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Comparison of celecoxib metabolism and excretion in mouse, rabbit, dog, cynomolgus monkey and rhesus monkey

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1. The metabolism and excretion of celecoxib, a specific cyclooxygenase 2 (COX-2) inhibitor, was investigated in mouse, rabbit, the EM (extensive) and PM (poor metabolizer) dog, and rhesus and cynomolgus monkey.

2. Some sex and species differences were evident in the disposition of celecoxib. After intravenous (i.v.) administration of [^{14}C]celecoxib, the major route of excretion of radioactivity in all species studied was via the faeces: EM dog (80.0%), PM dog (83.4%), cynomolgus monkey (63.5%), rhesus monkey (83.1%). After oral administration, faeces were the primary route of excretion in rabbit (72.2%) and the male mouse (71.1%), with the remainder of the dose excreted in the urine. After oral administration of [^{14}C]celecoxib to the female mouse, radioactivity was eliminated equally in urine (45.7%) and faeces (46.7%).

3. Biotransformation of celecoxib occurs primarily by oxidation of the aromatic methyl group to form a hydroxymethyl metabolite, which is further oxidized to the carboxylic acid analogue.

4. An additional phase I metabolite (phenyl ring hydroxylation) and a glucuronide conjugate of the carboxylic acid metabolite was produced by rabbit.

5. The major excretion product in urine and faeces of mouse, rabbit, dog and monkey was the carboxylic acid metabolite of celecoxib.

Introduction

Celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulphonamide, is a specific inhibitor of the inducible form of cyclooxygenase (COX-2) and does not inhibit COX-1 at plasma concentrations that are therapeutic in arthritis (Penning *et al*. 1997, Isakson *et al*. 1998, Simon *et al*. 1998). Constitutive COX-1 is widely expressed in most all tissues and appears responsible for most of the physiological prostaglandin production associated with gastric lining cytoprotection (O'Neill and Ford-Hutchinson 1993, Seibert *et al*. 1994). COX-2 expression is induced in inflammation (Crofford *et al*. 1994, Seibert *et al*. 1994). It has been hypothesized that COX-2 inhibitors will have the benefits of classical non-steroidal anti-inflammatory drugs (NSAID) without the risk of gastrointestinal toxicity (Isakson *et al*. 1998). Celecoxib was recently approved in the USA for the treatment of the signs of symptoms of rheumatoid arthritis and osteoarthritis.

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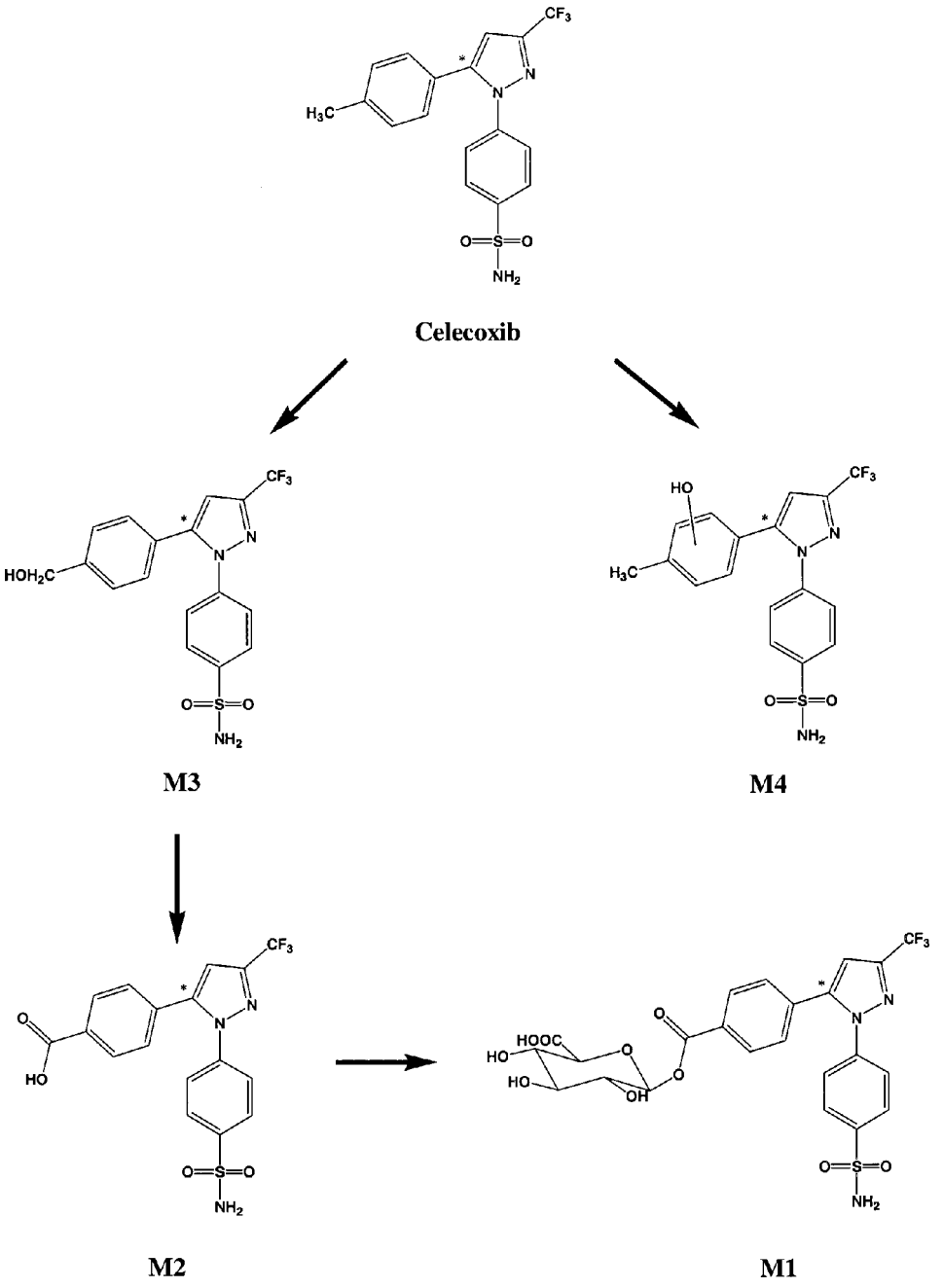

Figure 1. Proposed metabolic pathway for celecoxib.

The chemical structure of celecoxib is given in figure 1. Celecoxib is extensively metabolized in humans via a single metabolic pathway that involves hydroxylation of the aromatic methyl group of celecoxib followed by further oxidation of the hydroxymethyl metabolite to the carboxylic acid (Paulson *et al*. 2000a). The major excretion product of celecoxib in humans is the carboxylic acid metabolite.

Glucuronide conjugation of the carboxylic acid metabolite is a minor pathway of elimination of the carboxylic acid metabolite in humans.

In dog, celecoxib is metabolized *in vitro* to the hydroxymethyl metabolite with subsequent oxidation to the carboxylic acid analogue. There is a polymorphism in the canine metabolism of celecoxib associated with the formation of the hydroxymethyl metabolite (Paulson *et al*. 1999). At least two distinct populations of dog that have been described designated PM for 'poor metabolizers' and EM for 'extensive metabolizers' of celecoxib. An equal distribution of the two phenotypes, EM and PM, exists within a population of the beagle dog. The polymorphism appears due to a difference in the rate of metabolism of celecoxib by liver cytochrome P450. The specific cytochrome(s) responsible for the polymorphism is not understood.

The present study investigated the metabolism and excretion of celecoxib in several animal species: mouse, rabbit, dog, cynomolgus monkey and rhesus monkey. Routes of excretion were determined; metabolites were isolated from urine and faeces and their structures elucidated.

Materials and methods

Chemicals

Celecoxib and radiolabelled celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl-5-^{14}C], were synthesized at Searle (Skokie, IL, USA). The chemical purity of celecoxib was >99%; chemical purity of the celecoxib metabolites was >95%. Radiochemical purity of the radiolabelled celecoxib was >96% and the specific activity of the radiolabelled celecoxib was 56 mCi/mmol. The hydroxymethyl and carboxylic acid metabolites of celecoxib as well as the 1-*O*-glucuronide conjugate of the carboxylic acid metabolite of celecoxib were synthesized at Searle and MS and NMR (figure 1) confirmed the structures. The hydroxymethyl metabolite was synthesized from celecoxib by photocatalyzed bromination (NBS) of the methyl group 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 Jones's chromic acid in acetone afforded the carboxylic acid metabolite. The carboxylic acid metabolite was condensed with the benzyl ester of TBDMS-protected glucal epoxide followed by protecting group removal to afford the glucuronide. All other chemicals and reagents were analytical grade and commercially available.

Animal studies

Mouse. CD-1 mice (n = 33/sex) with body weights 20–40 g (Charles River Breeding Laboratory, Portage, MI, USA) were administered [^{14}C]celecoxib in a solution of polyethylene glycol 400:water (2:1 w/v) by oral gavage at 35 mg/kg (175 μCi/kg). Mice were fasted overnight before dosing and for at least 4 h afterwards. They were housed in individual metabolism cages designed for the separation and collection of urine and faeces. Blood samples (~1 ml) were collected from the auricular artery or marginal ear vein (n = 30/sex) into chilled, heparinized vacutainers at 0.5, 2, 4, 8, 24, 48, 72, 96, 120 or 168 h after dosing. Three blood samples were collected for each time point and only one sample was collected from each mouse. All urine and faeces were collected from mice (n = 3/sex) at −18 to 0 h predose and at 24-h intervals for 7 consecutive days after dose administration. Urine was collected into containers surrounded by dry ice. Faeces were collected at ambient temperature into Stomacher bags and frozen immediately on dry ice at the end of each collection interval. Urine and faeces were stored at about −20 °C until analyses for radioactivity and metabolic profile.

Rabbit. Three female New Zealand White (Hra:SPF) rabbits with body weights of 2.8–3.2 kg (Hazelton Research Products, Denver, PA, USA) were administered [^{14}C]celecoxib in a solution of polyethylene glycol 400:water (2:1 w/v) by oral gavage at 10 mg/kg (60 μCi/kg). Rabbits were fasted overnight before dosing and for at least 4 h afterwards. They were housed in individual metabolism cages designed for the separation and collection of urine and faeces. Blood samples (~5 ml) were collected from the auricular artery or marginal ear into chilled, heparinized vacutainers at 0.5, 2, 8 and 24 h after dosing. All urine and faeces were collected at −18 to 0 h predose and at 24-h intervals for 7 consecutive days after dose administration. Urine was collected into containers surrounded by dry ice. Faeces were collected into at ambient temperature into Stomacher bags and frozen immediately on dry ice at the end

of each collection interval. Urine and faeces were stored at about −20 °C until analyses for radioactivity and metabolic profile.

Monkey. One female cynomolgus and one female rhesus monkey were administered i.v. [^{14}C]celecoxib in a solution of polyethylene glycol 400:water (2:1 w/v) at 1 mg/kg (20 μCi/kg) via the jugular vein. Monkeys were fasted overnight before dosing and for at least 4 h afterward. They were housed in individual metabolism cages designed for the separation and collection of urine and faeces. Blood samples (~5 ml) were collected from the femoral vein into chilled, heparinized vacutainers at 5, 15, 30 min, 2, 3, 4 and 6 h for the cynomolgus monkey, and at 3 and 4 h after dosing for the rhesus monkey. All urine and faeces were collected from the monkeys at −18 to 0 h predose and at 24-h intervals for 7 consecutive days after dose administration. Urine was collected into containers surrounded by dry ice. Faeces were collected at ambient temperature and frozen immediately on dry ice at the end of each collection interval. Urine and faeces were stored frozen at about −20 °C until analyses for radioactivity and metabolic profile.

Dog. Six pure-bred beagle dogs (n = 2 male, 4 female) (Hazelton Research Product, Inc., Cumberland, VA, USA) weighing 10–14 kg were characterized as PM or EM as described (Paulson *et al.* 1999). Briefly, dogs were administered a single 5 mg/kg i.v. bolus injection of celecoxib in a solution of polyethylene glycol 400:water (2:1 w/v). Venous blood samples were collected into chill tubes containing sodium heparin at ~6, 12 and 24 h post-dose. Plasma was analyzed for celecoxib concentrations. Dogs with a 24-h plasma celecoxib concentration below the limit of detection of the assay (0.010 μg celecoxib/ml plasma) were EM of celecoxib; dogs with a 24-h plasma celecoxib concentration above the limit of detection of the assay were PM of celecoxib. Dogs were allowed at least 1 week to recover from the pre-screen procedure. After recovery, dogs were administered i.v. [^{14}C]celecoxib in a solution of polyethylene glycol 400:water (2:1 w/v) at 5 mg/kg (20 μCi/kg) via the cephalic vein. The dogs (n = 3 EM, 3 PM) were fasted overnight before dosing and for at least 4 h afterward. They were housed in individual metabolism cages designed for the separation and collection of urine and faeces. Blood samples (~5 ml) were collected from the jugular vein into chilled, heparinized vacutainers predose and at 5, 15, 30 min, 1, 2, 4, 8, 24, 48, 72 and 96 h after dosing. All urine and faeces were collected from the dogs at −18 to 0 h predose and at 24-h intervals for the 7 consecutive days after dose administration. Urine was collected into containers surrounded by dry ice. Faeces were collected at ambient temperature and frozen immediately on dry ice at the end of each collection interval. Urine and faeces were stored at about −20 °C until analyses for radioactivity and metabolic profile.

Radioactivity analyses

Plasma. Blood samples were centrifuged at ~1000 *g* at 4 °C for 10 min. Aliquots of plasma (0.1–0.5 ml) were placed in 20-ml glass scintillation vials along with liquid scintillation cocktail (Ultima Gold XR, Radiomatic, Meriden, CT; or Aquassure™, Packard Instrument Co., Downers Grove, IL, USA) and radioactivity was determined in a Model 1500 or 1900 TF liquid scintillation counter (LSC) (Packard).

Urine. Total volumes of the urine samples were recorded. Aliquots of urine were placed in liquid scintillation vials along with Aquassure™ or Ultima Gold XR liquid scintillation cocktail and radioactivity was determined by LSC.

Faeces. Faecal samples were thawed at ambient temperature and weighed. About two-to-three times the sample weight of methanol:water (50:50 or 80:20 v/v) was added to the faecal samples and the samples were homogenized either using a probe-type homogenizers or in a Stomacher™ bag using a Stomacher™ Laboratory Blender 400 (A. J. Seward, London, UK). Duplicate or triplicate aliquots (0.3–0.6 g) of each faecal homogenate were placed in a Combustocone™ with pads (Packard) and allowed to dry overnight. Samples were combusted using a Packard Sample Oxidizer. The combustion products were trapped in 7 ml Carbosorb™ and mixed with 9 ml Permafluor™ (Packard) for determination of radioactivity by LSC.

The percentage of dose excreted in each urine and faecal sample was calculated as: (total radioactivity recovered in each urine and faecal sample divided by total radioactivity dosed) × 100.

Quantitative metabolite profiling

Plasma. The mouse, rabbit and monkey plasma samples (1 ml) were acidified with ~0.2 vol. 1 N phosphoric acid and extracted by a 1-ml 100-mg C18 Bond Elut™ solid-phase extraction (SPE) column (Varian, Harbor City, CA, USA) preconditioned with 2-ml aliquots of acetonitrile, methanol and water. After loading the plasma sample, the column was washed with 2 ml water, then eluted with 2 ml acetonitrile. The eluate was dried under a stream of nitrogen and reconstituted into 0.2 ml solvent A of the mobile phase used in the HPLC analysis. The recoveries of radioactivity following extraction were 91.4, 87.4, 98.1 and 90.0% for mouse, rabbit, cynomolgus monkey and rhesus monkey respectively.

Dog plasma samples (~ 1 ml) were extracted with an equal volume of acetonitrile, mixed by vortexing and then centrifuged at 1000 *g* for 10 min. Supernatants were removed to a separate tube and 1 ml acetonitrile : water (50 : 50 v/v) was added to the pellet. The pellet and acetonitrile : water were vortexed and centrifuged as indicated above. Supernatants from the two extractions were combined. The recoveries of radioactivity following extractions were 108% for EM and 105% for PM dog.

Urine. The metabolic profile of [^{14}C]celecoxib in urine was determined by direct injection of urine samples (100 μl) onto the HPLC system. The percentage of the dose excreted as each metabolite in urine was calculated as : % dose recovered in the urine sample × the fraction of total HPLC radioactivity of each metabolite peak.

The urine samples from each dog (5% of the 0–72-h samples) were pooled. An aliquot (10 ml) of each pooled urine sample was concentrated under nitrogen to 1.5 ml. Acetonitrile (200 μl) was added to the concentrated urine. Samples were vortexed, sonicated for 10 min and then centrifuged at 1000 *g* for 15 min. The supernatant was removed and water : acetonitrile (80 : 20 v/v, 300 μl) was added to the pellet. The mixture was sonicated for 10 min and then centrifuged as above. The supernatants were removed and combined with the previous supernatant. The recoveries of radioactivity following extractions were 97.1% for EM and 95.3% for PM dog.

Faeces. To determine the distribution of radioactivity in faeces from mouse, rabbit and monkey, aliquots (~ 0.2 g) of faecal homogenates were pooled on a percentage weight basis and extracted with 15 ml methanol by end-over-end rotation for 1.5 h at room temperature. The faecal extracts were centrifuged at ~ 2000 *g* for 10 min at 5 °C using a Sorvall RT6000D centrifuge (Dupont Co., Wilmington, DE, USA). The supernatant volume was measured, aliquots taken and radioactivity determined by LSC. The pellet was resuspended in 15 ml methanol, vortexed briefly and 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 methanol and centrifuged at ~ 2000 *g* 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. The recovery of radioactivity from the extraction procedures were > 95, 80, ~ 100 and ~ 100% for mouse, rabbit, cynomolgus monkey and rhesus monkey respectively.

Dog faecal homogenate samples (~ 5 g) were acidified with 0.1 N HCl and extracted several times with acetonitrile or methanol at a ratio of 1 : 1. Samples were vortexed to mix, sonicated for 10 min and then centrifuged at 1000 *g* for 15 min at 5 °C. Supernatants from each extraction were combined. The recovery of radioactivity from the extraction procedure was 99.2% for EM and 99.3% for PM dog.

The percentage of the dose excreted as each metabolite in faeces was calculated as: % of dose recovered in the faecal sample × the fraction of total HPLC radioactivity of each metabolite peak.

HPLC

HPLC analyses of plasma, urine and faecal samples was performed using an HP1050 autosampler and HP1050 pump (Hewlett Packard, Wilmington, DE, USA) equipped with a Novapak C18 column (3.9×150 mm, 4 μm, Waters, Marlborough, MA, USA) and a Waters Novapak C18 guard column. A linear gradient was employed from 20% acetonitrile in 0.025 M sodium acetate buffer, pH 4.5 (mobile phase A) to 60% acetonitrile in 0.025 M sodium acetate buffer, pH 4.5 (mobile phase B) over 20 min and held at mobile phase B for 5 min before being re-equilibrated at mobile phase A for 20 min. The flow rate of mobile phase was 1 ml/min. Eluates from the HPLC column following injection of urine samples and faecal extracts were mixed with Ultima Flo™ (Packard) at 1 : 3 (v/v) and analysed for radioactivity using a Flo-One• Beta A-500 radioactivity detector (Packard). Plasma extracts were injected on the HPLC and 0.5-min fractions were collected using a Foxy 200 (ISCO, Inc., Lincoln, NE, USA) fraction collector. Each fraction was mixed with 10 ml Ultima Gold scintillation cocktail (Radiomatic, Meriden, CT, USA) and ^{14}C content was determined by LSC.

LC-MS/MS

Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analyses were performed using a series 200 LC autosampler (Perkin-Elmer, Norwalk, CT, USA), and two LC-10A HPLC pumps (Shimadzu, Tokyo, Japan) coupled to a API-III Plus triple quadrupole mass spectrometer (PE Sciex, Concord, Ont., Canada). Separation was performed on a Waters NovaPak C18 HPLC column (3.9×150 mm, 4 μm, Marlborough, MA, USA) that was eluted isocratically at 1.0 ml/min, with mobile phase C (acetonitrile: 0.025 M ammonium acetate, 20 : 80, pH 4.5) for 5 min, then programmed with a linear gradient system to mobile phase D (acetonitrile : 0.025 M ammonium acetate, 60 : 40, pH 4.5) over 20 min and held at mobile phase D for another 5 min. About 20% of the flow was diverted to the mass spectrometer using a 1 : 4 T-splitter. The mass spectral analyses were performed in a negative-ion turbo-ionspray ionization mode set at 400 °C with the turbo-ionspray interface and orifice voltages maintained at −3700 and −65 V respectively. The nitrogen gas for nebulization was set at 50 psi, whereas the

nitrogen auxiliary and curtain gases were flow controlled at 2 and 1.8 l/min respectively. Collision-induced dissociation (CID) studies were performed using a collision energy of −30 eV and at a collision gas thickness of 250×1013 molecules/cm² argon.

Results

Radioactivity in urine and faeces

The excretion data after i.v. administration of [^{14}C]celecoxib to dog and monkey are given in tables 1 and 2 respectively. The total recovery of radioactivity in urine and faeces collected 0–168 h after dosing to EM dog, PM dog, cynomolgus monkey and rhesus monkey was 85.3±1.67, 87.3±0.87, 91.4 and >100% respectively. For dog, the majority of the radioactivity was excreted in the faeces (80.0±1.1% for EM; 83.4±0.7% for PM), with < 6% excreted in the urine (5.10±0.77% for EM; 3.47±0.41% for PM). A greater percentage of the radioactivity was excreted in urine of monkey (27.9% for cynomolgus; 24.9% for rhesus) with the remainder of the dose excreted in faeces (63.5% for cynomolgus; 83.1% for rhesus).

Data on the excretion of radioactivity after oral administration of [^{14}C]celecoxib to mouse and rabbit are given in tables 3 and 4 respectively. Total mean (±SEM) percentage of administered radioactivity recovered was 93.5±1.9% for the male mouse (22.4±5.4% in urine; 71.1±3.7% in faeces) and 92.4±1.3% for the female mouse (45.7±0.8% in urine; 46.7±0.7% in faeces). In rabbit, total mean (±SEM) percentage of administered radioactivity recovered was 97.2±0.4%, with 25.0±4.4% excreted in the urine and 72.2±4.6% excreted in the faeces.

Metabolites of celecoxib present in plasma, urine and faeces

Plasma. The plasma metabolite profiles for the rabbit revealed the presence of two metabolic pathways for celecoxib: oxidation of the methyl group or hydroxylation in the phenyl ring of celecoxib to form hydroxymethyl (M3) and hydroxylphenyl (M4) metabolites respectively (figure 1 and 2, and table 5). A carboxylic acid metabolite (M2), formed from further oxidation of M3, as well as its glucuronide (M1) were also in rabbit plasma. The plasma metabolic profiles of [^{14}C]celecoxib differed between the male and female mouse (table 5). In the male mouse, celecoxib was the major circulating radioactive component and only minor amounts of M3 (< 11%) and M2 (< 7%) metabolites were in plasma. In the female mouse, celecoxib represented 54.4% (0.5 h) and 26.6% (8 h) of the radioactive material in plasma. The female mouse had higher circulating levels of M2 and M3 than the male. Following i.v. administration of [^{14}C]celecoxib, M2 was the major circulating radioactive component for both the cynomolgus and rhesus monkey after 15 min (table 6). The plasma metabolic profiles were different between the EM and PM dog. The plasma of the PM dog contained primarily celecoxib with only minor amounts of M3, whereas the plasma of the EM dog contained M1–4 as well as celecoxib. Although, the plasma HPLC profile from the EM dog had radioactivity eluting at a position similar to M4 (hydroxyphenyl metabolite of rabbits), the structure was not confirmed by LC/MS. There was no evidence for phenyl ring hydroxylation in mouse, the PM dog or monkey.

Urine and faeces. Following i.v. administration of radiolabelled celecoxib to dog and monkey, M2 was the major excretion product accounting for 96.2, 71.4, 79.3

Table 1. Excretion of radioactivity in urine and faeces after a single i.v. dose of 5 mg/kg [^{14}C]celecoxib to the EM and PM dog.

Collection interval (h)	Radioactivity (% of administered dose)			
	EM (n = 3)		PM (n = 3)	
	Urine	Faeces	Urine	Faeces
0–24	4.52±0.56	50.0±8.96	2.63±0.33	55.6±5.73
24–48	0.44±0.23	24.6±8.28	0.57±0.15	23.1±4.00
48–72	0.07±0.02	4.31±3.90	0.13±0.01	3.30±1.42
72–96	0.03±0.01	0.97±0.87	0.05±0.00	1.04±0.65
96–120	0.01±0.00	0.08±0.04	0.05±0.01	0.25±0.14
120–144	0.02±0.01	0.07±0.05	0.03±0.01	0.08±0.03
144–168	0.02±0.00	0.02±0.01	0.01±0.00	0.03±0.00
0–168	5.10±0.77	80.0±1.10	3.47±0.41	83.4±0.70
Cage wash	0.21		0.42	
Total	85.3±1.67		87.3±0.87	

EM, extensive metabolizer of celecoxib; PM, poor metabolizer of celecoxib.

Table 2. Excretion of radioactivity in urine and faeces after a single i.v. dose of 1 mg/kg [^{14}C]celecoxib to cynomologus and rhesus monkey.

Collection interval (h)	Radioactivity (% of administered dose)			
	Cynomolgus monkey (n = 1)		Rhesus monkey (n = 1)	
	Urine	Faeces	Urine	Faeces
0–24	18.9	0.02	21.6	18.5
24–48	8.1	6.4	0.5	56.8
48–72	0	34.2	2.6	7.2
72–96	0.6	13.0	0.1	0.5
96–120	0.1	7.9	0	0.1
120–144	0.1	1.8	0	0
144–168	0.1	0.2	0.1	0
0–168	27.9	63.5	24.9	83.1
Total	91.4		>100	

Table 3. Excretion of radioactivity in urine and faeces after a single oral dose of 35 mg/kg [^{14}C]celecoxib to mouse.

Collection interval (h)	Radioactivity (% of administered dose)			
	Male (n = 3)		Female (n = 3)	
	Urine	Faeces	Urine	Faeces
0–24	19.0±6.8	62.8±4.3	40.2±0.9	42.0±1.1
24–48	1.1±0.3	6.4±3.2	2.7±0.7	3.3±0.5
48–72	0.43±0.17	0.9±0.5	0.9±0.1	0.6±0.2
72–96	0.07±0.01	0.3±0.2	0.2±0.1	0.3±0.2
96–120	0.17±0.04	0.4±0.1	0.2±0.1	0.2±0.1
120–144	0.03±0.01	0.1±0.0	0.05±0.01	0.1±0.04
144–168	0.27±0.06	0.1±0.0	0.4±0.3	0.1±0.05
Cage wash	0.17±0.04		1.04±0.3	
0–168	22.4±5.4	71.1±3.7	45.7±0.8	46.7±0.7
Total	93.5±1.9		92.4±1.3	

Table 4. Excretion of radioactivity in urine and faeces after a single oral dose of 10 mg/kg [^{14}C]celecoxib to female rabbit (n = 3).

Time (h)	Radioactivity (% of administered dose)	
	Urine	Faeces
0–24	19.0±1.5	27.0±10.2
24–48	5.19±3.09	38.2±6.4
48–72	0.46±0.20	6.05±1.20
72–96	0.26±0.09	0.69±0.06
96–120	0.03±0.00	0.17±0.02
120–144	0.01±0.01	0.04±0.01
144–168	0.01±0.00	0.02±0.01
0–168	25.0±4.4	72.2±4.60
Total	97.2±0.4	

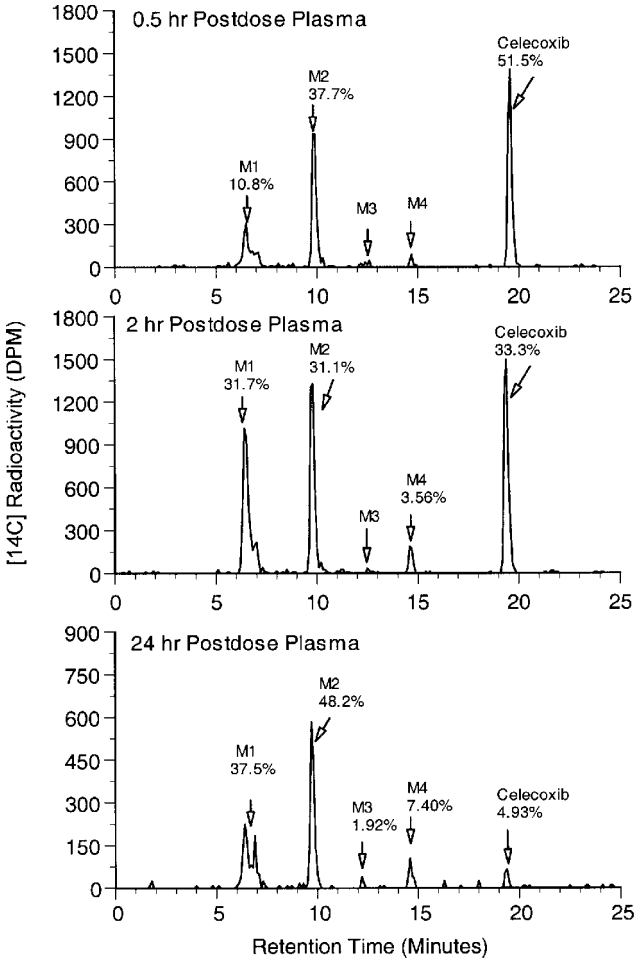

Figure 2. Representative radiochromatogram of an extract of plasma obtained from a female rabbit administered 10 mg/kg [^{14}C]celecoxib.

Table 5. Metabolite profiles in mouse and rabbit plasma after oral administration of [^{14}C]celecoxib.

		Metabolite (%)			
Sampling time (h)	Celecoxib	M1	M2	M3	M4
Female mouse[a]					
0.5	54.4	nd	13.9	30.0	nd
2	33.7	nd	22.0	39.2	nd
4	48.5	nd	17.9	30.4	nd
8	26.6	nd	39.6	26.6	nd
24	na	na	na	na	na
Male mouse[a]					
0.5	87.9	nd	2.32	7.3	nd
2	86.0	nd	1.66	10.4	nd
4	87.6	nd	1.74	8.93	nd
8	84.0	nd	6.48	2.77	nd
24	na	na	na	na	na
Female rabbit[b]					
0.5	51.5	10.8	37.7	nd	nd
2	37.8	33.1	25.4	0.71	2.85
4	na	na	na	na	na
8	28.9	7.40	33.0	nd	9.38
24	4.93	37.5	48.2	1.92	7.40

[a] Values are from pooled samples from $n = 3$ animals.
[b] Values for the 0.5-, 4-, 8- and 24-h samples are from pooled samples from $n = 3$ animals. The value for the 2-h sample represents mean of samples from $n = 3$ animals.
na, Sample was not analyzed due to low levels of radioactivity in mouse plasma at 24 h. A 4-h plasma sample was not collected for rabbit.
nd, no radioactivity detected in peak.

Table 6. Metabolite profiles in plasma after i.v. administration of 1 mg/kg [^{14}C]celecoxib to rhesus and cynomolgus monkey and i.v. administration of 5 mg/kg [^{14}C]celecoxib to EM and PM dog.

		Metabolite (%)			
Sampling time (h)	Celecoxib	M1	M2	M3	M4
Cynomolgus monkey					
0.083	72.6	nd	27.4	0	nd
0.25	34.4	nd	64.0	1.57	nd
0.5	28.6	nd	70.1	1.31	nd
2	34.2	nd	64.8	0.624	nd
3	20.5	nd	79.5	0	nd
4	24.7	nd	75.3	0	nd
6	9.2	nd	90.1	0	nd
Rhesus monkey					
3	36.7	nd	59.1	0	nd
4	30.2	nd	69.8	0	nd
EM dog					
0.5	78.4	2.24[a]	5.30	9.47	0.36[b]
4	50.3	2.36[a]	5.25	27.5	nd
PM dog					
0.5	94.0	nd	nd	1.77	nd
4	93.8	nd	nd	5.78	nd

[a] Metabolite elutes at a similar position to M1.
[b] Metabolite elutes at a similar position to M4.
nd, no radioactivity detected in peak.

Table 7. Percentage of the dose excreted as celecoxib or metabolites following a single i.v. dose given to dog, cynomolgus and rhesus monkey.

Species	Collection interval (h)	Metabolite (%)				
		Celecoxib	M1	M2	M3	M4
% of the Radioactive doses in urine						
Dog (EM)	0–72	nd	0.15[b]	4.6	nd	0.07[a]
Dog (PM)	0–72	nd	0.07[b]	3.0	0.03	0.07[a]
Cynomolgus monkey	0–24	nd	nd	18.7	nd	nd
Rhesus monkey	0–24	nd	nd	21.1	nd	nd
% of the Radioactive dose in faeces						
Dog (EM)	0–72	nd	nd	78.5	1.1	nd
Dog (PM)	0–72	nd	nd	76.3	2.0	nd
Cynomolgus monkey	0–96	nd	nd	52.7	nd	nd
Rhesus monkey	0–48	nd	nd	75.1	nd	nd

EM, extensive metabolizer of celecoxib; PM, poor metabolizer of celecoxib.
[a] Metabolite elutes at a similar position on the radiochromatogram as M4.
[b] Metabolite elutes at a similar position on the radiochroamtogram as M1.
nd, no radioactivity detected in peak.

Table 8. Percentage of the dose excreted as celecoxib or metabolites following oral administration of a single dose to mouse and rabbits.

Species	Collection interval (h)	Metabolite (%)				
		Celecoxib	M1	M2	M3	M4
% of the Radioactive dose in urine						
Mouse (M)	0–48	nd	nd	19.6	nd	nd
Mouse (F)	0–48	nd	nd	42.1	nd	nd
Rabbit	0–48	nd	1.6	21.9	nd	nd
% of the Radioactive dose in faeces						
Mouse (M)	0–48	8.3	nd	58.4	1.2	nd
Mouse (F)	0–48	0.4	nd	42.9	0.7	nd
Rabbit	0–48	0.5	0.1	63.9	nd	nd

nd, no radioactivity detected in peak.

and 83.1% of the dose in urine and faeces of rhesus and cynomolgus monkey, and PM and EM dog respectively (table 7). Minor amounts of M3 (< 2%) and the M1 (< 1%) were excreted in dog faeces. Dog, cynomolgus or rhesus monkey excreted no unchanged drug.

M2 was also a major excretion product of male and female mouse and rabbit, accounting for ~78.0, 85.0 and 85.8% of the dose excreted in the first 48 h respectively (table 8). Minor amounts (~1%) of M3 was excreted in faeces of both the male and female mouse. The female mouse differed from the male regarding the pathway of excretion of celecoxib and its metabolites. In the male, M2 represented 19.6% of the dose excreted in urine and 58.4% of the dose excreted in faeces, whereas in the female, 42.1% of the dose excreted in urine and 42.9% of the dose excreted in faeces was M2. Celecoxib (8.3% of the dose) was excreted unchanged in the faeces of the male, representing either unabsorbed dose or biliary excretion of the unchanged drug. Only 0.4% of the dose was excreted as celecoxib following oral administration to the female.

Structure elucidation of metabolites

The identity of celecoxib and its metabolites was elucidated by MS/MS. Because the rabbit produced all of the metabolites of celecoxib, details of the structure elucidation for only that species are presented. Representative radiochromatograms of plasma and urine samples from a rabbit administered [^{14}C]celecoxib are shown in figures 2 and 3. Ionspray ionization mass spectral data for celecoxib metabolites are shown in table 9.

Celecoxib. The HPLC retention time of the celecoxib peak was identical to that of an authentic celecoxib standard. The deprotonated molecular ion at 380 daltons in its negative-ion mass spectrum was consistent with that of the celecoxib standard. The CID spectrum of the negative-ion at *m/z* 380 generated a series of product ions identical to those in the CID spectrum of the celecoxib standard.

M1. The HPLC retention time of M1 was identical to that of an authentic standard of the 1-*O*-glucuronide conjugate of M1. The mass spectrum of M1 was identical to the mass spectrum of the synthetic standard of carboxylic acid celecoxib glucuronide (figure 1 and table 9). The HPLC and MS data indicate that M1 consisted of glucuronide conjugates of the carboxylic acid metabolite of celecoxib. The four peaks of glucuronide conjugates of M1 observed in the HPLC profiles of rabbit urine are likely to be positional isomers (Spahn-Langguth and Benet 1992, Sidelmann *et al*. 1996).

M2. M2 had the same HPLC retention time as the authentic standard of the carboxylic acid metabolite of celecoxib. The deprotonated molecular ion of M2 at *m/z* 410 in its negative-ion mass spectrum, 30 daltons higher than the parent compound celecoxib, suggested that M2 was a carboxylic acid metabolite. The CID spectrum of the negative-ion at *m/z* 410 generated a series of product ions similar to those in the CID spectrum of the synthetic carboxylic acid celecoxib (figure 1 and table 9). These data indicate that M2 was a carboxylic acid metabolite of celecoxib.

M3. M3 had the same HPLC retention time as the hydroxymethyl celecoxib standard. The deprotonated molecular ion of M3 at *m/z* 396 in its negative-ion mass spectrum was consistent with that of the synthetic hydroxymethyl metabolite of celecoxib. The CID spectrum of M3 (*m/z* 396) generated a series of product ions matching those in the CID spectrum of the synthetic hydroxymethyl celecoxib (figure 1 and table 9). These results confirmed that M3 was a monohydroxylated metabolite of celecoxib in which the hydroxylation occurred on the methyl moiety of the 5-(4-methyl)phenyl group.

M4. The HPLC retention time of the M4 peak was ~2 min longer than that of M3. The deprotonated molecular ion of M4 at *m/z* 396 is 16 daltons higher than that of celecoxib, indicating that another monohydroxylated metabolite of celecoxib, different from M3, was formed. The CID spectrum of M4 (*m/z* 396) produced a series of product ions at *m/z* 332, 317, 312, 302, 297, 277, 178, 160 and 69, which was also different from the CID spectrum of M3. The sequential losses of 64 (SO_2), 15 (NH), 20 (HF) and 20 (HF) daltons from the *m/z* 396 produced the product ions at *m/z* 332, 317, 297 and 277 respectively. The base peak at *m/z* 302 was formed by a loss of 30 (CH_3) daltons from the *m/z* 317 ion, suggesting that the hydroxyl group

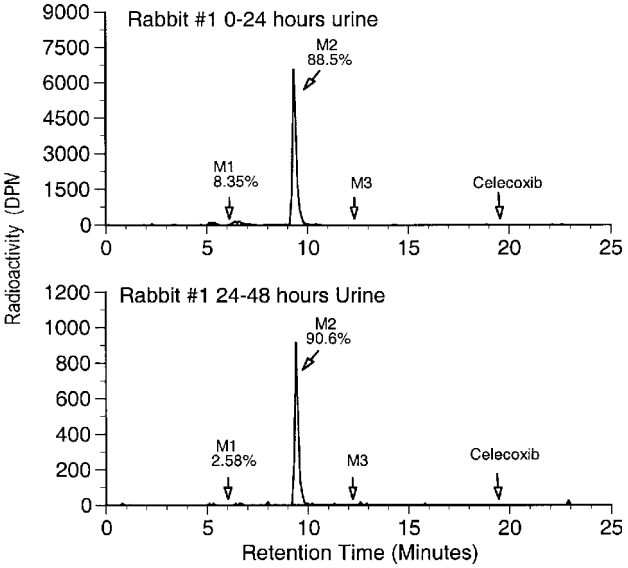

Figure 3. Representative radiochromatogram of urine from a female rabbit administered 10 mg/kg [^{14}C]celecoxib.

Table 9. Ionspray inonization mass spectral data for celecoxib metabolites.

Metabolite	$[M–H]^-$	CID mass spectral data, m/z (%)
M1	586	410 (28), M-H-$C_6H_8O_6$; 366 (60), M-H-$C_6H_80_6$-CO_2; 302 (28), M-H-$C_6H_8O_6$-CO_2-SO_2; 282 (7), M-H-$C_6H_8O_6CO_2$-SO_2-HF; 175 (5), $C_6H_7O_6$; 113 (100), $C_6H_7O_6$-CH_2OHCH_2OH.
M2	410	366 (32), M-H-CO_2; 302 (100), M-H-CO_2-SO_2; 282 (74), M-H-CO_2-SO_2-HF; 262 (98), M-H-CO_2-SO_2-2HF; 233 (52), M-H-CO_2-SO_2-CF_3; 179 (48), M-H-SO_2NH-$OCH_2C_6H_4C$-HF; 159 (26), M-H-SO_2NH-$OCH_2C_6H_4C$-2HF; 69 (82), CF_3.
M3	396	396 (12), M-H 366 (6), M-H-CH_2O; 302 (100), M-H-CH_2O-SO_2; 282 (48), M-H-CH_2O-SO_2-HF; 262 (45), M-H-CH_2O-SO_2-2HF; 233 (25), M-H-CH_2O-SO_2-CF_3; 179 (23), M-H-SO_2NH-$OCH_2C_6H_4C$-HF; 159 (10), M-H-SO_2NH-$OCH_2C_6H_4C$-2HF; 69 (35), CF_3.
M4	396	396 (9), M-H 332 (11), M-H-SO_2; 317 (35), M-H-SO_2-NH; 312 (11), M-H-SO_2-HF; 302 (100), M-H-SO_2-CHOH; 297 (24), M-H-SO_2-CH_3-HF; 277 (8), M-H-SO_2-CH_3-2HF; 178 (15), M-H-SO_2NH-$CH_3C_6H_3OHC$-HF; 160 (10), M-H-SO_2NH-$CH_3C_6H_3OHC$-2F; 69 (23), CF_3.

was at the phenyl moiety of the 5-(4-methyl)phenyl group. The product ion at m/z 69 was related to the CF_3 group. The MS results suggested that M4 was a monohydroxylated metabolite of celecoxib with the hydroxylation occurring on the phenyl moiety of the 5-(4-methyl)phenyl group (figure 1).

Discussion

The metabolism and excretion of celecoxib was investigated following i.v. administration to dog, cynomolgus and rhesus monkey, and after oral administration to mouse and rabbit. Celecoxib was extensively metabolized by all species studied. Only minor species differences in the metabolic pathway for celecoxib were observed. A gender difference in the disposition of celecoxib was observed in mouse. Two phase I oxidative pathways for the metabolism of celecoxib were demonstrated (figure 1). The major phase I pathway that occurred in mouse, rabbit, cynomolgus and rhesus monkey, and dog involved oxidation of the aromatic methyl group of celecoxib to form a hydroxymethyl metabolite (M3). M3 underwent further oxidation to the carboxylic acid metabolite (M2). M2 was produced by all the species studied. Oxidation of the aromatic methyl group of celecoxib was the metabolic pathway for elimination of celecoxib in humans and rat (Paulson *et al*. 2000a, b). A minor phase I pathway was the ring hydroxylation of the methylphenyl group of celecoxib to form M4, which was shown to occur only in rabbit and, possibly, EM dog.

In rabbit, the carboxylic acid metabolite of celecoxib (M2) was conjugated with glucuronic acid to form the *O*-glucuronide conjugate (M1). M1 was only a minor metabolite of celecoxib in the rabbit representing $< 2\%$ of the dose excreted. M1 was also only a minor metabolite in humans accounting for $< 2\%$ of the dose excreted (Paulson *et al*. 2000a). Conjugation of the hydroxy and the carboxylic acid metabolite of celecoxib also occurs in rat. Rat also produced a glucuronide conjugate of the hydroxymethyl metabolite (Paulson *et al*. 2000b).

There are two populations of dogs that have been reported as being PM or EM of celecoxib (Paulson *et al*. 1999). Qualitatively the metabolism of celecoxib was similar between the two populations with the primary metabolic pathway being hydroxylation of the aromatic methyl group. The polymorphism was associated primarily with the formation of M3 catalysed via liver microsomal CYP450. However, interestingly the EM dog contains a metabolite in plasma that elutes at a position similar to M4 (ring-hydroxylation) on a HPLC radiochromatogram that was not observed in plasma of the PM dog. Greater amounts of metabolites were found circulating in the EM dog than in the corresponding PM animal.

A gender difference in the disposition of celecoxib was observed in mouse. Although the metabolic pathway for celecoxib was the same for the male and female, metabolism was slightly more extensive in the female, with 0.4% of the dose excreted as unchanged celecoxib compared with 8.3% excreted as unchanged drug in the male. The male also had higher circulating levels of metabolites than the female.

Monkeys metabolize celecoxib via a single pathway with the formation of M3 and M2. The carboxylic acid metabolite (M2) appears to be the major circulating component of plasma and the major excretion product. However, since only a single cynomolgus monkey and rhesus monkey were studied, there can be no assessment of interanimal variability in this species.

After i.v. dosing to monkey and dog, excretion of the radioactivity was near complete in rhesus monkey and EM and PM dog by 48 h after dose administration. In the cynomolgus monkey, most of the dose was excreted by 5 days after dose administration. Most of the radioactivity was excreted in the faeces, suggesting that for dog and monkey the major route of elimination for celecoxib is metabolism followed by excretion of the metabolites in bile. Radiolabeled celecoxib was administered by oral gavage to mouse and rabbit. Excretion of radioactivity was near complete by 48 h in these species. Higher urinary excretion occurred in female (45.7%) compared with male (22.4%) mouse.

In conclusion, celecoxib was eliminated primarily by a single metabolic pathway, i.e. hydroxylation of the aromatic methyl group of celecoxib and further oxidation of the hydroxylated metabolite to a carboxylic acid metabolite. The major route of elimination of celecoxib was metabolism followed by excretion of the metabolites in the faeces, with the remainder of the metabolites excreted by the kidney.

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References

Crofford, L. J., Wilder, R. L., Ristimaki, A. P., Sano, H., Remmers, E. F., Epps, H. R. and Hla, T., 1994, Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues: effects of interleukin-1β, phorbol ester, and corticosteroids. *Journal Clinical Investigation*, **93**, 1095–1101.

Isakson, P., Zweifel, B., Masferrer, J., Koboldt, C., Seibert,. K, Hubbard, R., Geis, S. and Needleman, P. 1998, Specific COX-2 inhibitors: from bench to bedside. In J. Vane and J. Botting (eds), *Selective COX-2 Inhibitors: Pharmacology, Clinical Effects and Therapeutic Potential* (London: Kluwer and William Harvery), 127–133.

O'Neill, G. P. and Ford-Hutchinson, A. W., 1993, Expression of messenger mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS Letters*, **330**, 156–160.

Paulson, S. K., Engel, L., Reitz, B., Bolten, S., Burton, E. G., Maziasz, T. J., Yan, B. and Schoenhard, G. L., 1999, Evidence for polymorphism in the canine metabolism of the cyclooxygenase 2 inhibitor, celecoxib. *Drug Metabolism Disposition*, **27**, 1133–1142.

Paulson, S. K., Hribar, J. D., Liu, N. W. K., Hajdu, E., Bible, R. H., Piergies, A. and Karim, A., 2000a, Metabolism and excretion of [^{14}C] celecoxib in healthy male volunteers. *Drug Metabolism Disposition*, **28**, 308–314.

Paulson, S. K., Zhang, J., Breau, A. P., Hribar, J. D., Liu, N. W. K., Jessen, S. M., Lawal, Y. M., Cogburn, J. N., Gresk, C. J., Markos, C. S., Schoenhard, G. L. and Burton, E. G., 2000b, Pharmacokinetics, tissue distribution, metabolism and excretion of celecoxib in rats. *Drug Metabolism and Disposition*, **28**, 514–521.

Penning, T. D., Talley, J. J., Bertenshaw, S. R., Carter, J. D., Collins, P. W., Docter, S., Graneto, M. J., Lee, L. F., Malecha, J. W., Miyashiro, J. M., Rogers, R. S., Rogier, D. J., Yu, S. S., Anderson, G. D., Burton, E. G., Cogburn, J. N., Gregory, S. A., Koboldt, C. M., Perkins, W. E., Seibert, K., Veenhuizen, A. W., Zhang, Y. Y. and Isakson, P. C., 1997, Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]bezenesulfonamide (SC-58635, celecoxib). *Journal Medical Chemistry*, **40**, 1347–1365.

Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J., Perkins, W., Lee, L. and Isakson, P. C., 1994, Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proceedings of the National Academy of Sciences, USA*, **91**, 12013–12017.

Sidelmann, U. G., Lenz, E. M., Spraul, M., Hofmann, M., Troke, J., Sanderson, P. N., Lindon, J. C., Wilson, A. D. and Nicholson, J. K., 1996, 750 MHz HPLC-NMR spectroscopic studies on the separation and characterisation of the positional isomers of the glucuronides of 6,11-dihydro-11-oxodibenz[*b*,*e*]oxepin-2-acetic acid. *Analytical Chemistry*, **68**, 106–110.

Simon, L. S., Lanza, F. L., Lipsky, P. E., Hubbard, R. C., Talwalker, S., Schwartz, B. D., Isakson, P. C. and Geis, G. S., 1998, Preliminary study of the safety and efficacy of SC-58635, a novel cyclooxygenase 2 inhibitor, *Arthritis Rheumatology*, **41**, 1591–1602.

Spahn-Langguth, H. and Benet, L., 1992, Acyl glucuronides revisited: Is the glucuronidation process a toxification as well as a detoxification mechanism? *Drug Metabolism Reviews*, **241**, 5–48.