Mass Balance Study of \[^{14}\text{C}]\text{Eribulin in Patients with Advanced Solid Tumors

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ABSTRACT:

This mass balance study investigated the metabolism and excretion of eribulin, a nontaxane microtubule dynamics inhibitor with a novel mechanism of action, in patients with advanced solid tumors. A single approximately 2 mg (approximately 80 \(\mu\)Ci) dose of \[^{14}\text{C}]\text{Eribulin acetate was administered as a 2 to 5 min bolus injection to six patients on day 1. Blood, urine, and fecal samples were collected at specified time points on days 1 to 8 or until sample radioactivity was \\
\leq 1\% of the administered dose. Mean plasma eribulin exposure (627 ng \cdot h/ml) was comparable with that of total radioactivity (668 ng Eq \cdot h/ml). Time-matched concentration ratios of eribulin to total radioactivity approached unity in blood and plasma, indicating that unchanged parent compound constituted almost all of the eribulin-derived radioactivity. Only minor metabolites were detected in plasma samples up to 60 min postdose, pooled across patients, each metabolite representing \leq 0.6\% of eribulin. Elimination half-lives for eribulin (45.6 h) and total radioactivity (42.3 h) were comparable. Eribulin-derived radioactivity excreted in feces was 81.5\%, and that of unchanged eribulin was 61.9\%. Renal clearance (0.301 l/h) was a minor component of total eribulin clearance (3.93 l/h). Eribulin-derived radioactivity excreted in urine (8.9\%) was comparable with that of unchanged eribulin (8.1\%), indicating minimal excretion of metabolite(s) in urine. Total recovery of the radioactive dose was 90.4\% in urine and feces. Overall, no major metabolites of eribulin were detected in plasma. Eribulin is eliminated primarily unchanged in feces, whereas urine constitutes a minor route of elimination.

Introduction

Eribulin (Fig. 1) is a synthetic analog of halichondrin B, a complex polyether macrolide first isolated from the marine sponge \textit{Halichondria okadai} Kadota by Hirata and Uemura (1986). In human tumor cell lines, halichondrin B proved to be highly cytotoxic, causing mitotic cell cycle arrest by inhibiting the polymerization of tubulin and microtubule assembly (Bai et al., 1991). The naturally low abundance of the molecule led to the development of complex methods for total synthesis and eventually to the discovery of eribulin (Jackson et al., 2009), a structurally simplified halichondrin B analog with similar or identical anticancer properties in preclinical models (Towle et al., 2001). In clinical studies, eribulin has demonstrated antitumor activity in extensively pretreated patients who had advanced or metastatic breast cancer (MBC) with manageable tolerability (Vahdat et al., 2008, 2009; Cortes et al., 2010). A multicenter, randomized, phase III study involving heavily pretreated patients with MBC showed a significant improvement in median overall survival by 2.5 months for the patients treated with eribulin (\(n = 508\)) compared with that of patients who received a treatment of the physician’s choice (\(n = 254\)) (Cortes et al., 2011). Eribulin mesylate was approved recently in the United States for the treatment of patients with MBC who have received previously at least two chemotherapy regimens for the treatment of metastatic disease. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or the metastatic setting.

Knowledge about the metabolism and excretion of a new drug is essential for its approval. Over the past few years, interest in drug metabolism has

ABBREVIATIONS: MBC, metastatic breast cancer; ACN, acetonitrile; AE, adverse event; AUC, area under the concentration-time curve; CL, clearance; CLr, renal clearance; Eot, end of the 2 to 5 min \[^{14}\text{C}]\text{Eribulin infusion; FDA, U.S. Food and Drug Administration; HPLC, high-performance liquid chromatography; ICH, International Conference on Harmonisation; LC-MS/MS, liquid chromatography tandem mass spectrometry; LLOQ, lower limit of quantitation; LOD, limit of detection; LSC, liquid scintillation counting; PEG, polyethylene glycol; TRA, total radioactivity; ULN, upper limit of normal; \(V_s\), apparent volume of distribution.
increased rapidly due to discussion on the potential contribution of drug metabolites to toxicity. Within this scope, the U.S. Food and Drug Administration (FDA) and the International Conference on Harmonisation (ICH) recently issued guidance for metabolites in Safety Testing, recommending additional safety assessments for major metabolites (Guidance for Industry, Safety Testing of Drug Metabolites, http://www.fda.gov/downloads/Drugs/Guidance/UCM079266.pdf; ICH, Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002720.pdf). Major metabolites are defined herein as metabolites with a systemic exposure >10% of the parent (FDA) or the total drug-related (ICH) exposure that are either identified only in human plasma or present at disproportionally higher levels in humans than in any preclinical test species.

Knowledge about drug excretion is particularly important in the treatment of patients with impaired renal or liver function, because clinical implications of urinary or biliary excreted drugs may be more pronounced for these patients, with potential need for dosiage adjustments.

Preclinical studies showed that eribulin is metabolized predominantly by CYP3A4 (Zhang et al., 2008). In clinical studies, eribulin was found to be minimally eliminated in its unchanged form in urine (Goel et al., 2009; Tan et al., 2009). The objective of this study was to determine the metabolism and excretion of eribulin after a single dose of [14C]eribulin in patients with advanced solid tumors. Pharmacokinetics and excretion of both unchanged drug and total radioactivity (comprising parent drug and metabolites) were determined using validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assays and liquid scintillation counting (LSC), respectively. Radiochromatography and high-resolution mass spectrometry were combined to detect and identify eribulin metabolites.

Materials and Methods

Compounds and Reagents. [14C]Eribulin acetate was manufactured by GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK) and provided by Eisai Ltd. (Hatfield, Hertfordshire, UK). Sterile water for injection, normal saline (0.9% NaCl), and distilled water for sample preparation originated from B. Braun (Melsungen, Germany). All of the solvents for sample preparation and high-performance liquid chromatography (HPLC) were analytical or HPLC grade. Methanol and acetonitrile (ACN) were obtained from Biosolve (Valkenswaard, The Netherlands). Ammonium acetate was purchased from Fluka (Buchs, Switzerland), and water for mobile phase preparation (LiChrosolv), isopropanol, EDTA, 30% hydrogen peroxide (w/w), and acetic acid originated from Merck (Darmstadt, Germany). Solvable and Ultima Gold liquid scintillation cocktail were obtained from PerkinElmer Life and Analytical Sciences (Waltham, MA).

Patients. Patients aged at least 18 years with a histologically or cytologically confirmed advanced solid tumor that had progressed after standard therapy or for which no standard therapy existed were eligible for this study. Prior chemotherapy other than eribulin, mitomycin C, or nitrosourea was allowed, as was radiation or biological therapy, provided that the last treatment was at least 3 weeks before study entry (4 weeks for investigational drugs and alternative therapies). Other eligibility criteria included: Eastern Cooperative Oncology Group performance status ≤ 2, adequate renal function [serum creatinine ≤ 135 μM (≤1.5 mg/dl) or creatinine clearance ≥ 40 ml/min], adequate bone marrow function (absolute neutrophil count ≥ 5 × 10^9 neutrophils per liter and platelet count ≥ 100 × 10^9 platelets per liter), adequate hepatic function (bilirubin ≤ 1.5 times the upper limit of normal (ULN) and alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase ≤ 3 times ULN or ≤ 5 times ULN in the case of liver metastases), and resolution of all chemotherapy- or radiotherapy-related toxicities to ≤ grade 1, except for stable sensory neuropathy ≤ grade 2 and alopecia.

Exclusion criteria included the presence of severe intercurrent illness or infection, significant cardiovascular impairment, known positive human immunodeficiency virus status, major surgery within 4 weeks before the start of treatment, pulmonary dysfunction requiring active treatment, treatment with warfarin or related compounds other than for line patency, pregnancy and breastfeeding, meningeal carcinomatosis, the pre-existing neuropathy > grade 2, organ allografts requiring immunosuppression, radiation therapy that encompassed >30% of marrow, and active brain metastases.

Study Design. Six patients with advanced solid tumors were enrolled into this phase I, open-label, nonrandomized, single-center (The Netherlands Cancer Institute, Amsterdam, the Netherlands) study. Patients received a total dose of 2 mg of [14C]eribulin acetate (approximately 80–90 μCi) on day 1 of cycle 1 after physical examination, vital signs, electrocardiogram, and complete laboratory tests for safety were completed. During the study phase (days 1–8), patients remained hospitalized for the collection of blood samples and excreta. Collection of urine and feces was continued until the radioactivity levels of 24-h collections were <1% of the administered dose. Patients received 1.4 mg/m^2 of unlabeled eribulin mesylate on day 8 of cycle 1. However, if sample collection was continued beyond day 8, then the day 8 dose was delayed until the completion of the sample collection. From cycle 2 onward, patients could enter the extension phase of the trial and receive 1.4 mg/m^2 of unlabeled eribulin mesylate on days 1 and 8 of each subsequent 21-day cycle. Patients were permitted to continue their participation in the study and receive eribulin treatment if they had stable disease or partial or complete response to treatment, assessed according to response evaluation criteria in solid tumors. The safety of eribulin was assessed by measuring vital signs, laboratory testing, physical examinations, and documenting concomitant medication and adverse events.

The study was conducted in accordance with the ICH guidelines for Good Clinical Practice (CPMP/I/135/95), the European Clinical Trials Directive (2001/20/EC), and the Declaration of Helsinki. The protocol was approved by The Netherlands Cancer Institute Independent Ethics Committee.

[14C]Eribulin Drug Formulation and Administration. Individual aseptic preparations of [14C]eribulin infusions were prepared in a laminar flow cabinet, situated in a laboratory suited for handling radiolabeled drugs. [14C]Eribulin acetate was provided in vials containing 0.5 ml of ethanol with 10 mg/ml eribulin acetate at a specific activity of 40 μCi/mg (chemical and radiochemical purity >99.6%). A volume of 0.2 ml was extracted from the vial, added to 5 ml of sterile water for injection, and combined with 45 ml of normal saline (0.9% NaCl). The solution was filtered through a 0.22-μm syringe filter (Millipore Corporation, Billerica, MA). A 0.5-ml aliquot was separated from the final dosing solution for analysis, and two 200-μl aliquots thereof were mixed with 10 ml of the liquid scintillation cocktail, analyzed using LSC, and used to calculate the total radioactivity (TRA) of the final dosing solution. The final dosing solution was administered to the patient by a syringe pump as a 2- to 5-min bolus infusion, which was followed by the administration of normal saline to flush the lines. Remaining radioactivity was determined in both tubing and syringe. This was done by flushing them with a known volume of normal saline and analysis of a 1-ml aliquot for TRA. The actual administered activity was calculated by subtracting the radioactivity remaining in the dosing system from the radioactivity in the final solution.
Sample Collection. Venous blood samples (6 ml) were collected predose, at the end of the 2 to 5 min [14C]eribulin infusion (EoI) and at 5, 15, 30 min, 1, 2, 4, 6, 8, 10, 24, 48, 72, 96, 120, 144, and 168 h after EoI. The blood samples were collected in lithium heparinized tubes, inverted gently, and centrifuged at 2000g and 4°C for 15 min. Two 0.2-ml aliquots of the plasma layer were transferred to scintillation vials for TRA determination, and the remainder was divided between two labeled polypropylene tubes for quantitative bioanalysis and for metabolite profiling and identification.

Whole-blood samples (4 ml) were collected predose, at EoI and 5, 15, 30 min, 1, 2, 4, 8, 24, 72, and 168 h after EoI. Two 0.2-ml aliquots were transferred to scintillation vials to measure TRA, and the remainder was divided between two labeled polypropylene tubes for quantitative bioanalysis. Urine samples were collected before [14C]eribulin administration, over 6-h periods for the first 48 h after administration, and then over 24-h periods until day 8 or longer (i.e., until the daily urinary recovery was <1% of the administered activity). Total mass and collection time were recorded, a 1-ml aliquot was transferred to a scintillation vial to determine TRA, and 5- and 25-ml aliquots were stored for quantitative bioanalysis and for metabolite profiling and identification.

Predose fecal samples were collected, and all of the fecal portions produced after administration were collected individually in preweighed containers until day 8 or longer (i.e., until the daily fecal recovery was <1% of the administered activity). Samples were refrigerated until homogenization with water at a 1:3 (w/v) ratio. Three aliquots of 0.2 ml were used to determine TRA, and 1-, 5-, and 25-ml aliquots were stored for quantitative bioanalysis and for metabolite profiling and identification.

All of the samples were stored at nominally −70°C. The samples were collected for the following: 1) measurement of TRA with LSC (to determine the concentrations of all of the eribulin-related 14C-labeled compounds combined); 2) quantitative bioanalysis with LC-MS/MS (to determine the concentrations of unchanged eribulin); 3) metabolite profiling with LC-LSC-MS/MS (to detect and quantify eribulin metabolites); and 4) metabolite identification with LC-LTQ Orbitrap MS.

TRA. After the exact sample sizes by mass were recorded, radioactivity was determined by LSC. Before the scintillation cocktail was added, whole-blood and fecal homogenate samples were prepared (dissolved and decolorized) similar to the procedures described in van den Bongard et al. (2002). One milliliter of Solvable, 1 ml of isopropanol, and 0.4 ml of 30% hydrogen peroxide were added to fecal homogenates (triplicate), and 1 ml of Solvable, 100 ml of 0.1 M EDTA, and 0.5 ml of 30% hydrogen peroxide were added to whole-blood samples (duplicate). Subsequently, both fecal homogenates and whole-blood samples, the latter only after at least 1 h of storage in the dark at room temperature, were shaken gently in a water bath (Salm en Kipp, Breukelen, the Netherlands) at 40 to 45°C until decolorization. After being cooled, these samples were treated identically to plasma (duplicate) and urine (singul- lar) samples: 10 ml of liquid scintillation cocktail was added, and samples were stored in the dark for at least 1 h. Counting was performed on a Tri-Carb 2800TR liquid scintillation counter (PerkinElmer Life and Analytical Sciences), using a 14C counting protocol with automatic quench correction and a maximum counting time of 60 min per sample.

Quantitative Bioanalysis. Concentrations of unchanged eribulin in plasma, whole blood, urine, and feces were measured using validated LC-MS/MS assays as described elsewhere (Dubbelman et al., 2011). In brief, liquid-liquid extraction was used for sample cleanup. Supernatants of plasma, whole blood, and urine were concentrated, and extracts of all of the matrices were filtered. Final extracts were injected onto a C18 column and eluted using a gradient with 0.1% formic acid in water and ACN. An electrospary ionization source produced positive ions that were detected with a triple-quadrupole mass spectrometer. Through the use of multiple reaction monitoring, the mass transition of m/z 730 → 712 was recorded for eribulin, and m/z 731 → 681 for the internal standard ER-076349 (Dubbelman et al., 2011). The validated ranges were 0.2 to 100 ng/ml for plasma, 0.5 to 100 ng/ml for urine and whole blood, and 0.1 to 25 μg/g (undiluted feces) for feces. Quality control samples were prepared and assayed with the study samples. Criteria for the acceptance of bioanalytical data during routine drug analysis, as described in the FDA guidelines (Guidance for Industry, Bioanalytical Method Validation, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf), were applied.

The ratio of [14C]eribulin to [12C]eribulin in the dosing solution, as determined by LC-MS, was 1.03. Because only [12C]eribulin was quantified with these bioanalytical assays, the concentrations were multiplied by 2.03 to obtain the total eribulin concentrations.

Metabolite Profiling and Metabolite Identification. Sample preparation. To check for the presence of major metabolites, plasma samples collected up to 60 min after EoI were pooled across patients to obtain a single 300-μl plasma sample for each time point, containing equal volumes from each patient. In addition, plasma samples of individual patients from EoI and 5, 15, 60 min, 2, 6, 24, 72, and 144 h after EoI were processed and analyzed if the radioactivity in the extracts was considered sufficient for radiochromatography. Both urine and fecal samples were pooled within patients over 0 to 24, 24 to 48, 48 to 72, and 72 to 168 h in proportion to the total mass of the excreta.

To process plasma samples for metabolite profiling, proteins were precipitated by adding 900 μl of methanol/ACN (50:50, v/v) to 300 μl of plasma. The mixture was vortex mixed for 30 s and centrifuged for 10 min at 23,100g. The clear supernatant was transferred to a clean 1.5-ml vial, evaporated to dryness under a gentle nitrogen stream at 40°C, and reconstituted in 100 μl of 20 mM ammonium acetate (pH 5)/ACN (70:30, v/v) by vortex mixing for 10 s, followed by sonication for 1 h. After centrifugation for 10 min at 23,100g, a 10-μl aliquot of the final supernatant was mixed with 4 ml of scintillation cocktail and assayed for radioactivity using LSC to calculate the recovery of the sample preparation. The remaining supernatant was transferred to an autosampler vial and used for LC-LSC-MS/MS analysis (described under LC-LSC-MS/MS and LC-LTQ Orbitrap MS). In addition, for each sample, the pellets formed after the first and second centrifugation steps were mixed with 1 ml of Solvable, dissolved by sonication for 1 h, and analyzed for TRA.

For metabolite profiling, pooled urine samples were injected directly without sample preparation. For metabolite identification, a single urine sample, collected 0 to 6 h after infusion, was lyophilized. To this end, a 25-ml sample of frozen urine was transferred to a freeze-dryer (Sniijders, Tilburg, the Netherlands) operating at −80°C. Vacuum was applied until the sample was dehydrated totally. The dried sample was reconstituted in 1 ml of water. After centrifugation for 10 min at 23,100g, the supernatant was used for LC-LTQ Orbitrap MS.

Fecal homogenates were prepared for metabolite profiling by extracting 100-μl aliquots of fecal homogenates with 100 μl of ACN. The mixtures then were vortex mixed for 30 s, shaken for 10 min at 1250 rpm on an automatic shaker (Labincio, Breda, The Netherlands), vortex mixed another 30 s, and centrifuged for 10 min at 23,100g. This process was repeated with the pellet remaining after the transfer of the clean supernatants to empty vials. After the second extraction, the pellet was dissolved and analyzed for radioactivity, identically to the plasma pellets. The supernatants of the first and second extractions were combined and vortex mixed after the addition of 300 μl of water. An aliquot of 50 μl was mixed with 4 ml of scintillation cocktail, and radioactivity was determined to calculate the recovery of the sample preparation. The remainder was distributed over autosampler vials for metabolite profiling. For metabolite identification, the fecal homogenate with the highest radioactive concentration was concentrated further by evaporation. Two 500-μl aliquots of fecal homogenate were mixed with 500 μl of ACN and shaken and centrifuged identically to the other fecal homogenates. In 4 h, the supernatants were evaporated partially at 45°C in a SpeedVac (SPD1010; Thermo Fisher Scientific, Waltham, MA). After being centrifuged for 5 min at 23,100g, the clear supernatants were combined, and the volume was adjusted to 500 μl with ACN.

LC-LSC-MS/MS and LC-LTQ Orbitrap MS. For both metabolite profiling and metabolite identification, chromatography was performed using a 1-h gradient containing two mobile phases (A and B) on a Synergi Polar RP column (150 × 4.6 mm, particle size 4 μm) (Phenomenex, Torrance, CA) preceded by an in-line filter (0.2 μm; Upchurch Scientific, Oak Harbor, WA). Mobile phase A consisted of 20 mM ammonium acetate at (pH 5), and mobile phase B consisted of 100% ACN. Starting at 3% mobile phase B, the percentage of mobile phase B linearly increased to reach 5% at 5.0 min, 10% at 15.0 min, 20% at 25 min, 40% at 35 min, 60% at 45 min, and 80% at 50 min, where it was maintained until 54.9 min. From 55 to 60 min, the column was re-equilibrated at 3% mobile phase B. The flow rate was set to 1.0 ml/min, the
also were determined. The system used for metabolite profiling consisted of an Accela HPLC pump coupled to a LTQ XL linear ion trap mass spectrometer equipped with an electrospray ionization probe (Thermo Fisher Scientific). The mass spectrometer operated in positive ion mode, with a spray voltage of 5.4 kV, a capillary temperature of 300°C, and a capillary voltage of 6.5 V. The sheath, auxiliary, and sweep gas flow were optimized to 60, 10, and 5 arbitrary units, respectively. Wideband activation was enabled, the scan range was 100 to 1100 atomic mass units, the isolation width was 2.0, and the normalized collision energy used for collision-induced dissociation was 35%. To collect MS² and MS³ spectra, data-dependent acquisition was performed based on a parent list. This list contained masses of eribulin and hypothetical metabolites (e.g., products of single or multiple hydroxylation) and was expanded when additional potential metabolites were found.

Radiochromatograms were generated using HPLC coupled to a postcolumn accurate flow splitter (LC Packings, Sunnyvale, CA), directing one fourth of the flow to a linear ion trap MS and three fourths to a fraction collector (LKB-FRAC-100; GE Healthcare) to collect fractions for radioactivity measurement using LSC. The fraction collector collected the eluent in 6-mL plastic LSC vials at a rate of 1 min per vial (or in 20-mL vials at a rate of 5 min per vial in regions containing low radioactivity levels). After the addition of 4 mL (or 10 mL) of liquid scintillation cocktail, the fractions were assayed for TRA for 20 min per vial with LSC. Radiochromatographic profiles were prepared by plotting the net disintegrations per minute against the time after injection.

For metabolite identification, high-resolution mass spectrometry was applied to the concentrated urine and fecal samples. The equipment used for these samples consisted of a Finnigan Surveyor MS Pump Plus that was coupled to a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific). Pump and mass spectrometer settings were identical to those described above, except for the injection volume was set to 50 \( \mu \text{L} \) (20 \( \mu \text{L} \) for high-resolution mass spectrometry), and during the first 4 min the eluent was directed to waste.

Pharmacokinetic Analysis. Pharmacokinetic parameters of eribulin and TRA were calculated using noncompartmental analysis with WinNonlin Professional (version 5.1.1; Pharsight Corporation, Mountain View, CA). For plasma radioactivity, the pharmacokinetic parameters determined included maximum concentration \( \left(C_{\text{max}}\right) \), the terminal phase half-life \( \left(t_{1/2}\right) \), and the area under the plasma concentration-time curve (AUC). For eribulin in plasma, the clearance (CL), renal clearance (CLr), and apparent volume of distribution (\( V_{\text{d}} \)) also were determined.

Results

Patients. Four male and two female patients were enrolled in this study. All of the patients were Caucasian, had an Eastern Cooperative Oncology Group performance status of 0 or 1, a median age of 60.5 years (range, 34–70 years), a median weight of 70.5 kg (range, 55.0–175 kg), a median height of 172.5 cm (range, 162.0–210.0 cm), and a median body surface area of 1.9 m² (range, 1.6–3.2 m²). The patients had metastatic cancer with the primary tumor in one of the following locations: lung, testis, nasopharynx, ovary, esophagus and prostate.

TRA and Quantitative Bioanalysis. Pharmacokinetics. Plasma concentration-time curves of unchanged eribulin and TRA are presented in Fig. 2, and Table 1 summarizes the pharmacokinetic parameters. Because the actual administered dose ranged from 1.75 to 3.01 mg, the dose-normalized values for \( C_{\text{max}} \), AUC, and AUC \( \text{max} \) also were calculated and presented in Table 1. To obtain a standard infusion time of 5 min, the dosing solution was administered at a flow rate of 10 mL/min. \( T_{\text{max}} \) was observed typically at the end of infusion. The values for dose-normalized \( C_{\text{max}} \) and exposure (AUC \( \text{max} \)) were comparable with those of TRA (224 ng Eq/ml/mg and 357 ng Eq · h/ml/mg, respectively). The \( t_{1/2} \) value for eribulin (45.6 h) was similar to that of the TRA (42.3 h). Figure 3 shows the time courses of eribulin and TRA distribution as the whole blood to plasma ratios. For eribulin as well as TRA, the whole blood to plasma ratio approximated unity.

Excretion. For all six patients, urine and feces were collected as planned during the first 168 h after the administration of \([^{14}\text{C}]\)eribulin. Thereafter, four of the patients continued collecting feces for up to a maximum of 312 h, until the daily recovery of radioactivity in feces was <1% of the administered radioactivity.

Figure 4 shows the cumulative excretion of unchanged eribulin and TRA in urine and feces. The mean (±S.D.) recovery of TRA in the combined excreta (urine and feces) after 312 h was 90.4 ± 11.7% of the administered activity (8.9 ± 4.0% in urine and 81.5 ± 13.4% in feces). The total contribution of unchanged eribulin was 68.6 ± 14.1% of the administered dose.

Within 168 h, the minimal excreta collection period for all of the patients, 86.5 ± 16.4% of the administered activity was excreted, with unchanged eribulin accounting for 68.0 ± 13.1%. Most of the administered radioactivity (77.6 ± 19.0%) was excreted via feces, and a minor part (8.9 ± 4.0%) was excreted via urine.

Metabolite Profiling. Plasma. The mean sample pretreatment recovery of radioactivity in pooled and individual plasma extracts used for metabolite profiling was 87.7 ± 4.6%. Similar amounts of non-
extractable radioactivity were found in the first (4.3 ± 1.8%) and second (4.1 ± 1.9%) pellets that were formed during sample preparation. Radiochromatograms of the pooled plasma samples collected up to 1 h after EoI revealed the presence of a single major radioactive component, accounting for >90% of the injected radioactivity. The mass spectrum at this retention time showed a protonated molecular ion at m/z 730 with a [14C] isotope peak at m/z 732 (Fig. 5A) that was not present in the predose plasma sample. MS² fragmentation of this ion was identical to the fragmentation pattern of the reference standard of eribulin (Fig. 5B), and therefore this peak was identified as eribulin. Major metabolites were not present in these samples, because

![FIG. 3. Mean (±S.D., n = 6) whole blood to plasma concentration ratio of TRA (by LSC) and eribulin (by LC-MS/MS) after a single intravenous dose of approximately 2 mg (approximately 80 µCi) of [14C]eribulin to cancer patients.](image)

**TABLE 1**

**Pharmacokinetic parameters (mean ± S.D.) of eribulin and total radioactivity in patients with cancer (n = 6) after a 2- to 5-min bolus injection of 2.14 ± 0.44 mg (85.5 ± 17.9 µCi) of [14C]eribulin.**

<table>
<thead>
<tr>
<th></th>
<th>Eribulin</th>
<th>Total Radioactivity</th>
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<tbody>
<tr>
<td>Pharmacokinetic parameters</td>
<td></td>
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</tr>
<tr>
<td>Cmax</td>
<td>444 ± 144 ng/ml</td>
<td>449 ± 137 ng eq/ml</td>
</tr>
<tr>
<td>t1/2</td>
<td>45.6 ± 8.68 h</td>
<td>42.3 ± 17.2 h</td>
</tr>
<tr>
<td>AUC0-t</td>
<td>627 ± 386 ng·h/ml</td>
<td>568 ± 392 ng·h/ml</td>
</tr>
<tr>
<td>CL</td>
<td>681 ± 425 ng·h/ml</td>
<td>753 ± 403 ng·h/ml</td>
</tr>
<tr>
<td>CLr</td>
<td>3.93 ± 2.10 l/h</td>
<td></td>
</tr>
<tr>
<td>Vz</td>
<td>0.30 ± 0.13 l/h</td>
<td></td>
</tr>
<tr>
<td>V18</td>
<td>247 ± 123 liters</td>
<td></td>
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<tr>
<td>Dose-normalized parameters</td>
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</tr>
<tr>
<td>Cmax</td>
<td>222 ± 76.4 ng/ml·mg</td>
<td>224 ± 74.1 ng eq/ml·mg</td>
</tr>
<tr>
<td>AUC0-t</td>
<td>301 ± 165 ng·h/ml·mg</td>
<td>269 ± 153 ng·h/ml·mg</td>
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<tr>
<td>AUCin</td>
<td>328 ± 189 ng·h/ml·mg</td>
<td>357 ± 148 ng·h/ml·mg</td>
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eq, equivalents; Cmax, maximum observed plasma concentration; t1/2, terminal half-life; AUC0-t, area under the plasma concentration-time curve; AUC0-inf, area under the plasma concentration-time curve from zero to infinity; CL, clearance; CLr, renal clearance; Vz, apparent volume of distribution in the terminal phase.

n = 5 (for one patient too few data points were above the limit of quantitation to estimate an terminal disposition rate constant for the total radioactivity, therefore t1/2 and AUC0-inf could not be calculated for this patient).

other radioactivity-containing metabolites constituted only a maximum of 0.6% of the eribulin peak.

In the individual plasma extracts, the radioactivity of the samples collected at 48 and 72 h after EoI was below the LLOQ for all patients and below the LOD for most patients. Therefore, only samples collected up to 24 h after EoI were analyzed with LC-LSC-MS/MS. Eribulin represented the main peak of the radiochromatogram of all these samples (representative example shown in Fig. 6A). Small amounts of radioactivity eluted shortly before and after eribulin, mainly in the samples drawn at EoI and 5 min after EoI. Four elution fractions contained a radioactivity level above the LLOQ in one or more patient samples and were designated as eribulin metabolites MP1 to MP4. Table 2 provides an overview of these metabolites, summarizing the retention times, the maximum radioactivity level above the LLOQ in one or more patient samples and the number of patients in which the metabolites were detected in plasma, the time points at which the metabolites were detected, and the maximum relative and absolute concentrations that were detected in a sample. The most prominent metabolites were MP2 and MP3, observed in plasma of 5 and 4 patients, respectively, and both representing maximally ≤1% of the eribulin concentration.

**Urine.** The radiochromatograms of the urine samples, of which Fig. 6B is a representative example, show very little metabolism, similar to the plasma samples. The main radioactive peak eluted after 43 to 44 min and was identified as eribulin based on the MS and MS² spectra. Only a single radioactive peak other than eribulin was above the LLOQ in two patients within the 0 to 24 h urine pools. This peak, designated MU1, had a maximum of 3.48% of the eribulin peak and accounted for a maximum of 0.42% of the administered dose (Fig. 7).

**Feces.** Sample preparation of fecal homogenates with an evaporation and reconstitution step, similar to the plasma samples, resulted in <80% recovery. It was not feasible to resolve the analytes from the dried residue using a solution compatible with the LC system. Therefore, fecal homogenates were extracted using a minimal extraction
volume, and the supernatant was diluted further with water until the final composition was compatible with the LC system. This approach increased the recovery to 87.5 ± 6.0%.

Only small amounts of radioactivity corresponding to metabolites were present in fecal homogenates (representative example shown in Fig. 6C), with the largest peak in all of the samples identified as eribulin. The metabolite peaks were not resolved, complicating their designation. At least four metabolites appeared to be present. These were designated MF1 to MF4 and accounted for a maximum of 0.61, 0.85, 2.79, and 0.61% of the administered dose (Fig. 7).
Metabolite Identification. To identify the m/z values of the metabolites, LC-MS spectra of predose and postdose samples were compared at the retention time of each radioactive peak. Postdose samples also were scanned for the specific isotope pattern (Fig. 5A) of two peaks of equal intensity with a difference of 2 in m/z value (due to the 14C isotope).

Plasma samples were concentrated by a factor 3 for plasma profiling, which resulted in insufficient concentration to obtain MS signals for metabolite identification. Therefore, the identities of the metabolites MP1 to MP4, each with a maximum concentration ≤1% of the eribulin concentration, were unresolved.

Because a large volume of urine was available for each collection interval, a considerable concentration step (25 times) was required for sufficient MS response. The urine sample with the highest MU1 concentration was concentrated 25 times and analyzed using LC-LTQ Orbitrap MS. At the retention time of MU1, the MS spectrum showed the specific [13C]eribulin/[14C]eribulin-related pattern, with a parent m/z value of 748.4244. The MS2 spectrum of MU1 showed the same fragmentation pattern as eribulin, with subsequent losses of water and m/z value of 746.4085. Similar to MU1, the MS2 spectra of MF2, MF3, and MF4 showed the same pattern as eribulin (Fig. 5B), with mass shifts of 16, 16, and 18 Da, respectively. Also, for these metabolites, the unspecific fragmentation in the MS2 spectrum impeded further structural elucidation.

The fecal homogenate containing the highest radioactivity level was concentrated twice. The concentration of MF1 was still insufficient for MS detection, but MF2, MF3, and MF4 were detected. On the basis of high-resolution mass determinations, they were identified as eribulin + O, eribulin + O, and eribulin + H2O, respectively. Similar to MU1, the MS2 spectra of MF2, MF3, and MF4 showed the same pattern as eribulin (Fig. 5B), with mass shifts of 16, 16, and 18 Da, respectively. Also, for these metabolites, the unspecific fragmentation in the MS2 spectrum impeded further structural elucidation.

Table 3 summarizes the main characteristics of the metabolites detected in urine and feces: retention times, number of patients in which the metabolites were detected, time periods in which they were detected, maximum percentage of administered dose recovered as this metabolite (compared among the six patients), m/z value, proposed identity, theoretical m/z value of the proposed identity, and the difference between theoretical and measured m/z values.

Safety. The [14C]eribulin dose was generally well tolerated. A total of four of six patients experienced at least one treatment-related adverse event (AE) during the study phase. The most common AE reported as treatment-related was fatigue (n = 3). The majority of AEs were of grade 1 or 2; grade 3 AEs were reported for one patient. There were no deaths or serious adverse events and no dose reductions or delays due to AEs, and no patients were withdrawn from study treatment due to an AE. No significant abnormalities in laboratory parameters or obvious changes in vital signs were observed. Five patients continued treatment with eribulin mesylate after the study phase at a dose of 1.4 mg/m2 on days 1 and 8 of 21-day cycles; one patient discontinued after the completion of the assessments of the study phase and before entering cycle 2.

Discussion

This study investigated the metabolism and excretion of eribulin in humans. It was found that eribulin undergoes limited metabolism and is excreted primarily unchanged via feces (61.3% within 168 h). Four minor metabolites were detected in plasma, one in urine, and four in feces. Those that were identified had mass differences of +16 (+O) and +18 (+H2O) relative to unchanged eribulin.

The plasma pharmacokinetic properties of eribulin are comparable with those reported in previous phase I studies (Goel et al., 2009; Tan et al., 2009). The plasma concentration-time curve shows that both TRA and unchanged eribulin are distributed rapidly and then eliminated slowly. This profile and the extensive volume of distribution were demonstrated previously (Goel et al., 2009). Also, the average t1/2 of 45.6 h for eribulin was within the range of previously reported values for terminal half-life of 36 to 48 h (Cigler and Vahdat, 2010). In addition, the recovery of unchanged eribulin in urine (8.1% after 168 h; Fig. 7) is consistent with that of other phase I studies, wherein 5 to 6% (Goel et al., 2009) and 7% (Tan et al., 2009) of the administered eribulin were recovered unchanged within 72 h after a single dose.

Fig. 7. Overview of the average mass balance of [14C]eribulin in 5 or 6 patients with advanced solid tumors during 168 h of excreta collection after an intravenous bolus injection of approximately 2 mg (approximately 80 μCi) of [14C]eribulin. BA, quantitative bioanalysis using LC-MS/MS; MP, metabolite profiling using LC-LSC; Max % of dose, maximum percentage of the administered dose that was excreted for a patient as this metabolite (assuming that excretion recovery was proportional for all of the radioactive material).
The plasma to whole blood ratio of both TRA and unchanged eribulin approximates unity, suggesting no preferential distribution of eribulin or eribulin-derived compounds to either red blood cells or plasma compartments. The comparable pharmacokinetics for eribulin and TRA is indicative of limited metabolism. This was confirmed by the metabolic profile of eribulin in plasma: metabolites constituted ≤0.6% of the parent compound concentration in pooled plasma samples after 60 min after EoI, and individual plasma samples revealed only four minor metabolites, MP1 to MP4, of which the most abundant comprised maximally 1% of the eribulin concentration. The low concentrations of the plasma metabolites impeded detection with LC-MS/MS; consequently, definite identification was not possible. On the basis of retention time only, MP1 could be identical to the urine metabolite MU1, and MP3 to the fecal metabolite MF4. Because the plasma metabolites were only detected in a few samples collected up to 15 min after EoI and had a concentration of maximally 1% of the eribulin concentration in one patient, their overall systemic exposure does not exceed 10% of the parent compound or 10% of the total drug-related exposure. This means that further tests to evaluate the safety of the metabolites may not be required according to the Metabolites in Safety Testing guidelines (Guidance for Industry, Safety Testing of Drug Metabolites, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079266.pdf ICH, Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002720.pdf).

The total recovery of the administered radioactivity, 86.5% at day 8 (168 h) slowly increasing to 90.4% at day 14 (312 h), is generally acceptable for a mass balance study (Beumer et al., 2006). Expired air was not analyzed for the presence of $^{14}$CO$_2$, but considering the stability of eribulin, it is not expected that the production of $^{14}$CO$_2$ has contributed significantly to the loss of radioactivity, even though the $^{14}$C label was not incorporated in the ring structure of eribulin.

The quantitation of unchanged eribulin in feces samples of one patient was not possible due to interference with large amounts of polyethylene glycol (PEG) in the samples. The PEG caused a dramatic ion suppression of eribulin in the quantitative LC-MS/MS assay, hampering reliable quantification. The source of the PEG was probably macrogol, which was used daily by this patient to prevent constipation. Because of the interference, the LC-MS/MS results for eribulin in the feces of this patient were not included to calculate mean recoveries. PEG did not interfere with the quantification of eribulin in the plasma and urine of this patient because it appeared to be unabsorbed.

The total radioactivity that was recovered in feces after 168 h ($77.6\%$ of the administered dose) is not completely accounted for by the presence of unchanged eribulin ($61.3\%$ of the dose) and the identified metabolites MF1 to MF4 (<5% of the dose) (Fig. 7). This difference can be explained by at least four factors: 1) unattributed radioactivity in the radiochromatograms; 2) difference in the LLOQ between bioanalysis and TRA analysis; 3) incomplete recovery of eribulin during bioanalysis; and 4) the presence of nonextractable metabolites. We will discuss shortly these factors and their quantitative impact.

First, not all of the radioactivity in the radiochromatograms of fecal samples was attributed to eribulin or one of the four identified metabolites. Because the peaks were not resolved completely, a part of the unattributed radioactivity could be eribulin, MF1, MF2, MF3, and/or MF4. The other part comprises cumulative amounts of radioactivity below the LLOQ and could contain additional metabolites. On average, 5% of the administered dose was recovered in feces but unattributed.

Second, the LLOQ for the (LC-MS/MS) quantification of unchanged eribulin (0.1 μg/g) was higher than the LLOQ for TRA (LSC, 1.52 ng Eq/g) in feces. Consequently, samples with a concentration just below the LLOQ for quantitative bioanalysis were included to calculate TRA but excluded when calculating the total amount of unchanged eribulin. Due to this difference in the LLOQ, the underestimation of unchanged eribulin recovery in feces would on average be 2% of the administered dose, assuming that the ratio of unchanged eribulin to TRA was equal for all of the samples of the same patient.

Third, the recovery of unchanged eribulin during the bioanalysis of feces samples may have been incomplete, which also may have resulted in an underestimation of the unchanged eribulin recovery. This would be the case if feces samples spiked with eribulin during assay validation have a higher extraction recovery than real-life patient samples or if extraction recovery was dependent on the substance of the original nonhomogenized fecal samples. At least the latter was suspected, because it was observed that for metabolite profiling the highest extraction recoveries were obtained from watery feces samples, whereas originally dry and hard feces samples resulted in lower extraction recoveries. To obtain an indication of the underestimation of unchanged eribulin recovery in feces due to incomplete eribulin recovery, the highest extraction recovery across all of the patients and the extraction recoveries of individual patients were used. This resulted in an average potential underestimation of 6% of the administered dose.

Finally, the feces samples could have contained unknown nonextractable metabolites. However, if these were present, then their quantitative contribution was limited, because the three above-mentioned factors already explained the main difference between TRA in feces and unchanged eribulin plus metabolites MF1 to MF4.

The metabolites detected in urine and feces differed by 16 and 18 mass units from eribulin. High-resolution mass analysis indicated the incorporation of O and H$_2$O, respectively, in eribulin. Metabolites MF2 and MF3, with molecular masses of eribulin + 16, may be products of hydroxylation. Eribulin metabolites with the same molecular masses were reported previously by Zhang et al. (2008) in an in vitro study with human liver microsomes. That study showed that

### TABLE 3

Eribulin metabolites detected in urine and feces

<table>
<thead>
<tr>
<th>Peak ID</th>
<th>Retention Time</th>
<th>n</th>
<th>Time Periods</th>
<th>Max. Contribution</th>
<th>Accurate Mass</th>
<th>Proposed ID</th>
<th>Theoretical Mass</th>
<th>Δppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MU1</td>
<td>35–36</td>
<td>2</td>
<td>0–24</td>
<td>0.42%</td>
<td>748.4244</td>
<td>Eribulin + H$_2$O</td>
<td>748.4267</td>
<td>−3.07</td>
</tr>
<tr>
<td>MF1</td>
<td>31–32</td>
<td>1</td>
<td>48–72</td>
<td>0.61%</td>
<td>746.4116</td>
<td>Eribulin + O</td>
<td>746.4110</td>
<td>0.80</td>
</tr>
<tr>
<td>MF2</td>
<td>33–34</td>
<td>1</td>
<td>48–72</td>
<td>0.85%</td>
<td>746.4111</td>
<td>Eribulin + O</td>
<td>746.4110</td>
<td>0.13</td>
</tr>
<tr>
<td>MF3</td>
<td>35–37</td>
<td>2</td>
<td>24–72</td>
<td>2.79%</td>
<td>748.4272</td>
<td>Eribulin + H$_2$O</td>
<td>748.4267</td>
<td>0.67</td>
</tr>
<tr>
<td>MF4</td>
<td>41–42</td>
<td>1</td>
<td>48–72</td>
<td>0.61%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ID, identification; n, number of patients for which this metabolite was observed in urine/feces; Δppm, deviation of accurate mass from theoretical mass in parts-per-million; unk, unknown.
eribulin was metabolized primarily by CYP3A4, resulting in the formation of at least four mono-oxygenated metabolites. The described product ion spectra were similar to those we found for MF2 and MF3 in this study and, likewise, only comprised fragments formed by the loss of water and/or methanol. Due to the limited information provided by MS² spectra, further structural elucidation was impossible. The metabolites MU1 and MF4, with a mass of eribulin + 18, may be products of hydration of an alkene group or products of hydroxylation with the reduction of the ketone at the C-1 position. Alternatively, they may have been formed by the hydrolysis of the ketal at the C-14 position. The exact structures of these metabolites remain undetermined. The results of the present study may help to explain findings in other clinical studies. For example, the limited contribution of metabolism to the elimination of eribulin can explain that although eribulin is metabolized predominantly by CYP3A4 in vitro (Zhang et al., 2008), coadministration of the CYP3A4 inhibitor ketoconazole with eribulin to patients has no effect on eribulin exposure (Devriese et al., 2011a), and neither do CYP3A4 inducers or inhibitors on eribulin systemic clearance (Reyderman et al., 2011). Furthermore, the small contribution of urinary excretion to total excretion of eribulin may help to explain why eribulin is well tolerated at full dose in patients with moderate and severe renal dysfunction (Syndol et al., 2010). Likewise, the important role of biliary excretion may explain why dose reductions of eribulin are supported by a population pharmacokinetic/AE model based on data from phase I and II studies in patients with moderate hepatic impairment (Devriese et al., 2011b). Hepatic impairment prolonged the elimination half-life of eribulin, and the dose-normalized exposures (AUC₀₋∞) of patients with mild and moderate hepatic impairment were higher by 1.75- and 2.79-fold, respectively, compared with normal hepatic function (Devriese et al., 2011b).

In conclusion, eribulin was distributed rapidly and eliminated slowly after a 2 to 5 min intravenous bolus injection of approximately 2 mg (approximately 80 μCi) of [¹⁴C]eribulin. Eribulin was excreted primarily unchanged in feces, and metabolism played only a minor role in the elimination. Renal clearance represented a minor component (<10%) in eribulin clearance. No major metabolites were found in plasma; each metabolite represented ≤0.6% of eribulin in pooled plasma samples.

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Contributed new reagents or analytic tools: Beijnen.
Performed data analysis: Dubbelman, Jansen, Mergui-Roelvink, Reyderman, and Lopez-Anaya.

Wrote or contributed to the writing of the manuscript: Dubbelman, Rosing, Jansen, Mergui-Roelvink, Huijema, Koetz, Lymphoura, Reyderman, Lopez-Anaya, Schellens, and Beijnen.

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