:45:0.15, by volume). The zone corresponding to ifetroban was located by visualization of the nonradioactive ifetroban reference standard with short wavelength UV light. The zone was removed from the plate by scraping, and mixed with 2 ml of methanol/water (1:1) and 15 ml of scintillation cocktail before scintillation counting. Concentrations of ifetroban in plasma were calculated from a standard curve prepared with spiked plasma samples of known concentrations of [ $^3\text{H}$ ]ifetroban. Spiked plasma samples ranged from 0.06 to 3000 ng/ml. Over this concentrations range, the TLC assay was linear and had acceptable accuracy (percent deviation  $< 10\%$ ) and precision (c.v.  $\leq 25\%$ ).

**Total Radioactivity.** Total radioactivity in urine samples (1 ml of urine added to 15 ml of Hionic-Fluor) was measured without any processing. Fecal samples were first homogenized with approximately 3 volumes of water and 0.2 g was combusted for measurement of total radioactivity in 8 ml of Permafluor. In the rat tissue distribution study, the total organ or a tissue sample (approximately 0.2 g) was accurately weighed and digested with an appropriate amount of Soluene-350 (Packard Instrument Company).

**Metabolic Profiling.** Ifetroban and its metabolites in urine and plasma were separated and detected using HPLC and flow-through radioactivity detection (FLO-SCINT II, 3 ml/min; Packard Instrument Co., Downers Grove, IL). Quantitative analyses of radioactive ifetroban and its metabolites in plasma were accomplished using HPLC with direct collection of the effluent into 7 ml of scintillation vials every 0.5 min for 45 min.

Representative (fixed percentage of the volume) pooled urine samples were prepared for analysis. Equal volumes of plasma for each collection time were pooled for analysis. Unless otherwise discussed, the extraction efficiency of samples prepared for metabolic profiling was greater than 90%.

For HPLC analysis, a 1.0-ml aliquot of each urine sample was applied to the activated C $_{18}$  cartridge (Waters Associates, Milford, MA). The cartridge was eluted with acidified methanol [95% methanol, 5% ammonium acetate buffer (0.01 M, pH 5.0);  $2 \times 1$  ml] and the eluate was collected and dried under a steady stream of nitrogen. The sample was reconstituted with 200  $\mu\text{l}$  of 0.01 M ammonium acetate buffer (pH 5.0). Pooled plasma samples were prepared for HPLC (Waters Associates) analysis by precipitation and multiple washing of the protein with ice-cold acidified methanol. The sample was passed through a 0.45- $\mu\text{m}$  filter. The filtrate was dried under a steady stream of nitrogen, and the residue was reconstituted in 200  $\mu\text{l}$  of 0.01 M ammonium acetate buffer (pH 5.0).

The reverse-phase gradient system used an octadecasilane (ODS) column (5- $\mu\text{m}$  particle size,  $4.6 \times 250$  mm; Beckman) fitted with an ODS precolumn [Zorbax (Hewlett Packard, Chadds Ford, PA); 5- $\mu\text{m}$  particle size,  $4.0 \times 12.5$  mm]. Ammonium acetate buffer (0.01 M, pH 5.0) was delivered by pump A and acetonitrile was delivered by pump B. Initial conditions were 80% A and 20% B, flow rate was 1 ml/min. A linear gradient that ran for 20 min to 60% A, 40% B was immediately started. These conditions were maintained for 14 min. An acetonitrile gradient was then applied; this consisted of a 1-min linear gradient to 100% B, which was maintained for 3 min. The column was then re-equilibrated to initial conditions. UV absorbance was monitored at 210 nm. Samples were then analyzed by liquid chromatography with tandem mass spectroscopy (MS/MS) to identify metabolites. This assay used the same HPLC system as described above, and the mass spectrometer was a Sciex API

III tandem quadrupole using argon as the collision gas and operating in the positive/negative ion mode.

To identify metabolite(s) in rat bile and in human urine, a semipreparative reverse-phase HPLC system was used. This semipreparative reverse-phase gradient system used an ODS column (5- $\mu$ m particle size, 10.0  $\times$  250 mm; Beckman) fitted with an ODS precolumn (Zorbax, 5- $\mu$ m particle size, 4.0  $\times$  12.5 mm). The mobile phase and detection parameters were the same as previously described; however, the flow rate was 4 ml/min. The human urine fractions that were collected were combined and underwent further purification by an isocratic partition system that used a semipreparative silica column (Zorbax, 5- $\mu$ m particle size, 9.4  $\times$  250 mm) fitted with a silica precolumn (Zorbax, 5- $\mu$ m particle size, 4.0  $\times$  12.5 mm). Ammonium acetate buffer (0.01 M, pH 5.0) was delivered by pump A and acetonitrile was delivered by pump B. Assay conditions were 80% A and 20% B, flow rate was 4 ml/min. UV absorbance was monitored at 210 nm. After collection of the purified peak from the partition system, the sample was dried under a steady stream of nitrogen, with the residue reconstituted in 50% acetonitrile, 50% 0.01 M ammonium acetate buffer (pH 5.0). NMR analyses were then performed on a Bruker AM500 instrument equipped with a microprobe cell. Analyses, including 2-D correlation spectroscopy and proton D<sub>2</sub>O exchange, were used to assist in the structure analysis.

#### Pharmacokinetic Analysis

Plasma concentrations versus time data for ifetroban were analyzed by noncompartmental methods (Gibaldi and Perrier, 1982). The terminal log-linear phase of the plasma concentration-time curve was identified by least-squares linear regression of data points that yielded a minimum mean square error. The area under the plasma concentration-time curve from 0 to infinity ( $AUC_{(INF)}$ ) was determined by a combination of trapezoidal and log-trapezoidal methods, or by the integration method of Lagrange (Yeh and Kwan, 1978), plus the extrapolated area. The extrapolated area was determined by dividing the observed concentration at the time of last nonzero plasma concentration by the slope ( $\beta$ ) of the terminal log-linear phase. The  $t_{1/2}$  of the terminal log-linear phase was calculated as  $\ln(2)$  divided by the absolute value of  $\beta$ . The maximum plasma concentration ( $C_{max}$ ) and the time at which  $C_{max}$  occurred ( $t_{max}$ ) were obtained from the observed data. Total clearance ( $CL_T$ ) and steady-state volume of distribution ( $VD_{ss}$ ) were calculated as  $CL_T = \text{Dose}(i.v.) / AUC_{(INF)}$  and  $VD_{ss} = \text{Dose}(i.v.) \times AUMC / (AUC_{(INF)})^2$ , where AUMC is the area under the first moment of plasma concentration versus time curve. The absolute bioavailability ( $F$ ) of ifetroban was estimated from the plasma  $AUC_{(INF)}$  data of unchanged ifetroban after i.v. and oral treatments.

### Results

**Plasma Time Course of Total Radioactivity and Pharmacokinetics of Ifetroban.** Mean (standard deviation) plasma concentration-time profiles of total radioactivity and/or unchanged ifetroban after i.v. and oral dosing in rats, dogs, monkeys, and humans are shown in Fig. 2. Mean (standard deviation) pharmacokinetic parameters of ifetroban for rats, dogs, monkeys, and humans are summarized in Table 1. Peak plasma concentrations of total radioactivity in all species were about an order of magnitude lower after oral dosing in comparison with i.v. dosing. The concentrations of ifetroban after i.v. dosing were similar to those for total radioactivity up to approximately 30 min in the animals, at which point the concentrations for ifetroban began to decline more rapidly than the concentrations of radioactivity. In contrast, the concentrations of ifetroban in the human began to decline more rapidly than the concentrations of total radioactivity almost immediately after the infusion was terminated.

The absolute oral bioavailability was 25, 35, and 23% in rats, dogs, and monkeys, respectively. Ifetroban was rapidly absorbed after oral dosing with peak concentration achieved within 20 min in all animal species studied. The  $t_{1/2}$  value ranged from 7.7 h (rats) to 27 h (monkeys). The total body clearance generally paralleled the hepatic blood flow rate (Davies and Morris, 1993). Ifetroban was extensively distributed into the extravascular space. A secondary peak was no-

ticeable in dogs and monkeys but was most prominent in the dog with the peak occurring around 12 h after dosing. This is thought to be due to biliary excretion of ifetroban and/or ifetroban conjugates after the dogs were fed. Therefore, parameters for  $AUC_{(INF)}$ ,  $CL_T$ , and  $VD_{ss}$  could not be calculated for the dog.

In humans, the mean absolute bioavailability was 48%. After oral dosing, ifetroban was absorbed rapidly with a peak concentration of 241 ng/ml achieved within 20 min of dosing. A secondary peak was observed starting around 4 h after dosing (when subjects were fed); it peaked around 6 h after dosing. The decline in plasma concentrations yielded a mean  $t_{1/2}$  of 22 h after both i.v. and oral dosing. Mean  $CL_T$  of ifetroban in humans was 6.4 ml/min/kg and mean  $VD_{ss}$  was 4.4 l/kg.

**Excretion of Radioactivity.** Mean percent recovery of total radioactivity in urine and feces is summarized in Table 2. After i.v. dosing in rats, dogs, and monkeys, a mean of 2.8, 3.7, and 27.4% of total radioactivity, respectively, was recovered in urine. After oral dosing, the recovery of total radioactivity in urine was 3.2, 2.7, and 14.2% of the dose, respectively. Fecal excretion accounted for 91.9, 86.5, and 61.9% after i.v. dosing in the rat, dog, and monkey, respectively. The percentage after oral dosing was 89.2, 85.8, and 63.0%, respectively. The majority of the administered dose was recovered in the first 24 h post dose in the rat and monkey, and within the first 48 h post dose in the dog. The less than 90% recovery in the monkey may be attributed to the formation of <sup>3</sup>H-water because, after 2 h, >50% of the radioactivity in plasma was volatile, and after 1 day >90% was volatile. This <sup>3</sup>H-water was probably formed during hydroxylation of ifetroban because the tritium was located in the pentyl side chain that was later found to be a major site of hydroxylation in monkeys.

Biliary excretion of total radioactivity in three SD rats after oral dosing accounted for 85% of the total dose in 12 h. There were minimal amounts (<0.3%) recovered in urine or the carcass. Approximately 6% was recovered from the GI tract and feces. The intestinal absorption of radioactivity was determined by the summation of radioactivity in bile, urine, and carcass and was estimated to be 86%.

In humans, the mean cumulative excretion of total radioactivity in urine over 120 h post dose was 34.0 and 26.7% after i.v. and oral doses, respectively. Approximately 90% of the radioactive dose recovered in urine was excreted over the first 48 h post dose. Fecal excretion in the human accounted for 50.5 and 54.4% of the i.v. and oral dose, respectively. Overall, 85 and 81%, respectively, of the total i.v. and oral radioactivity was recovered in urine and feces over 120 h post oral dosing. The <90% recovery was due to three or four subjects who had low recovery. The other subjects had >90% recovery of radioactivity. The reason for the low recovery in these few subjects is unknown.

**Distribution of Radioactivity in Tissues and Fluids.** Figure 3 depicts the concentrations of total radioactivity in selected tissues in SD rats after oral and i.v. administrations. Highest concentrations of radioactivity after either oral or i.v. dosing were observed at 5 min post dose in most tissues except for the large intestine where peak concentrations occurred at 8 h post dose. Except for heart and brain, all other tissues had higher concentrations of total radioactivity than plasma. Also, radioactivity in heart and brain were below the limit of detection before 8 h, whereas the other tissues had detectable levels at 24 h. The limit of detection (20% counting error) was 10 ng/g for brain and about 2 ng/g for other tissues and fluids. The decline in concentration in all tissues seemed to parallel the decline in plasma. Concentrations in all tissues measured tended to be comparable between i.v. and oral dosing.

**Metabolite Identification.** HPLC chromatograms of rat, dog, and human plasma and urine are shown in Fig. 4. The retention times of

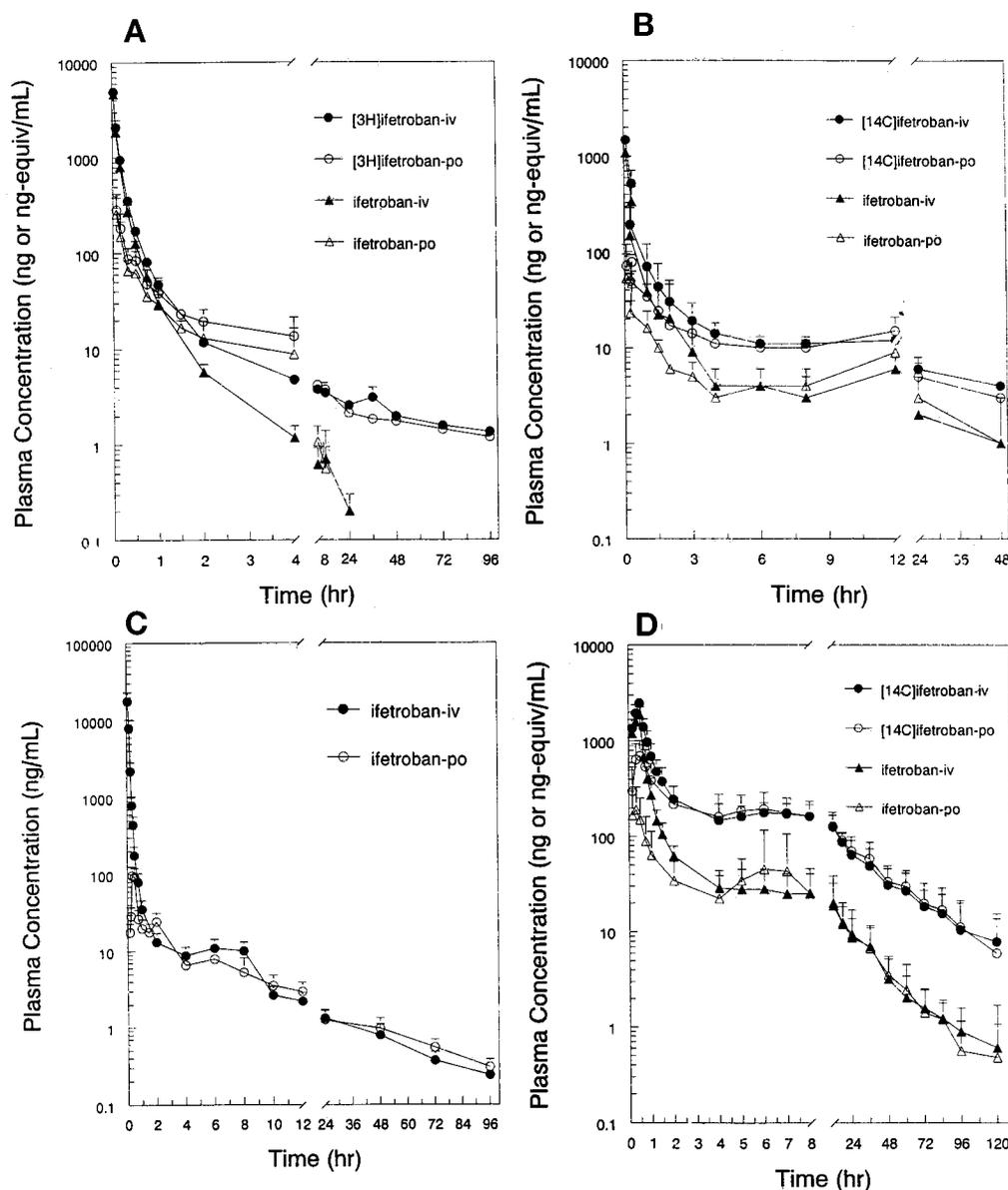


FIG. 2. Mean (standard deviation) plasma concentration-time profiles of total radioactivity and/or unchanged ifetroban after i.v. and oral (p.o.) administrations of 3 mg/kg of [ $^3\text{H}$ ]ifetroban in rats (A), 1 mg/kg of [ $^{14}\text{C}$ ]ifetroban in dogs (B), 1 mg/kg of [ $^{14}\text{C}$ ]ifetroban in monkeys (C), and 50 mg of [ $^{14}\text{C}$ ]ifetroban in humans (D).

TABLE 1

Mean (standard deviation) pharmacokinetic parameters for ifetroban in SD rats, dogs, African green monkeys, and humans after i.v. and oral administrations

Species	Dose, Route	$C_{\max}$	$t_{\max}^a$	$\text{AUC}_{(0-\infty)}^b$	$t_{1/2}$	$\text{CL}_T$	$\text{VD}_{ss}$	$F$
		ng/ml	min	ng · h/ml	h	ml/min/kg	l/kg	%
Rat	3 mg/kg, i.v.			578 (35)	7.7 (0.8)	87.5 (10.3)	2.8 (1.3)	
	3 mg/kg, p.o.	259 (55)	5	143 (13)				
Dog	1 mg/kg, i.v.			475 (120)	20 <sup>c</sup>	— <sup>d</sup>	— <sup>d</sup>	
	1 mg/kg, p.o.	66 (28)	7	158 (60)				
Monkey	1 mg/kg, i.v.			957 (146)	27 <sup>c</sup>	19.1 (3.5)	5.5 (1.1)	
	1 mg/kg, p.o.	56 (46)	20	217 (75)				
Human	50 mg, i.v.			1828 (317)	22 (7)	6.4 (1.4)	4.4 (2.3)	
	50 mg, p.o.	241 (152)	20	915 (563)				

<sup>a</sup> Median;  $t_{\max}$ , time to maximum concentration.

<sup>b</sup> AUC for dog is  $\text{AUC}_{(0-24)}$ .

<sup>c</sup> The mean terminal slope was used to calculate  $t_{1/2}$ .

<sup>d</sup> Could not be accurately determined because  $\text{AUC}_{(0-\infty)}$  could not be determined.

known standards (Fig. 1) are contrasted to observed in vivo peaks. The chromatograms from human plasma and urine are quite different from those from rat and dog. Table 3 summarizes the concentrations

of ifetroban and identified metabolites in rat, dog, and human plasma after oral administration of [ $^{14}\text{C}$ ]ifetroban.

*Plasma.* In the rat after oral administration, approximately 50% of

TABLE 2

Mean (standard deviation) percent cumulative recovery of total radioactivity (rat = 168 h postdose; dog = 96 h postdose; monkey = 360 h postdose; human = 168 h postdose) after i.v. and oral administration of [<sup>3</sup>H]ifetroban in male monkeys (n = 3) and [<sup>14</sup>C]ifetroban in male SD rats (n = 4), male dogs (n = 4), and health male subjects (n = 12)

Species	Dose	Matrix	Intravenous	Oral
Rat	5 mg/kg	Urine	2.8 (1.2)	3.2 (2.2)
		Feces	91.9 (3.0)	89.2 (2.4)
		Carcass	0.2 (0.0)	0.1 (0.2)
		Total	93.8 (3.6)	92.5 (3.2)
Dog	1 mg/kg	Urine	3.7 (1.0)	2.7 (0.8)
		Feces	86.5 (2.6)	85.8 (2.0)
		Total	90.3 (2.4)	88.6 (3.0)
Monkey <sup>a</sup>	1 mg/kg	Urine	27.4	14.2
		Feces	61.9	63.0
		Total	89.3	77.2
Human	50 mg	Urine	34.0 (6.3)	26.7 (8.5)
		Feces	50.5 (15.6)	54.4 (12.7)
		Total	84.5 (17.0)	81.0 (17.5)

<sup>a</sup> After the first study period, the collection time was extended from 96 to 360 h. Therefore, for the second period, n = 1 for i.v. and n = 2 for oral.

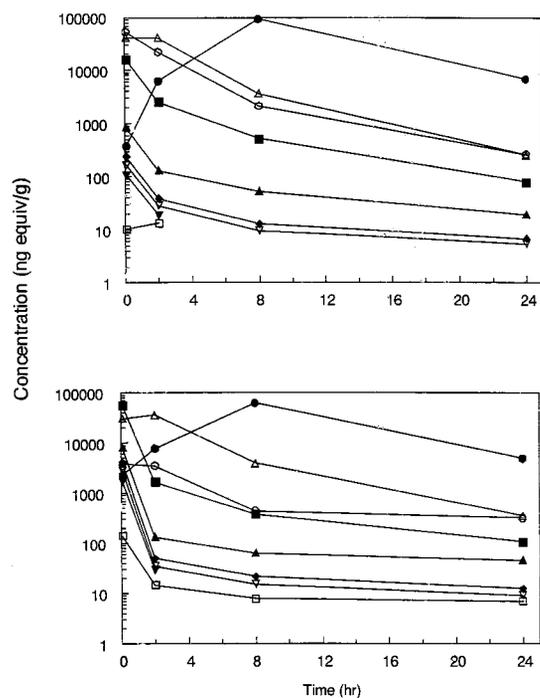


FIG. 3. Mean concentrations of total radioactivity in selected tissues from SD rats after oral (top) and i.v. (bottom) administration.

●, large intestine; △, small intestine; ○, stomach; ■, liver; ▲, kidney; ◆, plasma; ∇, blood; ▼, heart; □, brain.

the radioactivity in the 1-h plasma samples was attributed to parent compound, 8% to the 14-hydroxy metabolite, and 3% to ifetroban acylglucuronide. The remaining 39% could not be identified. The 3- and 6-h rat plasma chromatograms were qualitatively similar to the 1-h sample. In the dog, approximately 43% of the radioactivity present in the 1-h plasma sample was attributed to parent compound and 18% to ifetroban acylglucuronide. The remaining 39% could not be identified. The 3-, 6-, and 24-h dog plasma chromatograms were qualitatively similar to the 1-h sample. In the 1-h human plasma sample, regardless of i.v. or oral administration of ifetroban, HPLC analysis indicated the majority (62%) of the radioactivity was present as ifetroban acylglucuronide, with a minor component (9%) present as parent compound. The remaining 29% of the radioactivity could not

be identified. The unidentified radioactivity in the rat, dog, and human plasma samples represents radioactivity that was either not extracted into methanol, not recovered from the column during HPLC analysis, or present in minor amounts, which were not sufficient to identify, in the chromatogram.

**Urine.** Because of the low urinary level of radioactivity present in rat and dog (Table 1), profiling consisted only of comparing retention times to reference standards. In rat urine, HPLC analysis indicated that the majority of the radioactivity was present as metabolites, with a minor component as parent compound. Treatment with  $\beta$ -glucuronidase/arylsulfatase indicated that none of the metabolites in the rat were conjugated. In dog urine, HPLC analysis indicated that the majority of the radioactivity was present as parent compound, and one of the additional peaks corresponded to ifetroban acylglucuronide. There were several additional unidentified peaks in the chromatogram. In human urine, HPLC analysis indicated that the majority of the radioactivity was present as ifetroban acylglucuronide. Analyses by MS and NMR indicated that the acylglucuronide conjugate was the 1- $\beta$ -acylglucuronide of ifetroban. Full scan MS analysis indicated that the metabolite had a molecular weight of 176 Da greater than the parent, which indicated the addition of a glucuronide functional group. MS/MS substructural information of the metabolite compared with parent was consistent with an acylglucuronide metabolite. <sup>1</sup>H NMR data showed a resonance at 5.5 ppm, corresponding to H-1. The observed chemical shift was consistent with the presence of an acyl-type glucuronic acid conjugate. A measured J value of 8.2 Hz indicated that substitution of the glucuronic acid portion was in the  $\beta$  position at H-1. The parent compound was a minor component of the human urine radioactivity, and very minor unidentified metabolites were present.

**Bile.** In rat bile, the major metabolite was the 14-hydroxy metabolite of ifetroban with the hydroxyl group in the endo position on the C-14 carbon. This identification was confirmed by MS/MS and NMR procedures. Full scan MS experiments indicated the molecular weight of the metabolite as 16 Da greater than the parent compound. Substructural MS/MS information obtained by analyzing strategic fragment ions indicated that the addition of the 16 Da had occurred on the oxabicycloheptyl or oxazole substructure. Both the metabolite and parent molecule underwent extensive fragmentation, including the facile loss of water. The elimination of three water molecules from the metabolite, compared with two for the parent compound, further indicated that the 16-Da increase resulted from hydroxylation. The exact location of the hydroxylation was obtained by NMR analysis. The observation of a singlet resonance at 8.17 ppm in the spectrum of ifetroban and the metabolite indicated that the oxazole remained unchanged in the metabolite. In the proton spectrum of the metabolite, the C-14 proton was shifted 2.5 ppm downfield, indicating that the hydroxyl group was at C-14. Furthermore, the proton-proton coupling constant ( $J_{14,15} = 5.8$  Hz) suggested that the configuration of the hydroxyl group at C-14 was endo. A minor component of the bile samples was parent compound, with additional unidentified peaks. It is possible that some or all of the unknown peaks represent either the acylglucuronide metabolite or rearrangement products of this metabolite.

## Discussion

The mean recovery of total radioactivity in urine and bile in the rat and in urine in humans after i.v. and oral administration of [<sup>14</sup>C]ifetroban suggests that ifetroban was almost completely absorbed from the GI tract after oral administration. Although the recovery from urine in the rat was only 3%, the recovery from bile after oral administration was 85%, suggesting that oral absorption was at least 88% complete.

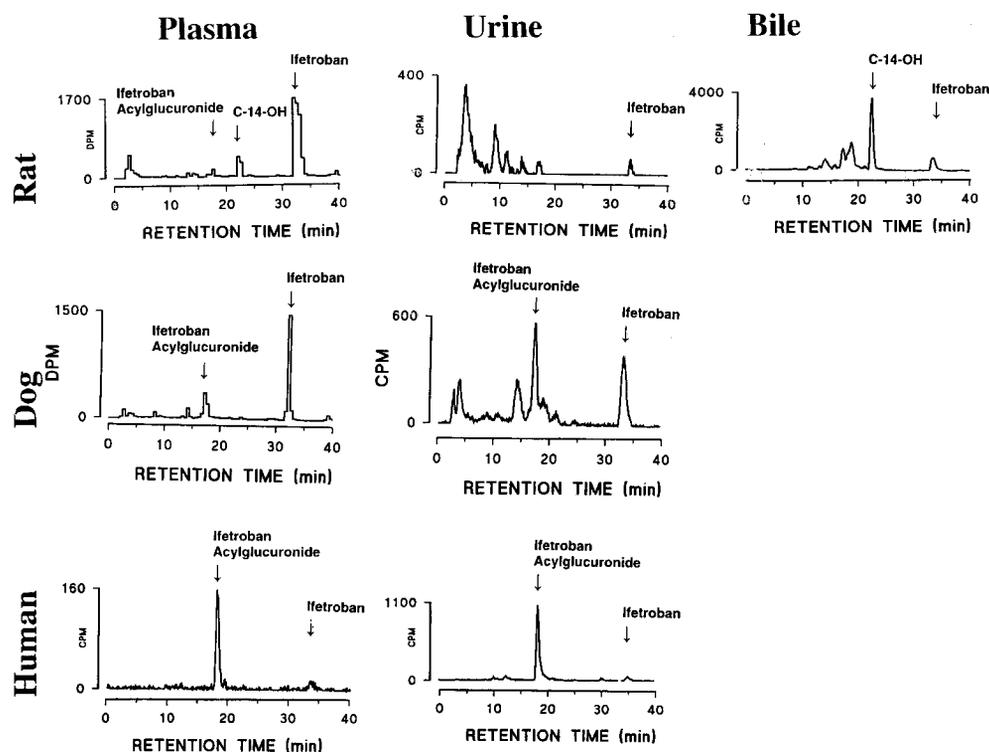


FIG. 4. Plasma and urine HPLC chromatograms of rat, dog, and human; 1-h pooled plasma samples and 0–8 h pooled rat urine, 0–1 h pooled rat bile, 0–24 h pooled dog urine, and 12–24 h pooled human urine.

TABLE 3

Concentrations of ifetroban and identified metabolites in plasma after oral administration of [ $^{14}$ C]ifetroban

Species	Sample	Total	Nonextractable <sup>a</sup>	Polar <sup>b</sup>	Ifetroban Acylglucuronide	C <sub>14</sub> Hydroxy Metabolite	Ifetroban
	<i>h</i>	<i>ng-equivalents of [<math>^{14}</math>C] radioactivity/ml plasma (% of total)<sup>c</sup></i>					
Rat	1	153	16 (10%)	18 (12%)	5 (3%)	12 (8%)	76 (50%)
	3	49	15 (31%)	7 (14%)	1 (2%)	2 (4%)	16 (33%)
	6	47	19 (40%)	8 (17%)	1 (2%)	2 (4%)	12 (26%)
Dog	1	61	4 (7%)	2 (3%)	11 (18%)		26 (43%)
	3	20	3 (15%)	2 (10%)	3 (15%)		4 (20%)
	6	20	3 (15%)	1 (5%)	3 (15%)		7 (35%)
	24	6	2 (33%)	1 (17%)	1 (17%)		2 (33%)
Human	1	468	33 (7%)		289 (62%)		43 (9%)
	6	223	28 (13%)		113 (51%)		23 (10%)

<sup>a</sup> Portion of radioactivity not soluble in methanol after protein precipitation.

<sup>b</sup> Unidentified polar metabolite(s) eluting at void volume of chromatogram.

<sup>c</sup> % of total is the percentage of total radioactivity in the plasma sample.

The excretion pattern in the dog was similar to that in the rat. Although there is no confirmatory data in the dog, the pattern suggests that absorption should be extensive in the dog as well. The absorption in the monkey appears to be 52%, but the i.v. data are from only one monkey. The absorption in humans appears to be similar to that observed in the rat, based on the mean recovery of total radioactivity in the urine, with at least 80% of the oral dose being absorbed. Despite the nearly complete absorption of the oral dose, the absolute bioavailability of ifetroban was 25, 35, 23, and 48% in rat, dog, monkey, and human, respectively. This suggests that a significant portion of the orally administered dose is subjected to first-pass metabolism. The majority of the i.v. dose in each species was recovered in the feces. Therefore, it seems that biliary excretion is the major route of excretion of ifetroban and/or its metabolites.

Absorption of ifetroban in rat, dog, monkey, and human after oral administration was rapid, as evident by attainment of  $C_{max}$  within 20

min. Levels of ifetroban in plasma fell quickly for about the next 2 h and slowly thereafter, suggesting rapid distribution into tissues. Concentrations of ifetroban in the plasma of dogs and humans showed secondary peaks at about 12 and 6 h, respectively, after oral or i.v. dosing, suggesting that the drug may undergo enterohepatic recirculation. Terminal elimination half-life values may represent only a small proportion of the dose, however, because most of the area under the plasma concentration versus time curves occurred within a few hours after dosing; the long terminal half-life may be a result of rate-limiting release of ifetroban from tissues.

Ifetroban appears to be extensively distributed into tissues, as indicated by its high volume of distribution in all species studied. After single 5-mg/kg oral and i.v. doses of [ $^{14}$ C]ifetroban to male SD rats, radioactivity was distributed throughout the body and there was no evidence that radioactivity tended to accumulate in any tissue. The increase in the concentration of radioactivity in the intestinal tract up

to 8 h after i.v. administration indicated the biliary secretion of the drug and/or metabolite(s). After oral administration, the concentrations in the intestinal tract probably represented any unabsorbed drug and the drug/metabolite(s) secreted into bile. After oral and i.v. doses to SD rats, the maximum concentrations of total radioactivity were generally observed at 5 min for all samples, with the exception of the large and small intestines, and the contents of the large and small intestines. Concentrations of total radioactivity were detectable in all of the tissues examined after oral and i.v. doses at least up to 8 h and in most cases up to 24 h after dosing, indicating extensive distribution of [<sup>14</sup>C]ifetroban, its metabolites, or both into most tissues. Little radioactivity was observed in brain, suggesting that ifetroban crosses the blood-brain barrier to only a small extent.

The main metabolite found in rat bile was the C<sub>14</sub>-hydroxy metabolite of ifetroban. This metabolite was also observed in rat plasma, but was not detected in dog or human plasma. The predominate amount of radioactivity in rat and dog plasma was attributed to parent compound with a small amount of ifetroban acylglucuronide (glucuronide ester of the carboxyl group) being present (3% in rat and 18% in dog). In contrast, the largest percent of radioactivity in human plasma was attributed to ifetroban acylglucuronide (62%) as opposed to parent drug (9%). The unaccounted for radioactivity in the chromatogram represents a combination of radioactivity present in the void volume of the analysis, radioactivity lost during the extraction procedure, and radioactivity present as minor components that were not possible to identify in the chromatogram. Although all of the radioactivity was not recoverable from plasma, it is most likely that the nonextractable radioactivity in plasma samples from different species may be due to covalent binding of ifetroban acylglucuronide. Such a phenomenon has been reported for other acylglucuronide metabolites (Spahn-Langguth and Benet, 1992), and a direct relationship was found between the amount irreversibly protein-bound and the extent of glucuronide present (Benet et al., 1993).

In summary, the disposition of ifetroban in animals (rats, dogs, and monkeys) and humans appears to be similar. Ifetroban was rapidly absorbed after oral administration in all species, with absolute bio-

availability ranging from 25 to 48% across species. Ifetroban is subject to significant first-pass metabolism with biliary excretion being the predominant route of elimination. [<sup>14</sup>C]Ifetroban shows extensive distribution into tissues. Approximately 40 to 50% of the radioactivity in rat and dog plasma was accounted for by parent drug whereas, in humans, approximately 60% of the plasma radioactivity was accounted for by ifetroban acylglucuronide.

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