PHARMACOKINETICS, TISSUE DISTRIBUTION, METABOLISM, AND EXCRETION OF CELECOXIB IN RATS


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ABSTRACT:

The pharmacokinetics, tissue distribution, metabolism, and excretion of celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide, a cyclooxygenase-2 inhibitor, were investigated in rats. Celecoxib was metabolized extensively after i.v. administration of [14C]celexib, and elimination of an unchanged compound was minor (less than 2%) in male and female rats. The only metabolism of celecoxib observed in rats was via a single oxidative pathway. The methyl group of celecoxib is first oxidized to a hydroxymethyl metabolite, followed by additional oxidation of the hydroxymethyl group to a carboxylic acid metabolite. Glucuronide conjugates of both the hydroxymethyl and carboxylic acid metabolites are formed. Total mean percent recovery of the radioactive dose was about 100% for both the male rat (9.6% in urine; 91.7% in feces) and the female rat (10.6% in urine; 91.3% in feces). After oral administration of [14C]celecoxib at doses of 20, 80, and 400 mg/kg, the majority of the radioactivity was excreted in the feces (88–94%) with the remainder of the dose excreted in the urine (7–10%). Both unchanged drug and the carboxylic acid metabolite of celecoxib were the major radioactive components excreted with the amount of celecoxib excreted in the feces increasing with dose. When administered orally, celecoxib was well distributed to the tissues examined with the highest concentrations of radioactivity found in the gastrointestinal tract. Maximal concentration of radioactivity was reached in most all tissues between 1 and 3 h postdose with the half-life paralleling that of plasma, with the exception of the gastrointestinal tract tissues.

Prostaglandins (PGs)2 are local mediators of cellular activity that produce biological responses that include the ability to induce pain, fever, and symptoms associated with inflammation (Davies et al., 1984; Needleman et al., 1986; Robinson, 1987). The first step in the synthesis of PGs is the conversion of arachidonic acid to PGH2, which is catalyzed by the enzyme cyclooxygenase (COX). Since the early 1970s, the mechanism of action of nonsteroidal anti-inflammatory drugs has been attributed to the blockade of the production of PGs by inhibition of COX (Smith and Willis, 1971; Vane, 1971). Two isoforms of COX are known to exist, COX-1 and COX-2, which differ in their regulation and tissue distribution (Merlie et al., 1988; Fu et al., 1990; Masferrer et al., 1990; Kujubu et al., 1991; Xie and Chipman, 1991; DeWitt and Smith, 1988). The gene for COX-1 is constitutively expressed and believed to be responsible for maintaining physiological processes in tissues such as the platelet and gastrointestinal (GI) tract. COX-2 is an inducible enzyme and the expression of the COX-2 gene has been shown to increase in certain inflammatory states. These data led to the hypothesis that specific COX-2 inhibitors would have the anti-inflammatory efficacy of traditional nonsteroidal anti-inflammatory drugs but not adverse effects in the GI tract and platelet (Masferrer et al., 1994; Isakson et al., 1998).

Celecoxib is an inhibitor of COX-2 that has analgesic and anti-inflammatory effects in patients with rheumatoid arthritis and no effect on COX-1 activity at therapeutic plasma concentrations (Penny et al., 1997; Isakson et al., 1998). Celecoxib was recently approved in the United States in 1998 for relief of symptoms of osteoarthritis and rheumatoid arthritis in adults.

Celecoxib is extensively metabolized in humans via a single oxidative pathway (Paulson et al., 2000). The metabolic pathway of celecoxib in humans involved oxidation of the methyl group of celecoxib to form a methylhydroxy metabolite, and then to its carboxylic acid. A glucuronide conjugate of the carboxylic acid metabolite is also produced in humans. The objective of the present study was to characterize the metabolic disposition of celecoxib in rats.

Materials and Methods

Chemicals. Celecoxib and radiolabeled celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-5-[14C]were synthesized at Searle (Skokie, IL) (Fig. 1). The specific activity of the [14C]celecoxib was approximately 44.2 μCi/mg. The methylhydroxy and carboxylic acid metabolites of

1 Current address: Genentech Inc., South San Francisco, CA.
2 Abbreviations used are: PG, prostaglandins; COX, cyclooxygenase; LSC, liquid scintillation counting; MS, mass spectrometry; SPE, solid-phase extraction; CID, collision-induced dissociation; AUC, area under the plasma concentration-time curve; Vp, volume of distribution; Cmax, maximum plasma concentration; NSAIDs, nonsteroidal anti-inflammatory drugs; GI, gastrointestinal; PEG, polyethylene glycol.

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celecoxib (Fig. 1) were synthesized at Searle, and the structures were confirmed by mass spectrometry (M5) and NMR. All other chemicals and reagents were analytical grade and commercially available.

Radiolabeled celecoxib was synthesized using Friedel-Crafts acylation of toluene with [1-14C]acetate chloride to produce 4',methyl[2-14C]acetophenone. The labeled intermediate was condensed sequentially with trifluoroacetic acid, catalyzed by sodium methoxide followed by 4-sulfonamidophenylhydrazine catalyzed by dilute hydrochloric acid to provide [14C]celecoxib in an overall yield of 37% after chromatography and crystallization.

The hydroxymethyl metabolite of celecoxib (M3) was synthesized from celecoxib by photocatalyzed bromination of the methyl group using N-bromosuccinimide followed by hydrolysis of the bromomethyl products. Sodium borohydride reduction of the hydrolysis mixture converted the mixture of hydroxymethyl and aldehyde products to the desired compound. Oxidation of the hydroxymethyl metabolite with Jone's chonic acid in acetone afforded the carboxylic acid metabolite of celecoxib (M2). All other chemicals and reagents were analytical grade and commercially available.

Animal Studies, i.e. pharmacokinetics. Twelve male and twelve female Sprague-Dawley rats were administered i.v. a single dose of celecoxib at 1 mg/kg in a solution of polyethylene glycol (PEG) 400/saline (2:1, v/v). Blood (approximately 1.0 ml) was collected from the jugular vein into chilled tubes containing sodium heparin at 5, 15, and 30 min, and 1, 2, 4, 8, and 24 h after administration of the dose. Each animal was bled twice. Three blood samples were collected for each time period. All animals were sacrificed by exsanguination 48 h after dosing. Plasma was prepared by centrifugation of blood. The plasma was stored at −20°C until analyzed for celecoxib concentrations.

Metabolism and excretion studies. Three male and three female Sprague-Dawley rats were administered i.v. a single dose of [14C]celecoxib at 1 mg/kg (44.2 μCi/kg). The [14C]celecoxib was administered as a solution in PEG 400/saline (2:1, v/v). After dosing, the animals were housed in individual glass metabolism cages designed for the separation and collection of urine and feces.

Urine and feces were collected at −18 to 0 h predose and at 24-h intervals for the five consecutive days after dose administration. Urine was collected into containers surrounded by dry ice. Feces were frozen immediately on dry ice at the end of each collection interval. Urine and feces were stored frozen at approximately −20°C until analyzed for radioactivity and metabolic profile.

Male and female Sprague-Dawley rats (n = 3/sex/dose) were administered by oral gavage a single dose of [14C]celecoxib at doses of 20, 80, and 400 mg/kg. At each dose, the animals were administered a radioactive dose of 87 μCi/kg. The [14C]celecoxib was administered in a suspension in 0.5% methylcellulose, 0.1% polysorbate 80. After dosing, the animals were housed in individual glass metabolism cages designed for the separation and collection of urine and feces. Urine and feces were collected at −18 to 0 h predose and at 24-h intervals for the seven consecutive days after dose administration. Urine was collected into containers surrounded by dry ice. Feces were frozen immediately on dry ice at the end of each collection interval. Urine and feces were stored frozen at approximately −20°C until analyzed for radioactivity and metabolic profile.

Bile duct-cannulated rat study. Cannulas were surgically inserted into the bile duct of male Sprague-Dawley rats (n = 6). A 100 μCi/kg dose of [14C]celecoxib was administered by oral gavage in a solution of PEG 400/saline (2:1, v/v) to the rats. Three rats were given radiolabeled celecoxib at a dose of 5 mg/kg, and the other three rats were administered a 20 mg/kg dose. Bile was collected from the rats for up to 8 h for the rats given 5 mg/kg and 6.5 h for the rats administered the 20 mg/kg dose. Bile samples were stored at approximately −20°C until they were analyzed for total radioactivity, celecoxib, and metabolites.

Tissue distribution study. Thirty male Long-Evans rats were administered a single oral dose of [14C]celecoxib at a dose of 2 mg/kg (18.4 μCi/animal). Long-Evans rats were used to evaluate the distribution of drug-related radioactivity to both pigmented and nonpigmented skin. The [14C]celecoxib was administered as a solution in PEG 400/water (2:1, v/v). Three animals each were sacrificed at 0.5, 1, 3, 8, 24, 48, 72, 96, 144, or 168 h after dose administration. One additional rat was sacrificed predose to provide control tissues for analysis. The following tissues and organs were collected at the time of sacrifice for determination of radioactivity: adrenal glands, aorta, blood, bone (femur, no marrow), bone marrow (femur), brain, cecum, eye lens, eye (remainder), fat (brown), fat (s.c.), heart, kidneys, lacrimal glands, large intestine, liver, lungs, lymph nodes, mesenteric, muscle (skeletal), pancreas, pituitary, plasma, prostate, red blood cells, skin (nonpigmented), skin (pigmented), small intestine, spinal cord, spleen, stomach, testes, thymus, thyroid, urinary bladder, and vena cava. Whole samples of aorta, bone marrow, eye lens, pituitary, thyroid and vena cava were analyzed. Adrenal glands, cecum, eye, lacrimal glands, lymph nodes, prostate, spinal cord, and urinary bladder were split into two aliquots and analyzed. Fat samples were minced and allowed to extract into scintillation cocktail before analysis. Brain, kidney, large intestine, liver, lungs, muscle, small intestine, stomach, and testes were homogenized with a probe homogenizer and duplicate aliquots (about 0.2 g)
were analyzed. Bone, heart, pancreas, spleen, and thymus were cut into small pieces, and the whole sample was analyzed in duplicate. Skin (pigmented and nonpigmented) were cut into small pieces and the samples were digested in 1 N NaOH at 40°C overnight. The digested skin samples were homogenized with probe homogenizer, and duplicate aliquots (about 0.2 g) were analyzed.

**Organ and Tissue Radioactivity Determinations.** All organ and tissue sample combustions were performed using a Packard model 306 or 307 oxidizer (Packard Instrument Company, Downers Grove, IL). Combusted samples were analyzed for radioactivity by liquid scintillation counting (LSC) using a Model 1900TR or 1500 liquid scintillation counter (Packard Instrument Company) using the external standardization and an instrument-stored quench curve to determine counting efficiency. Samples were counted for at least 5 min or until 100,000 counts accumulated. Radioanalytical procedures were validated by analyzing aliquots of control tissues fortified with known amounts of radioactivity. The mean recovery of radioactivity was 98.2%.

Tissue levels of radioactivity were expressed as microgram equivalents per gram of tissue and were calculated by dividing the dpm per gram of tissue by the specific activity of the [14C]celecoxib administered. Pharmacokinetic parameters [time to maximal plasma concentration (Cmax), tmax, B, t1/2, area under the plasma concentration-time curve (AUC0-∞)] for mean radioactivity levels in tissues were calculated. The tissue radioactivity concentration-time curve was plotted and the elimination rate constant (β) was determined from the slope of the regression line of the terminal phase. The half-life was calculated as ln 2/β, AUCt→∞ was calculated by trapezoidal summation, and the AUC0-∞ was calculated by dividing the concentration at time t by the elimination rate constant. AUC0-∞ was the sum of AUCt→∞ and AUCt→0.

**Urine and Feces Radioactivity Determinations.** Urine samples were analyzed for radioactivity by LSC after samples were mixed with Ultima-Gold scintillation cocktail (Packard Instruments). Approximately 2 to 3 times the fecal sample weight of ethanol was added. The resulting mixture was weighed and then homogenized using a probe homogenizer. Duplicate weighed aliquots (approximately 0.5 g) were combusted in a Packard Oxidizer. The resulting 14CO2 was trapped using Carbosorb (Packard Instruments). Perma-Flour E (Packard Instruments) was added and samples were analyzed for radioactivity by LSC. The percentage of dose excreted in urine or feces was calculated as dpm in urine or feces divided by total dpm dosed × 100.

**Quantitative Metabolic Profiling of Urine and Feces.** To determine metabolic profiles, urine samples were subjected to the same solid-phase extraction (SPE) procedure as described below for bile and the same HPLC procedure as described for feces below.

To determine the distribution of radioactivity in feces, aliquots (approximately 0.2 g) of fecal homogenates were pooled on a percentage of weight basis and extracted with 15 ml of methanol by end-over-end rotation for 1.5 h at room temperature. The fecal extracts were centrifuged at approximately 2000g for 10 min at 4°C using a Sorvall RT6000D centrifuge (DuPont, Wilmington, DE). The supernatant volume was measured, aliquots were taken, and the radioactivity was determined by LSC. The pellet was resuspended in 15 ml of methanol, vortexed briefly, and then extracted as described above.

The extracts were combined and evaporated in a heated water bath under a stream of nitrogen. The residues were reconstituted in 3.0 ml of methanol and centrifuged at approximately 2000g for 10 min at 4°C. Aliquots of the reconstituted residues were evaporated in a heated water bath under a stream of nitrogen, and reconstituted in 15% acetonitrile containing 0.025 M sodium acetate, pH 4.5, and directly injected onto the HPLC system. Extraction efficiency was greater than 95%.

HPLC analyses of urine and fecal samples were performed using a Beckman System Gold autosampler, UV detector, and pump (Beckman Instruments, Fullerton, CA) equipped with a Novapak C18 column (3.9 × 150 mm, 5 μm; Waters Associates, Milford, MA), and a Novapak C18 guard column (Waters Associates). A linear gradient was used from 25% acetonitrile in 0.01 M sodium phosphate buffer, pH 7.4 (mobile phase A) to 70% acetonitrile in 0.01 M sodium phosphate buffer, pH 7.4 (mobile phase B) over 15 min followed by a linear gradient from mobile phase B to mobile phase A over 1 min and equilibration for 4 min at mobile phase A. The flow rate was 1 ml/min. Eluates from the HPLC column after injection of urine samples and fecal extracts were mixed with Ready Flow III (Beckman Instruments) at a ratio of 1:3 (v/v) and analyzed for radioactivity using a Beckman model 171 radioactivity detector (Beckman Instruments).

**Extraction and HPLC Profiling of Bile.** Bile was extracted using a C-18 Bond Elut SPE column (size 3 cc; Varian, Harbor City, CA), which was preconditioned with 6 ml of acetonitrile and 6 ml of methanol. The SPE columns were rinsed with 6 ml of water and left hydrated before application of the sample. After application of the sample, the column was washed with 6 ml of water. The radioactive compounds retained on the column were eluted with 6 ml of acetonitrile followed by 3 ml of methanol. The extracts were evaporated to dryness in a heated water bath under a gentle stream of nitrogen. The residues were reconstituted in 20% acetonitrile in 0.025 M ammonium acetate, pH 4.5, before being injected on the HPLC for determination of metabolic profile.

HPLC analysis of bile extracts was performed a 1050 series autosampler and pump (Hewlett-Packard, Wilmington, DE) equipped with a Novapak C18 column (3.9 × 150 mm, 4 μm; Waters Instruments, Marlborough, MA). A linear gradient system was run from acetonitrile/0.025 M ammonium acetate, pH 4.5 (20:80) to acetonitrile/0.025 M ammonium acetate, pH 4.5 (60:40) over a 20-min period. Fractions were collected at 5 to 6.5, 6.5 to 7.5, 7.5 to 8.5, and 8.5 to 11.2 min, respectively. The fractions were evaporated to dryness under a stream of nitrogen in preparation for LC/MS.

**MS, LC/MS Analysis.** MS analysis was carried out using a Perkin-Elmer ISS 200 LC autosampler (Perkin-Elmer, Norwalk, CT), a 1050 HPLC pump (Hewlett-Packard, Naperville, IL), and a API-III Plus triple quadrupole mass spectrometer (PE Sciex, Thornhill, Ontario, Canada). The separation was performed on an Inertsil ODS-3 HPLC column (2 × 150 mm, 5 μm; MetaChem Technol-}

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**TABLE 1**

<table>
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<td></td>
<td>h</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
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<tr>
<td>Male</td>
<td>0–24</td>
<td>8.76 ± 2.34</td>
<td>71.8 ± 7.5</td>
<td>80.0 ± 7.0</td>
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<td></td>
<td>24–48</td>
<td>0.74 ± 0.12</td>
<td>17.4 ± 4.8</td>
<td>18.1 ± 4.8</td>
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<td></td>
<td>48–72</td>
<td>0.08 ± 0.04</td>
<td>2.24 ± 1.68</td>
<td>2.32 ± 1.71</td>
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<td></td>
<td>72–96</td>
<td>0.03 ± 0.01</td>
<td>0.27 ± 0.15</td>
<td>0.30 ± 0.16</td>
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<td></td>
<td>96–120</td>
<td>0.02 ± 0.00</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td></td>
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<tr>
<td>Female</td>
<td>0–24</td>
<td>9.63 ± 2.48</td>
<td>91.7 ± 2.0</td>
<td>0.14</td>
<td>101 ± 1</td>
</tr>
<tr>
<td></td>
<td>24–48</td>
<td>6.14 ± 2.00</td>
<td>26.4 ± 6.9</td>
<td>32.5 ± 7.9</td>
<td></td>
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<tr>
<td></td>
<td>48–72</td>
<td>2.92 ± 0.79</td>
<td>39.2 ± 6.5</td>
<td>42.2 ± 5.8</td>
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<tr>
<td></td>
<td>72–96</td>
<td>0.99 ± 0.32</td>
<td>19.7 ± 5.3</td>
<td>20.7 ± 5.3</td>
<td></td>
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<tr>
<td></td>
<td>96–120</td>
<td>0.42 ± 0.21</td>
<td>4.62 ± 1.02</td>
<td>5.04 ± 1.23</td>
<td></td>
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<tr>
<td></td>
<td>0–120</td>
<td>0.14 ± 0.09</td>
<td>1.36 ± 0.67</td>
<td>1.50 ± 0.76</td>
<td></td>
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<tr>
<td></td>
<td>10.6 ± 3.3</td>
<td>91.3 ± 4.0</td>
<td>0.43</td>
<td>102 ± 1</td>
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</tbody>
</table>

*Percentage of the dose recovered in cage wipes and washes.

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**Note:** All values are presented as mean ± S.D. The samples were collected over 8 h at 4°C following the administration of [14C]celecoxib to rats. The samples were collected in duplicate, and the radioactivity was determined using a Beckman model 171 radioactivity detector (Beckman Instruments).
adjusted to a constant flow rate of 1.8 liters/min. For collision-induced dissociation (CID) experiments, the collision energy was −30 electron volts and collision gas thickness was set at 250 × 1013 molecules/cm².

**Assay for Plasma Celecoxib.** Rat plasma (0.3 ml) containing an internal standard, was treated with 100 µl 1.0 N phosphoric acid. The plasma sample was extracted with a cation exchange/hydrophobic mixed mode SPE column (Jones Chromatography, Lakewood, CO) preconditioned with 2×1 ml acetoniitride followed by 2×1 ml water. The sample was eluted from the SPE column with 1.0 ml of 0.6% ammonium hydroxide in methanol. The extract was evaporated under nitrogen, and the sample was dissolved into 200 µl HPLC mobile phase. An aliquot of the sample extract was injected onto a Novapak TM C18 HPLC column (3.9 × 150 nm, 4 µM; Waters Associates) using a 3.2 × 15 mm, 7 µM RP-18 New Guard Cartridge (Brownlee Labs, Inc., Santa Clara, CA). The mobile phase, acetoniitride/0.01 M sodium phosphate buffer (pH 9) (50:50, v/v), was run at 1.0 ml/min. The analyte was quantified by peak height comparison to the internal standard using a FL2000 fluorescence detector (Thermo Separation Product, Schaumburg, IL) with excitation at 240 nm and emission at 380 nm. The analyte was compared against a standard curve of rat plasma fortified at concentrations of 0.01 to 10 µg celecoxib/ml, prepared as described above.

**Pharmacokinetic Calculations.** The celecoxib plasma concentration-time curves after i.v. administration were analyzed by a one compartment model using NONLIN for final parameter estimates (Metzler et al., 1974). The curve was described by the equation ($C_t = Ae^{-k_{el}t}$) where $C_t$ is the plasma concentration at time $t$, $A$ is the coefficient, and $k_{el}$ is the first order rate constant for the elimination phase. The volume of distribution ($V_d$) was calculated from $V_d = Dose/AUC_{0-\infty}$. Clearance (Cl) was calculated from $Cl = Dose/AUC_{0-\infty}$. The half-life ($t_{1/2}$) was calculated as $ln2/k_{el}$.

**Results**

**Pharmacokinetics.** There was a gender difference in the pharmacokinetics of celecoxib. Celecoxib was eliminated from plasma approximately four times faster in the male ($t_{1/2} = 3.73$ h) than in the female ($t_{1/2} = 14.0$ h) animal (Fig. 2). The clearances of celecoxib in the male and female were 7.76 and 1.99 ml/min/kg, respectively. The $V_d$ of celecoxib in male (2.51 liters/kg) and female (2.42 liters/kg) animals were similar. The AUC$_{0-\infty}$ was higher in female (8.38 µg/ml·h) compared with male (2.15 µg/ml·h) rats.

**Metabolism and Excretion Study. i.v. route.** The primary route of excretion of the administered [14C]celecoxib was biliary excretion and/or intestinal secretion, because 91.7% of the dose for male and 91.3% of the dose for female was recovered in the feces (Table 1). The percentage of the radioactive dose excreted in urine was 9.63% for male rat and 10.6% for female rat. However, there was a sex-related difference in the rate of excretion of the radioactive dose. In male rats, about 80.6% of the dose was excreted within the first 24 h compared with 32.5% for their female counterparts during the same time period. Excretion of the administered radioactivity appeared to be complete for both sexes by 120 h after dose administration.

[14C]Celecoxib was extensively metabolized after i.v. administration with less then 2% of the dose recovered in urine or feces as parent drug (Table 2). Representative radiochromatograms of urine and fecal extracts are in Figs. 3 and 4, respectively. The majority of the urinary and fecal radioactivity consisted of the carboxylic acid metabolite of celecoxib (M2), which accounted for 92.5% of the dose for the male rat, and 80.0% of the dose for female rat. The remainder of the radioactive dose was excreted as the hydroxymethyl metabolite of celecoxib (M3), which accounted for 3.2% of the dose in male rats and 5.2% of the dose in female rats.

**Oral.** The cumulative excretion of drug-related radioactivity after single oral dose administration is given in Table 3. After single oral doses of [14C]celecoxib ranging from 20 to 400 mg/kg to male rats, approximately 7% of the dose was recovered in the urine, 88 to 94% of the dose was recovered in feces, for a total recovery of 95 to 101%. After single oral doses of [14C]celecoxib ranging from 20 to 400 mg/kg to female rats, approximately 9 to 10% of the dose was recovered in the urine, 88 to 94% of the dose was recovered in feces, for a total recovery of about 99%.

The radiolabeled components in urine and feces collected after oral administration of [14C]celecoxib were analyzed for celecoxib and its metabolites by HPLC (Table 4). The radioactivity excreted in urine

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**TABLE 2**  
Percentage of the radioactivity excreted as celecoxib and metabolites in urine and feces of rats administered i.v. [14C]celecoxib at 1 mg/kg

<table>
<thead>
<tr>
<th>Sex</th>
<th>Collection Interval (h)</th>
<th>Matrix</th>
<th>Percentage of Radiolabeled Dose Excreted as Celecoxib</th>
<th>Methylhydroxy metabolite M5</th>
<th>Carboxylic acid metabolite M4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0–24</td>
<td>Urine</td>
<td>0.04</td>
<td>0.04</td>
<td>8.64</td>
</tr>
<tr>
<td></td>
<td>0–48</td>
<td>Feces</td>
<td>0.57</td>
<td>3.19</td>
<td>83.9</td>
</tr>
<tr>
<td>Female</td>
<td>0–24</td>
<td>Urine</td>
<td>0.02</td>
<td>0.05</td>
<td>5.97</td>
</tr>
<tr>
<td></td>
<td>0–72</td>
<td>Feces</td>
<td>1.77</td>
<td>5.18</td>
<td>74.1</td>
</tr>
</tbody>
</table>

**FIG. 3.** Representative radiochromatogram of a extracted rat urine sample.

**FIG. 4.** Representative radiochromatogram of an extracted rat fecal sample.
was primarily the carboxylic acid metabolite of celecoxib (M2). No unchanged drug was excreted in the urine. The excretion of celecoxib and its metabolites was dose-dependent. The amount of celecoxib excreted in the feces increased with dose, and the amount of the carboxylic acid metabolite of celecoxib (M2) was dose-dependent. The amount of celecoxib and/or its metabolites to melanin. The tissues with the lowest exposure were eye lens (0.025 mg equivalent/g). By 96 h post dose, concentrations of radioactivity in most tissues were below the limit of detection.

**Tissue Distribution Study.** After oral administration of 2 mg/kg [14C]celecoxib to male Long-Evans rats, radioactivity was shown to be distributed to all 35 tissues examined (Table 5, Fig. 5). Maximum levels of radioactivity were seen in the majority of tissues by 1 h, with the exception of large intestine and cecum, where peak radioactivity was observed at 8 h. The GI tract tissue exhibited the highest exposures. The exception of large intestine and cecum, where peak radioactivity was seen in the majority of tissues by 1 h, with the exception of large intestine and cecum, where peak radioactivity was observed at 8 h. The GI tract tissue exhibited the highest exposures. The GI tract tissue exhibited the highest exposures.

**Identification of Biliary Metabolites.** The HPLC chromatogram of bile extract contained four peaks containing radioactivity eluting at 5 to 6.5 min (M1) contained two peaks. The negative ion spray mass spectra of the two peaks showed the same deprotonated molecular ion (M−H)− at m/z 586, which was 176 Da higher than that of M2, suggesting that they are glucuronide conjugates of the carboxylic acid metabolite of celecoxib. The CID spectrum of m/z 410 generated a series of product ions at m/z 366, 302, 282, 263, 179, and 159, which were similar to those of an authentic standard of the carboxylic acid metabolite of celecoxib. The sequential losses of 44 (CO2), 64 (SO2), 20 (HF), and 20 (HF) daltons from m/z 410 generated the product ions at m/z 366, 302, 282, and 262, respectively. The product ion at m/z 233 was generated by a loss of a CF3 from m/z 302. Based on these data, M2 was identified as a carboxylic acid metabolite of celecoxib.

The LC/MS chromatogram of the fraction eluting from 5 to 6.5 min (M1) contained two peaks. The negative ion spray mass spectra of the two peaks showed the same deprotonated molecular ion (M−H)− at m/z 586, which was 176 Da higher than that of M2, suggesting that they are glucuronide conjugates of the carboxylic acid metabolite of celecoxib. The CID spectrum of m/z 410 generated a series of product ions at m/z 366, 302, 282, 263, 179, and 159, which were similar to those of an authentic standard of the carboxylic acid metabolite of celecoxib. The sequential losses of 44 (CO2), 64 (SO2), 20 (HF), and 20 (HF) daltons from m/z 410 generated the product ions at m/z 366, 302, 282, and 262, respectively. The product ion at m/z 233 was generated by a loss of a CF3 from m/z 302. Based on these data, M2 was identified as a carboxylic acid metabolite of celecoxib.

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**Identification of Biliary Metabolites.** The HPLC chromatogram of bile extract contained four peaks containing radioactivity eluting at 5 to 6.5, 6.5 to 7.5, 7.5 to 8.5, and 8.5 to 11.2 min that were further analyzed by LC/MS (Fig. 6). A summary of the mass spectral data are given in Table 6.

The peak eluting from 8.5 to 11.2 min (M2) had the same HPLC retention time as that of the carboxylic acid metabolite of celecoxib. The deprotonated molecular ion (M−H)− of M2 at m/z 410 in its negative ion mass spectrum, 30 Da higher than the parent compound celecoxib, suggested that M2 was a carboxylic acid metabolite of celecoxib. The CID spectrum of m/z 410 generated a series of product ions at m/z 366, 302, 282, 263, 179, and 159, which were similar to those of an authentic standard of the carboxylic acid metabolite of celecoxib. The sequential losses of 44 (CO2), 64 (SO2), 20 (HF), and 20 (HF) daltons from m/z 410 generated the product ions at m/z 366, 302, 282, and 262, respectively. The product ion at m/z 233 was generated by a loss of a CF3 from m/z 302. Based on these data, M2 was identified as a carboxylic acid metabolite of celecoxib.

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<tr>
<td></td>
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<td>%</td>
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<tr>
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<td>98.2 ± 0.38</td>
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<td>9.42 ± 2.61</td>
<td>88.5 ± 2.93</td>
<td>0.90</td>
<td>98.9 ± 0.90</td>
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* Percentage of the dose recovered in cage wipes and washes.
carboxylic acid metabolite of celecoxib (M1), respectively. The CID spectrum of m/z 572 gave a series of product ions at 510, 396, 380, 316, 302, and 113. The sequential losses of 62 (CH₃OHCH₂OH), 176 (dehydroglucuronic acid), and 94 (O) daltons from m/z 586 was not obtained. The CID spectra of m/z 586 were very similar to that of M1. This result demonstrated that the 8.5- to 11.2-min fraction contained a positional isomer of M1, a glucuronide conjugate of the carboxylic acid metabolite of celecoxib with the glucuronide attached at the carboxylic acid group.

Discussion

There is a gender difference in the clearance of celecoxib in rats with elimination of parent drug from plasma occurring faster in male compared with female animals. As a result of the gender difference in celecoxib pharmacokinetics, female rats achieve a higher exposure to the drug than the males when administered the same dose. Sex differences in pharmacokinetics of xenobiotics that undergo metabolism are not unusual for the rat and have been, in part, attributed to sex-specific expression of rat CYP2C and CYP3A genes (Waxman et al., 1985). There were no gender differences in celecoxib pharmacokinetics in healthy subjects or in patients as determined either by classical or population pharmacokinetic methods (Dr. Aziz Karim, personal communication).

After i.v. or oral administration, total recovery of the radioactive dose of celecoxib was about 100% for both male and female rats. The majority of the i.v. administered dose (approximately 90%) was recovered in the urine. For both sexes, the total excretion in the feces for both sexes, indicating that the primary route of excretion was biliary. Approximately 10% of the i.v. dose represented renal elimination for both sexes. The excretion of the radioactivity after i.v. administration was also faster in male than in female animals. Approximately 81% of the dose was excreted in the first 24 h for the male rats, whereas only about 33% was excreted in the same time period for the female animals. In male rats, 98% of the dose was excreted by 48 h after dosing, whereas the excretion of the same percentage took 96 h for female rats. After oral administration, the percentage of the dose was excreted primarily in the feces (about 90%) with the remaining percentage was excreted in the urine. The pattern of excretion of radioactivity after oral administration was the same over the 40-fold dose range tested.

Both male and female rats extensively metabolized celecoxib after
an i.v. dose, with less than 2% of the radioactive dose excreted as unchanged drug by either sex. After oral administration, the majority of the radioactivity is excreted as celecoxib and its carboxylic acid metabolite in the feces, with the amount of celecoxib excreted increasing with dose. The celecoxib excreted in the feces at the higher oral doses likely represents unabsorbed drug.

The metabolic pathway for celecoxib involved oxidation of the aromatic methyl group to form a hydroxymethyl metabolite, which underwent additional oxidation to a carboxylic acid metabolite (Fig. 1). Glucuronide conjugates of both the hydroxymethyl and the carboxylic acid metabolites of celecoxib were minor metabolites found only in bile. Four glucuronide conjugates of the carboxylic acid metabolite were produced. One of the biliary acyl glucuronide metabolites was a 1-O-glucuronide. The position of the glucuronide on the other acyl glucuronides was not established. It is possible that the other three glucuronide conjugates of the carboxylic acid metabolite are positional isomers formed as the result of acyl migration (Spahn-Langguth and Benet, 1992). No glucuronide conjugates were present in feces, suggesting that hydrolysis of the acyl and ether glucuronide metabolites occurred in the GI tract. The carboxylic acid was the major metabolite of celecoxib excreted in urine and feces of rats, representing 80% of the dose excreted by female animals and 92% of the dose excreted by male animals. A minor portion of the dose was excreted as the hydroxymethyl celecoxib in the feces of male (5%) and female (3%) rats.

Celecoxib is also extensively metabolized in humans. The metabolism of celecoxib in humans is similar to rat occurring primarily through a single metabolic pathway with the formation of the methylhydroxy (M3) and carboxylic acid (M2) metabolites (Paulson et al., 2000). M2 is also the major metabolite of celecoxib excreted in humans. The carboxylic acid glucuronide (M1) is only a minor metabolite in humans, representing less than 2% of an oral dose excreted. Unlike the rat, the carboxylic acid glucuronide (M1) is found in human urine and was present only as the 1-O-glucuronide (Paulson et al., 2000).
Celecoxib is well distributed to tissues as indicated by most tissue to plasma ratios greater than one for most of the tissues examined in the tissue distribution study. The distribution to tissues is rapid with the maximal concentration occurring in most tissues at about 1 h. The highest concentrations of radioactivity were found in the GI tract. Total radioactivity concentrations were below background in most tissues by 96 h postdose, indicating that there was no retention of drug and/or metabolites in the animals.

In conclusions, celecoxib is readily distributed to tissues. Celecoxib is eliminated by a single metabolic pathway, i.e., hydroxylation of the aromatic methyl group of celecoxib and additional oxidation of the hydroxymethyl metabolite to a carboxylic acid metabolite. The major route of elimination of celecoxib is metabolism followed by excretion of the metabolites in feces (90%), with the remainder of the metabolites excreted by the kidney.

Acknowledgments. Bioanalytical assays for celecoxib were performed by CEDRA Corporation (Austin, TX). Radioactivity measurements were conducted by Covance Labs (Madison, WI). Radiolabeled compounds were synthesized by Scott Harring Ph.D., at the Radiochemistry Department at G.D. Searle.

References


