PHARMACOKINETICS AND METABOLISM OF LUMIRACOXIB IN HEALTHY MALE SUBJECTS

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ABSTRACT:

Lumiracoxib (Prexige; 2-[(2-fluoro-6-chlorophenyl)amino]-5-methyl-benzeneacetic acid) is a novel, chemically distinct cyclooxygenase-2 selective inhibitor, which has been developed for the treatment of osteoarthritis, rheumatoid arthritis, and acute pain. The absorption, metabolism, disposition, and mass balance of [14C]lumiracoxib were investigated in four healthy male subjects after a single 400-mg oral dose. Serial blood and complete urine and feces were collected for 168 h postdose. Lumiracoxib was rapidly absorbed, achieving mean plasma concentrations >1 μg/ml within 1 h of dosing. Unchanged drug in plasma accounted for 81 to 91% of radioactivity up to 2.5 h postdose, suggesting a modest first-pass effect; unchanged drug was the major circulating component in plasma, accounting for ~43% of the AUC∞ to 24h. The terminal half-life of lumiracoxib in plasma was 6.5 h. Major plasma metabolites were the 5-carboxy, 4’-hydroxy, and 4’-hydroxy-5-carboxy derivatives. Excretion involved both renal (54.1%) and fecal (42.7%) routes, and dose recovery was almost complete (96.8%). Lumiracoxib was extensively metabolized before excretion, with little unchanged drug in urine (3.3% of dose) or feces (2.0% of dose). The major metabolic pathways of lumiracoxib were oxidation of the 5-methyl group and hydroxylation of the dihaloaromatic ring. Glucuronic acid conjugates of lumiracoxib metabolites (and to a minor extent lumiracoxib itself) were identified, although there was no evidence of cysteine, mercapturic acid, or glutathione conjugates. In summary, orally administered lumiracoxib is rapidly absorbed and undergoes extensive metabolism before excretion via urine and feces, with no evidence of formation of potentially reactive metabolites.

Traditional nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) are the mainstay of therapy for osteoarthritis, rheumatoid arthritis, and acute pain, although their long-term use is associated with gastrointestinal (GI) ulceration and serious GI complications such as perforation and bleeding (Hernandez-Diaz and Garcia-Rodriguez, 2001). This is thought to be due to local inhibition of the cyclooxygenase (COX)-1 enzyme, which generates prostaglandins that are normally cytoprotective in the GI tract (Crofford, 1997; Warner et al., 1999). The identification of a second isoform, COX-2, which is up-regulated as a result of inflammation (Fu et al., 1990), has been shown to have efficacy comparable with NSAIDs but a superior GI safety profile during long-term use (Bombardier et al., 2000; Goldstein et al., 2001).

Lumiracoxib (Prexige; 2-[(2-fluoro-6-chlorophenyl)amino]-5-methyl-benzeneacetic acid) is a COX-2 selective inhibitor (Marshall et al., 2002), which has been developed for the treatment of osteoarthritis, rheumatoid arthritis, and acute pain. Lumiracoxib is chemically distinct from other COX-2 selective inhibitors in that it has a carboxylic acid group that confers weakly acidic properties (pKa 4.7; Fig. 1). This structure may be the reason for its distinct pharmacokinetic and pharmacodynamic profile. For example, lumiracoxib is characterized by rapid absorption (tmax 2 h) and sustained higher concentrations in synovial fluid versus plasma (2- to 3-fold from 12 to 24 h postdose) in patients with rheumatoid arthritis (Reynolds et al., 2003), the latter being a characteristic typically associated with the acidic nature of traditional NSAIDs (Rainsford et al., 1981; Bruno et al., 1988; Day et al., 1999).

Lumiracoxib has been shown to demonstrate dose-proportional and time-independent pharmacokinetics in single- and multiple-dose studies in healthy subjects and patients with osteoarthritis, at doses up to 800 mg once daily (Rordorf et al., 2002; Scott et al., 2002a,b, 2003a). In addition, near dose-proportional pharmacokinetics have been observed in patients with rheumatoid arthritis at doses up to 1200 mg once daily, with no accumulation and maintenance of COX-2 selectivity (Scott et al., 2003b). Lumiracoxib also demonstrates good oral bioavailability of 74% (Hartmann et al., 2003) and is rapidly and
efficiently absorbed from all regions of the GI tract (Wilding et al., 2004). In vivo, lumiracoxib is extensively metabolized by the hepatic cytochrome P450 isozyme CYP2C9 (Novartis data on file). In vivo, lumiracoxib showed no significant pharmacokinetic interaction with R- or S-warfarin, a particularly sensitive CYP2C9 substrate (Bonner et al., 2003), and fluconazole, a potent CYP2C9 inhibitor, had no clinically significant effect on the pharmacokinetics of lumiracoxib (Yih et al., 2003). The present study was conducted to characterize the absorption, metabolism, disposition, and mass balance of a single 400-mg oral dose of [14C]lumiracoxib in healthy male subjects.

Materials and Methods

Study. Four healthy male, nonsmoking subjects, aged 39 to 51 years, participated in this open-label study. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki (1964 and subsequent revisions), and all subjects gave written informed consent before participation. Subjects were genotyped with regard to hepatic cytochrome P450 2C9, with genotypes corresponding to “poor metabolizer” status excluded from the study. Subjects were admitted to the study site at least 20 h before dosing. Blood was collected by either direct venipuncture or an indwelling cannula into lithium heparin tubes predose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h postdose. Plasma was prepared within 30 min of sampling by centrifugation between 3°C and 5°C for 15 min at approximately 800 × g. Urine was collected predose and at 0 to 8, 8 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h postdose. Fecal samples were collected predose and thereafter up to study completion. Blood, plasma, urine, and feces were stored at −20°C until analysis.

Study Medication. All subjects received a single 400-mg oral dose of [14C]lumiracoxib (prepared by the Isotope Laboratory, Novartis Pharmaceuticals Corporation, East Hanover, NJ), containing a mean dose of radioactivity of 87.2 μCi, in the form of two capsules. The 14C label was uniformly incorporated into the dhabitroic ring, as shown in Fig. 1. The radioactive label had a specific activity of 0.21 μCi/mg and a radiochemical purity of 98%. No medication other than study drug was allowed from 14 days before lumiracoxib dosing until all study completion evaluations were complete.

Analysis of Unchanged Lumiracoxib. Plasma concentrations of unchanged lumiracoxib were measured using a validated HPLC assay (range, 10–5000 ng/ml). Acidified plasma (0.5 ml) was extracted using pentane/methyl chloride (2:1, v/v). The organic layer was dried and reconstituted in 200 μl of water/methanol (80:20, v/v). Chromatographic separation was achieved at 40°C on a 5-μm fluoro SEP RP 18 column (3.2 mm × 150 mm). A linear elution gradient (24–80% acetonitrile in 0.05% phosphoric acid) was run over 15 min at 1.0 ml/min, and the analytes were monitored using UV detection at 270 nm. A derivative of lumiracoxib was used as an internal standard. Noncompartmental pharmacokinetic parameters were derived using standard methods.

Total Radioactivity Measurement and Sample Preparation for Characterization of Lumiracoxib Metabolites. For blood, plasma, urine, and feces, levels of total radioactivity were determined in aliquots of each sample by liquid scintillation counting (LSC). LSC for plasma, urine, and other clear samples used Quickszint 1 liquid (Zinsser Analytic GmbH, Frankfurt, Germany) or Flo-Scint II (PerkinElmer Life and Analytical Sciences, Boston, MA) scintillation fluid. Whole blood and fecal samples were combusted before LSC (Packard Tri-Carb 306 sample oxidizer, Carbo-Sorb O2, absorbing fluid, and Permaflor E+ scintillation fluid). For extracts, extraction efficiency was calculated based on the total radioactivity in the original aliquot and the resulting extract.

Plasma. For metabolite profiles, equal volumes of plasma from each subject per time point were pooled and extracted twice with acetonitrile (6 ml and 3 ml, respectively). The combined extracts were evaporated to dryness with a stream of nitrogen at 37°C (Turbo-vap LV; Caliper Life Sciences, Hopkinton, MA), reconstituted with water/methanol/acetonitrile (8:1:1, v/v, 150 μl), vortex-mixed, and centrifuged. An aliquot of 20 μl was used to determine total radioactivity using a Packard 2550TR liquid scintillation counter, and 100 to 120 μl was analyzed by HPLC to obtain the metabolite profile.

Urine. Frozen urine samples were thawed at room temperature and filtered through a 0.45-μm nylon filter. Aliquots of 200 μl were assayed in duplicate for total radioactivity using the Packard 2550TR liquid scintillation counter. An aliquot of 5 ml (from 0- to 8-h and 8- to 24-h samples) and 10 ml (24- to 48-h samples) from each subject was lyophilized in a FLEXI-DRY freeze-dryer (FTS/Kinetics Thermal Systems, Stone Ridge, NY). The residue was reconstituted with methanol/water (2:8, v/v, 1 ml), vortex-mixed, and centrifuged at approximately 2000 × g at 5°C for 5 min. A 120-μl aliquot was analyzed by HPLC to obtain the metabolite profile.

Feces. For metabolite profiles, aliquots of dried fecal samples for each subject were pooled to encompass ≥95% of the total fecal radioactivity excretion. Each pooled sample was then extracted with methanol (three times, ~150 ml) by shaking vigorously for 5 min. Each sample was centrifuged between extractions, and the extract was separated from the post-extract solid by decanting. The methanol extracts were combined and partitioned with an equal volume of hexane to remove fatty material. Duplicate aliquots of the methanol layer were then analyzed for total radioactivity using the Packard 2550TR liquid scintillation counter. Each methanol extract was then evaporated to dryness and then reconstituted with ~8 ml of methanol, transferred to plastic centrifuge tubes, vortex-mixed, and sonicated for ~3 min. One milliliter of the suspension was transferred to a clean tube, assayed in duplicate for radioactivity, and then centrifuged at ~2000g at 18°C for 5 min. An aliquot of the supernatant was then analyzed by HPLC to obtain the metabolite profile.

Metabolite Analysis by HPLC. Metabolites in plasma, urine, and fecal extract samples were resolved using a Waters Alliance HPLC System, model 2690, with Millenium32 software (Waters, Milford, MA). The HPLC system incorporated an Inertsil ODS-2 (4.6 × 250 mm, 5 μm column; GL Sciences, Inc., Tokyo, Japan); mobile phases, A = 0.02 M ammonium acetate (pH 6–9), B = acetonitrile; flow rate, 1.0 ml/min; ultraviolet detector wavelength, 280 nm. The HPLC apparatus was connected in series to a Waters photodiode array detector (model 996) and an IN/US bRAM radioactivity detector (IN/US Systems, Tampa, FL) or a Gilson fraction collector (model FC204; Gilson, Inc., Middleton, WI). The gradient elution program was as follows (all gradient steps were linear): 0 to 2 min, 0% solvent B; 2 to 5 min, 0 to 15% solvent B; 5 to 10 min, 15% solvent B; 10 to 25 min, 15 to 30% solvent B; 25 to 40 min, 30 to 50% solvent B; 40 to 45 min, 50 to 100% solvent B; 45 to 55 min, 100% solvent B. Total HPLC flow was divided (1:1) into the MS electrospray ionization source and radioactivity detector after diversion of the first 2 min to waste.

After the injection of plasma or urinary extracts, column eluent was sampled at 0.2 min/well intervals using the fraction collector directly into 96-Deep Well Lumaplates (PerkinElmer Life and Analytical Sciences). The plates were dried in a SPE DRY-96 (Argonaut, Foster City, CA) at 45°C using a stream of nitrogen. Each plate was assayed for radioactivity at 10 min/well in a Packard TopCount radioactivity detector (model NXT). Fecal extracts were analyzed using HPLC with online radioactivity detection. The column effluent (1 ml/min) was mixed with liquid scintillant at a flow rate of 3 ml/min (Flo-Scint II; PerkinElmer Life and Analytical Sciences).

Metabolite Identification by Liquid Chromatography/MS and NMR. Metabolites were identified in pooled human urine, feces, and plasma samples using either a Waters Alliance HPLC 2690 equipped with a Finnigan LCQ (ion trap) MS and an IN/US bRAM radioactivity detector or a Shimadzu HPLC equipped with a Sciex API-3000 (triple quadrupole) MS (PerkinElmer/Sciex).
Instruments, Boston, MA). The chromatographic separation was the same as that used for generating the metabolite profiles.

The LCQ-MS was operated in negative ion mode with capillary temperature at 225°C, spray needle voltage at −4.0 kV, and sheath gas pressure and auxiliary gas flow at 30 and 30 arbitrary units, respectively. The Sciex API-3000 MS was operated in the positive ion mode with a proton spray needle voltage at 225°C, spray needle voltage at 4.0 kV, and sheath gas pressure and auxiliary gas flow at 80 and 30 arbitrary units, respectively. The LCQ-MS was operated in negative ion mode with capillary temperature at 225°C, spray needle voltage at 4.0 kV, and sheath gas pressure and auxiliary gas flow at 80 and 30 arbitrary units, respectively. The LCQ-MS was operated in negative ion mode with capillary temperature at 225°C, spray needle voltage at 4.0 kV, and sheath gas pressure and auxiliary gas flow at 80 and 30 arbitrary units, respectively. The LCQ-MS was operated in negative ion mode with capillary temperature at 225°C, spray needle voltage at 4.0 kV, and sheath gas pressure and auxiliary gas flow at 80 and 30 arbitrary units, respectively.

Confirmatory identification of 4'-hydroxy (4'-OH) metabolites (M5 and M23) in selected lyophilized plasma, urine, and feces samples was performed using HPLC coupled to 19F NMR and proton NMR (1H NMR). The HPLC system used for 19F NMR consisted of a Bruker LC22C pump, a Bischoff UV detector, and a Bruker Peak Sampling Unit; the 19F NMR analysis of lumiracoxib samples and reference lumiracoxib analogs was accomplished using a Bruker DMX500 spectrometer equipped with a 4-mm inverse triple (1H, 19F, 31P) flow probe with a shielded Z-axis gradient coil. Proton spectra were acquired using a composite pulse presaturation experiment. The spectral width was set for 12,000 Hz, and 1H 90° pulse was 8.75 μs at a power level of 7 dB. For 19F data collection, the 1H channel of the probe was tuned for the observation of 19F. The spectral width was set for 37,593 Hz, the 19F 90° pulse was 5 μs at 4 dB, and 32K data points were collected using 3-4 recycle delay.

An evaluation of lumiracoxib metabolites was performed using a Discovery RP-Amide C16 analytical column (4.6 × 150 mm, 5 μm; Supelco, Bellefonte, PA). The mobile phases were: A = 0.1% trifluoroacetic acid in deuterium oxide, B = 0.1% trifluoroacetic acid in deuterated acetonitrile (1.0 mL/min) with UV detection at 260 nm. The gradient elution program was as follows (all gradient steps were linear): 0 to 35 min, 25 to 75% solvent B; 35 to 40 min, 75 to 95% solvent B; 40 to 45 min, 95% solvent B. The HPLC separation was interrupted every 30 s (time-slicing technique) to acquire 19F data. Identification of metabolites was based on the evaluation of the 19F and 1H data collected by this technique. Reference standards of lumiracoxib analogs were analyzed in the same way to facilitate the interpretation of the 19F and 1H metabolite spectra.

Results

Four male subjects took part in this study with a mean age of 46 ± 6 years (range 39–51 years) and a mean weight of 76.8 ± 8.8 kg (range 71–87.6 kg). All subjects were of the CYP2C9 *1/*1 genotype. No adverse events or clinically significant changes in vital signs, clinical chemistry, hematology, or urinalysis were observed during the course of the study.

Blood and Plasma Concentrations of Radioactivity. Mean blood and plasma concentrations of radioactivity and key pharmacokinetic parameters are summarized in Table 1 and Fig. 2. The mean AUC0–t (max) of blood and plasma radioactivity was 89.6 μg Eq/g and 240 μg Eq/h/ml, respectively. The mean Cmax (at ~4 h postdose) of radioactivity in blood and plasma was 6.88 μg Eq/g and 12.0 μg Eq/ml, respectively. The apparent terminal half-life of the mean total radioactivity in blood and plasma ranged from 7.1 to 9.8 h in blood and 136 to 240 h in plasma. Extraction recovery of radioactivity in plasma, urine, and feces was 68% to 98%, 82% to 100%, and 74% to 83%, respectively.

Plasma Lumiracoxib Concentrations. These data show that a single oral dose of [14C]lumiracoxib (400 mg) is rapidly absorbed, achieving mean plasma concentrations >1 μg/ml within 1 h postdose and a mean peak plasma concentration (Cmax) of 7.28 μg/ml by a median of 4 h (range 2.5–4.0 h) postdose (Table 1). Unchanged lumiracoxib was the major drug-related circulating component in plasma, accounting for ~1% of the radioactivity up to 2.5 h postdose, and approximately 43% of total plasma radioactivity AUC0 to 24h, 38% of AUC0 to 48h, and 26% of the AUC0 to 168h. The apparent plasma clearance (CL/F) for lumiracoxib was 8.36 l/h, and the mean AUC0–t(max) value for plasma lumiracoxib was 48.4 ± 6.12 μgEq/g·h/ml. Using radiolabeled lumiracoxib in capsule form, the terminal plasma half-life of lumiracoxib ranged from 5.4 to 8.6 h (mean 6.5 h). Lumiracoxib pharmacokinetics in blood paralleled that of plasma; the blood/plasma radioactivity ratio was approximately 0.53.

Excretion and Mass Balance in Urine and Feces. Approximately
equal proportions of radioactivity were recovered in urine (mean recovery 54.1% of initial dose) and feces (mean recovery 42.7% of initial dose) in all four subjects (Fig. 3). The majority of urinary excretion (>85%) occurred during the first 24 h postdose and overall dose recovery was almost complete (96.8 ± 0.8%) within the 168-h collection period. Only a small percentage of the dose was excreted as unchanged drug in urine (3.3%, range 2.5–4.4%) and feces (1.9%, range 0.5–4.1%).

Metabolism of Lumiracoxib. A number of glucuronic acid conjugates of lumiracoxib metabolites (and to a minor extent, lumiracoxib, itself) were identified, but there was no evidence of glutathione-derived metabolites (glutathione, cysteine, or mercapturic acid conjugates) in either plasma or excreta. In plasma, lumiracoxib was the major circulating component with three major metabolites (M5, 4′-hydroxy-5-carboxy; M11, 5-carboxy; and M23, 4′-hydroxy) and at least two minor metabolites (M8 and M15) (Fig. 4A). The characteristics and structure of the main metabolites are shown in Table 2. Relative plasma exposure was highest for the M11 and M5 metabolites. In urine, at least 20 metabolites were identified, with most accounting for <2% of the dose. Metabolites M5 and M11 were the most prominent, representing 7.8% and 6.3% of the dose, respectively (Fig. 4B). Metabolite profiles in feces were less complex than in urine, although metabolites M5 and M11 were the major components, accounting for 20.5% and 8.28% of the dose, respectively (Fig. 4C).

MS identification of metabolites derived through 4′-hydroxylation was confirmed by 19F NMR and 1H NMR as illustrated in Fig. 5 and comparison to an available reference standard. The metabolic pathway of lumiracoxib in humans is shown in Fig. 6.

Discussion

After administration of a single oral dose of [14C]lumiracoxib, lumiracoxib was rapidly absorbed, with peak plasma concentrations of both unchanged lumiracoxib and radioactivity being reached around 4 h postdose. The concentration of unchanged lumiracoxib in plasma up to 2.5 h postdose was equivalent to 81 to 91% of the radioactive dose, with the level of unchanged lumiracoxib demonstrating an
**Fig. 5.** $^{19}$F NMR and $^1$H NMR spectra of metabolite M5.

**Fig. 6.** Metabolism of lumiracoxib in humans.
Lumiracoxib (radioactivity) was primarily excreted in the urine (54.1%) and feces (42.7%). The majority of urinary excretion occurred during the first 24 h postdose, and overall dose recovery was complete (96.8%) within 168 h of dosing. The observation that more occurred during the first 24 h postdose, and overall dose recovery was consistent with previously reported levels (Reynolds et al., 2003), suggesting that this metabolite is unlikely to contribute significantly to efficacy. Further phase II conjugation of metabolites (and to a minor extent, lumiracoxib) was observed. Cyclization to the corresponding lactam occurred with several metabolites, whereas direct glucuronic acid conjugation of lumiracoxib (acyl glucuronide formation) was a relatively minor metabolic pathway, accounting for approximately 2.5% of the dose.

Given the extensive metabolism of lumiracoxib by the hepatic cytochrome P450 isozyme CYP2C9, the potential exists for hepatic dysfunction, or the coadministration of other drugs metabolized by the same enzyme system, to adversely affect lumiracoxib pharmacokinetics. However, studies show that lumiracoxib pharmacokinetics remain unchanged in patients with moderate hepatic impairment (Kalbag et al., 2002), and no clinically significant pharmacokinetic interactions have been found between lumiracoxib and the sensitive CYP2C9 substrate, warfarin (Bonner et al., 2003) or the potent CYP2C9 inhibitor, flucloxacilone (Yih et al., 2003). These findings may suggest that compensatory pathways for metabolism exist.

In summary, orally administered lumiracoxib was well tolerated and rapidly absorbed, with unchanged lumiracoxib accounting for the majority of drug present in plasma. Lumiracoxib undergoes extensive metabolism before excretion via urine and feces, with no evidence of formation of potentially reactive metabolites.

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