Metabolism and Disposition of Dasatinib after Oral Administration to Humans

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ABSTRACT:

SPRYCEL (dasatinib; BMS-354825; Bristol-Myers Squibb, Princeton, NJ), a multiple kinase inhibitor, is currently approved to treat chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia tumors in patients who are resistant or intolerant to imatinib mesylate (Gleevec; Novartis, Basel, Switzerland). After a 100-mg single p.o. dose of [14C]dasatinib to healthy volunteers, the radioactivity was rapidly absorbed (<0.5 h). Both dasatinib and total radioactivity (TRA) plasma concentrations decreased rapidly with elimination half-life values of <4 h. Dasatinib was the major drug-related component in human plasma. At 2 h, dasatinib accounted for 25% of the TRA in plasma, suggesting that metabolites contributed significantly to the total drug-related component. There were many circulating metabolites detected that included hydroxylated metabolites (M20 and M24), an N-dealkylated metabolite (M4), an N-oxide (M5), an acid metabolite (M6), glucuronide conjugates (M8a,b), and products of further metabolism of these primary metabolites. Most of the administered radioactivity was eliminated in the feces (85%). Urine recovery accounted for <4% of the dose. Dasatinib accounted for <1 and 19% of the dose in urine and feces, respectively, suggesting that dasatinib was well absorbed after p.o. administration and extensively metabolized before being eliminated from the body. The exposures of pharmacologically active metabolites M4, M5, M6, M20, and M24 in patients, along with their cell-based IC50 for Src and Bcr-Abl kinase inhibition, suggested that these metabolites were not expected to contribute significantly toward in vivo activity.

SPRYCEL (dasatinib; Bristol-Myers Squibb, Princeton, NJ) is a multiple kinase inhibitor that potently inhibits Bcr-Abl, Src family (Src, Lck, Yes, Fyn), c-Kit, EPHA2, and platelet-derived growth factor receptor β kinases (Lombardo et al., 2004; Shah et al., 2004; Das et al., 2006). It is currently approved in the United States and European Union to treat chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia tumors in patients who are resistant or intolerant to imatinib mesylate (Gleevec, Novartis, Basel, Switzerland). Unlike imatinib mesylate, which binds to the closed confirmation of Bcr-Abl kinase, dasatinib was designed to bind to both the open and closed form of the enzyme (Shah et al., 2004; Tokarski et al., 2006). Because of this binding property and the ability to inhibit multiple kinases, including Src, dasatinib is effective in tumors that are resistant to imatinib mesylate (O’Hare et al., 2005; Schittenhelm et al., 2006). Clinical studies have shown that dasatinib shows clinical response in patients with CML or Philadelphia chromosome-positive acute lymphoblastic leukemia who are resistant or intolerant to imatinib mesylate treatment (Cortes et al., 2006; Hochhaus et al., 2006; Talpaz et al., 2006; Quintas-Cardama et al., 2007).

Numerous in vitro and in vivo studies have been conducted with dasatinib in nonclinical species to understand its absorption, distribution, metabolism, and excretion (ADME) properties and gauge the suitability of these species as toxicological models (Christopher et al., 2008; Kamath et al., 2008). The metabolic profiles from in vitro studies in liver microsomes, and hepatocytes showed good correlation with the in vivo profiles generated after a single p.o. dose of [14C]dasatinib to rats and monkeys. The primary metabolites of dasatinib

ABBREVIATIONS: dasatinib, BMS-354825, [N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-y1amino)thiazole-5-carboxamide]; CML, chronic myelogenous leukemia; ADME, absorption, distribution, metabolism, and excretion; HPLC, high-performance liquid chromatography; TRA, total radioactivity; LC/MS/MS, liquid chromatography/tandem mass spectrometry; LSC, liquid scintillation counting; IS, internal standard; GI, gastrointestinal.
identified were products of hydroxylation on the phenyl ring (M20 and M24), N-oxidation on the piperazine ring (M5), N-dealkylation of the hydroxyethyl moiety (M4), and oxidation of the hydroxyethyl moiety to a carboxylic acid (M6) (Christopher et al., 2008). In rat and monkey, only a small fraction of the radioactivity in bile and urine was attributed to the parent compound, suggesting that metabolism plays a major role in the elimination of dasatinib in these species (Christopher et al., 2008). In vitro studies with human liver microsomes and expressed enzyme systems showed that multiple enzymes were responsible for the oxidative metabolism of dasatinib with CYP3A4 as the primary enzyme involved.

The value and utility of doing ADME studies with radiolabeled compound is well understood. In drug development, these studies are performed with either C-14 or tritium-labeled material to provide detailed quantitative information on circulating metabolites, the extent of metabolism, and routes of excretion for drug and its metabolites (Marathe et al., 2004). This study describes the metabolism and disposition of [14C]-dasatinib after a single p.o. dose to healthy human subjects. Furthermore, based on the metabolic profile in plasma from the human ADME study and in vitro kinase activities of the metabolites found in the human study, the primary oxidative metabolites M4, M5, M6, M20, and M24 were monitored in plasma samples from the human ADME study and in vitro kinase activities of the metabolites. Furthermore, based on the metabolic profile in plasma from a subsequent clinical study after administration of a single 180-mg p.o. dose of dasatinib. This article describes the metabolism of dasatinib in humans along with the pharmacokinetics of its primary oxidative metabolites.

Materials and Methods

Chemicals. Reference standards for high-performance liquid chromatography (HPLC) and mass spectrometry, namely, dasatinib, N-(2-chloro-6-methylphenyl)-2-(2-(6-piperazin-1-yl)pyrimidin-4-ylamino)-thiazole-5-carboxamide (N-dealkylated amine of dasatinib, M4), 4-(6-(5-(2-chloro-6-methylphenylcarbamoyl)thiazol-2-ylamino)-2-methylpyrimidin-4-yl)-1-(2-hydroxyethyl)piperazine 1-oxide (piperazine N-oxide of dasatinib, M5), and 2-(4-(6-(5-(2-chloro-6-methylphenylcarbamoyl)thiazol-2-ylamino)-2-methylpyrimidin-4-yl)piperazin-1-yl)acetic acid (carboxylic acid metabolite of dasatinib, M6) were synthesized at Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ). Reference standards N-(2-chloro-4-hydroxy-6-methylphenyl)-2-(6-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (4-phenyl-hydroxy metabolite of dasatinib, M20) and N-(2-chloro-6-hydroxyphenyl)methyl)-2-(6-((4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-yl)aminoo)thiazole-5-carboxamide (benzylhydroxy metabolite of dasatinib, M24) were isolated from a microbial biotransformation reaction of dasatinib with Streptomyces (Li et al., 2008). [14C]-Dasatinib (17.0 μCi/mg), labeled on the 2-carbon of the thiazole ring, was synthesized by the Radiochemistry Group of the Department of Chemical Synthesis, Bristol-Myers Squibb Pharmaceutical Research Institute (New Brunswick, NJ) (Allentoft et al., 2008). Ecolite scintillation mixture was obtained from MP Biomedicals (Irvine, CA), and Emulsifier-Safe and Permafluor E+ scintillation fluid were obtained from PerkinElmer Life Sciences, Inc. (Boston, MA), respectively. Reagents and solvents of analytical or HPLC grade were purchased from commercial manufacturers.

Dosing and Sample Collection in the Human ADME Study. The human ADME study with [14C]-dasatinib was an open-label, nonrandomized, single-dose study. Eight healthy male subjects were enrolled into the study. All the subjects received a single p.o. dose of 100 mg of [14C]-dasatinib solution in 25 mL of fasting (Norland, West Chester, PA). Blood samples were collected into Vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ) containing EDTA as an anticoagulant. Plasma was prepared from the blood samples by centrifuging for 15 min at 1000g and 5°C and then stored at −20°C until analysis.

Representative pooled urine and fecal samples were prepared by combining a constant percentage of urine volume or fecal homogenate by weight across subjects. Pooled samples from 0 to 168 h were used for radioactivity profiling and liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis. Plasma samples were segregated by collection time, and equal volumes of plasma were combined from each subject. All the pooled plasma, urine, and fecal samples for metabolite profiling were stored at −80°C before analysis.

Analyses of Radioactivity. Samples of plasma (100 μl) and urine (300–350 μl), in triplicate, were mixed with Emulsifier-Safe (PerkinElmer Life Sciences, Inc.) scintillation mixture for the analysis of radioactivity by liquid scintillation counting (LSC). The fecal samples were transferred to tared containers and homogenized with water. The weight of the fecal homogenate was recorded for each sample. Accurately weighed duplicate portions (−0.2–0.4 g each) of the fecal homogenates were combusted to 14CO2. Blood samples (0.1 ml each) were directly combusted to 14CO2. Combustion of the blood and fecal samples was carried out in a model 307 or model 387 sample oxidizer (PerkinElmer Life Sciences, Inc.), and the 14CO2 was trapped with Carbo-Sorb E (PerkinElmer Life Sciences, Inc.). Permafluor E+ (PerkinElmer Life Sciences Inc.) scintillation mixture was added to the 14CO2-containing Carbo-Sorb E samples. 14C-labeled standards prepared in blank fecal matrix were combusted along with the study samples to determine combustion efficiency.

Radioactivity in all the samples was determined in triplicate by LSC in a model LS 6000 or LS6500 liquid scintillation counter (Beckman Instruments, Fullerton, CA) equipped with automatic background subtraction and quench correction. All the samples were counted for 10 min each to an error of 5%. The limits of quantitation ranged from 132 to 152 ng-Eq/ml for plasma (100 μl), 46.6 to 48.3 ng-Eq/ml for urine (300–350 μl), and 56.0– 2.3 ng-Eq/g for feces (0.2–0.4 g).

Analysis of Dasatinib and M5 in Plasma Samples from the Human ADME Study. The plasma concentrations of dasatinib and M5 were determined by a validated LC/MS/MS method. The internal standard (IS, BMS proprietary compound) was a close structural analog of dasatinib. The IS (200 ng/ml plasma) in 0.5% phosphoric acid was added to 0.1 ml of plasma sample before loading onto an Oasis HLB μElution 96-well solid-phase extraction plate (Waters, Milford, MA). Sample components were eluted from the plate with 50 μl of methanol. The extracts were diluted with 75 μl of water and vortexed, and a 10-μl portion of the sample was injected onto the LC/MS/MS system. The HPLC system consisted of a Shimadzu LC-10ADVP pump (Shimadzu Corporation, Columbia, MD) and a PerkinElmer Series 200 autosampler (PerkinElmer Life Sciences, Inc.). The column used was a Phenomenex Luna Phenyl-Hexyl (2 × 50 mm, 3 μm) (Torrance, CA). The mobile phase flow rate was 0.3 ml/min, and the column was maintained at 50°C. The components were eluted from the column with an isocratic solvent system (45% water, 55% acetonitrile). Solvent A and B were 0.1% formic acid in 0.5 M ammonium acetate, pH 3.0/methanol (80/20, v/v/s). Solvent B was a mixture of 10 mM ammonium acetate, pH 3.0/methanol (20/80, v/v). The HPLC system was interfaced to a Sciex API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) that was operated in the positive ion electrospray ionization mode. The analysis time was 3 min with dasatinib, M5, and the IS eluting at 1.3, 1.67, and 1.0 min, respectively. For multiple-reaction monitoring, the transitions monitored were m/z 488 to 401 for dasatinib, m/z 504 to 387 for M5, and m/z 474 to 387 for the IS. The standard curve ranges in plasma were 1 to 1000 ng/ml for both dasatinib and M5.

Dosing and Sample Collection in a Phase 1 Clinical Study. As part of a Phase 1 dose-escalation exploratory study in patients with chronic phase CML who showed hematologic resistance to imatinib mesylate (Gleevec), serial blood samples at 0.5, 1, 2, 3, 4, 5, 6, 8, and 24 h were collected after the first dose of 180 mg of dasatinib from three cancer patients treated once daily (q.d.) on a 5-day-on/2-day-off schedule (q.5d.). Dasatinib was administered as a tablet formulation in 50- and 5-mg strengths. All the blood samples were collected into Vacutainer tubes (BD Diagnostics) containing EDTA as an anticoagulant. Plasma was prepared from the blood samples by centrifuging for 15 min at 1000g and 5°C and then stored at −20°C until the time of analysis.

Analysis of Dasatinib and Metabolites M4, M5, M6, M20, and M24 in Plasma Samples from a Phase 1 Clinical Study. The plasma samples were stored at −20°C until analysis. The entire urinary and fecal output for each subject was collected over the study period. The day 1 urine collection was in 12-h collection increments; thereafter, urine collection was in 24-h intervals. Feces were collected in 24-h intervals. Individual urine and fecal samples were stored at −20°C or below until analysis.
analyzed for dasatinib and M5 with the validated LC/MS/MS method described above. Analysis of metabolites M4, M6, M20, and M24 were conducted with an LC/MS/MS method that was not fully validated. The assays for these metabolites were conducted in an exploratory mode where quality control samples were not included as would be done with a validated method. A 10-point standard curve was run in duplicate with an appropriate internal standard for each of the analyses. Metabolites M4 and M6 were analyzed together in one analytical assay, whereas metabolites M20 and M24 were analyzed separately. The LC/MS/MS method that was used for the determination of dasatinib and M5 in human plasma was adapted for measurement of M4 and M6. The same solid-phase extraction procedure and chromatography conditions used for dasatinib and M5 were used for M4 and M6. The mass spectrometer instrument parameters were optimized for the detection of M4 and M6 in 0.1 ml of human EDTA plasma. The internal standard was the same as that used in the analysis of dasatinib and M5. Chromatographic separation was achieved isocratically on a Phenomenex Luna phenyl-hexyl analytical column (2 × 50 mm, 3 μm). The mobile phase contained formic acid, water, methanol, and ammonium acetate. Detection was by positive ion electrospray tandem mass spectrometry on a Sciei API 3000 system (Applied Biosystems). The analysis time was 3 min, with M4, M6, and the IS eluting at 1.3, 1.8, and 1.0 min, respectively. The ion transitions monitored were m/z 444 to 401 for M4, m/z 502 to 401 for M6, and m/z 474 to 387 for the IS. The standard curves ranged from 1 to 1000 ng/ml for M4 and from 1 to 10,000 ng/ml for M6.

For extraction of metabolites M20 and M24 the same solid-phase extraction procedure was used as described for dasatinib and M5. The mobile phase for the HPLC method was the same except the pH of mobile phase A was 5.0 instead of pH 3.0. Gradient chromatography was used, where mobile phase B was 35% at t = 0, and increased from 35% to 65% from t = 0 to t = 2.0 min at a flow rate of 0.3 ml/min and a total cycle time of 4 min. The mass spectrometer instrument parameters were optimized for the detection of M20 and M24 in 0.1 ml of human EDTA plasma. A [13C4,15N2] stable labeled M4 was used as the IS. After the addition of IS (200 ng/ml of plasma) in 0.5% phosphoric acid to 0.1 ml of each sample and calibration standard, the samples were loaded onto an Oasis HLB μElution 96-well solid-phase extraction plate. The components were eluted from the plate with 50 μl of methanol. After the addition of water to the methanol eluate and vortexing, the extracts were injected into the LC/MS/MS system. Chromatographic separation by gradient HPLC was achieved on a Luna phenyl-hexyl analytical column (2 × 50 mm, 3 μm). Detection was by positive ion electrospray tandem mass spectrometry on a Sciei API 3000 system (Applied Biosystems) operating the Analyst version 1.4 software (Applied Biosystems). The analysis time was 4.4 min with M20, M24, and the IS eluting at 2.0, 2.2, and 2.4 min, respectively. The ion transitions monitored were m/z 504 to 417 for M20, m/z 504 to 399 for M24, and m/z 450 to 407 for the IS. The standard curves ranged from 1 to 1000 ng/ml for M20 and M24.

**Pharmacokinetic Analysis Methods.** The pharmacokinetic parameters of dasatinib, M4, M5, M6, TRA in the human ADME study were determined from plasma concentration versus time and urinary excretion data by a noncompartmental method using Kinetta 4.28 in the eToolbox (InnaPhase Corporation, Philadelphia, PA). The single-dose pharmacokinetic parameters assessed included Cmax (maximum observed concentration), Tmax (time of maximum observed concentration), AUC(INF) (area under the concentration-time curve from time 0 extrapolated to infinite time), AUC(0-T) (area under the concentration-time curve from time 0 to the last measurable sample time), T-HALF (terminal phase half-life); %UR (percentages of the [14C]dasatinib dose excreted as dasatinib, M5, and TRA in the urine), CLR (renal clearance), %FE (percentage of TRA excreted in the feces), and %TOTAL (percent of TRA excreted in feces and urine combined). The Cmax and Tmax were obtained from experimental observations. The terminal log-linear phase of the concentration-time curve was identified by least-square linear regression of at least three data points that yielded a maximum G-criterion, which is also referred to as adjusted R-squared, using no weighting factor. The t1/2 was calculated as Ln2/Kel, where Kel was the absolute value of the slope of the terminal log-linear phase. The AUC(0-T) was calculated using the mixed log-linear trapezoidal algorithm in Kinetta (InnaPhase Corporation). The AUC(INF) was estimated by summing AUC(0-T) and the extrapolated area, computed by the quotient of the last observable concentration and Kel. The amount of dasatinib, M5, and TRA excreted in urine and TRA in feces during each collection interval was calculated by multiplying the concentration of each analyte by the volume or weight of matrix collected over that interval. The total urinary recovery (UR) or fecal recovery (FE) was calculated as the cumulative amount excreted over all the collection periods and expressed as a percentage of the administered [14C]dasatinib dose (%UR or %FE). CLR was calculated by dividing the cumulative amount excreted in the urine by the AUC(INF).

Pharmacokinetic parameters of dasatinib, M4, M5, M6, M20, and M24 in patients administered 180 mg p.o. dose were derived from plasma concentration-time data by a noncompartmental method using Kinetta 4.2 in the eToolbox (InnaPhase Corporation). The single-dose pharmacokinetic parameters assessed included Cmax (maximum observed plasma concentration), Tmax (time of maximum observed plasma concentration), and AUC(0-T) (area under the plasma concentration-time curve from zero to the last time of the last quantifiable concentration). The Cmax and Tmax were obtained from experimental observations. The AUC(0-T) was calculated using the mixed log-linear trapezoidal algorithm in Kinetta (InnaPhase Corporation). AUC(0-T) was calculated for the metabolites as the circulating concentrations were low and could not be measured in all the 24-h samples.

**Preparation of Samples for Radioactivity Profiling.** Plasma. Pooled plasma samples obtained from the human ADME study were extracted with methanol/acetonitrile (1:1, v/v) at a ratio of 2 volumes solvent to 1 volume of plasma. The mixtures were vortex-mixed, sonicated for 5 min, and centrifuged at 3500 rpm for 10 min. The supernatants were transferred into new tubes. The remaining pellets were extracted two more times with 3 ml of methanol/ acetonitrile (1:1, v/v)). The supernatants were combined and evaporated to dryness in a Savant Speed-Vac (Savant Instruments, Inc., Holbrook, NY). The residue was reconstituted in 0.35 to 0.45 ml of 30% acetonitrile in water, vortex-mixed, and centrifuged at 13,000 rpm for 5 min (American Scientific Products Biofuge A centrifuge, Heraeus Sepatech GmbH, Osterode, Germany). The supernatant was subjected to HPLC analysis. Aliquots of the reconstituted samples (30 μl) were also counted using LSC to determine the radioactivity extraction recovery.

**Urine.** A pooled 0- to 168-h human urine (5 ml) sample was extracted with 2 volumes of acetonitrile/acetone (1:1, v/v). The mixtures were vortex-mixed, sonicated, and centrifuged at 3500 rpm for 10 min. The supernatants were evaporated to dryness. The residue was suspended in 0.3 to 0.5 ml of 30% acetonitrile in water and centrifuged at 13,000 rpm for 5 min. Aliquots of the supernatants were subjected to HPLC analysis or analyzed by LSC for radioactivity recovery.

**Feces.** Human 0- to 168-h pooled fecal homogenate (5.0 g) was extracted by addition of 3 volumes of acetonitrile/methanol (1:1, v/v) and mixed on a vortex mixer. The mixture was sonicated for 5 min and then centrifuged at 3000 rpm for 10 min. The supernatants were removed and saved. The extraction was repeated two additional times, and the supernatants were combined and evaporated to dryness on a Savant Speed-Vac (Savant Instruments Inc.). The residue was suspended in 0.5 ml of 35% acetonitrile in water, vortex-mixed for 5 min, and centrifuged at 13,000 rpm for 10 min. An aliquot of the supernatant was injected onto the HPLC for metabolite profiling, and a portion was analyzed by LSC for radioactivity extraction recovery.

**HPLC for Biotransformation Analysis.** Metabolite profiling was performed on an Agilent 1100TM system equipped with binary pumps, autoinjector, and a column heater (Agilent Technologies, Santa Clara, CA). A Phenomenex Synergi Polar-RP column (4.6 × 250 mm, 5 μm) was used for metabolite separation. All the HPLC analyses were performed at 1 ml/min flow rate at 35°C. The HPLC mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The linear gradient was as follows: 0 min, 15% B; 3 min, 15% B; 5 min, 32% B; 5.5 min, 32% B; 6.0 min, 90% B; and 63 min, 90% B. The column was re-equilibrated at 15% B for 6 min before the next injection.

For quantification of radioactivity, the HPLC effluent was collected in 15-s intervals into 96-well Packard Luminaplates (PerkinElmer Life Sciences, Inc.) with a Gilson Model FC 204 fraction collector (Gilson, Middleton, WI). Each fraction of column eluent was evaporated to dryness on a Savant Speed-Vac (Savant Instruments Inc.) and counted for radioactivity with a Packard Top Count microplate scintillation analyzer (PerkinElmer Life Sciences, Inc.). Radiochromatographic metabolite profiles were prepared by plotting the net counts per minute values obtained from the Top Count versus time after injection with Microsoft Excel (Microsoft, Redmond, WA). The relative abun-
Results

Pharmacokinetics of TRA, Dasatinib, and M5 in Healthy Subjects. The mean TRA, dasatinib, and M5 plasma concentration versus time profiles following p.o. administration of 100 mg of [14C]dasatinib (120 µCi) are shown in Fig. 1. The last quantifiable time point for TRA was different from the last quantifiable time point for dasatinib and M5 because the TRA assay was less sensitive compared with the LC/MS/MS assays. On p.o. administration, radioactivity was rapidly absorbed with a median $T_{\text{max}}$ of 0.5 h. The concentrations of TRA were substantially higher than unchanged dasatinib in the plasma. After the completion of the absorption phase, concentrations of dasatinib decreased rapidly with a mean terminal phase half-life of 3.6 h. By 24 h postdose, the mean concentrations of dasatinib were ~1% of the mean $C_{\text{max}}$ values. The pharmacokinetic parameters, determined by a noncompartmental method, are summarized in Table 1. Metabolite M5 was rapidly formed after administration of dasatinib as $C_{\text{max}}$ was attained at a median $T_{\text{max}}$ of 1.5 h. The plasma concentration of M5 decreased with a mean terminal half-life of 3.1 h. At 2 h, dasatinib accounted for 25% of the TRA in plasma, suggesting that metabolites contributed significantly to the total drug-related species.

Recovery of Radioactivity in Healthy Subjects Dosed with [14C]Dasatinib. The recovery of radioactivity in urine and feces from eight healthy human volunteers administered a single 100-mg (120 µCi) p.o. dose of [14C]dasatinib was 3.58 ± 1.17 and 85.32 ± 17.28%, respectively (data not shown). Only one of the eight subjects at 9 days postdose showed low recovery (45.89%). The reason for the low recovery for this one patient is not known. The majority of TRA that was recovered in urine and feces was recovered within 24 and 72 h after dosing, respectively.

Biotransformation Profile in Plasma. The recovery of radioactivity after an extraction and a concentration step was >90%. The 2-h plasma sample was the only sample with sufficient radioactivity for profiling and analysis of metabolites. The biotransformation profile of the human 2-h plasma sample is shown in Fig. 2, and the distribution of metabolites is summarized in Table 2. In addition to dasatinib, multiple circulating metabolites were detected by radioactivity in human plasma. Dasatinib was the major circulating species and represented approximately 26% of total radioactivity in the 2-h plasma sample. Metabolite M20, a phenyl hydroxylated metabolite, and its sulfate conjugate (M21) were also detected in significant amounts in human plasma.

Biotransformation Profile in Urine. Excretion of radioactivity in urine accounted for 4% of the radioactive dose. The biotransformation profile of pooled human urine (0–168 h) is shown in Fig. 2, and the distribution of urinary metabolites is summarized in Table 2. The recovery of radioactivity after an extraction and a concentration step was 92%. M5, a piperoxone N-oxide, was the predominant metabolite accounting for 1.4% of the radioactive dose (Table 2). Dasatinib accounted for <1% of the dose. Multiple metabolites, in addition to dasatinib and M5, including products from both oxidative and conjugated pathways, were also identified in urine, but none of these metabolites accounted for >1% of the radioactive dose.

Biotransformation Profile in Feces. The biotransformation profile of pooled fecal extract (0–168 h) is shown in Fig. 2, and the distribution of fecal metabolites is summarized in Table 2. After extraction, recovery of radioactivity was 75% in the pooled human fecal sample. Unchanged parent drug was a prominent component in the fecal profile accounting for 19% of the dose. Metabolites M20 and M6 were also detected in significant amounts. Other fecal metabolites included M23a,b, M24, and M4. No conjugated or N-oxide metabolites were observed in feces.

LC/MS/MS Analysis of Metabolites. Metabolites in plasma, urine, and fecal homogenates were detected by LC/MS/MS analysis. The identity of the primary metabolites M4, M5, M6, M20, and M24 was based on a match of their retention times and mass spectrometric fragmentation patterns to synthetic standards. The rest of the metabolites were identified based on their MS/MS fragmentation (data not shown). Detailed identification of the metabolites of dasatinib is described in a separate article (Christopher et al., 2008).
both the cell lines, producing inhibitory IC\textsubscript{50} values of 0.7 and 2.7 nM in the K562 and mSRC-A4 cell lines, respectively (Table 3). The metabolites exhibited a range of potency against both cell lines (Table 3), with M4 being the most potent (approximately equivalent to dasatinib), M5 and M20 of intermediate potency (10–20-fold less potent than dasatinib), and M6 and M24 the least potent (\textgreek{H}100\textsuperscript{22}50-fold less potent than dasatinib).

Pharmacokinetics of Dasatinib, M4, M5, M6, M20, and M24 in Patients. The plasma concentration versus time profiles for dasatinib and metabolites M4, M5, M6, M20, and M24 after a single p.o. dose of 180 mg to three patients are shown in Fig. 3, and the pharmacokinetic parameters are summarized in Table 4. There was a dose-dependent increase in exposures to dasatinib and M5 when compared with the 100-mg dose in the human ADME study. All five metabolites had mean \( C_{\text{max}} \) and mean AUC(0-T) values less than dasatinib. M20 was the most prominent of the metabolites with an AUC value 45\% of that of dasatinib. Median \( T_{\text{max}} \) values for dasatinib and the five metabolites ranged from 1 to 3 h.

**Discussion**

In healthy human subjects administered a single p.o. solution dose of \textsuperscript{[14]C}dasatinib, the drug was rapidly absorbed as indicated by the

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>( C_{\text{max}}/\text{Geometric Mean (CV %)} )</th>
<th>AUC(0-T)/Geometric Mean (CV %)</th>
<th>( T_{\text{max}}/\text{Median (Min, Max)} )</th>
<th>T-HALF/\text{Mean (S.D.)}</th>
<th>CLR/\text{Mean (S.D.)}</th>
<th>UR/\text{Mean (S.D.)}</th>
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<tbody>
<tr>
<td>TRA (n = 7)</td>
<td>224.6 (23)</td>
<td>\textsuperscript{a}</td>
<td>400.4 (41)</td>
<td>0.5 (0.25, 1.5)</td>
<td>3.58 (1.17)</td>
<td></td>
</tr>
<tr>
<td>Dasatinib (n = 8)</td>
<td>104.5 (29)</td>
<td>313.9 (42)</td>
<td>298.8 (44)</td>
<td>0.5 (0.25, 1.5)</td>
<td>3.6 (1.0)</td>
<td>404.8 (168.7)</td>
</tr>
<tr>
<td>M5 (n = 8)</td>
<td>2.9 (55)</td>
<td>15.3 (53)</td>
<td>8.9 (74)</td>
<td>1.5 (0.75, 3)</td>
<td>3.2 (1.2)</td>
<td>1.2 (0.49)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} n = 7 because one subject had no quantifiable levels of TRA in plasma. The limits of quantitation in plasma for radioactivity ranged from 132 to 152 ng-Eq/ml.

\textsuperscript{b} The AUC(INF) and T-HALF for radioactivity was not calculated because radioactivity was measurable only up to 4 h.

\textsuperscript{c} n = 6 because one subject had no quantifiable levels of TRA in plasma and the extrapolated AUC could not be estimated in a second subject.
Relative percent distribution of radioactive metabolites in pooled plasma, urine, and fecal samples after p.o. administration of [14C]dasatinib to humans

<table>
<thead>
<tr>
<th>Metabolite ID</th>
<th>Plasma (2 h)</th>
<th>Urine (0-168 h)</th>
<th>Feces (0-168 h)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% Radioactivity</td>
<td>% Radioactivity</td>
<td>% Radioactivity</td>
</tr>
<tr>
<td>M3a,b⁺</td>
<td>3.3</td>
<td>6.8</td>
<td>0.2</td>
</tr>
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<td>M4</td>
<td>M</td>
<td>1.3</td>
<td>0.05</td>
</tr>
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<td>M5</td>
<td>4.5</td>
<td>39.8</td>
<td>1.4</td>
</tr>
<tr>
<td>M6</td>
<td>3.6</td>
<td>1.3</td>
<td>0.05</td>
</tr>
<tr>
<td>M7</td>
<td>3.3</td>
<td>2.1</td>
<td>0.08</td>
</tr>
<tr>
<td>M8a</td>
<td>3.4</td>
<td>5.5</td>
<td>0.2</td>
</tr>
<tr>
<td>M8b, M23a,b⁻</td>
<td>1.4</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>M9</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>M20</td>
<td>12.5</td>
<td>4.1</td>
<td>0.2</td>
</tr>
<tr>
<td>M21</td>
<td>9.5</td>
<td>7.8</td>
<td>0.3</td>
</tr>
<tr>
<td>M23a,b</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>M24</td>
<td>3.1</td>
<td>6.0</td>
<td>0.2</td>
</tr>
<tr>
<td>M30</td>
<td>6.9</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>M31</td>
<td>3.6</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>M34</td>
<td>1.1</td>
<td>2.3</td>
<td>0.08</td>
</tr>
<tr>
<td>M35a</td>
<td>3.6</td>
<td>4.2</td>
<td>0.2</td>
</tr>
<tr>
<td>M36</td>
<td>N.D.</td>
<td>4.4</td>
<td>0.2</td>
</tr>
<tr>
<td>M37a,b⁻</td>
<td>4.1</td>
<td>2.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>25.5</td>
<td>3.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Total</td>
<td>89.4</td>
<td>91.7</td>
<td>3.4</td>
</tr>
</tbody>
</table>

+ M5, mass spectrometry; N.D., not detected.

⁺ Metabolites (M3a,b), (M23a,b), and (M37a,b) were pairs of positional isomers that were not well resolved on HPLC.

⁻ Metabolites M8b and M23a,b were not well resolved on HPLC. The percentage of distribution is the total percentage of all three metabolites.

Inhibitory activities of dasatinib and five of its metabolites on the in vitro growth of mSRC-A4 mammary carcinoma and K562 CML cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>IC₅₀ / Geometric Mean (S.D.)</th>
<th>AUC₀-T / Geometric Mean (S.D.)</th>
<th>Tₘ₅ₓ / Median (Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dasatinib</td>
<td>274.3 (52.0)</td>
<td>1151 (213)</td>
<td>1.5 (0.5, 2.0)</td>
</tr>
<tr>
<td>M4</td>
<td>10.4 (6.8)</td>
<td>53 (53)</td>
<td>2.1 (2.0, 4.9)</td>
</tr>
<tr>
<td>M5</td>
<td>11.2 (3.5)</td>
<td>56 (22)</td>
<td>2.0 (2.0, 2.0)</td>
</tr>
<tr>
<td>M6</td>
<td>21.9 (7.2)</td>
<td>115 (45)</td>
<td>3.0 (1.5, 5.0)</td>
</tr>
<tr>
<td>M20</td>
<td>200.2 (101.6)</td>
<td>513 (188)</td>
<td>1.5 (1.0, 1.5)</td>
</tr>
<tr>
<td>M24</td>
<td>71.4 (26.7)</td>
<td>288 (102)</td>
<td>1.0 (1.0, 2.0)</td>
</tr>
</tbody>
</table>

⁺ Derived from a mammary carcinoma from a transgenic mouse harboring the activated SRC oncogene.

⁻ Proliferation of mSRC-A4 mammary carcinoma and K562 CML cells is dependent on Src and Bcr-Abl kinases, respectively.

Most of the radioactive dose was recovered in feces (85%) with a small amount (<4%) recovered in urine for a mean total recovery of 88.9%. One subject showed a recovery value (<46%) significantly lower than the other subjects. If this subject was excluded, the overall recovery improved to 95%. Because the bulk of the TRA was in the feces, it was thought that the low recovery in this subject could be the result of incomplete homogenization of the fecal samples. However, rehomogenization and reanalysis of the fecal homogenate from the low-recovery subject gave similar results to the initial values. Therefore, the reason for the low recovery is not known. Interestingly, the same subject had radioactivity counts in plasma below lower limits of quantitation at all the time points even though LC/MS/MS analysis of plasma samples showed exposure to both dasatinib and M5 that was similar to other subjects (data not shown).

Intact dasatinib accounted for approximately 19% of the recovered dose. These data suggest that dasatinib is well absorbed after p.o. administration. The presence of dasatinib in human feces could be caused by a combination of one or more of the following, namely, 1) incomplete absorption of the p.o. dose, 2) reduction of the N-oxide metabolite M5 to dasatinib by the gastrointestinal (GI) bacteria, and 3) hydrolysis of the conjugated metabolites of dasatinib (M8a,b) in the GI tract. In rat, M5 was a prominent metabolite in bile but was not observed in the feces. Instead, dasatinib was observed in rat feces, indicating that M5 was reduced during transit through the GI tract by the gut bacteria (Christopher et al., 2008). Reduction of N-oxide metabolites by the GI gut bacteria is known and has been reported for a number of compounds (Powis et al., 1979; Jaworski et al., 1991; Mitchell et al., 1997).

The results of the current study indicate that dasatinib is extensively...
metabolized in vivo. The proposed metabolic pathway for dasatinib in humans is shown in Fig. 4. Dasatinib was the major drug-related component in human plasma. The many circulating metabolites of dasatinib in humans included products of hydroxylation, N-dealkylation, N-oxidation, alcohol oxidation, sulfate, and glucuronide conjugation and products of further metabolism of the primary metabolites. All the human circulating metabolites identified here were also present in monkey plasma and many in rat plasma (Christopher et al., 2008). One interesting interspecies difference in metabolite profiles was the lack of a direct sulfate conjugate in monkey and human, whereas rat produced significant amounts of this metabolite. This interspecies difference in sulfation of primary alcohols has been observed before (Nakano et al., 2004).

The metabolites identified in plasma were also present in human microsomal and hepatocyte incubations (Christopher et al., 2008). Similar to the human plasma profile metabolites, M20 and M24 were the most abundant oxidative metabolites in liver microsome and hepatocyte incubations. Metabolite M21, a prominent metabolite in human plasma, was also a major metabolite in human hepatocyte incubations. The other minor metabolites of dasatinib identified in human hepatocytes and microsomal incubations were also identified as minor metabolites in human plasma. These data suggest a good correlation between in vitro and in vivo metabolism profiles for dasatinib. A similar correlation between in vitro and in vivo profiles was also observed in rat and monkey. These data support the utility of microsomal and hepatocyte system to predict in vivo metabolism.

Both imatinib and dasatinib bind to the ATP-binding site of Bcr-Abl kinase. An X-ray crystal structure of dasatinib bound to the Bcr-Abl kinase and docking studies to ATP-binding site of Src kinase showed that the piperazine ring and the terminal ethyl alcohol group were not in the active site of either enzyme (Lombardo et al., 2004; Shah et al., 2004; Das et al., 2006). Thus, it was not surprising that metabolic modifications on the piperazine ring yielded compounds that retained Src and Bcr-Abl kinase activity. This was confirmed with metabolites M4, M5, and M6, which also showed activity toward Src and Bcr-Abl kinases in the nanomolar range (Table 3). Metabolite M4 was approximately equipotent to dasatinib, whereas the other metabolites M5 and M6 were >10-fold less active than dasatinib (Table 3). M20 and M24 were modified on a portion of the dasatinib molecule expected to be in the active site; however, these metabolites still maintained reasonable potency. In general, among the metabolites and dasatinib, the rank order of potency was similar for Src and Bcr-Abl, indicating that the Src/Abl dual-kinase inhibitory activity of parent was also maintained by the metabolites.

Because only the 2-h plasma sample in the human ADME study contained sufficient radioactivity for profiling, no exposure versus time data were generated for metabolites M4, M6, M20, and M24 from the 14C-profiling experiments. M5 was analyzed by a validated LC/MS/MS assay, as mentioned above, and found to be a minor circulating metabolite. Exposure values for metabolites M4, M6, M20, and M24 were obtained with specific LC/MS/MS assays from three patients dosed with a single p.o. dose of 180 mg of dasatinib. These data were used to make an informed decision with regard to monitoring metabolites in future clinical studies. The results showed that M20 and M24 were prominent circulating metabolites, whereas M4, M5, and M6 were relatively minor circulating metabolites (Fig. 3). These data along with the relative potency values suggest that these metabolites are unlikely to contribute significantly to the pharmacological activity in humans. Moreover, because this study provided sufficient data regarding exposure to the prominent human metabolites, a decision was made not to monitor them in future clinical studies.

FIG. 4. Proposed biotransformation pathway based on metabolites identified in plasma, urine, and fecal samples from humans administered a single p.o. dose of [14C]dasatinib (100 mg, 120 μCi).
The current study clearly shows that the major route of clearance for dasatinib is via oxidative biotransformation. Along with the in vitro data, these data suggest that CYP3A4 will play a major role in dasatinib clearance. Furthermore, key drug-drug interaction studies have shown that perturbation of the CYP3A4 pathway strongly impacts the pharmacokinetics of dasatinib. Coadministration of dasatinib with ketoconazole increased the exposures of dasatinib by 4- to 5-fold, whereas rifampin reduced the exposure of dasatinib by ~80%.

In summary, the human ADME study of dasatinib showed that the drug was well absorbed and extensively metabolized in vivo, with all the circulating metabolites detected in human plasma also present in plasma of at least one of the species used in toxicological evaluations. LC/MS/MS monitoring of key primary metabolites, which had in vitro kinase activity, showed that these metabolites were not expected to contribute significantly toward in vivo pharmacological activity.

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References


Hochhaus A, Kim DW, Rousselot P, Dohiuchi-Luccioni PE, Milone J, Francis S, Bleckardt E, and Kantarjian H (2006) Dasatinib (SPRYCEL(R)) 50mg or 70mg BID versus 100mg or 140mg QD in patients with chronic myeloid leukemia in chronic phase (CML-CP) resistant or intolerant to imatinib: results of the CA180–034 phase III study. ASH Annual Meeting Abstracts 108:05.

References


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