THE ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION OF ROFECOXIB, A POTENT AND SELECTIVE CYCLOOXYGENASE-2 INHIBITOR, IN RATS AND DOGS

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ABSTRACT:

Absorption, distribution, metabolism, and excretion studies were conducted in rats and dogs with rofecoxib (VIOXX, MK-0966), a potent and highly selective inhibitor of cyclooxygenase-2 (COX-2). In rats, the nonexponential decay during the terminal phase (4- to 10-h time interval) of rofecoxib plasma concentration versus time curves after i.v. or oral administration of [14C]rofecoxib precluded accurate determinations of half-life, AUC∞ (area under the plasma concentration versus time curve extrapolated to infinity), and hence, bioavailability. After i.v. administration of [14C]rofecoxib to dogs, plasma clearance, volume of distribution at steady state, oral (2, 5, and 10 mg/kg) administration, except in rats where no additional increase was observed between the 5 and 10 mg/kg doses. Radioactivity distributed rapidly to tissues, with the highest concentrations of the i.v. dose observed in most tissues by 5 min and by 30 min in liver, skin, fat, prostate, and bladder. Excretion occurred primarily by the biliary route in rats and dogs, except after i.v. administration of [14C]rofecoxib to dogs, where excretion was divided between biliary and renal routes. Metabolism of rofecoxib was extensive. 5-Hydroxyrofecoxib-O-β-D-glucuronide was the major metabolite excreted by rats in urine and bile. 5-Hydroxyrofecoxib, rofecoxib-3′,4′-dihydrodiol, and 4′-hydroxyrofecoxib sulfate were less abundant, whereas cis- and trans-3,4-dihydrorofecoxib were minor. Major metabolites in dog were 5-hydroxyrofecoxib-O-β-D-glucuronide (urine), trans-3,4-dihyro-rofecoxib (urine), and 5-hydroxyrofecoxib (bile).

Aspirin has been marketed since the latter part of the nineteenth century as an anti-inflammatory agent, but its mechanism of action remained obscure until 1971 when Sir John Vane proposed that aspirin served as an inhibitor of prostaglandin (PG) synthesis (Vane, 1971). This inhibition occurred by an irreversible acetylation of cyclooxygenase (COX), the key enzyme involved in PG biosynthesis (Roth et al., 1975).

COX [E.C. 1.14.99], also known as prostaglandin endoperoxide synthase (PGHS), is a bifunctional, membrane-bound hemeprotein that catalyzes the addition of two molecules of molecular oxygen to arachidonic acid to form PGH2, followed by reduction of the endoperoxide moiety to give PGG2 (Smith and Marnett, 1991; Smith and DeWitt, 1996). The enzyme exists as two isoforms: a constitutive form, designated as COX-1, and an inducible form, referred to as COX-2 (Fu et al., 1990; Masferrer et al., 1990, 1992). The constitutive COX-1 is expressed in most tissues, generating PGs that function to protect the gastric mucosal lining, regulate blood flow to the kidney, and support platelet aggregation. In contrast, COX-2 is constitutively expressed in limited healthy tissues, e.g., kidney. However, when induced, the PGs produced by this isoform are associated with the pain and swelling of inflammation. Expression of COX-2 is stimulated by growth factors, cytokines, phorbol esters, and mitogens, but is inhibited by steroid anti-inflammatories, e.g., glucocorticoids, which have little or no effect on COX-1 levels (reviewed by Vane and Botting, 1996; Donnelly and Hawkey, 1997; Jouzeau et al., 1997).

Currently marketed NSAIDs generally are nonselective COX-1/COX-2 inhibitors whose therapeutic utility is due to inhibition of COX-2, but whose side effect profile (i.e., gastrointestinal irritation and/or bleeding) results from inhibition of COX-1. Clearly, the development of highly selective inhibitors of COX-2 are an attractive target, because such agents retain the anti-inflammatory, analgesic, and antipyretic properties of current NSAIDs, while reducing the risk of gastrointestinal side effects (Vane and Botting, 1996; Donnelly and Hawkey, 1997; Jouzeau et al., 1997).

Rofecoxib (3-phenyl-4-[4-(methysulfonyl)phenyl]-2-(5H)-furanone, VIOXX, MK-0966; Fig. 1), a potent and highly selective inhibitor of COX-2 (Prasit et al., 1999), has been approved by the Food and Drug Administration for the treatment of osteoarthritis and pain. The high selectivity of rofecoxib as an inhibitor of COX-2 has been examined in several in vitro preparations and in vivo rodent models (Chan et al., 1999). In clinical trials, rofecoxib showed effec-
tive analgesia in a dental pain model (Ehrich et al., 1999) and effective antipyretic activity in monkeys and humans (Schwartz et al., 1999).

The objectives of the present investigation were to determine the absorption, distribution, metabolism, and excretion (ADME) of rofecoxib in rats and dogs, the two species used in the toxicological evaluation of this compound.

Materials and Methods

Chemicals and Dosing Solutions. Chemicals. [Furanone-4-14C]rofecoxib was prepared as outlined in Fig. 2. All chemicals for the synthesis were obtained from Aldrich Chemical Co. (Milwaukee, WI), except that [14C]sodium acetate was obtained from ChemSyn Laboratories (Lenexa, KS). The label was introduced by Friedel–Crafts acylation of thioanisole (1) using [14C]acetyl chloride (prepared from [14C]sodium acetate) to give acetonaphene. Oxidation of 2 was achieved using monoperophosphoric acid magnesium salt to afford sulfone 3, which was brominated via formation of the enol triflate. The resulting bromosulphone 4 was esterified using phenylacetic acid to yield ketoester 5. Cyclization was effected by treatment with disopropylethylamine. The crude product was purified by preparative HPLC and crystallized from ethyl acetate. The overall radiochemical yield for the synthesis was 30% from [14C]sodium acetate. Structural identification of the tracer was confirmed by chromatographic coelution with unlabeled standard. After purification, chemical and radiochemical purity were at least 99% (as determined by HPLC chromatographic coelution with unlabeled standard. After purification, chem-ical and radiochemical purity were at least 99% (as determined by HPLC analysis), and the specific activity of the stock material was 12.62 mCi/mmol (40.14 μCi/mg). Unlabeled rofecoxib was synthesized by the Process Research group (Merck and Company, Inc., Rahway, NJ), whereas rofecoxib metabolites and the internal standard (the 4'-methylphenyl analog of rofecoxib, Fig. 1) were synthesized by Medicinal Chemistry (Merck Forest Canada, Kirkland, Quebec). Due to the sensitivity of rofecoxib to natural light, the parent compound, the internal standard, and all biological samples were handled under yellow lights.

Other chemicals. β-Glucuronidase (Helix pomatia, type H-5) was obtained from Sigma Chemical Co. (St. Louis, MO), trifluoroacetic acid (TFA) was purchased from Aldrich Chemical Co., and dimethyl sulfoxide (DMSO), ammonium hydroxide (NH4OH), sodium acetate, acetonitrile (CH3CN), and methanol (CH3OH) were obtained from Fisher (Pittsburgh, PA).

Dosing solutions. [14C]Rofecoxib or unlabeled rofecoxib was administered to rats and dogs for absorption, metabolism, excretion, and pharmacokinetic studies, and to rats for the tissue distribution study.

[14C]Rofecoxib was used without dilution of the specific activity for i.v. administration to rats. The weighed material was dissolved directly in DMSO before dosing. The specific activity of [14C]rofecoxib was diluted with unlabeled rofecoxib for i.v. administration to dogs and oral administration to rats and dogs. For these solutions, the radiolabeled and unlabeled compounds were combined and dissolved in organic solvent (either toluene or methylene chlo-ride). After evaporation of the solvent to dryness (N2, 44°C), the residue was either dissolved in DMSO for i.v. dosing or suspended in 0.5% methylcellulose (w/v) for oral dosing. The concentrations were 4 mg/ml (rats) or 10 mg/ml (dogs) for the i.v. solutions or 1 mg/ml for the oral dosing suspensions. The specific activities ranged from 0.56 to 40.1 μCi/mg, and each animal received approximately 20 to 25 μCi. For those studies requiring only unlabeled rofecoxib, the i.v. solutions were prepared in DMSO, and the oral solutions were prepared as 0.5% methylcellulose suspensions at the required concentrations.

Animal Studies. Male Sprague–Dawley rats weighing approximately 225 to 350 g were obtained from Taconic Farms (Germantown, NY). Male beagle dogs weighing approximately 8 to 10 kg were obtained from Marshall Farms (North Rose, NY). All procedures were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. Rats and dogs were housed in temperature-controlled rooms with a 12-h light/dark cycle. Animals were surgically prepared with a cannula in the jugular vein and/or bile duct for sample collection. For all studies described below, except the tissue distribution study in dogs and the food effect study in dogs, the animals were deprived of food 14 to 18 h before dosing but were allowed free access to water. Food was provided to rats and dogs 6 to 8 h after dosing. Blood samples were collected in heparinized syringes, and plasma subsequently was obtained by centrifugation. With the exception of blood samples, which were stored at 4°C, all biological samples were stored at −20°C until analyzed. Finally, all biological samples were centrifuged before analysis to remove particulate matter.

Disposition and metabolism of [14C]rofecoxib in rats and dogs. [14C]Rofecoxib was administered i.v. to rats (n = 4) by tail vein injection (0.5 ml/kg) at a dose of 2 mg/kg. The oral dose (5 mg/kg) was administered to a second group of animals (n = 4) by oral gavage (5 ml/kg). In dogs (n = 4), the i.v. dose (2 mg/kg) of [14C]rofecoxib was administered as a 10-min infusion (0.2 ml/kg) via either the cephalic or saphenous veins. After a 3-week wash-out period, the same dogs received an oral dose (5 mg/kg) of [14C]rofecoxib by gavage. In both studies, blood was collected at time points of 0, 0.08 (i.v. only), 0.17 (i.v. only), 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, 48, 72, and 96 h. Urine and feces were collected at time intervals of 0 to 8, 8 to 24, 24 to 48, 48 to 72, and 72 to 96 h. The urine samples from 0 to 8 and 8 to 24 h were collected over dry ice, and the remaining samples were collected at room temperature.

Biliary excretion of [14C]rofecoxib in rats and dogs. [14C]Rofecoxib was administered to bile duct-cannulated rats (n = 3–4 per group) and dogs (n = 1 per group) as i.v. (2 mg/kg) or oral (5 mg/kg) doses. Bile samples were collected on dry ice at hourly intervals for 6 h (rat) or 8 h (dog) and then until 24 h. Dose dependence of rofecoxib in rats and dogs. Rats (n = 4) and dogs (n = 4) received i.v. doses of unlabeled rofecoxib of 1, 2, and 4 mg/kg, and oral doses of 2, 5, and 10 mg/kg. Blood samples were collected for 48 h.

Effect of food on absorption of rofecoxib in dogs. The effect of food on the absorption kinetics of rofecoxib was studied in dogs (n = 4) at an oral dose of 5 mg/kg. The fasted study was carried out as described above. After a 1-week wash-out period, dogs were fed 1 h before and 10 h after a second oral dose. Blood was collected at specific time points to 48 h.

Tissue distribution of [14C]rofecoxib in rats. The tissue distribution study was performed by Covance Laboratories Inc. (Madison, WI). [14C]Rofecoxib was administered as an i.v. dose (2 mg/kg) to rats (n = 12) by tail vein injection. Groups of three animals were sacrificed by exsanguination under halothane anesthesia at 5 min, and at 0.5, 2, and 24 h postdose. Blood (4–10 ml) was collected via cardiac puncture at the time of sacrifice. Specified tissues were excised, rinsed with water, blotted dry, weighed, and placed on ice until transferred to a freezer.

Metabolism of rofecoxib in rats after administration of a high oral dose. Rats (n = 3 per group) received either unlabeled or radiolabeled rofecoxib (100 mg/kg, 5 ml/kg, 0.5% methylcellulose) by oral gavage. Urine was collected over dry ice for 24 h.

Radioactivity Measurements. Total radioactivity in plasma, bile, and urine was determined directly by adding aliquots of sample (0.025–1.0 ml) to polyethylene vials containing scintillation cocktail (15 ml, Ready Safe, Beckman Instruments, Fullerton, CA). Fecal samples were homogenized in 4 to 5 volumes of water using a homogenizer (Omni International, Waterbury, CT). Aliquots of the homogenate (~1 g) were weighed into tared combustion cups, and, after drying overnight, the samples were combusted in a Packard Tricarb sample oxidizer (model B306; Packard Instruments, Downers Grove, IL). The resultant carbon dioxide was trapped in a Carbosorb/Permafluor mixture.

![Fig. 1. Chemical structures of rofecoxib and internal standard. The asterisk indicates the location of the 14C radiolabel.](image-url)
interday precision, expressed as the coefficient of variance (%CV), was defined as the mean percentage of recovery, was studies) or 1.0 to 250 ng/ml (unlabeled rofecoxib studies). The assay accuracy, and radiochemical detection. To aliquots of bile or urine (10
ml) for the fecal samples. Skin samples were digested in 1 N sodium hydroxide at 40°C, homogenized, and counted. Plasma from this study was analyzed directly by liquid scintillation counting (model 1900TR; Packard Instruments). Quench correction was automatic and employed the H-number method.

**Rofecoxib Assay.** Concentrations of rofecoxib in rat and dog plasma from all the kinetics studies were determined by HPLC with fluorescence detection of a product resulting from postcolumn photochemical activation (Woolf et al., 1999). Briefly, an aliquot of plasma (0.15 ml) was added to 0.85 ml of buffer (0.1 M sodium acetate, pH 5), followed by the addition of internal standard (25 µl of 150 ng/ml solution in CH3CN). Liquid-liquid extraction was carried out with a 50:50 mixture of methylene chloride and hexane (4 ml). After centrifugation (3000g, 5 min), the tubes were placed in an acetone/dry ice bath to freeze the aqueous layer. The organic phase was isolated and evaporated to dryness under a flow of nitrogen and mild heat (44°C, Reacti-Therm heating module). The residue was reconstituted in HPLC mobile phase (35% aqueous CH3CN) immediately before analysis.

HPLC analysis was carried out on a Hewlett-Packard 1090 instrument equipped with a photochemical reactor (Aura Industries, Staten Island, NY) and fluorescence detector (Perkin-Elmer LC 240, Norwalk, CT). The mobile phase, consisting of 0.1% aqueous TFA (TFA aq )/NH4 OH (pH 3, 2 ml). Pooled urine (1 ml, containing $85\%$ of total radioactivity was recovered. The solvent was evaporated to dryness under nitrogen, and the residue was reconstituted in 15% CH3CN in 0.1% TFAa/NH4OH (pH 3) and injected for radiochromatography as described above.

**Isolation and Characterization of Rofecoxib Metabolites.** Metabolite isolation and purification by HPLC. Metabolites of rofecoxib were isolated for identification from urine that was collected as part of low dose (2 mg/kg, i.v.) radiolabeled studies in rats and dogs and a high dose (100 mg/kg, p.o.) unlabeled study in rats. During initial HPLC analysis to determine the retention times of drug-related material in these samples, metabolites from the radiolabeled studies were monitored by a combination of UV and radiochemical detection, whereas metabolites from the high dose unlabeled study were followed by UV and fluorescence detection. For isolation and purification of metabolites from biological samples, fluorescence detection was not employed.

From the radiolabeled i.v. study in rats, urine from three animals was pooled and subjected to solid phase extraction before chromatographic separation and isolation of metabolites. The solid phase extraction cartridges (C18, Baker) were preconditioned by sequential additions of CH3OH (1 ml) and 0.1% TFAa/NH4OH (pH 3, 2 ml). Pooled urine (1 ml, containing $281,000$ dpm) was transferred to the cartridge. The cartridge was washed with 0.1% TFAa/NH4OH (pH 3, 1 ml), the analytes were eluted with CH3OH (2 ml), and $88\%$ of total radioactivity was recovered. The solvent was evaporated to dryness under nitrogen, and the residue was reconstituted in 10% CH3CN in 0.1% TFAa/NH4OH (pH 3, 1 ml) before HPLC analysis. Urine from other studies were only filtered and/or centrifuged to remove particulate material before HPLC analysis.

Rat urine (1 ml) was injected onto a semipreparative C18 column (9.4 × 250 mm). The mobile phase consisted of 0.1% TFAa/NH4OH (pH 3, solvent A) and CH3CN (solvent B) and was delivered at a flow rate of 3 ml/min. A gradient elution began with 10% solvent B and increased to 60% solvent B at a rate of 2%/min, followed by a hold (8-min) at 60% solvent B, to give a total run time of 33 min. Analysis of dog urine was carried out as described above, except that a C18 analytical column was used, solvent A was 0.1% TFAa (pH 1.8), and the

**Radiochromatographic Analysis of Urine and Bile for Metabolite Profiles.** Urine and bile from rats and dogs were analyzed by HPLC with both UV and radiochemical detection. To aliquots of bile or urine (10 µl to 1 ml), four volumes of CH3CN were added, and after centrifugation and evaporation of solvent, the residues were reconstituted in mobile phase (10–200 µl) for injection directly onto the HPLC column. In the study where rats received a high oral dose (100 mg/kg) of [14C]rofecoxib, the urine sample (1 ml) was adjusted to pH 2 with TFA (20 µl).

The chromatographic system included a Hewlett-Packard 1050 instrument equipped with a Zorbax RX C8 analytical column (4.6 × 250 mm, 5 µm) heated to 40°C. The mobile phase, consisting of 0.1% aqueous TFA (TFAa)/NH4OH (pH3, solvent A) and CH3CN (solvent B), was delivered at a flow rate of 1 ml/min, starting at 15% solvent B and increasing to 60% solvent B as a linear gradient (1%/min) for a total run time of 45 min. The effluent was monitored by a photodiode array UV detector, set at 280 as well as 220 nm (i.e. samples) or 225 nm (p.o. samples), and by an in-line radiochemical detector using a 3 ml/min flow rate for the scintillation cocktail. For profiles of rat urine from the 100 mg/kg oral dose of [14C]rofecoxib, the gradient began at 20% solvent B and increased to 40% solvent B (0.5%/min) for a total run time of 40 min. Percentage of recovery of radioactivity from rat samples ranged from 39 to 100% (average ~70%) and from dog samples were 60 and 43% after i.v. and oral dosing, respectively.

**β-Glucuronidase Treatment of Urine and Bile.** β-Glucuronidase (2 mg/ml) was dissolved in 0.2 M sodium acetate (pH 5). Aliquots of urine (1 ml) and bile (20–100 µl diluted to 1 ml with acetate buffer), obtained after i.v. or p.o. administration of [14C]rofecoxib to rats and dogs, were incubated at 37°C with a final β-glucuronidase concentration of 1 mg/ml (600 U/ml). Concomitantly, samples were incubated in acetate buffer in the absence of enzyme to serve as controls. The reaction was terminated after ~16 h by the addition of four volumes of CH3CN. After centrifugation, the supernatants were evaporated to dryness under nitrogen. The residues were reconstituted in 15% CH3CN in 0.1% TFAa/NH4OH (pH 3) and injected for radiochromatography as described above.

![Fig. 2. Radiochemical synthesis of [14C]rofecoxib.](image_url)
estimated from the dose-normalized ratios of \(\text{AUC}_0-\infty\) concentration versus time curve from time zero to infinity. Bioavailability was determined by incubating the compound with pooled plasma from rat and dog. \(^{14}\text{C}\) Rofecoxib, dissolved in DMSO (1% v/v), was added to plasma samples (adjusted to pH 7.4 with 1 M sodium phosphate buffer, pH 4.0, and equilibrated to 37°C) and incubated for 15 to 30 min. The samples were transferred to Centrifree ultrafiltration units (Amicon/ Millipore, Bedford, MA), and, after centrifugation of the sample (2000 rpm for 37°C), the ultrafiltrates were removed and analyzed by liquid scintillation counting. The free fraction of drug was determined by dividing the amount (dpm) of drug in the ultrafiltrate by the amount in the original plasma sample. The percentage of protein bound values were not corrected for nonspecific binding (±5%).

Blood-to-Plasma Ratio. The partitioning of rofecoxib into erythrocytes was determined by incubating radiolabeled drug with fresh, heparinized whole blood from rat and dog. \(^{14}\text{C}\) Rofecoxib, dissolved in DMSO (1% v/v), was added to blood (1 ml) to give a final concentration of 1 \(\mu\text{g/ml}\), and the samples were incubated at 37°C for 15 min. Plasma was separated by centrifugation, and aliquots were taken for scintillation counting.

Pharmacokinetic Analysis. The plasma concentration versus time curves from both i.v. and oral studies in rats displayed nonexponential decay behavior during the terminal phase (4- to 10-h interval), which precluded calculation of the elimination half-life \((t_{1/2})\), and hence, area under the plasma concentration versus time curve extrapolated to infinity \((\text{AUC}_0-\infty)\), plasma clearance \((\text{Cl}_p)\), volume of distribution at steady state \((V_d)\), and bioavailability (F). Due to interanimal variability observed in the terminal phase of the plasma concentration data, the partial AUC from time zero to the last quantifiable time point, \(C_{\text{max}}\), and \(T_{\text{max}}\) values were estimated from mean plasma concentration data. The plasma concentration at time zero \((C_0)\) after the i.v. dose in rats was obtained by back extrapolation of the mean plasma concentration versus time curve. The pharmacokinetic parameters in dogs were determined using model-independent methods. The terminal elimination rate constant \((\beta)\) for each animal was determined from the slope of the unweighted regression line fitted to the terminal phase of the log-linear plasma concentration versus time data using the method of least-squares. The half-life was obtained by dividing \(\ln 2\) by \(\beta\). The plasma clearance \((\text{Cl}_p)\) was calculated as

\[
\text{Cl}_p = \frac{\text{Dose}}{\text{AUC}_0-\infty} \tag{1}
\]

The steady-state volume of distribution was estimated by the following equation:

\[
V_d = \frac{\text{Dose}_{\text{iv}} \times \text{AUMC}_0-\infty}{(\text{AUC}_0-\infty)} \tag{2}
\]

where \(\text{AUMC}_0-\infty\) is the total area under the first moment of the drug concentration versus time curve from zero to infinity. Bioavailability was estimated from the dose-normalized ratios of \([\text{AUC}]_{0-\infty, \text{p.o.}}\) to \([\text{AUC}]_{0-\infty, \text{i.v.}}\).

Results

Absorption and Disposition of \(^{14}\text{C}\) Rofecoxib. Mean plasma concentration versus time curves of rofecoxib and total radioactivity after a single i.v. (2 mg/kg, Fig. 3A) or oral (5 mg/kg, Fig. 3B) dose of \(^{14}\text{C}\) Rofecoxib to rats exhibited a nonexponential decline over the time interval of 4 to 10 h, precluding a reliable estimate of the elimination rate constant, and hence, half-life, AUC\(_{\text{0-\infty}}\) plasma clearance, volume of distribution, and bioavailability. The time to peak plasma concentration (205 ng/ml) after oral dosing was 30 min. Comparison of partial AUC values of unchanged rofecoxib and total radioactivity after i.v. or oral dosing showed that parent drug represented 62 or 29%, respectively, of circulating drug-related material. The corresponding AUC values for rofecoxib and total radioactivity after each dose were 1357 and 2195 ng·h/ml (0–10 h, i.v.) and 687 and 2368 ng·h/ml (0–24 h, p.o.).

The plasma concentration of rofecoxib and total radioactivity in dogs (Fig. 4), unlike rats, declined exponentially after i.v. (2 mg/kg) and oral (5 mg/kg) administration. After a short distribution phase after i.v. dosing (Fig. 4A), the decline in concentrations of parent drug and total radioactivity was essentially monoeponential with an elimination half-life of 2.6 h. Total plasma clearance and steady-state volume of distribution values were 33.6 ml/min/kg and 0.8 l/kg, respectively. The \(C_{\text{max}}\) (1114 ng/ml) after the oral dose occurred at 1.5 h, and half-life and bioavailability were 3.3 h and 26.1%. Plasma concentrations of unchanged rofecoxib after i.v. or oral dosing comprised 66 and 43%, respectively, of total radioactive drug equivalents in the systemic circulation of dogs. The AUC\(_{\text{0-\infty}}\) values for rofecoxib and total radioactivity were 9,225 and 13,116 ng·h/ml (i.v.) and 5,997 and 13,708 ng·h/ml (p.o.).
the T\textsubscript{max} which ranged from no effect to a 2- to 4-fold increase over not noted that, in individual dogs, the effect of food led to variability of respectively, higher than values observed in fasted animals. It was dogs that were fed 1 h before the oral dose were 34 and 13%, whereas 69% was recovered after an oral dose (5 mg/kg).

oral dosing had a modest impact on the absorption kinetics of rofex-

coxib.

The Effect of Dose on Rofecoxib Kinetics. The mean pharmacokinetic parameters for rats and dogs that received unlabeled doses of rofecoxib at 1, 2, and 4 mg/kg (i.v.) and 2, 5, and 10 mg/kg (p.o.) are presented in Table 2. As had been noted with the 2 mg/kg, i.v. dose of \([^{14}C]\)rofecoxib in rats, an estimate of t\textsubscript{1/2} for the 1 mg/kg, i.v. dose in this dose dependence study could not be determined due to the nonexponential decline over the 2- to 10-h interval. However, for the 2- and 4-mg/kg doses, an estimate of t\textsubscript{1/2} was 6.6 and 6.8 h, respectively. In both rats and dogs the increase in AUC values suggested that the pharmacokinetics were nearly linear with increasing i.v. doses over the range of 1 to 4 mg/kg. After administration of the oral doses to rats, the increase in AUC values was greater than proportional between the low and middle doses, with no significant difference in AUC or C\textsubscript{max} values between the middle and high oral doses. The AUC and C\textsubscript{max} values in dogs increased with all three oral doses, but the increases were less than proportional.

Tissue Distribution of \([^{14}C]\)Rofecoxib in the Rat. Radioactivity was widely distributed to most tissues of rats after i.v. administration of \([^{14}C]\)rofecoxib at 2 mg/kg (Table 3). The maximum concentration of radioactivity in plasma was 2.25 \(\mu\text{g}\) equivalents/g at 5 min postdose, and the level declined to 0.051 \(\mu\text{g}\) equivalents/g at the 24-h sampling time. Although concentrations of radioactivity were highest in most tissues at 5 min postdose, maximum levels in the urinary bladder, prostate, skin, and fat occurred at 30 min. Concentrations in the liver were sustained at time to 24 h, with the amount remaining in the body confined primarily to the gastrointestinal tract and liver.

Plasma Protein Binding and Blood-to-Plasma Ratio. \([^{14}C]\)Rofecoxib was highly bound to plasma proteins from rat and dog and showed species dependence. At \([^{14}C]\)rofecoxib concentrations ranging from 0.05 to 5.0 \(\mu\text{g}\)/ml, the bound value percentages were 93 and 82%, respectively. \([^{14}C]\)Rofecoxib partitioned into erythrocytes at a blood-to-plasma ratio of 0.76, indicating that blood clearance would be higher than plasma clearance.
Rats. Rofecoxib was extensively metabolized in rats as indicated by the absence of unchanged drug in radiochromatograms of urine and bile (Fig. 6). The profiles of urine (Fig. 6A) and bile (Fig. 6B) from rats after i.v. (2 mg/kg) or oral (5 mg/kg) administration were qualitatively and quantitatively similar, showing two radioactive components. The metabolites were identified by NMR spectroscopy as 5-hydroxyrofecoxib (major) and 5-hydroxyrofecoxib (minor). The most notable change in the NMR spectra of the two metabolites as compared with a reference spectrum of synthetic rofecoxib occurred at the C-5 protons (Hd) of the furanone ring. A significant downfield shift from 6.59 or 6.87 ppm and the loss of one proton supported the conclusion that hydroxylation had occurred at this position. The five additional signals appearing in the spectrum of the major urinary and biliary metabolites were identified by NMR data for the identified metabolites are presented in Table 4. At the time of these studies, liquid chromatography tandem mass spectrometry analyses in the positive ionization mode showed that rofecoxib ionized poorly under these conditions, limiting the use of this analytical technique to support the identification of rofecoxib metabolites. Reference standards of some of the metabolites were prepared by chemical synthesis, which allowed further confirmation of the pathway of rofecoxib metabolism in laboratory animals (Fig. 5).

**Identification of Rofecoxib Metabolites in Urine and Bile of Rats and Dogs.** Metabolites of rofecoxib were isolated from urine of rat and dog and from rat bile after i.v. or oral administration of rofecoxib. After further purification, the metabolites were characterized by the combined application of HPLC, UV, and NMR. The 1H NMR data for the identified metabolites are presented in Table 4. At the time of these studies, liquid chromatography tandem mass spectrometry analyses in the positive ionization mode showed that rofecoxib ionized poorly under these conditions, limiting the use of this analytical technique to support the identification of rofecoxib metabolites. Reference standards of some of the metabolites were prepared by chemical synthesis, which allowed further confirmation of the pathway of rofecoxib metabolism in laboratory animals (Fig. 5).

**Metabolism of [14C]Rofecoxib (2 mg/kg, i.v. and 5 mg/kg, p.o.).**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>AUC_{0-24} (ng·h/ml)</th>
<th>CI_{0-24} (ml/min/kg)</th>
<th>Vd_{ss} (l/kg)</th>
<th>t_{1/2} (h)</th>
<th>C_{emax} (ng/ml)</th>
<th>T_{max} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 1</td>
<td>1</td>
<td>409</td>
<td>0.35</td>
<td>7.08</td>
<td>45.5</td>
<td>18.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Rat 2</td>
<td>1156</td>
<td>2271</td>
<td>6.6</td>
<td>6.8</td>
<td>5</td>
<td>400</td>
<td>2.3</td>
</tr>
<tr>
<td>Dog 1</td>
<td>4458</td>
<td>3.8</td>
<td>0.7</td>
<td>2.2</td>
<td>5195</td>
<td>798</td>
<td>1.8</td>
</tr>
<tr>
<td>Dog 2</td>
<td>9255</td>
<td>3.6</td>
<td>1.0</td>
<td>2.5</td>
<td>7490</td>
<td>1096</td>
<td>1.6</td>
</tr>
<tr>
<td>Dog 3</td>
<td>26174</td>
<td>2.6</td>
<td>0.7</td>
<td>3.2</td>
<td>11214</td>
<td>1506</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* The AUC value for rats is a partial value from 0 to 10 h (i.v.) or 0 to 24 h (p.o.).
* ND, not detectable.
* For the 5 mg/kg oral dosing study in dogs, n = 8.

**TABLE 2**

The effect of intravenous and oral dose levels on the pharmacokinetics of rofecoxib in rats (n = 4) and dogs (n = 4)

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>AUC_{0-24} (ng·h/ml)</th>
<th>CI_{0-24} (ml/min/kg)</th>
<th>Vd_{ss} (l/kg)</th>
<th>t_{1/2} (h)</th>
<th>C_{emax} (ng/ml)</th>
<th>T_{max} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>1</td>
<td>409</td>
<td>0.35</td>
<td>7.08</td>
<td>45.5</td>
<td>18.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Rat</td>
<td>2</td>
<td>1156</td>
<td>6.6</td>
<td>6.8</td>
<td>5</td>
<td>400</td>
<td>2.3</td>
</tr>
<tr>
<td>Dog</td>
<td>1</td>
<td>4458</td>
<td>3.8</td>
<td>0.7</td>
<td>5195</td>
<td>798</td>
<td>1.8</td>
</tr>
<tr>
<td>Dog</td>
<td>2</td>
<td>9255</td>
<td>3.6</td>
<td>1.0</td>
<td>7490</td>
<td>1096</td>
<td>1.6</td>
</tr>
<tr>
<td>Dog</td>
<td>4</td>
<td>26174</td>
<td>2.6</td>
<td>0.7</td>
<td>11214</td>
<td>1506</td>
<td>1.3</td>
</tr>
</tbody>
</table>

**TABLE 3**

Tissue distribution of total radioactivity in male rats (n = 3 per time point) following i.v. administration of [14C]rofecoxib at 2 mg/kg
metabolite were characteristic of protons corresponding to a glucurosyl moiety with a $\beta$ configuration. The structure of the glucuronide conjugate was confirmed by treatment of urine and bile with $\beta$-glucuronidase and subsequent HPLC analyses, which showed the disappearance of the glucuronide conjugate peak and an increase in the area of the 5-hydroxyrofecoxib peak. The UV and NMR spectra, as well as HPLC retention time of the 5-hydroxy metabolite were identical with those of its synthetic reference standard. The retention time of the glucuronide conjugate was variable, ranging from 11 to 17 min in these studies (Figs. 6 and 7, where the same chromatographic conditions were used). These shifts were attributed to batch-to-batch changes in the HPLC column stationary phase, although biological matrix effects could not be ruled out.

**Dogs.** Radiochromatographic analysis of urine (0–24 h) from dogs (Fig. 7) that received either an i.v. (2 mg/kg) or oral (5 mg/kg) dose of [14C]rofecoxib showed a more complex metabolite profile than that observed in rats. Similar to rats, rofecoxib was extensively metabolized and unchanged parent drug was not detected in urine (Fig. 7A) or bile (Fig. 7B). 5-Hydroxyrofecoxib was barely detected in urine (Fig. 7A) but was the most abundant component in bile (Fig. 7B). The

### TABLE 4

**$^1$H NMR data for rofecoxib and metabolites isolated from rat and dog urine (500 MHz, CD$_3$OD)**

<table>
<thead>
<tr>
<th>Protons</th>
<th>Rofecoxib</th>
<th>5-Hydroxyrofecoxib</th>
<th>5-Hydroxyrofecoxib-O-(\beta)-d-glucuronide</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>7.93 (AA'XX'', 2H, $J = 8.6$)</td>
<td>7.92 (AA'XX'', 2H, $J = 8.6$)</td>
<td>7.88 (AA'XX'', 2H, $J = 8.6$)</td>
</tr>
<tr>
<td>b</td>
<td>7.62 (AA'XX'', 2H, $J = 8.6$)</td>
<td>7.67 (AA'XX'', 2H, $J = 8.6$)</td>
<td>7.73 (AA'XX'', 2H, $J = 8.6$)</td>
</tr>
<tr>
<td>c'</td>
<td>7.39 (m, 5H)</td>
<td>7.39 (m, 5H)</td>
<td>7.41 (m, 5H)</td>
</tr>
<tr>
<td>c''</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>c'''</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>d</td>
<td>5.34 (s, 2H)</td>
<td>6.59 (s, 1H)</td>
<td>6.87 (s, 1H)</td>
</tr>
<tr>
<td>d'</td>
<td>3.12 (s, 3H)</td>
<td>3.12 (s, 3H)</td>
<td>3.12 (s, 3H)</td>
</tr>
<tr>
<td>e</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>f</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>g</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>h</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>i</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>j</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

### TABLE 4

<table>
<thead>
<tr>
<th>Protons</th>
<th>trans-3,4-Dihydro-rofecoxib</th>
<th>Rofecoxib-trans-3',4'-dihydrodiol</th>
<th>3'-Hydroxyrofecoxib Sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>7.90 (AA'XX'', 2H, $J = 8.4$)</td>
<td>7.96 (AA'XX'', 2H, $J = 8.5$)</td>
<td>7.96 (AA'XX'', 2H, $J = 8.5$)</td>
</tr>
<tr>
<td>b</td>
<td>7.60 (AA'XX'', 2H, $J = 8.4$)</td>
<td>7.66 (AA'XX'', 2H, $J = 8.5$)</td>
<td>7.64 (AA'XX'', 2H, $J = 8.5$)</td>
</tr>
<tr>
<td>c'</td>
<td>7.22 (m, 2H, ortho)</td>
<td>6.20 (t, 1H, $J = 2.0$)</td>
<td>7.26 (AA'XX'', 2H, $J = 8.8$)</td>
</tr>
<tr>
<td>c''</td>
<td>7.31 (m, 1H, para)</td>
<td>5.85 (d, 1H, $J = 9.9, 2.4$)</td>
<td>7.20 (AA'XX'', 2H, $J = 8.8$)</td>
</tr>
<tr>
<td>c'''</td>
<td>7.27 (m, 1H, meta)</td>
<td>5.54 (d, 1H, $J = 9.9, 2.0$)</td>
<td>—</td>
</tr>
<tr>
<td>d</td>
<td>4.76 (dd, 1H, $J = 8.4, 8.7$)</td>
<td>5.17, 5.06 (AB, 2H, $J = 17.3$)</td>
<td>5.36 (s, 2H)</td>
</tr>
<tr>
<td>d'</td>
<td>4.41 (dd, 1H, $J = 10.6, 8.7$)</td>
<td>3.08 (s, 3H)</td>
<td>3.25 (s, 3H)</td>
</tr>
<tr>
<td>e</td>
<td>4.08 (s, 3H)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>f</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>g</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>h</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>i</td>
<td>4.26 (d, 1H, $J = 12.4$)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>j</td>
<td>4.12 (d, 1H, $J = 12.4, 10.6, 8.4$)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$ Splitting patterns: s = singlet; d = doublet; t = triplet; dd = doublet of doublets; ddd = doublet of doublet of doublets; m = multiplet; AA''XX'' = multiplet characteristic of a para-substituted benzene ring. Coupling constants ($J$) are expressed in Hz.

$^b$ —, signals were not detected.

$^c$ —, signals were not detected.
presence of 5-hydroxyrofecoxib-O-β-D-glucuronide in dog urine was supported by comparison of its UV spectrum with that of the metabolite identified in rat urine. Isolation and purification of metabolites from the 8- to 24-h urine sample led to the identification of only one metabolite by NMR spectroscopy, namely \textit{trans}-3,4-dihydro-rofecoxib (Fig. 7A). The $^1$H NMR spectrum showed loss of the signal for the pair of protons at C-5 of rofecoxib (H$_d$, 5.34 ppm) and the appearance of four novel signals at 4.76 (H$_a$), 4.41 (H$_b$), 4.26 (H$_c$), and 4.12 (H$_f$) consistent with a CH$_2$-CH-CH spin system. The splitting patterns and coupling constants (12.6 Hz) of the two new proton signals led to identification of this metabolite as 3,4-dihydro-rofecoxib with protons H$_a$ and H$_f$ in a \textit{trans}-orientation (Table 4). The characteristic drug-related UV absorbance band at 280 to 300 nm was absent in the UV spectrum of the \textit{trans}-dihydro derivative due to loss of the extended conjugation of the \textit{p}-electrons. Only a very weak absorbance band with some fine structure at 260 to 270 nm could be observed. The UV and NMR spectra and HPLC retention time of this metabolite were closely similar to those of the synthetic standard. The cluster of metabolites that eluted between 15 and 18 min were not characterized due to poor stability of these components during isolation. However, work is ongoing to resolve, isolate, and characterize these metabolites.

**Metabolism of Rofecoxib in Rats (100 mg/kg, p.o.)**. Two additional metabolites of rofecoxib were isolated from rat urine (Fig. 8A) after oral administration of unlabeled rofecoxib at 100 mg/kg, one of the doses used in toxicological studies. Fluorescence detection of products from postcolumn photoirradiation of the HPLC effluent, which provided a highly sensitive analytical method for the determination of rofecoxib concentrations in plasma samples (Woolf et al., 1999), was exploited to detect urinary metabolites in studies where unlabeled drug was administered. Fluorescence detection and UV absorption (photodiode array spectra) both were utilized to identify drug-related material. Those components that gave only UV peaks were considered to be endogenous material. Unlike the three metabolites described above, in which metabolism perturbed the proton signals of the furanone ring, the NMR spectral data for these two components indicated that the unsubstituted phenyl ring was the site of metabolism (Table 4). The metabolites were identified as rofecoxib-3,4\textit{trans}-dihydrodiol and 4\textit{trans}-hydroxyrofecoxib sulfate (retention times of 17 and 19 min, respectively, Fig. 8A). In the NMR spectrum of the dihydrodiol derivative, the phenyl ring resonances of the rofecoxib H$_c$ multiplet were conspicuously absent, and three novel signals at 6.20, 5.85, and 5.54 ppm, consistent with a conjugated diene system, appeared. In addition, two CH$_2$-(OH) signals were evident at 4.39 ppm. The large vicinal coupling (11.3 Hz) between these two methine protons indicated a \textit{trans} configuration, supporting the identification of this metabolite as rofecoxib-3,4\textit{trans}-dihydrodiol.

The NMR spectrum of the second metabolite indicated that metabolism occurred at the \textit{para} position of the unsubstituted phenyl ring of rofecoxib. The AA′-XX′ multiplet signal of the aromatic protons (characteristic of \textit{para} substitution), in addition to the absence of a
signal for the phenolic OH proton (8–12 ppm), supported the identification of this metabolite as 4'-hydroxyrofecoxib sulfate.

When [14C]rofecoxib was administered orally to rats at the same high dose (100 mg/kg), the radiochromatogram of urine (0–24 h) showed the presence of two major and several minor radioactive peaks (Fig. 8B). The HPLC gradient conditions were modified from those used for the profiles of rat and dog urine and bile (Figs. 6 and 7), resulting in a slight shift of retention times for the known metabolites in this profile. Two of the four late-eluting peaks were characterized as 5-hydroxyrofecoxib (retention time = 25 min) and unchanged rofecoxib (retention time = 32 min) by comparing their respective HPLC retention times and UV spectra with those of authentic standards. UV Spectra of the two small peaks that eluted between 5-hydroxyrofecoxib and parent drug were identical with one another and displayed the same weak absorbance band at 260 to 270 nm that was observed with authentic trans-3,4-dihydro-rofecoxib. The retention times of these two minor radiochemical peaks at 26 and 29 min corresponded to those of reference cis- and trans-3,4-dihydro-rofecoxib, respectively, suggesting that both isomers were formed in vivo. A more rigorous characterization of the cis-isomer will be described elsewhere.4

Discussion

The pharmacokinetics and metabolism of rofecoxib displayed distinct species differences in rats and dogs. After oral administration, the drug was well absorbed in rats, but less so in dogs. In rats, the nonexponential decay during the terminal phase that was observed in plasma concentration versus time curves after administration of radiolabeled rofecoxib (i.v. or p.o.) or unlabeled rofecoxib (1 mg/kg, i.v.) did not permit determination of the elimination rate constant, and consequently, calculation of such pharmacokinetic parameters as the terminal half-life, or Clp, Vdss, AUC0–', and bioavailability. The appearance of secondary plasma concentration peaks at approximately 8 h after either i.v. or oral administration at the doses examined suggested the involvement of enterohepatic recirculation. That these secondary peaks appeared after i.v. as well as oral routes of administration ruled out the possibility of localized sites of absorption. In addition, the absence of rofecoxib in bile after an i.v. dose excluded the possibility of re-absorption of unchanged drug in the lower gastrointestinal tract. The glucuronide conjugate identified in rat bile (Fig. 6B) was that of 5-hydroxyrofecoxib, not of unchanged drug, indicating that re-entry of rofecoxib into the systemic circulation involved more than hydrolysis of a glucuronide conjugate with subsequent re-absorption of parent drug. A preliminary study in which 5-hydroxyrofecoxib was administered orally (5 mg/kg) to a rat provided evidence that the metabolite underwent reversible metabolism to rofecoxib. The extensive studies undertaken to examine this phenomenon and its role in the mechanism by which these secondary peaks occurred in rats are the subject of a separate report.5

The plasma concentration versus time curves of rofecoxib in dogs did not display the secondary peaks observed in rats. In fact, the decay curves were essentially nonexponential after a short distribution

4 R. Zamboni, unpublished data, Merck Frosst Canada, Kirkland, Quebec.

5 T. A. Baillie and coworkers, manuscript in preparation, Merck Research Laboratories, West Point, PA.
Metabolism of rofecoxib was more complex in dogs than in rats, with several polar metabolites observed in urine (Fig. 7A). 5-Hydroxyrofecoxib, its O-β-glucuronide, and trans-3,4-dihydro-rofecoxib were identified in dog urine, whereas 5-hydroxyrofecoxib was the most abundant metabolite in dog bile (Fig. 7B). Attempts to isolate and characterize several components in dog urine proved futile due to their instability either on the HPLC column or during workup after isolation. However, work is ongoing to isolate and characterize these metabolites.

Two additional metabolites of rofecoxib were identified in rat urine with the aid of fluorescence and radiochemical detection (Fig. 8). Rofecoxib-3',4'-dihydrodiol and 4'-hydroxyrofecoxib sulfate were products of epoxidation at the 3',4'-position on the unsubstituted phenyl ring of rofecoxib. The relative abundance of these metabolites could not be determined, because synthetic standards were not available. The corresponding oral high dose study with [14C]rofecoxib revealed the presence of two novel minor metabolites in rat urine, namely, cis- and trans-3,4-dihydro-rofecoxib. These metabolites, resulting from reduction of the furanone ring double bond, were not fluorescent due to loss of extended π-electron conjugation.

Drugs that contain a substituted 2(5H)-furanone ring similar to that of rofecoxib are limited. Digoxin, a cardiac glycoside used in the treatment of congestive heart failure, is the most noted. In addition to the sequential metabolic removal of its three 2,6-dideoxyglucoside groups, digoxin undergoes reduction of the furanone ring double bond to yield inactive 20,22-dihydrodigoxin (Brown et al., 1962; Watson et al., 1973; Shomo et al., 1988). This highly stereoselective reduction leads to the creation of a chiral center at the steroidal C-20 of digoxin. Only the R-enantiomer has been identified in human urine (Reuning et al., 1985). Unlike the monosubstituted furanone ring of digoxin, reduction of the disubstituted furanone ring of rofecoxib yields a pair of diastereomers, wherein the substituted and unsubstituted aromatic rings are cis and trans to each other. The absolute stereochemistry of the two reduced metabolites observed in this study, cis- and trans-3,4-dihydro-rofecoxib, has not been determined.

Losigamone, in early development as an anticonvulsant drug, possesses a 4,5-disubstituted 2(5H)-furanone ring and is a racemic mixture with two chiral centers (Torchin et al., 1996). The in vitro metabolism studies with (+)-losigamone and its (+)- and (−)-enantiomers in human liver microsomal preparations or with recombinant P450 enzymes yielded five metabolites, two of which were characterized as phenolic derivatives by mass spectrometry (Torchin et al., 1996). Although one of the unidentified UV-absorbing metabolites was thought to be nonphenolic due to its lack of electrochemical response, no evidence was presented in this report to support the possibility that losigamone had undergone reduction of the double bond.

In conclusion, rofecoxib displayed notable species differences in pharmacokinetic and metabolic behavior in laboratory animals. The role of reversible metabolism and subsequent enterohepatic recirculation in the disposition of rofecoxib in rats has been examined and will be described in a subsequent report.

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phenyl analog of rofecoxib). We also thank Dr. K. M. Schultz for assistance in preparing this manuscript.

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