Metabolism and Disposition of Oral Dabrafenib in Cancer Patients: Proposed Participation of Aryl Nitrogen in Carbon-Carbon Bond Cleavage via Decarboxylation following Enzymatic Oxidation

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ABSTRACT

A phase I study was conducted to assess the metabolism and excretion of \([^{14}C]\)dabrafenib (GSK2118436; \(N\)-(3-[5-(2-amino-4-pyrimidinyl)-2-(1,1-dimethylethyl)-1,3-thiazol-4-yl]-2-fluorophenyl)-2,6-difluorobenzene sulfonamide, methanesulfonate salt), a BRAF inhibitor, in four patients with BRAF V600 mutation-positive tumors after a single oral dose of 95 mg (80 \(\mu\)Ci). Assessments included the following: 1) plasma concentrations of dabrafenib and metabolites using validated ultra-high-performance liquid chromatography—tandem mass spectrometry methods, 2) plasma and blood radioactivity, 3) urinary and fecal radioactivity, and 4) metabolite profiling. Results showed the mean total recovery of radioactivity was 93.8%, with the majority recovered in feces (71.1% of administered dose). Urinary excretion accounted for 22.7% of the dose, with no detection of parent drug in urine. Dabrafenib is metabolized primarily via oxidation of the t-butyl group to form hydroxy-dabrafenib. Hydroxy-dabrafenib undergoes further oxidation to carboxy-dabrafenib, which subsequently converts to desmethyl-dabrafenib via a pH-dependent decarboxylation. The half-lives for carboxy- and desmethyl-dabrafenib were longer than for parent and hydroxy-dabrafenib (18-20 vs. 5-6 hours). Based on area under the plasma concentration-time curve, dabrafenib, hydroxy-, carboxy-, and desmethyl-dabrafenib accounted for 11%, 8%, 54%, and 3% of the plasma radioactivity, respectively. These results demonstrate that the major route of elimination of dabrafenib is via oxidative metabolism (48% of the dose) and biliary excretion. Based on our understanding of the decarboxylation of carboxy-dabrafenib, a low pH-driven, nonenzymatic mechanism involving participation of the aryl nitrogen is proposed to allow prediction of metabolic oxidation and decarboxylation of drugs containing an aryl nitrogen positioned \(\alpha\) to an alkyl (ethyl or t-butyl) side chain.

Introduction

Dabrafenib (TAFINLAR, GSK2118436; \(N\)-(3-[5-(2-amino-4-pyrimidinyl)-2-(1,1-dimethylethyl)-1,3-thiazol-4-yl]-2-fluorophenyl)-2,6-difluorobenzene sulfonamide, methanesulfonate salt) is an oral, potent, and selective RAF kinase inhibitor. The RAS/RAF/MEK/ERK pathway is a critical proliferation pathway in many human cancers, and BRAF mutations have been identified in approximately 40%–60% of melanoma (Davies et al., 2002; Rubinstein et al., 2010). Dabrafenib has been approved for the treatment of BRAF V600E mutation-positive tumors as monotherapy, and is currently under development for use in combination therapy with trametinib, an allosteric noncompetitive inhibitor of MEK1/MEK2 activation and kinase activity (Flaherty et al., 2012).

The pharmacokinetics (PK) of dabrafenib after single and repeat dosing have been investigated as part of a first-time-in-human study, where it was shown that, after single-dose administration, dabrafenib concentrations peaked about 2 hours postdose and declined thereafter, with a terminal half-life of approximately 5 hours (Falchook et al., 2012). Most of the human dabrafenib studies conducted to date have included an assessment of parent drug concentration, along with the concentration of the three main circulating metabolites, hydroxy-, carboxy-, and desmethyl-dabrafenib, to better define the clinical pharmacokinetics of the drug. These three metabolites were identified as being of potential clinical importance based on preclinical pharmacology, as they were shown to be active in preclinical models, albeit with...
different potency, and metabolic data suggested that they represented a major pathway of elimination. Preliminary human metabolite information obtained using plasma samples from the first-time-in-human study confirmed the importance in terms of exposure of these three metabolites.

The metabolite profile and routes of excretion following oral administration have yet to be fully elucidated in humans. Here, we report our findings from a study of the metabolism and excretion of [14C]dabrafenib in subjects with BRAF V600 mutation–positive tumors after a single radiolabeled oral dose of 95 mg. In addition to metabolite profiling and determination of total radioactivity concentrations in plasma, assessment of the concentrations of dabrafenib and its three main metabolites using validated ultra-high-performance liquid chromatography (UHPLC)–tandem mass spectrometry (MS/MS) methods allowed for characterization of the full PK profiles, calculation of the PK parameters, and determination of the fraction of the area under the curve (AUC) accounted for by dabrafenib combined with these three metabolites, relative to the total concentration of radioactivity in plasma. Results of in vitro experiments to understand the pH dependency of the decarboxylation of carboxy-dabrafenib to desmethyl-dabrafenib are presented, and a mechanism is proposed for metabolic oxidation and decarboxylation of drugs containing an aryl nitrogen α to an ethyl or t-butyl side chain. Taken together, these results provide a full understanding of dabrafenib disposition in humans.

**Materials and Methods**

**Chemicals and Reagents**

[14C]Dabrafenib (nonmicronized, radiochemical purity of 96.9% and specific activity of 111.2 μCi/mg) was synthesized by Isotope Chemistry (GlaxoSmithKline, Stevenage, UK). A single [14C] label was positioned in the thiazole moiety. Unlabeled dabrafenib and metabolite standards, hydroxy-dabrafenib [M7], carboxy-dabrafenib [M4], and desmethyl-dabrafenib [M8], were prepared by Product Development (GlaxoSmithKline, King of Prussia, PA) and by Pharmaron (Beijing, China) (Adams et al., 2009; Rheault et al., 2013). Chemicals and solvents of reagent or high-performance liquid chromatography (HPLC) grade were purchased from commercial sources.

**Subjects and Study Design**

The study (ClinicalTrials.gov registration identifier NCT01262963) was a phase 1, single-center, open-label study in subjects with BRAF V600 mutation–positive tumors. Male or female subjects were eligible to enroll in the study if they were at least 18 years of age and had a BRAF V600 mutation–positive tumor as determined via relevant genetic testing. Additional relevant inclusions were prepared by Product Development (GlaxoSmithKline, King of Prussia, PA) and by Pharmaron (Beijing, China) (Adams et al., 2009; Rheault et al., 2013). Chemicals and solvents of reagent or high-performance liquid chromatography (HPLC) grade were purchased from commercial sources.

**Radiation Safety of Subjects**

The expected radiation exposure of a subject receiving an 80-μCi oral dose of [14C]dabrafenib was estimated prognostically according to regulatory guidelines (Code of Federal Regulations (revised as of April 1, 2013)). Data from male and female rat whole-body autoradiography and excretion studies following oral administration of [14C]dabrafenib were subject to relative organ mass scaling and physiological time scaling, and were fit using noncompartmental analysis. The human absorbed dose estimates were calculated following the medical internal radiation dose schema using the MIRDSE3.1 software package (Oak Ridge Associated Universities, Oak Ridge, TN). Oral administration of 80 μCi of [14C]dabrafenib to adult male and female subjects was expected to result in an effective dose to the whole body of less than 100 mRem (1 mSv), within risk category IIa in accordance with the International Commission on Radiological Protection. Hence, the radiation risks in the current study were estimated to be low.

**Dosing and Sample Collection**

Subjects received the equivalent of 95 mg of dabrafenib free base, which was administered as methanesulfonate salt in an oral suspension of 0.5% hydroxypropyl methylcellulose and 0.1% polysorbate 80. Each dose contained approximately 80 μCi of radiolabeled carbon. The radioactivity of the dose ranged from 98.9% to 99.5%. The single oral dose was administered in the morning of day 1.

Blood samples were collected on day −1, day 1, predose (for determination of background radioactivity), and then at various times postdose (0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours) after determination of blood radioactivity, plasma radioactivity, and plasma concentrations of dabrafenib and its three main metabolites (hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib), with additional plasma obtained at select time points (2, 6, 10, and 24 hours postdose) for radioprofiling. Urine (0–6-hour, 6–12-hour, and 12–24-hour intervals followed by 24-hour intervals) and feces (24-hour intervals) were collected predose and for a minimum of 96 hours and a maximum of 240 hours after dosing. Collection was stopped after 96 hours if >90% of the administered dose was recovered and total radioactivity in feces and urine was ±1% of the administered dose in two consecutive collections.

**Sample Analysis for Radioactivity**

Analysis of total radioactivity in blood and plasma at all time points was conducted internally at GlaxoSmithKline (King of Prussia, PA). Triplicate aliquots of blood (0.2–0.4 g) were combusted in a Model 307A Oxidizer (PerkinElmer Inc., Waltham, MA). The resulting 14CO2 was trapped and scintillation cocktail (Carbosorb E/Permafluor E+; PerkinElmer) was added. Triplicate aliquots of plasma (0.1–0.3 g) were transferred to scintillation vials and 10 ml of scintillation cocktail (Ultima Gold; PerkinElmer) was added. Samples were assayed for radioactivity by liquid scintillation counting (LSC) using a PerkinElmer Tri-Carb 3170 TR low-level liquid scintillation counter. The total blood and plasma radioactivity concentrations were calculated by converting the LSC data (disintegrations per minute) to concentrations (nanogram of dabrafenib equivalent per gram or nanomolar) using the specific activity (0.84 μCi/mg or 0.44 μCi/μmol, respectively) of [14C]dabrafenib. It was assumed that 1 g of plasma is equivalent to 1 ml of plasma, based on historic experimental precedent.

Analyses of total radioactivity content in collected excreta samples were conducted at Covance Laboratories Inc. (Madison, WI). Triplicate aliquots of fecal homogenate sample (0.3 g per aliquot) were combusted in a Model 307 Sample Oxidizer (Packard Instrument Co., Downers Grove, IL). The resulting 14CO2 was trapped, scintillation cocktail added, and the radioactivity content was quantified by LSC. Triplicate aliquots of urine sample (1.0 g per aliquot) were transferred to scintillation vials, and scintillation cocktail was added. The vials were then counted in a scintillation counter for 5 minutes or 100,000 counts.

**Quantification of Dabrafenib, Hydroxy-dabrafenib, Desmethyl-dabrafenib, and Carboxy-dabrafenib in Plasma**

Two separate validated assays were used to measure dabrafenib and its three metabolites. Concentrations of dabrafenib, hydroxy-dabrafenib, and desmethyl-
dabrafenib in plasma samples at all time points were determined with a validated analytical UHPLC-MS/MS method, over a range of 1–1000 ng/ml. Dabrafenib, hydroxy-dabrafenib, and desmethyl-dabrafenib were extracted from 50 μl of human plasma by liquid-liquid extraction with 1 ml of ethyl acetate after the addition of isotopically labeled internal standards ([2H6]dabrafenib, [2H6 13C2]-hydroxy-dabrafenib, and [2H6 13C2]-desethyl-dabrafenib). Extracts (4 μl) were injected onto a Waters Acuity BEH C18 column (1.7 μm, 50 × 2.1 mm; Waters, Milford, MA) maintained at 55°C. The mobile phase consisted of water/formic acid (0.1%, v/v; solvent A) and acetonitrile (solvent B). A flow rate of 0.8 ml/min was maintained for the entire run. The following 1.4-minute linear gradient was used: start at 30% B, increase to 50% B over 0.5 minute, maintain 50% B for 0.3 minute, increase to 80% B over 0.2 minute, and maintain at 80% B for 0.3 minute. The column was re-equilibrated after each injection. Detection was performed by positive-ion MS/MS using a Turbolon-Spray interface on an API 4000 mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Ontario, Canada) with positive-ion MS/MS using a TurboIon-Spray interface on an API 4000 mass spectrometer. The ratio of blood-to-plasma total radioactivity concentrations at each PK time point was calculated to assess partitioning of dabrafenib-related material into red blood cells. Plasma ratio of AUC0–t to AUC0–∞ for dabrafenib and metabolites (corrected for differences in molecular weight) to total radiocarbon was determined.

Sample Preparation for Metabolite Profiling

Plasma. The plasma samples collected for metabolite profiling at selected time points (2, 6, 10, and 24 hours) were analyzed individually. Each sample (2 ml) was first extracted with 0.5 volume of methanol, vortex mixed, and sonicated in a Covaris sonicator (E210 series; Covaris Inc., Woburn, MA). Then, 0.5 volume of acetonitrile was added to each sample, which was then vortex mixed, sonicated, and centrifuged at 3500 gav at room temperature for 10 minutes. The supernatants were removed and combined. The pellet was then extracted once more with 0.5 volume of methanol and acetonitrile in an identical procedure with the resulting supernatants combined with the first extracts. Duplicate aliquots were removed from the combined extract for LSC to determine its radioactivity content and extraction recoveries. The combined extract was then concentrated to 3 ml using a vacuum centrifuge. The samples were laterally mixed for 30 minutes at 750 rpm and at 10°C, sonicated using the Covaris sonicator, and centrifuged at 3500 gav at room temperature for 10 minutes. Duplicate aliquots were removed from the concentrated supernatants for LSC to determine concentration efficiency prior to analysis by quantitative radio-HPLC (radio-HPLC, described later).

Urine. Urine samples from collections representing ≥2% of the administered dose were pooled for each patient based on the total sample weight collected from each interval to produce a single representative sample for the patient. Aliquots of the pooled samples were centrifuged at 3500 gav at room temperature for 5 minutes. The supernatants were then analyzed by radio-HPLC.

Feces. Fecal homogenate samples from collections representing ≥2% of the administered dose were pooled for each patient based on the total sample weight collected from each interval to produce a single pooled representative sample for the patient. The pooled fecal homogenate samples were extracted with 3 volumes of 3:1 (v/v) methanol:water, sonicated at room temperature for 15 minutes, vortex mixed at room temperature for 20 minutes, and centrifuged at 3000 gav at room temperature for 5 minutes. The supernatants were removed and combined. The residual pellets were extracted twice more with the same solvent in an identical procedure with the resulting supernatants combined with the first extracts. The combined extract was then evaporated to dryness under a stream of nitrogen gas and reconstituted in 1:1 (v/v) methanol:water. The reconstituted sample was sonicated for 15 minutes, vortex mixed for 20 minutes, and centrifuged at 3000 gav at room temperature for 5 minutes prior to analysis by radio-HPLC.

Quantitative Radio-HPLC Analysis

Radio-HPLC analysis was conducted on selected urine, fecal, and plasma samples on Agilent 1100 and 1200 systems (Agilent Technologies, Palo Alto, CA) consisting of a pump (G1312A binary), an autosampler (G1329A), and a Phenomenex Synergi Polar RP column (4.6 by 250 mm, 4 μm; Phenomenex, Torrance, CA). Aliquots (≥900 μl) of samples were injected and were eluted at 1 ml/min with 10 mM ammonium acetate, pH 5.5 (solvent A) and acetonitrile (solvent B). The following gradient was used: start at 0% B, increase to 10% B over 1 minute, maintain 10% B for 2.5 minute, increase to 20% B over 0.2 minute, and maintain at 20% B for 0.4 minute. The column was re-equilibrated after each injection. Detection was performed by positive-ion MS/MS using a Turbolon-Spray interface on an API 4000 mass spectrometer over a range of 5–5000 ng/ml. Carboxy-dabrafenib was extracted from 25 μl of human plasma by protein precipitation using 80/20 ethyl alcohol/MilliQ water (EMD Millipore, Danvers, MA) containing an isotopically labeled internal standard ([2H6 13C2]-carboxy-dabrafenib). Extracts (4 μl) were injected onto a Waters Acuity BEH Phenyl column (1.7 μm, 50 × 2.1 mm) maintained at 55°C. The mobile phase consisted of water-formic acid (0.1%, v/v; solvent A) and acetonitrile (solvent B). A flow rate of 1 ml/min was maintained for the entire run. The following 1.4-minute linear gradient was used: start at 30% B, increase to 50% B over 0.5 minute, maintain 50% B for 0.3 minute, increase to 80% B over 0.2 minute, and maintain at 80% B for 0.3 minute. The column was re-equilibrated after each injection. Detection was performed by positive-ion MS/MS using a Turbolon-Spray interface on an API 4000 mass spectrometer with multiple reaction monitoring (m/z 520–267 for dabrafenib, m/z 529–267 for [2H6]dabrafenib, m/z 536–233 for hydroxy-dabrafenib, m/z 544–331 for [2H6 13C2]-hydroxy-dabrafenib, m/z 506–277 for desethyl-dabrafenib, and m/z 514–280 for [2H6 13C2]-desethyl-dabrafenib). Concentrations of carboxy-dabrafenib in plasma samples were determined with a separate validated analytical UHPLC-MS/MS method over a range of 5–5000 ng/ml. Carboxy-dabrafenib was extracted from 25 μl of human plasma by protein precipitation using 80/20 ethyl alcohol/MilliQ water (EMD Millipore, Danvers, MA) containing an isotopically labeled internal standard ([2H6 13C2]-carboxy-dabrafenib). Extracts (4 μl) were injected onto a Waters Acuity BEH Phenyl column (1.7 μm, 50 × 2.1 mm) maintained at 55°C. The mobile phase consisted of water-formic acid (0.1%, v/v; solvent A) and acetonitrile (solvent B). A flow rate of 1 ml/min was maintained for the entire run. The following 1.4-minute linear gradient was used: start at 30% B, increase to 50% B over 0.5 minute, maintain 50% B for 0.3 minute, increase to 80% B over 0.2 minute, and maintain at 80% B for 0.3 minute. The column was re-equilibrated after each injection. Detection was performed by positive-ion MS/MS using a Turbolon-Spray interface on an API 4000 mass spectrometer with multiple reaction monitoring (m/z 550–506 for carboxy-dabrafenib and m/z 558–514 for [2H6 13C2]-carboxy-dabrafenib).

Pharmacokinetic Analysis

PK parameters were determined for blood and plasma total radioactivity and plasma dabrafenib, hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib concentrations. PK parameters were calculated by standard noncompartamental analysis with WinNonlin Pro 5.2 (Pharsight, Mountain View, CA) by ICON Development Solutions (Marlow, UK). All calculations of noncompartamental parameters were based on actual sampling times. The PK endpoints of interest were area under the plasma-concentration time curve (AUC0–t and AUC0–∞), maximum plasma concentration (Cmax), time to Cmax (Tmax), and half-life (t1/2). Metabolite-to-parent ratios were calculated after correcting for differences in molecular weight.

The ratio of blood-to-plasma total radioactivity concentrations at each PK sampling time point was calculated to assess partitioning of dabrafenib-related material into red blood cells. Plasma ratio of AUC0–t to AUC0–∞ for dabrafenib and metabolites (corrected for differences in molecular weight) to total radiocarbon was determined.

Mass Spectroscopic Analysis

LC/MS® (Liquid Chromatography/Tandem Mass Spectrometry) was used to analyze representative samples of plasma extracts and urine and fecal homogenate extracts according to the conditions described previously. During the LC separation, a postcolumn split was used to direct approximately 15% of the sample to an LTQ-Orbitrap XL (ThermoFisher, San Jose, CA) mass spectrometer equipped with an electrospray ionization source, or an LTQ XL (ThermoFisher) mass spectrometer equipped with an electrospray ionization source, implementing data-dependent scanning by using a parent mass list. The parent mass list consisted of masses of all known and probable metabolites. A full-scan mass spectrum (at resolution 30,000 for the Orbitrap) was collected and the data interrogated in real time to identify mass peaks corresponding to masses in the parent mass list. If present, the parent mass peaks were selected as target peaks for subsequent MS3 scans. The remaining LC elute from the postcolumn split was directed into a Gilson 215 liquid handler (Gilson, Middleton, WI) with a collection time of approximately 11 seconds per well. All instruments used a LEAP autosampler (LEAP Technologies, Carborro, NC) for sample introduction. Data were acquired and processed using Xcalibur software (version 2.1; Thermo Scientific, Waltham, MA).

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Effect of pH on the Conversion of Carboxy-Dabrafenib to Desmethyl-Dabrafenib

Carboxy-dabrafenib (20 μg/ml) was incubated at 37°C in buffers of varying pH (7, 5, 4, and 3) for up to 1 hour. Each buffer (4.9 ml) was added to separate 15-ml tubes and preheated in a ThermoFisher reciprocal shaking bath for 15 minutes at 37°C. An aliquot (0.1 ml) of a 1 mg/ml carboxy-dabrafenib solution was added to each tube. Aliquots of the incubates were removed immediately and after approximately 1 hour and were then analyzed by LC/UV/MS. Peak areas from the UV signals corresponding to the carboxy- and desmethyl-dabrafenib retention times and MS signals versus authentic standards were determined and are presented as a percentage of the total amount of carboxy-dabrafenib in the pH 7 incubate aliquot at t = 0 or t = 1 hour.

Results

Subject Demographics and Safety. Four subjects were enrolled and completed the study. Two subjects remained in the study for 11 days after dosing, whereas the other two subjects were discharged on day 9 and day 10 after >90% of the total dose was recovered, although one subject did not meet the early stopping criteria of two consecutive 24-hour excreta collections containing ≤1% of the dose (the final two 24-hour fecal collections for this subject contained 1.62% and 0.23%). All subjects were Caucasian, three male and one female, with ages ranging from 25 to 57 years and body weights ranging from 57.0 to 94.9 kg. All subjects had stage IV, metastatic BRAF V600 mutation-positive melanoma with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 (n = 1) or 1 (n = 3) at screening.

All adverse events were grade 1 or grade 2 except for one adverse event of dehydration, which was grade 3 and not considered related to the study drug. The most common adverse event was diarrhea, which was reported in two out of four subjects. There were no serious adverse events, no withdrawals due to adverse events, and no adverse events related to changes in clinical laboratory parameters, electrocardiograms, or vital signs.

Pharmacokinetics. The PK parameters for dabrafenib and its metabolites and for total radioactivity (blood and plasma) are summarized in Table 2; the PK profiles are shown in Fig. 1. The median time to peak dabrafenib concentration was 1.0 hour after administration of the oral suspension. The median \( T_{\text{max}} \) for radioactivity in blood and plasma was delayed relative to dabrafenib in plasma, consistent with the formation and circulation of metabolites. The maximum plasma concentrations of metabolites hydroxy-, carboxy-, and desmethyl-dabrafenib were achieved at 3.0, 10, and 30 hours postdose, respectively. The geometric mean plasma half-life of dabrafenib and its primary metabolite hydroxy-dabrafenib were similar (5.3 and 5.7 hours, respectively). Metabolites carboxy-dabrafenib and desmethyl-dabrafenib had longer half-lives (17.5 and 20.4 hours, respectively) relative to the parent (Fig. 1, upper panel). The metabolite-to-parent AUC\(_{\text{0-\infty}} \) ratios for hydroxy-, carboxy-, and desmethyl-dabrafenib were 0.75, 4.9, and 0.26, respectively, with carboxy-dabrafenib being the most abundant metabolite in circulation.

The contribution of the parent drug and its metabolites to total plasma radioactivity is illustrated in Fig. 1 (lower panel), which shows the plasma concentration-time profiles of dabrafenib, the total concentration of dabrafenib plus its three main metabolites with metabolite concentrations adjusted for differences in molecular weights, and total radioactivity. As shown, dabrafenib and its three metabolites account for the majority of the plasma radioactivity profile. Using the geometric mean plasma AUC\(_{\text{0-\infty}} \) ratios of dabrafenib to total plasma radioactivity, the percentage of total radioactivity in the form of parent compound was approximately 11%. The metabolites hydroxy-, carboxy-, and desmethyl-dabrafenib accounted for approximately 8%, 54%, and 3% of plasma radioactivity, respectively.

The mean blood-to-plasma ratio of total radioactivity concentration ranged from 0.58 to 0.70 across the four subjects, suggesting minimal association of dabrafenib drug-related material (DRM) with red blood cells.

Metabolite Profiles in Plasma. The relative amounts of dabrafenib and quantifiable metabolites in plasma after oral administration of \(^{[14C]}\)dabrafenib are shown in Table 2. Recovery of radioactivity from the plasma following extraction with organic solvent was high (range of 88%–99%). Consistent with the concentration data determined using the validated UHPLC-MS/MS assay, dabrafenib, hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib were notable radiolabeled components in plasma extracts from all four subjects. Dabrafenib was the predominant circulating component at 2 hours postdose, with carboxy-dabrafenib becoming the predominant component by 10 hours postdose. At 24 hours postdose, concentrations of dabrafenib represented less than 1.4% of circulating DRM, with carboxy-dabrafenib as the predominant component in all subjects (44.8%). The remaining radioactivity not accounted for by dabrafenib and its three main metabolites can be accounted for by circulating downstream metabolites of desmethyl-dabrafenib (M26, M28, M29, M30, and M31), observed mostly at the 24-hour time point, plus any trace metabolites not detected by HPLC radioprofiling.

There were no significant qualitative or quantitative differences between the radioprofiles of plasma samples from subjects 401, 403, and 404, with representative profiles depicted in Fig. 2. Subject 406 had a different radioprofile, notable for additional oxidative metabolites and higher levels of the downstream metabolites of desmethyl-dabrafenib at the later plasma time points. Moreover, at 24 hours postdose, carboxy-dabrafenib, desmethyl-dabrafenib, and two additional metabolites (M26 and M31) were all predominant circulating species (10%–29% DRM) in this subject. Several minor metabolites (below the lower limit of quantitation), derived from the further

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( T_{\text{max}} )</th>
<th>( C_{\text{max}} )</th>
<th>AUC(_{\text{0-\infty}} )</th>
<th>( T_{\text{1/2}} )</th>
<th>Ratio M/P</th>
<th>Ratio C/TR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood radioactivity</td>
<td>5.0 (2.0–8.0)</td>
<td>1616 (27)</td>
<td>37,028 (53)</td>
<td>20.8 (56)</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Plasma radioactivity</td>
<td>3.0 (2.0–8.0)</td>
<td>2364 (18)</td>
<td>59,447 (48)</td>
<td>26.4 (44)</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Plasma dabrafenib</td>
<td>1.0 (0.6–2.0)</td>
<td>1662 (31)</td>
<td>6535 (28)</td>
<td>5.3 (28)</td>
<td>N.A.</td>
<td>0.11 (35)</td>
</tr>
<tr>
<td>Plasma hydroxy-dabrafenib</td>
<td>3.0 (2.0–4.0)</td>
<td>666 (29)</td>
<td>5022 (31)</td>
<td>5.7 (34)</td>
<td>0.75 (15)</td>
<td>0.08 (23)</td>
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<tr>
<td>Plasma carboxy-dabrafenib</td>
<td>10.0 (6.1–12.0)</td>
<td>1283 (23)</td>
<td>33,948 (57)</td>
<td>17.5 (36)</td>
<td>4.91 (50)</td>
<td>0.54 (17)</td>
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<tr>
<td>Plasma desmethyl-dabrafenib</td>
<td>30.0 (12.0–36.0)</td>
<td>36 (87)</td>
<td>1674 (59)</td>
<td>20.4 (21)</td>
<td>0.26 (53)</td>
<td>0.03 (35)</td>
</tr>
</tbody>
</table>

\( C: \) compound (parent or metabolite) AUC\(_{\text{0-\infty}} \), M, metabolite AUC\(_{\text{0-\infty}} \), N.A., not applicable; P, parent (dabrafenib) AUC\(_{\text{0-\infty}} \), TR, total radioactivity in plasma AUC\(_{\text{0-\infty}} \).
S.D. recovery of 71.1% related material was predominantly excreted in feces, with a mean across all four subjects. Other subjects, but the metabolite-to-parent AUC ratios were similar 12 versus 24 hours and 16 versus 20 hours. 

The sequential metabolic transformation in subject 406 appeared to be more efficient, with more rapid appearance of carboxy- and desmethyl-dabrafenib. The percentage of the dose, with the other radiocomponents each accounting for less than 3.5% of the dose in these 3 subjects. There were minimal differences, qualitatively and quantitatively, in the radioprofiles between subjects 401, 403, and 404. In subject 406, carboxy-dabrafenib, M28/M29, and M31 were the predominant urinary radiocomponents (3.7%–5.9% of the dose), with the other radiocomponents each accounting for <2% of the dose.

**Metabolite Profiles in Urine.** Representative radio-HPLC chromatograms from human urine are shown in Supplemental Fig. 1. Individual and mean quantification data for the major metabolites, expressed as a percentage of the administered dose, are given in Supplemental Table 1. Unchanged dabrafenib was not detected in urine, whereas M3 (a glucuronide of hydroxy-dabrafenib) was detected only in urine. In subjects 401, 403, and 404, carboxy-dabrafenib was the predominant radiocomponent in the pooled urine samples (6%–12% of the dose), with the other urinary radiocomponents each accounting for less than 3.5% of the dose in these 3 subjects. There were minimal differences, qualitatively and quantitatively, in the radioprofiles between subjects 401, 403, and 404. In subject 406, carboxy-dabrafenib, M28/M29, and M26 were the predominant urinary radiocomponents (3.7%–5.9% of the dose), with the other radiocomponents each accounting for <2% of the dose.

**Metabolite Profiles in Feces.** Representative radio-HPLC chromatograms obtained from analysis of human fecal extracts are shown in Supplemental Fig. 2. Individual and mean quantification data for the major metabolites are provided in Supplemental Table 2. Average levels of circulating dabrafenib and metabolites in humans after a single oral administration of 95 mg of [14C]dabrafenib Data are the mean (n = 4).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>2 h</th>
<th>6 h</th>
<th>10 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dabrafenib</td>
<td>64.6</td>
<td>22.8</td>
<td>7.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Hydroxy-dabrafenib</td>
<td>19.4</td>
<td>18.4</td>
<td>10.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Carboxy-dabrafenib</td>
<td>4.1</td>
<td>34.2</td>
<td>51.1</td>
<td>44.8</td>
</tr>
<tr>
<td>Desmethyl-dabrafenib</td>
<td>N.D.</td>
<td>6.6</td>
<td>11.6</td>
<td>13.0</td>
</tr>
<tr>
<td>M28</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>M28/M29</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D./N.Q</td>
</tr>
<tr>
<td>M30</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D./N.Q</td>
</tr>
<tr>
<td>M31</td>
<td>2.6</td>
<td>4.7</td>
<td>6.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Total quantified</td>
<td>88.1</td>
<td>81.9</td>
<td>82.5</td>
<td>69.1</td>
</tr>
</tbody>
</table>

N.D., not detected; N.Q., below level of quantitation by radioprofiling.

* Dabrafenib was quantifiable by radioprofiling only in subjects 401 (3.4%) and 404 (2.2%) at 24 hours and was not quantifiable by radioprofiling in other subjects.

* Hydroxy-dabrafenib was quantifiable by radioprofiling only in subjects 401 (3.6%) and 404 (2.6%) at 24 hours and was not quantifiable by radioprofiling in other subjects.

* Carboxy-dabrafenib was quantifiable by radioprofiling only in subjects 403 (5.3%) and 406 (11.2%) at 2 hours and was not quantifiable by radioprofiling in other subjects.

* M26 was quantifiable by radioprofiling only in subject 406 at 10 (1.9%) and 24 hours (10.1%) and was not quantifiable by radioprofiling in other subjects.

* M31 was quantifiable by radioprofiling only in subject 406 at 10 hours (3.4%) and was not quantifiable by radioprofiling in other subjects.

**Mass Balance.** The mean cumulative percentage of radioactive dose recovered in urine and feces is illustrated in Fig. 3. [14C]Dabrafenib-related material was predominantly excreted in feces, with a mean ± S.D. recovery of 71.1% ± 8.1% of the administered dose. Urinary excretion accounted for 22.7% ± 4.7% of the dose. Total recovery of radioactivity in all excreta varied from 88.4% to 100% (mean recovery of 93.8%), with the majority recovered within the first 4 days (85.6%).
metabolites carboxy-dabrafenib and hydroxy-dabrafenib were detected in fecal samples from all four subjects. Four minor metabolites, products of further oxidation of desmethyl-dabrafenib (i.e., M26, M28, M29, and M30), were only detected in the feces of subject 406, with each accounting for <5% of the dose. As the synthetic standard of carboxy-dabrafenib has been shown to undergo decarboxylation at a low pH or in the presence of aprotic solvents, it is possible that ex vivo decarboxylation of carboxy-dabrafenib to form desmethyl-dabrafenib may have contributed to the levels of desmethyl-dabrafenib detected in feces.

Fig. 2. Representative HPLC radiochromatograms of plasma extracts after a single oral administration of [14C]dabrafenib (95 mg). CDAB, carboxy-dabrafenib; DAB, dabrafenib; DDAB, desmethyl-dabrafenib; HDAB, hydroxyl-dabrafenib.
Structural Characterization of Metabolites. Metabolite structures were characterized by LC/MS\textsuperscript{n} analysis from plasma, urine, and fecal homogenate extracts (a summary of metabolite structures and MS\textsuperscript{n} fragmentation data are available in Supplemental Table 2). Similarities in HPLC retention times and mass spectrometric fragmentation patterns with those obtained from the available standards facilitated the identification of carboxy-dabrafenib, hydroxy-dabrafenib, and desmethyl-dabrafenib. Structures of other metabolites were proposed based on their mass spectrometric fragmentation patterns relative to dabrafenib or these known metabolites.

M30 was characterized as an oxidation product of desmethyl-dabrafenib. MS/MS generated a fragment ion 44 atomic mass units less than the protonated molecular ion, indicating a loss of carbon dioxide (carboxy-dabrafenib exhibited a similar fragmentation). Further fragmentation generated an ion with an additional loss of 220 atomic mass units. This corresponds to cleavage of the isopropyl and the difluorophenyl sulfone moieties; accurate mass analysis of this ion does not indicate the presence of any oxygen molecules. Therefore, M30 results from oxidation of both isopropyl terminal methyl groups, one to the alcohol and the other to the acid.

M31 can be assigned as the unsaturated (propenyl) derivative, since the isopropyl moiety is the only position that would support the loss of two hydrogen atoms. There was insufficient spectroscopic data for definitive structural assignments of the other downstream oxidative metabolites of desmethyl-dabrafenib.

Effect of pH on Carboxy-Dabrafenib to Desmethyl-Dabrafenib Conversion. While carboxy-dabrafenib was stable at pH 7, this metabolite decarboxylated to generate desmethyl-dabrafenib at pH 4 and pH 3, with some conversion noted at pH 5 (Fig. 4). Conversion of carboxy-dabrafenib to desmethyl-dabrafenib was greater following incubation for 1 hour at 37°C.

Discussion

Disposition of Dabrafenib in Humans. The human biotransformation of dabrafenib is illustrated in Fig. 5. Following oral absorption, dabrafenib is primarily transformed via oxidation of the t-butyl group to form hydroxy-dabrafenib. Hydroxy-dabrafenib is further oxidized to the carboxylic acid derivative, carboxy-dabrafenib, and, based on urinary data, can also undergo phase II conjugation to generate hydroxy-dabrafenib O-glucuronide (M3). Carboxy-dabrafenib circulates until it undergoes decarboxylation to form desmethyl-dabrafenib via a nonenzymatic process or is excreted in urine or bile. Following biliary secretion, it is possible that carboxy-dabrafenib may also undergo decarboxylation in the gut to form desmethyl-dabrafenib, which is either excreted in feces or potentially absorbed back into the bloodstream. Circulating desmethyl-dabrafenib is further metabolized to downstream oxidative metabolites.

The main circulating drug-related components were dabrafenib, hydroxy-dabrafenib, carboxy-dabrafenib, and desmethyl-dabrafenib, which together account for 76% of circulating radioactivity in humans. As demonstrated in the PK profiles, dabrafenib was the predominant circulating component at earlier times postdose, with hydroxy-dabrafenib becoming the predominant component at later time points. The terminal phase of hydroxy-dabrafenib parallels that of the parent, suggesting that it is a formation rate–limiting metabolite. In contrast, for carboxy-dabrafenib, its concentrations peaked later, it exhibited a longer half-life, and it is considered to be elimination rate–limited. The desmethyl-dabrafenib terminal phase parallels that of carboxy-dabrafenib, and is thought to be formation rate–limited relative to carboxy-dabrafenib. Both carboxy- and desmethyl-dabrafenib are predicted to accumulate with repeat dosing.

The pharmacology of dabrafenib metabolites was evaluated using preclinical assays in BRAF V600 mutant enzymes or cell lines (Adams et al., 2009). Relative potency generally ranked in the following order: dabrafenib > hydroxy-dabrafenib ~ desmethyl-dabrafenib > > carboxy-dabrafenib. Based on our current understanding, the relative contribution of metabolites to clinical activity depends on the steady-state exposure of metabolites and the parent. Dabrafenib, hydroxy-dabrafenib, and desmethyl-dabrafenib may contribute to clinical activity, whereas contribution of carboxy-dabrafenib is assumed to be minimal despite high plasma exposure at steady state.
These metabolites were identified early in the program in preclinical studies and have been measured in patients starting with the first-time-in-human study (study BRF112680) to better understand their disposition and contribution to the clinical efficacy and safety profile of dabrafenib. The biotransformation of dabrafenib was qualitatively similar between humans and the nonclinical toxicology species (i.e., rat and dog); however, to establish coverage for the main circulating metabolites, additional studies were conducted in the mouse (Richards-Peterson et al., 2013).

Excretion of Dabrafenib in Humans and Clinical Implications. Following a single oral administration of [14C]dabrafenib, fecal excretion is the major route of elimination, accounting for 71.1% of radioactive dose, whereas urinary excretion accounted for 22.7% of administered radioactivity. The predominant radiolabeled component in urine was carboxy-dabrafenib, accounting for 7% of the dose, whereas M28 and M29 were predominant in one subject (6% of the dose, together). In addition, hydroxy-dabrafenib, desmethyl-dabrafenib, M3, M26, and M30 were detected in urine samples (<2.1% for each), whereas unchanged dabrafenib was not observed in urine.

In feces, dabrafenib was predominant, accounting for 21.8% of the radiolabeled suspension dose, whereas desmethyl-dabrafenib, carboxy-dabrafenib, and hydroxy-dabrafenib accounted for 14.4%, 9.5%, and 4.5% of the dose, respectively. Other metabolites including M26, M28/M29, and M30, each representing 2.1%–3.1% of the administered dose, were detected in feces in only one subject. Of the total amount of unchanged parent excreted in the feces, it is unclear what proportion was due to unabsorbed drug versus biliary elimination of dabrafenib. The elimination of dabrafenib occurred predominantly by oxidative metabolism (48% of the dose), based on the total recovery of oxidative metabolites in urine and feces. Thus, renal impairment is unlikely to significantly impact exposure to parent dabrafenib, whereas hepatic impairment may alter its exposure.

Investigation of the Decarboxylation of Carboxy-Dabrafenib to Desmethyl-Dabrafenib. The first two steps of the metabolism of dabrafenib are oxidative (i.e., hydroxy-dabrafenib, then carboxy-dabrafenib formation) and mediated by cytochrome P450 enzymes (GlaxoSmithKline, 2013), whereas the final step (decarboxylation) does not require oxidative enzymes. The decarboxylation of
carboxy-dabrafenib to generate desmethyl-dabrafenib was investigated in vitro by incubation of the synthetic carboxy-dabrafenib standard in buffers of pH 7, 5, 4, and 3. Although some conversion was seen immediately at pH 3 and 4, extensive conversion was detected following a 1-hour incubation, as well as some conversion at pH 5 (Fig. 4). Decarboxylation in the range of pH 3–4 corresponds to the pKa of the carboxylic acid of carboxy-dabrafenib, calculated to be 4.24 (ChemAxon v5.4.1.1; ChemAxon, Budapest, Hungary). Therefore, decarboxylation of carboxy-dabrafenib is catalyzed by acidic, but not neutral, aqueous conditions. Based on these data, we have proposed an acid-catalyzed mechanism of carboxy-dabrafenib decarboxylation to generate desmethyl-dabrafenib (Fig. 6) involving participation of the aryl nitrogen of the thiazole ring as a Lewis base for the carboxylic acid proton (available only near or below its pKa). A 6-membered cyclic transition state would drive the decarboxylation of the protonated carboxylic acid. This proposed mechanism is a novel variant of the accepted mechanism for decarboxylation of 1,3-dicarboxylic acids or β-keto acids. Carboxy-dabrafenib was stable in cofactor-fortified human liver microsomes, yet was converted to desmethyl-dabrafenib in hepatocytes from human and nonclinical species (data not shown). Thus, the decarboxylation likely occurs nonenzymatically in an acidic hepatocellular compartment (e.g., lysosome) (Chan et al., 2006); however, contribution from cytosolic enzymes cannot be ruled out.

Another example of nonenzymatic decarboxylation of a carboxylic acid metabolite was described for LC15-0133 (1-(5-tert-butyl-[1,3,4] oxadiazole-2-carbonyl)-4-fluoro-pyrrolidin-1-yl)-2-(2-hydroxy-1,1-dimethyl-ethylamino)-ethanone), a t-butyl oxadiazole-containing compound previously in development for treatment of diabetes, using in vitro liver metabolism systems (Yoo et al., 2008). When incubated with D2O, the uptake of deuterium occurred on the resulting isopropyl methine carbon in rat liver microsomes. Also, the isolated carboxylic acid metabolite of LC15-0133 decarboxylated nonenzymatically in solution at a high pH or under heated conditions, although we believe that the proposed base-catalyzed mechanism is unlikely to occur in a biological system. Rather, a possible mechanism of LC15-0133 metabolite decarboxylation would involve protonation of the carboxylic acid, and participation of the oxadiazole nitrogen as a Lewis base, analogous to what we have proposed for carboxy-dabrafenib. Indeed, the microsomal incubation was quenched with 1% acetic acid, potentially catalyzing some decarboxylation postincubation. Further evidence of nonenzymatic, acid-catalyzed decarboxylation (at room temperature) has been described in the chemistry literature for benzo-thiazol-2-yl glyoxalic acid, and the proposed mechanism involves participation of the adjacent thiazole nitrogen (Baudet and Otten, 1970).

Carbon-carbon bond cleavage of an aryl-alkyl side chain was also described for torcetrapib in preclinical species, in which an aryl-ethy!- to aryl-methyl conversion occurred from an intermediate metabolite containing a quinoline ring (Dalvie et al., 2008). The authors proposed a mechanism of oxidation of the terminal carbon of the ethyl group to a carboxylic acid intermediate with subsequent decarboxylation. We suggest that this intermediate is well positioned for a 6-membered cyclic decarboxylation reaction to generate desmethyl-dabrafenib (Fig. 6) involving participation of the aryl nitrogen.

In the absence of an aryl nitrogen, C-demethylation of an alkyl side chain following enzymatic oxidation may still occur, as demonstrated for a prostaglandin antagonist, CP-533,536 [(3-[(4-tet-butylbenzyl)-(pyridine-3-sulfonyl)-amino]-methyl-phenoxy)-acetic acid], but would proceed via an alternative mechanism not involving decarboxylation (Prakash et al., 2008). An example of aryl t-butyl carboxylic acid formation without subsequent C-demethylation is illustrated by terfenadine (Seldane), for which a t-butyl moiety is metabolically oxidized to a stable carboxylic acid, fexofenadine (Allegra), in the absence of a nitrogen in the adjacent aryl ring (Garteiz et al., 1982).

These examples illustrate that a drug structure containing an aryl nitrogen moiety with an alkyl (ethyl or t-butyl) side chain in the α position can undergo carbon-carbon bond cleavage of the alkyl terminal methyl. This occurs by enzymatic oxidation with subsequent pH-dependent decarboxylation via a 6-membered cyclic transition state involving the aryl nitrogen.

**Oxidative Metabolism of Desmethyl-Dabrafenib.** Downstream oxidative metabolites of desmethyl-dabrafenib were detected at low concentrations in human plasma, urine, and/or feces. The formation of M31, the propenyl-substituted thiazole, is likely derived from oxidation of the isopropyl methine carbon in a manner analogous to that described for ezlopitant (Obach, 2001), whereas M30 results from oxidation of the terminal isopropyl methyls to an alcohol and a carboxylic

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**Fig. 6.** Proposed mechanism of carbon-carbon bond cleavage of dabrafenib via pH-dependent decarboxylation following metabolic oxidation.
acid. The remaining oxidative metabolites (M26, M28, and M29) could not be definitively identified with the available data.

**Conclusions.** In conclusion, the metabolic pathway and excretion of dabrafenib, including characterization of its major circulating metabolites, was described and is based on results from this study. Dabrafenib is sequentially oxidized to hydroxy- and carboxy-dabrafenib, which then undergoes decarboxylation to form desmethyl-dabrafenib. The proposed pH-dependent decarboxylation mechanism follows metabolic oxidation of the t-butyl methyl of dabrafenib allows for the prediction of alkyl carbon-bond cleavage (C-demethylation) for drugs containing an aryl nitrogen positioned α to an ethyl or t-butyl side chain. Furthermore, the thorough understanding of human biotransformation and disposition this study provided was crucial in guiding the nonclinical and clinical development of dabrafenib, especially in establishing the relative pharmacokinetic exposures of the metabolites in relevant nonclinical toxicology species. This understanding allowed for more complete management of the clinical usage of this agent for disease treatment, robust modeling for the prediction of drug-drug interactions with concomitant medications, and informed recommendations for hepatic or renal impairment patients.

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**Authorship Contribution**

**Participated in research design:** Ouellet, Carson, Blackman, Morrison, Gorycki, Richards-Peterson.

**Conducted experiments:** Bershas, Mamari-Fishman, Morrison, Jurusik, Knecht.

**Performed data analysis:** Bershas, Ouellet, Mamari-Fishman, Nebot, Adams, Jurusik, Gorycki, Richards-Peterson.

**Wrote or contributed to the writing of the manuscript:** Bershas, Ouellet, Mamari-Fishman, Nebot, Carson, Blackman, Morrison, Adams, Jurusik, Knecht, Gorycki, Richards-Peterson.

**References**


Code of Federal Regulations (Revised as of April 1, 2013). Radioactive drugs for certain research uses. 21CFR361.1.


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