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PHASE I STUDIES

Metabolism of patupilone in patients with advanced solid tumor malignancies

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Summary A phase 1, open-label, non-randomized, single center study was conducted to determine the pharmacokinetics, distribution, metabolism, elimination, and mass balance of patupilone in patients with advanced solid tumors. Five patients with advanced solid tumors received 10 mg/m^2 (1.1 MBq) of ^{14}C–radiolabeled patupilone at cycle 1 as a 20-minute intravenous infusion every 3 weeks until disease progression. Sequential samples of blood/plasma were taken for 3 weeks and urine and fecal samples were collected for seven days after the first dose of patupilone. Patupilone blood levels decreased rapidly after the infusion. The compound showed a large volume of distribution (V_{ss}: 2242 L). The main radiolabeled component in blood was patupilone itself, accompanied by the lactone hydrolysis products that are unlikely to contribute to the pharmacological effect of patupilone. The blood clearance of patupilone was relatively low at 14 L/h. The administered radioactivity dose was excreted slowly (46 % of dose up to 168 h) but ultimately accounted for 91 % of the dose by extrapolation. The fecal excretion of radioactivity was 2–3 times higher than the urinary excretion consistent with hepato-biliary elimination. Three patients had progressive disease and two patients had stable disease as their best response. Patupilone was generally well tolerated in patients with advanced solid tumors with no newly occurring safety events compared to previous clinical studies. In adult solid tumor patients, intravenous radiolabeled patupilone undergoes extensive metabolism with fecal excretion of radioactive metabolites predominating over renal excretion.

Keywords Human mass balance · ADME · EPO906 · Patupilone

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Introduction

Patupilone (EPO906) is an epothilone, a group of macrolide compounds that have shown potent antitumor activity [1, 2]. Patupilone binds to and stabilizes microtubulin, suppressing microtubule dynamics, thereby causing mitotic cell cycle arrest and eventual apoptosis in human cancer cells [3]. The mechanism of action is similar to that of the taxanes (paclitaxel and docetaxel), but patupilone is 3–20 times more potent in vitro [4]. Consistent with this, patipulone inhibits the growth of a broad range of human tumors in vitro (including taxane resistant cell lines) and induces

regression in nude mouse human xenograft models of lung, breast, colon, prostate and ovarian carcinomas [5–9].

A major limitation of taxane therapy is the development of resistance. Resistance can occur due to over-expression of the drug efflux pump, P-glycoprotein or due to mutations of β-tubulin [10, 11]. An important advantage of the epothilones is their ability to overcome taxane resistance. In contrast to paclitaxel, patupilone is equally cytotoxic to paclitaxel-sensitive and paclitaxel-resistant cells [4].

In mice and dogs, patupilone rapidly distributed to tissues with a large volume of distribution and also permeated the blood-brain barrier. The apparent half-life of patupilone in tumor tissues was approximately 4 days. The elimination of patupilone from all tissues is generally slow compared with its plasma half-life, resulting in high tissue-to-blood ratios of unchanged patupilone . In humans, after 20 minutes of infusion of patupilone, blood concentrations of patupilone declined rapidly in a multiphasic manner, with a terminal half-life ($t_{1/2}$) of approximately 4 days. The apparent volume of distribution at steady state was 1350 L, consistent with preclinical findings of extensive tissue binding [5]. The mean total body clearance for all patients was 13 L/h [12].

While the pharmacokinetics of patupilone has been studied in humans, disposition and metabolism of patipulone in human remained largely unknown. The objective of this study was to investigate the metabolism, including the routes of elimination and the excretion mass balance following a single intravenous dose of 10 mg/m^2 radiolabeled [^{14}C] patupilone in patients with advanced solid tumors. The major and many minor metabolites of patupilone were identified in blood, urine, and feces.

Patients and methods

Patient selection

Patients with solid malignancies for whom standard treatments were ineffective or for whom adequate therapy was not available were eligible for this study. Relevant eligibility criteria included: at least 18 years of age; World Health Organization (WHO) performance status score of 0 or 1; life-expectancy of at least 3 months; adequate hematological laboratory parameters within 72 hours prior to first dose of study treatment; (absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L; hemoglobin ≥10 g/dL; platelet count ≥100,000/mm^3 (100×10^9/L); and adequate renal (creatinine ≤1.5 mg/dL) and hepatic function (bilirubin ≤1.5 mg/dL, aspartate transaminase (AST) ≤2.5 times upper limit of normal (ULN), alkaline phosphatase (ALP) ≤3 x ULN and alanine transaminase ≤2.5 x ULN). Female patients with fertile potential must have had a negative serum pregnancy test at screening and had to agree to use an effective method of contraception during the study and for 3 months following termination of treatment.

Study exclusion criteria included: severe and/or uncontrolled medical disease (i.e., uncontrolled diabetes, congestive heart failure, chronic renal disease, or active uncontrolled infection); severe cardiac insufficiency (New York Heart Association grade III or IV), with uncontrolled and/or unstable cardiac or coronary artery disease; known diagnosis of human immunodeficiency virus (HIV) infection; presence of any other active or suspected acute or chronic uncontrolled infection; symptomatic brain metastases or leptomeningeal disease; history of colostomy procedure; evidence of concurrent drug/alcohol abuse; peripheral neuropathy > grade 1; unresolved diarrhea of any grade, within the last 7 days before treatment; use of known diarrheogenic agents (patients were required to stop treatment with these agents within 7 days prior to enrollment in the study, although they could continue to receive stool softeners); use of hematopoietic growth factors except erythropoietin (or equivalent); use of warfarin, with the exception of low dose warfarin (1 mg or less daily) administered prophylactically for maintenance of in-dwelling lines or ports; prior administration of epothilone; chemotherapy, biologics, immunotherapy, vaccine, cytokine therapy < 3 weeks (6 weeks for nitrosoureas or mitomycin-C) prior to study entry; major surgery for any cause within < 3 weeks prior to study entry and not fully recovered (signs and symptom resolution) from surgery; minor surgery for any cause within < 1 week prior to study entry and not fully recovered (signs and symptom resolution) from surgery; wide field radiation within < 3 weeks prior to study entry (palliative radiotherapy of metastasis at > 2 weeks prior to study entry was allowed; however, lesions within the radiation field could not be used to evaluate disease response); investigational compounds < 3 weeks or while participating in the study and female patients who were pregnant or breast-feeding.

Trial design

Based on the results of several Phase I and II clinical studies, an optimum dose of 10 mg/m^2 of patupilone (once every 3 weeks) as a single intravenous infusion over approximately 20 minutes was selected for use in this study. At cycle 1, radiolabeled [^{14}C]patupilone (radioactivity dose approximately 1.1 MBq) was administered, and for subsequent cycles, non-radiolabeled patupilone was administered. Patients benefiting from patupilone treatment continued to receive additional cycles of treatment with normal non-radiolabeled patupilone every 3 weeks until disease progression was noted or the patient was withdrawn from study due to other reasons. The study was conducted according to the ethical principles of the Declaration of Helsinki and the protocol was reviewed by the University of Texas Health Science Center San Antonio, Institutional Review Board. Informed consent was obtained from each subject in writing

before the start of the study. Side effects were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.

The radioactivity dose of 1.1 MBq was estimated to be the minimum dose required for sensitive analysis of collected blood and plasma samples. Dosimetry calculations performed according to the principles of the International Commission for Radiological Protection (ICRP) indicated that this dose is equivalent to a whole-body radiation dose of 3.5 mSv.

Study compound

Patupilone was specifically radiolabeled with ^{14}C, in the carbon-2″ position of the thiazole ring, and in the thiazole methyl group, by Novartis Pharma AG, Isotope Laboratory, Basel, Switzerland. The specific radioactivity was 60 kBq/mg and radiochemical purity was 99.3 %. The radiolabeled drug product was the same as standard, non-radiolabeled patupilone, as far as technically feasible. For the extension phase of the study Novartis supplied non-radiolabeled patupilone as a clear, colorless concentrated solution for injection, in dosage strength of 10 mg/4 mL.

Statistical methods

No formal statistical analysis was performed. Data was summarized in a descriptive fashion with respect to demographic and baseline characteristics, safety observations, drug effect measurements, and pharmacokinetic measurements. Adverse events (AEs) were summarized by the number and percentage of patients who had any AE and by primary system organ class and preferred term.

Pharmacokinetic studies

Pharmacokinetic (PK) sampling were performed immediately prior to infusion of patupilone on cycle 1 day 1 and post infusion of cycle 1 day 1 patupilone at 0.33, 0.66, 1, 2, 4, 7, 10, 24, 72, 120, 168, 336 and 504 hours (start of infusion = time zero). Urine and fecal samples were collected quantitatively up to day 8 during cycle 1. Additionally prior to infusion on cycle 1, day 1 a predose blank sample of feces and at least 100 mL urine were collected. An additional 8-hour urine and portion fecal samples were collected on Days 15 (336 h) and 22 (504 h) within cycle 1 only.

Determination of radioactivity in blood, plasma, urine, feces and vomitus

Dosimetry calculations indicated that a ^{14}C-radioactivity dose of 1.1 MBq (30 μCi) could be administered to patients. This dose was lower than used normally in absorption, distribution, metabolism and excretion (ADME) studies, and therefore classical radiometric analysis by Liquid Scintillation Counting (LSC) was not sufficiently sensitive to analyze blood and plasma. These samples were analyzed by LSC on a low-level counter with long counting times.

Blood and plasma Weighed aliquots of blood (200 μL) and plasma (300 μL) were assayed for radioactivity (single determinations) on a Tri-Carb 3170TR/SL low-level counter from Packard BioScience after solubilization and bleaching (blood only) by a standard procedure and addition of scintillation cocktail (Irgasafe Plus, Zinsser Analytic).

Urine/feces/vomitus Radioactivity in urine, feces homogenates and vomitus homogenates was determined on a Tri-Carb 2700TR liquid scintillation counter from Packard Instruments in duplicates (urine) or quadruplicates (feces and vomitus homogenates) using weighed aliquots of 2 mL for urine samples or 250 μL for feces and vomitus homogenates.

Determination of patupilone in blood

Patupilone in blood was measured using LC-MS/MS (Novartis Pharma SA, Rueil Malmaison, France) as described by Rubin et al. [12].

Determination of metabolite profiles in blood, urine, and feces

Reference compounds used in the analysis of metabolite profiles and metabolite structures were ADB251 and ACX335 (Fig. 2).

Blood At the time of analysis, equal volumes of blood, obtained from the five patients at the same sampling time, were combined. A 3 mL aliquot of blood pool (1.2 mL at 0.67 h) was added to 7 mL of deionized water and the mixture was vortexed for 30 s. The diluted blood pool was spiked with 5 nmol of unlabeled patupilone and ADB251 as chromatographic retention time markers. Thereafter, 30 mL of acetonitrile was added and the mixture was stirred for 30 min, followed by centrifugation at 2000*g* for 10 min. After removing the supernatant, the pellet was extracted another time with 10 mL of water/acetonitrile 1:1 (v/v) by stirring for 30 min and centrifugation at 2000*g* for 10 min. The 4 h blood pool was extracted a third time as described for the second extraction. Aliquots proportional to the volumes of the different supernatants (of a given blood pool) were combined and evaporated to dryness by a stream of nitrogen. The residue was reconstituted in water/acetonitrile 2:1 (v/v), transferred into an ultracentrifuge tube and re-

evaporated to dryness under a stream of nitrogen. The dry residue was reconstituted by sonication in 70 μL of acetonitrile and diluted with 130 μL of water, followed by an additional 500 μL of 10 mmol/L ammonium acetate buffer pH 4.7. The suspension was ultracentrifuged at 110000*g* for 10 min, and the supernatant was separated from the pellet. An aliquot of this supernatant was analyzed by HPLC with UV- and radioactivity detection. The recovery of radioactivity after sample preparation was 86±7 % (mean±SD).

Urine Urine samples were pooled (for each patient separately) across the 0–72 h and the 72–168 h time intervals by combining identical percentages of the volumes of the individual urine fractions. A 1000 μL aliquot of each urine pool was ultracentrifuged at 50000*g* for 5 min and the supernatant was injected for HPLC with radioactivity detection. The recovery of radioactivity after centrifugation was complete.

Feces Fecal samples were homogenized after addition of approximately 3 weights of water before freezing. After thawing, the feces samples were mixed and pooled (for each patient separately) across the 0–168 h time interval by combining 0.5 % weight of each individual feces homogenate. To 3 g of feces pool 30 mL of acetonitrile was added. The suspension was stirred for 30 min and centrifuged at 2000*g* for 10 min. After removing the supernatant, the pellet was extracted two more times, once with 10 mL of acetonitrile, once with 25 mL of methanol, both times with stirring for 30 min and centrifugation at 2000*g* for 10 min. Aliquots proportional to the volumes of the different supernatants were combined and evaporated to dryness by a stream of nitrogen. The residue was reconstituted by sonication in 250 μL of water/acetonitrile 2:1 (v/v) and transferred into an ultracentrifuge tube. The suspension was ultracentrifuged at 110000*g* for 10 min and the supernatant was analyzed by HPLC with radioactivity detection. The recovery of radioactivity after sample preparation was 78±5 % (mean±SD).

Radioactivity measurements for determination of recoveries Recoveries of radioactivity after sample preparation for metabolite profile analysis were determined by LSC as follows: blood samples, blood pellets, inhomogeneous blood extracts, feces homogenates, feces pellets and inhomogeneous feces extracts were solubilized (and bleached in case of blood-derived samples) according to standard procedures and subsequently mixed with Irgasafe Plus scintillation cocktail. Homogeneous blood extracts, urine aliquots and homogeneous feces extracts were measured directly following addition of Irgasafe Plus. The measurements were performed in a low-level counter (Tri-Carb 3170TR/SL). For quench correction, a standard addition method was used.

Structural characterization of metabolites

[^{14}C]Patupilone-related components in the following biological samples were structurally characterized by LC-MS and/or LC-MS/MS: Blood, pooled across all 5 patients, 1 h and 72 h samples combined, urine, 0-72 h, pooled across 2 patients and feces, 0–168 h, pooled across all 5 patients. Before LC-MS(/MS) analysis, the components were isolated by HPLC with fraction collection. The preparation of the biological samples and the chromatography were performed in a similar way as for the metabolites profiles. Aliquots of 50 μL of the fractions were transferred onto LumaPlates, which were dried and measured in a TopCount NXT microplate scintillation counter to obtain radiochromatograms. Aliquots of the fractions constituting a single peak in the radiochromatogram were combined, concentrated by partial evaporation by a stream of nitrogen and supplemented with water and methanol to reach an organic solvent content of approximately 5 %. Aliquots of 10–20 μL of these solutions, containing between 2 and 150 pmol of analyte, were injected for LC-MS(/MS) analysis. The samples were injected onto a 150×1 mm Lichrospher 100RP-18ec column, packed with 5 μm particles, kept at 25°C. The components were eluted with a gradient of 0.1 % (v/v) formic acid (mobile phase A) versus methanol (mobile phase B). After the column, the effluent was split in a 1:4 ratio. The smaller part of the effluent (~ 10 μL/min) was directed into the electrospray LC-MS interface of a Waters Synapt HDMS mass spectrometer.

Efficacy assessments

Efficacy evaluation was a secondary objective in this trial. Evidence of neoplastic activity was as a function of tumor response. Definitions of tumor assessments were based on RECIST criteria in patients with measurable/evaluable disease. Overall assessment of all measurable and non-measurable disease was to be obtained at screening (within 30 days prior to first dose of patupilone) and at the end of study visit. Patients who had stable or responding disease at the completion of cycle 1 and met extension protocol eligibility criteria were permitted to enter the extension study. In the extension protocol, response was to be assessed every even cycle (starting at the end of cycle 2, approximately every 6 weeks), prior to the next dose of patupilone.

Results

General and demographical characteristics

Five patients (1 Hispanic/Latino, 4 Caucasian), two males and three females were enrolled and completed the core

phase of the study. Their mean age was 56 years (range 45–63 years), height was 171±12 cm, (mean±S.D.; range 156–185 cm), and weight was 88.8±25.2 kg (range 59.9–122.7 kg), and body surface area was 2.0±0.35 m^2 (range 1.6–2.5 m^2). Two patients had adenocarcinoma of the colon, while the other three patients had breast, ovarian and pancreatic cancer. The patient with breast cancer was previously treated with cyclophosphamide, doxorubicin and capecitabine while the patient with ovarian cancer received carboplatin, paclitaxel, gemcitabine, topotecan and doxorubicin. Fluorouracil and gemcitabine were prior treatment regimens administered to the patient with pancreatic cancer. Both patients with colon cancer received prior treatment with fluorouracil, oxaliplatin, irinotecan and cetuximab. After the core phase, one of these patients discontinued due to consent withdrawal, and the other four patients entered the extension phase of the study. Three patients subsequently discontinued due to disease progression and one discontinued due to adverse events.

Safety and efficacy

All treated patients were assessed for safety. All patients in the core phase of the study experienced at least one adverse event (AE). Diarrhea was the most commonly observed AE, reported in all five patients. All diarrhea AEs and most gastrointestinal events were attributed a suspected relationship to the study drug according to the investigator. This observation is consistent with previous clinical experience with patupilone. No deaths were reported in the core or extension phases of the study. During the extension phase of the study, pleural effusions as a SAE (serious adverse event) were reported in two patients. In one of the patients, the study drug was discontinued due to the event. However both patients had a history of pleural effusions prior to starting study medication and therefore this SAE was not considered study drug related. There were no newly occurring safety events compared to previous clinical studies, and no worsening ECG abnormalities or relevant changes in vital signs. For efficacy, three patients were categorized as having progressive disease and two patients had stable disease. One patient with pancreatic adenocarcinoma had stable disease lasting 4 cycles but then discontinued due to adverse events after 5 cycles. Another patient with ovarian cancer had stable disease lasting 2 cycles but then withdrew consent.

Pharmacokinetics of patupilone

Based on the blood concentration-time profile of patupilone determined by LC-MS/MS, the $AUC_{0\text{-}inf}$ and C_{max} were 846±393 h·nmol/L and 52±16 nmol/L, respectively. The large steady state volume of distribution (2244±926 L) and the low blood clearance of patupilone (14.04±5.31 L/h) were consistent with its long terminal half-life (137±70 h).

Radioactivity concentrations in blood and plasma and derived pharmacokinetic parameters

Concentrations of ^{14}C-radioactivity (i.e. total radiolabeled components) in plasma were approximately 1.4 times higher than in blood (Fig. 1a). In both matrices, the radioactivity concentrations dropped three fold during the first 20 minutes post infusion, followed by a very slow decrease. Half-lives, estimated from concentrations between 72 h and the last sampling time, amounted to 326±72 h in blood and 327±116 h in plasma (mean±SD of n=5). Coefficients of variation of the blood and plasma concentrations of radioactivity were 15–39 % and 10–55 %, respectively.

Metabolite structures and metabolic pathways

Partial or complete metabolite structures were derived from LC-MS and LC-MS/MS spectra, in comparison with the respective spectra of the reference compounds patupilone, ADB251 and ACX335. Proposed metabolic pathways of patupilone are shown in Fig. 2. It was not possible to characterize each metabolite in each biological matrix by mass spectrometry. Therefore, some metabolites were assigned to peaks in the radiochromatograms (Fig. 1b, c and d) based on chromatographic retention times only. The protonated reference compounds patupilone, ADB251 and ACX335 underwent numerous analogous fragmentations upon collisional activation which were found also in the mass spectra of the metabolites isolated from the biological samples. These fragmentations allowed determination of regions of metabolic changes in the molecules. The identity of metabolite M13 with the reference compound ADB251, of metabolite M9 with the reference compound ACX335 and of patupilone in the biological samples with the reference compound patupilone was confirmed by identical retention times and mass spectra.

The metabolites M13 (ADB251) and M15 showed indistinguishable mass spectra. Therefore, M15 is proposed to be a stereoisomer of M13. The formation of M15 might occur by an *O*-alkyl cleavage at the lactone function of patupilone, resulting in a carbonium ion intermediate at carbon number 3, stabilized by the neighboring thiazolylvinyl function, followed by non-stereoselective addition of water to form either M13 or M15. Sefkow, et al. proposed a similar first step to explain the formation of rearrangement products of epothilone A and B (epothilone B is identical to patupilone) [13]. An alternative structure of M15 with a contracted macrocycle (migration of the carboxyl group from carbon 3 to carbon 1 with opening of the epoxide ring), as found by Sefkow, et al. [13], seems less likely. A further alternative structure of M15 with intact lactone macrocycle but

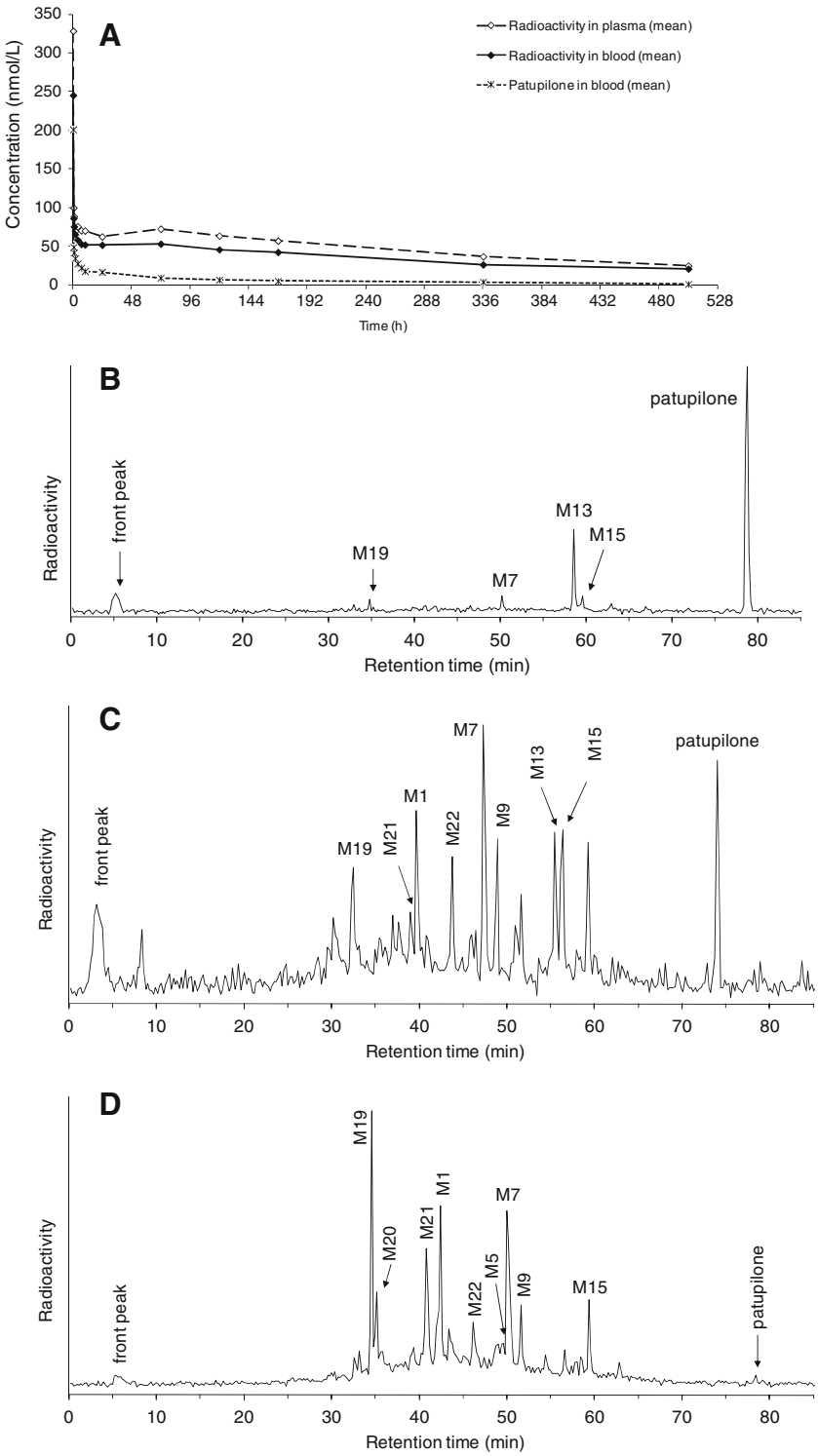

Fig. 1 **a** Blood and plasma concentration-time profiles. **b** HPLC-radioactivity chromatogram of blood sampled at the 4 h time-point (pool of five patients). **c** HPLC-radioactivity chromatogram of urine sampled from 0 to72 h form patient #4. D) HPLC-radioactivity chromatogram of feces sampled from 0 to 168 h from patient #4

hydrolyzed epoxide function could be excluded on the basis of the mass spectral fragmentation. Metabolite M13 could also be formed by *O*-acyl cleavage.

Similar to the M13 and M15 metabolites, M7 and M9 (ACX335) produced indistinguishable mass spectra and are also proposed to be stereoisomers of each other. However, in this case, the difference in absolute configuration might be either at carbon 1, 3 or 16 (the stereochemistry of ACX335 at carbon 1 and 16 is unknown). We propose that M9 (ACX335) is formed by epoxide hydrolysis of M13 (ADB251) while M7 may be formed by the same reaction from either M13 or M15.

M21 and M22 are proposed to be oxidation products of M7 or M9 and of M13 or M15, respectively. Their

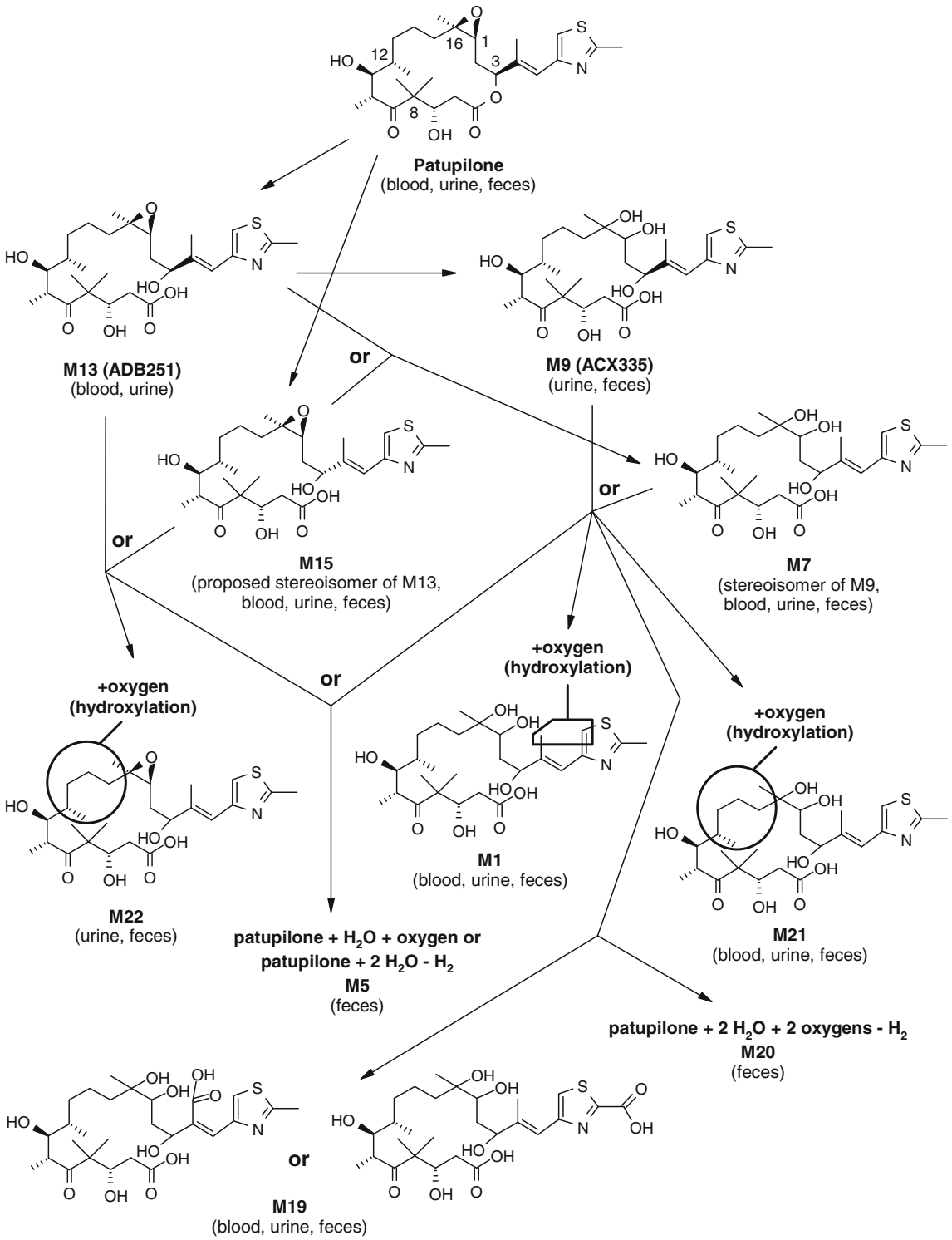

Fig. 2 Proposed metabolic pathways of patupilone

formation involved a hydroxylation in the region of carbon number 12-15 or at one of the methyl groups bound to carbon number 12 and 16. M1, a second oxidation product of either M7 or M9, had undergone hydroxylation within the substituent at carbon number 3. Many other positions could be excluded as sites of hydroxylation based on the mass spectral fragmentation or for chemical reasons, leaving the two positions indicated in Fig. 2 as the only likely sites of hydroxylation. M19, a further downstream product of M7 or M9, underwent a threefold oxidation of a methyl- to a

carboxylic group, as indicated by prominent collision-induced losses of CO_2 from different fragment ions, not observed with the other metabolites. The fragmentations of protonated M19 allowed oxidation at either one of the two methyl groups of the substituent at carbon number 3. The two possible structures could not be differentiated by mass spectrometry.

For the two additional minor metabolites M5 and M20, only the $[M+H]^+$ ions could be observed by mass spectrometry. According to exact mass data, M5 is proposed to be either an oxygenation product of M13 or M15 or a dehydrogenation product of M7 or M9 while M20 is proposed to be the product of a double oxygenation plus a dehydrogenation of M7 or M9.

Metabolite profiles

Blood

Metabolite profiles in blood (Fig. 1b), analyzed at selected time points up to 72 h using pools across the five patients, showed patupilone was the most abundant radiolabeled component in blood. Among the circulating metabolites retained on the HPLC column, the hydrolysis product M13 was most abundant, followed by its isomer M15 (based on $AUC_{0\text{-}72h}$). Other metabolites, including M19, M21, M1 and M7, were present in traces only. The patupilone concentrations in blood, derived from the metabolite profiles in a semi-quantitative manner, were comparable to those determined by the quantitative LC-MS/MS assay. The blood concentrations of M13 were highest at 0.33 h (end of infusion) and decreased rapidly during the following 20 min, followed by a very slow decrease. Therefore M13 may be rapidly formed while patupilone was present predominantly in the blood, prior to its extensive tissue distribution.

Metabolite M15 was formed more slowly than M13 and was present in highest blood concentration at the latest time point analyzed (72 h). A front peak, representing highly polar material not retained on the HPLC column, peaked at 72 h. This material could not be structurally characterized but can be assumed to represent one or several small cleavage products of patupilone still containing the ^{14}C-radiolabeled carbon atoms. The concentrations and AUC values of patupilone and metabolites in blood are summarized in Table 2.

Urine

The metabolite profiles in urine (0–72 h and 72–168 h pools) and in feces (0–168 h pools) were analyzed for each patient individually. The profiles in urine up to 168 h covered on average approximately 14 % of the dose while, by extrapolation to infinity, a total urinary excretion of radioactivity of approximately 25 % was estimated. Both the urinary excretion of radioactivity and the metabolite profiles in urine showed a high variability between the five patients. In contrast, the profiles in the 0–72 h and the 72–168 h urine fractions in each individual patient were similar. This suggests that the composition of the radiolabeled material excreted in urine after 168 h was similar to that excreted between 0 h and 168 h. In contrast to the metabolites, unchanged patupilone was of higher relative abundance in the 0–72 h urine, compared to the 72–168 h urine. The hydrolysis products M7, M9, M13 and M15 and the oxidized hydrolysis products M1, M19, M21 and M22 were among the most abundant urinary metabolites. However, none of them exceeded 1 % of dose, on average (Fig. 1c). The oxidized hydrolysis product M20 was a minor urinary metabolite. A significant front peak (on average 1.8 % of dose in urine 0–168 h) was observed as well, representing, as in blood, highly polar material not retained on the HPLC column. Unchanged patupilone in urine 0–168 h accounted for only 0.3 % of dose, on average.

Feces

The metabolite profiles in the feces 0–168 h covered, on average, approximately 25 % of the dose, whereas the extrapolation to infinity suggested a total fecal excretion of radioactivity of approximately 66 %. As for urine, both the fecal excretion of radioactivity and the metabolite profiles in feces showed a high inter-individual variability. However, in the same patient, the fecal metabolite profile resembled that in urine. On average, the most abundant metabolites in feces were the hydrolysis products M7, M9 and M15 and the oxidized hydrolysis products M1, M19, M20, M21 and M22, accounting for between 0.9 % and 2.4 % of dose in feces 0–168 h (Fig. 1d). The oxidized hydrolysis product M5 and the highly polar component(s) constituting the front peak were minor in feces 0–168 h (on average 0.5 % and 0.2 % of dose, respectively) and unchanged patupilone was found in traces only (0.1 % of dose on average). All oxidation products of patupilone identified in urine or feces has undergone hydrolysis as well.

Excretion of radioactivity

Radioactivity was excreted very slowly in urine and feces (Table 1). Up to 168 h after administration, 31±8 % of the dose appeared in the feces and 14±8 % in the urine. By extrapolation to infinity, a total excretion of radioactivity of 66±22 % of dose in feces and of 25±7 % of dose in urine was estimated, adding up to an essentially complete recovery of radioactivity in the excreta (91±18 %). Only negligible amounts of radioactivity were excreted in vomitus, as expected after intravenous administration (Table 2).

Table 1 Cumulative excretion of radioactivity in urine and feces of five patients

Time interval	Excretion of radioactivity								
	Urine			Feces			Total excretion		
(h)	Mean (% dose)	SD (% dose)	CV (%)	Mean (% dose)	SD (% dose)	CV (%)	Mean (% dose)	SD (% dose)	CV (%)
0–24	4.37	2.68	61	1.97	4.37	222	6.34	3.52	56
0–48	6.84	4.66	68	5.26	4.35	83	12.1	7.26	60
0–72	8.76	5.70	65	11.1	6.97	63	19.9	11.0	55
0–96	10.6	6.52	61	16.7	11.4	68	27.4	12.8	47
0–120	12.1	7.03	58	23.5	8.98	38	35.6	12.0	34
0–144	13.4	7.51	56	28.2	7.18	25	41.6	8.85	21
0–168	14.4	7.81	54	31.3	7.72	25	45.8	9.67	21
0–∞	25.4	7.33	29	65.6	22.2	34	91.0	17.8	20

Discussion

Blood patupilone and radioactivity concentration-time profile declined bi-phasically showing a rapid and a slow elimination phase following intravenous infusion. The concentrations of radioactivity in plasma were slightly higher than those in blood (*approx.* 1.4-fold) and decreased approximately in parallel to the blood concentrations. The inter-individual variability of the patupilone and radioactivity concentrations in blood was moderately high with average coefficients of variations of 41 and 27 %, respectively. The pharmacokinetics of patupilone in cancer patients is characterized by a large volume of distribution (*approx.* 41-fold the total body water) and low total body clearance (*approx.* 13 % of hepatic blood flow) consistent with an extensive tissue uptake of patupilone that was also observed in tumor-bearing mice and rats [5]. The pharmacokinetic parameter V_{ss}, CL and $t_{1/2}$ of patupilone in this study were in good agreement with those observed in a previous Phase I dose-finding study in 91 patients with advanced solid tumors [12].

The patients were systemically exposed mainly to patupilone itself, followed by the two isomeric lactone hydrolysis products M13 and M15 and to one or several highly polar metabolites of probably low molecular weight. This highly polar material was formed very slowly and was likely responsible for the higher terminal half-life of radioactivity, compared to that of the parent compound. M13 (ADB251) has been found previously to be inactive with respect to microtubule protein polymerization and to show no inhibitory activity

Table 2 Concentrations and AUC values of patupilone and metabolites in blood, derived from metabolite profiles in pools across five patients

Sampling time (h)	Concentration (nmol/L)						$AUC_{0-72\ h}$	
	0.33	0.67	1	4	10	72	(nmol·h/L)	(% AUC of radioactivity)
Front peak	5.4	4.1	3.9	5.6	7.0	20.1	896	22.8
M19	n.d.	n.d.	1.0	1.4	n.d.	n.d.	8.1	0.21
M21	n.d.	n.d.	1.4	n.d.	n.d.	n.d.	2.3	0.06
M1	n.d.	n.d.	1.2	n.d.	n.d.	n.d.	2.1	0.05
M7	n.d.	n.d.	1.8	1.8	n.d.	n.d.	11.1	0.28
M13 (ADB251)	60.0	8.8	5.0	7.6	3.5	6.3	379	9.66
M15	n.d.	n.d.	1.4	2.1	3.0	4.8	263	6.69
Patupilone	146	58.6	56.6	32.9	27.3	10.8	1575	40.1
Sum of additional metabolites	7.2	3.6	n.d.	0.06	n.d.	n.d.	3.9	0.10
Total components detected	218	75.0	72.6	51.4	40.8	42.1	3140	80.0
Lost during sample processing	27.0	11.2	3.0	7.0	11.5	11.2	787	20.0
Total radiolabeled components in original sample	245	86.2	75.6	58.5	52.3	53.2	3927	100

n.d.: not detected

on growth of epidermal carcinoma cells in vitro (unpublished data), suggesting that an opening of the lactone macrocycle of patupilone leads to loss of pharmacological activity. This renders it likely that none of the circulating metabolites contributed to the intended pharmacological effect of patupilone. The metabolite profiles in urine and feces were highly complex and showed substantial inter-individual differences. The small amounts of unchanged patupilone found in urine (on average 0.3 % of dose over 0–168 hours) and in feces (on average 0.1 % of dose over 0–168 hours) suggest that patupilone is eliminated almost exclusively by metabolism, rather than by direct excretion. All metabolites identified in blood and excreta appeared to have undergone hydrolysis of the lactone function, some in combination with hydrolysis of the epoxide function and/or oxidation. This suggests that hydrolysis (in particular of the lactone function), rather than oxidation, was the initial metabolic reaction and therefore the main elimination pathway of patupilone. The human enzymes involved in the hydrolysis of patupilone were identified as the carboxylesterase isoforms hCE-1 and hCE-2 (unpublished data).

The excretion of radioactivity occurred very slowly and to a higher extent via the feces (on average 31 % of dose up to 168 hours) than via the urine (on average 14 % of dose up to 168 hours). Even though the recovery of radioactivity in the excreta up to 168 hours was incomplete (on average 46 % of dose), an extrapolation, based on estimated half-lives of the urinary excretion of radioactivity, suggested an essentially complete excretion of radioactivity up to infinity (on average 91 % of dose with 66 % of dose in feces and 25 % of dose in urine). The interindividual variability of the excretion of radioactivity was moderate. The CV of the estimated excretion zero to infinity amounted to 29 % for urine, 34 % for feces, 20 % for total excretion.

Patupilone was generally well tolerated in patients with advanced solid tumor malignancies. All study patients had at least one AE. The AE seen in the study were as expected for this population and this class of drug with diarrhea as the most commonly observed AE. The patient number was low and did not allow conclusive assessment of safety trends.

The safety and efficacy of patupilone has now been studied in various tumor types including ovarian, colon and prostate cancer. Three different dose schedules of patupilone administered every three weeks were evaluated in a phase 1 study of patupilone colon cancer [14]. While no DLTs occurred in the twenty minute infusion arm, three patients in a twenty four hour continuous infusion arm and two in a sixteen hour intermittent infusion arm experienced DLTs. Additionally 4 partial responses were seen in the twenty minute infusion arm suggesting that this schedule is well tolerated and associated with clinical activity. Clinical activity has also been seen in other tumor types including patients with metastatic castration-resistant prostate cancer and advanced ovarian cancer [15, 16]. Additionally patupilone is well tolerated in combination with radiation therapy in central nervous system cancers [17]. Consistently, the pharmacokinetic analysis across these studies shows a large volume of distribution, low blood clearance and long terminal half-life of patupilone.

In conclusion, this study with intravenous administration of [^{14}C]patupilone (10 mg/m^2) to patients with advanced solid tumor malignancies showed the parent compound as the main radiolabeled component in blood, accompanied by the M13 and M15 lactone hydrolysis products and one or several highly polar metabolites. The circulating metabolites are unlikely to contribute to the pharmacological effect of the compound. The apparent terminal half-life of radioactivity was longer than that of patupilone, probably due to the highly polar metabolites. The elimination of patupilone appeared to occur almost exclusively by hydrolytic metabolism. Oxidative processes were observed only in combination with hydrolysis. Hydrolysis and oxidation of patupilone resulted in a large number of different biotransformation products in the excreta. Excretion of administered radioactivity occurred slowly but appeared to reach completeness.

Conflict of interest Markus Zollinger, Frédéric Lozac'h, Eugene Tan, Felix Waldmeier, Patrick Urban, Suraj Anand, Yanfeng Wang and Piet Swart are employees of Novartis Pharmaceuticals Corporation.

Kevin R. Kelly, Alain Mita, Chris Takimoto and Monica Mita do not have a conflict of interest to declare.

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