Anti-Cancer Drugs 2002, **13,** pp. 807–814

Clinical report

An excretion balance and pharmacokinetic study of the novel anticancer agent E7070 in cancer patients

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E7070 is a novel sulfonamide anticancer agent that arrests the G_1/S phase of the cell cycle. Preclinical and phase I studies have demonstrated non-linear pharmacokinetics of the drug. The objective of this study was to quantify the excretion of E7070 and the metabolite 1,4-benzene-sulfonamide (M1) in cancer patients. E7070 (1000 mg) radiolabeled by ^{14}C in the benzene disulfonamide moiety (cohort 1, $n=6$) or in the indole moiety (cohort 2, $n=7$) was i.v. infused over 1 h. The levels of radioactivity in plasma, red blood cells, urine and feces were determined by liquid scintillation counting, and the E7070 and M1 concentrations in plasma, urine and feces were determined by coupled liquid chromatography–tandem mass spectrometry (LC/ESI-MS/MS). In plasma, the mean area under the concentration–time curve (AUC) based on radioactivity measurements (32.5 and 28.9 h · mM in cohorts 1 and 2, respectively) was substantially higher than the mean AUC of E7070 (3.8 h · mmol/l) and M1 (0.1 h · mmol/l) in all patients. The excretion of radioactivity (mean ± SD) as a percentage of administered radioactivity was higher in urine [63.7 ± 9.8% (cohort 1) and 61.5 ± 5.5% (cohort 2)] than in feces [22.7 ± 2.6% (1) and 21.1 ± 3.1% (2)] during a mean collection period of 11 days. In both cohorts, the contribution of urinary and fecal recovery of E7070 (2.3 and 2.7%, respectively) and M1 (5.3 and 5.1%, respectively) was low. Subsequent HPLC analysis with online radioisotope detection of urine showed that the high radioactivity levels are caused by compounds other than E7070 and M1. The major metabolite is formed by glucuronidation of a hydroxylated metabolite of E7070. In conclusion, the excretion of the benzene sulfonamide and the indole moieties of E7070 was the same with a higher renal than gastrointestinal excretion. E7070 is extensively converted into currently unidentified metabolites. Glucuronidation is a major metabolic pathway. [© 2002 Lippincott Williams & Wilkins.]

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Key words: Anticancer agent, benzene disulfonamide derivative, ^{14}C-labeled E7070, excretion, pharmacokinetics.

Introduction

Several sulfonamide derivatives have been synthesized to develop novel cytotoxic agents against solid tumors.[1,2] Sulfonamide agents are well known to have a variety of pharmacological activities including antibacterial, carbonic anhydrase-inhibitory, antidiabetic, diuretic and antithyroid activities.[3] The first sulfonamide derivative that has been studied for its antitumor activity is E7010 (*N*-[2-[(4-hydroxyphenyl)amino]-3-pyridinyl]-4-methoxybenzene sulfonamide), an antimitotic agent that inhibits tubulin polymerization.[4,5] A follow-up compound, E7070 [*N*-(3-chloro-7-indolyl)-1,4-benzene disulfonamide], is another sulfonamide derivative that has been studied for its antitumor activity. E7070 exhibited a potent antitumor activity in *in vitro* (in murine and human tumor cell lines) and *in vivo* (in human xenografts) studies. Most antitumor activity was observed in colorectal and lung cancer xenografts (HCT116 colorectal cancer and LX-1 lung cancer models). The drug arrests the transition of the G_1/S phase of the cell cycle by inhibiting the phosphorylation step of cyclin E and activation of cyclin-dependant kinase 2.[6–8]

To determine the toxicity and maximum tolerated dose of E7070 in humans, phase I trials were initiated using four different infusion schedules in 127

patients with solid tumors.[9,10] The dose-limiting toxicity was mainly hematological. Partial and minor responses were observed in patients with breast, endometrial, renal and ovarian carcinoma. Non-compartmental pharmacokinetic analysis of these phase I trials revealed non-linear pharmacokinetics with a more than dose-proportional increase of the AUC at higher dose levels.[9,10] A population pharmacokinetic analysis of E7070 in three phase I studies (n=91) revealed that the concentration–time data could best be fitted to a three-compartment model with saturable transport to one compartment.[11] Phase II studies with the dosing schemes of 1-h infusion every 3 weeks (700 mg/m^2) and 5-day continuous infusion every 3 weeks (96 mg/m^2) are currently ongoing in patients with solid tumors. To provide a better understanding of the excretion pathway of E7070 in humans, which is pivotal information for the further development of the drug, a mass balance study was performed following a single dose of radiolabeled [^{14}C]E7070 in patients with solid tumors.

Materials and methods

Patients

This prospective, non-randomized, single-center study was performed between November 1999 and August 2000 at the Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute (Amsterdam, The Netherlands). The Institutional Medical Ethics Committee approved the study. All patients were required to give written informed consent prior to participation in the trial.

Patients with a histologically or cytologically confirmed diagnosis of a solid tumor not amenable to established forms of treatment were eligible for the study. Prior chemotherapy other than E7070, immunotherapy or radiotherapy was allowed, provided that the last treatment was at least 4 weeks prior to study entry (6 weeks for nitrosoureas and extensive radiotherapy) and any resulting toxicities were resolved. Patients of child-bearing potential had to use adequate contraceptives and in fertile females a pregnancy test had to be performed within 1 week before study entry. Other eligibility criteria included: age $\geqslant$18 years, life expectancy of $\geqslant$3 months, performance status $\leqslant$2 according to the WHO scale, acceptable bone marrow function (absolute neutrophil count $\geqslant$1500/mm^3, platelet count $\geqslant$100 000/mm^3), adequate renal function [serum creatinine $\leqslant$120 μmol/l (1.4 mg/dl) or creatinine clearance $\geqslant$50 ml/min], adequate hepatic function [serum bilirubin $<$25 μmol/l (1.5 mg/dl), serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leqslant$2.5 times the upper normal limit or $\leqslant$5 times the upper normal limit in the case of liver metastases]. Exclusion criteria included the presence of active infections, the presence of symptomatic brain metastases, or glaucoma, presence of alcoholism, treatment with sulfonylurea agent for diabetes, any anti-arrhythmic agent or cisapride, pregnancy or breast-feeding, clinical signs of urinary and/or fecal incontinence.

Trial treatment

The patient population consisted of two cohorts because E7070 was radiolabeled at two different locations in the molecule. In the first cohort it was radiolabeled at the benzene sulfonamide moiety and in the second cohort it was radiolabeled at the indole moiety to study the elimination routes of each part of the molecule. In the first cohort each patient received a single dose of 1000 mg E7070 containing 3.7 MBq ^{14}C-labeled E7070 ([benzene sulfonamide-^{14}C]ER-35744) (Figure 1A) over 1 h infused i.v. through a central venous catheter. In the second cohort a single dose of 1000 mg E7070 containing 3.7 MBq ^{14}C-labeled E7070 ([indole ring-U-^{14}C]ER-35744) (Figure 1B) was i.v. administered over 1 h through a central venous catheter. Subsequent courses consisted of 700 mg/m^2 E7070 i.v. over 1 h every 3 weeks. Eligibility criteria with regard to performance status, hematological, hepatic, renal

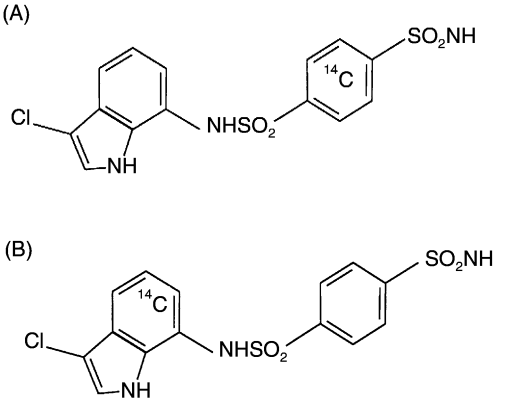

Figure 1. Chemical structure of E7070 [benzene sulfonamide-^{14}C]ER-35744 (A) and [indole ring-U-^{14}C]ER-35744 (B).

functions, intercurrent complications and medication were checked for re-treatment. Toxicity evaluation was performed every course according to the National Cancer Institute Common Toxicity Criteria (NCI CTC). If toxicity occurred, the patient was retreated upon recovery. Treatment was continued at the same dose provided no serious toxicity and no progressive disease was observed.

E7070 was supplied (Eisai, London, UK) as a white to pale yellow freeze-dried cake, in glass vials containing 100 mg of E7070 with 380 mg of meglumine and 475 mg of mannitol. Vials were stored at 2–8°C in the dark. Ten E7070 vials were reconstituted by adding 5 ml of water to each vial. The E7070 radiolabeled compounds ([benzene sulfonamide-^{14}C]ER-35744 and [indole ring-U-^{14}C]ER-35744) were supplied in glass vials each containing 3.7 Mbq ^{14}C-labeled compound with a specific activity of 2.52 and 2.74 GBq/mmol, respectively (Amersham International, Little Chalfont, UK). One vial containing radiolabeled compound and 10 reconstituted vials (1000 mg E7070) were added to saline (final volume of 1000 ml) for i.v. infusion under sterile conditions at the radioisotope laboratory in the Netherlands Cancer Institute. Quality control included endotoxin, sterility testing and radio-chemical purity testing with HPLC, and was conducted before the administration to the patients.

Sample collection

Blood samples of each 10 ml were collected from a peripheral venous catheter during the first course. Blood samples for E7070 analysis were collected in heparinized tubes immediately pre-dose (0 h), at 30 min after the start of the infusion, at the end of the infusion, at 10 and 30 min, and 1, 2, 4, 6, 8, 12, 24 and 36 h after the end of the infusion, and at 2, 3, 4, 5, 6,7, 8, 13 and 20 days after the day of the infusion. Blood samples were centrifuged (5 min, 3000 r.p.m.) and the plasma layer was separated and immediately stored at −20°C in polypropylene tubes until analysis. The buffy coats with the leukocytes were removed carefully with sufficient margins and discarded, followed by transfer of the red blood cells in polypropylene tubes. After one wash step with ice-cold isotonic phosphate-buffered saline (PBS) the red blood cells were centrifuged at 3000 r.p.m. for 15 min and the supernatant was discarded. The washed red blood cells were immediately stored in polypropylene tubes at −20°C until analysis.

Urine was collected pre-dose and at 12 h intervals between time 0 and 96 h after the start of the E7070 infusion followed by additional 24 h cumulative collections. The urine collection was continued until the E7070 concentration in urine and feces had returned to below 1% in combination with a cumulative excretion percentage higher than 80% of the administered dose (1000 mg). The actual times of the pre-dose urine collection, the times of the urine collection intervals and the volumes of the urine cumulative collections were recorded. Three aliquots of urine (20 ml) were removed, frozen and stored at −20°C until analysis.

One pre-dose feces portion and all feces produced following the administration of E7070 were collected, and the actual time and weight of each collection were recorded. The fecal samples were homogenized in distilled water (1:3, w/v) and stored at −20°C.

Bio-analysis

The detection of β radiation in plasma, red blood cells and PBS was performed by a liquid scintillation counter (LSC; Tri-CARB 2100 CA; Packard, Meriden, CA) with an energy range of 0–2000 keV. Prior to the infusion of E7070 to all patients, a 25-μl aliquot of the infusion solution was removed followed by accurate determination of the radioactivity level by the LSC. Each sample was mixed with 10 ml of Ultima Gold LSC cocktail (Packard) in a plastic vial. The counting time was 5 min per vial. Before the measurement of radioactivity in red blood cells and feces, red blood cell samples (200 μl) were dissolved and decolorized using 1 ml Solvable (Packard Bioscience, Groningen, The Netherlands), 100 μl 0.1 M ethylenediaminetetraaceticacid (EDTA, Titriplex; Merck, Darmstadt, Germany) and 500 μl hydrogen peroxide (Perhydrol; Merck). Fecal homogenates were decolorized using 1 ml Solvable, 1 ml isopropanol (2-propanol; Biosolve, Valkenswaard, The Netherlands) and 0.4 ml hydrogen peroxide (Perhydrol). The samples were analyzed together with calibration standards and QC standards in the LSC. Calibration curves were fitted using least-squares regression analysis. The equation of the calibration curve was used to calculate the radioactivity concentration in all samples in d.p.m. The lower and upper limits of quantification were 1 and 1000 μg/ml E7070, respectively.

The concentrations of E7070 and the metabolite 1,4-benzene sulfonamide (M1) in plasma, urine and fecal homogenates were measured using

high-performance liquid chromatography coupled to an electrospray ionization tandem mass spectrometer (LC/ESI-MS/MS).[12] Before shipment from the C laboratory to the non-radioactive laboratory, the plasma, urine and fecal homogenate samples were diluted with blank plasma, blank urine and distilled water, respectively, to a radioactivity concentration of less than 100 Bq/ml. Samples were stored at −20°C until analysis.

Pre-treatment of the plasma and urine samples involved solid-phase extraction (SPE) on 60 mg Oasis cartridges (Waters, Milford, MA). Fecal homogenates were extracted twice with ethylacetate. Reconstituted extracts were injected onto an Apex Octyl column (Jones Chromatography, Hengoed, UK) and a water/acetonitrile gradient containing 2.5 mM ammonium acetate was used to transfer the analytes to the TurboIonSpray sample inlet (Sciex, Thornhill, Ontario, Canada). Negative ions were created at atmospheric pressure and the parent ions were fragmented in the API 365 triple quadrupole mass spectrometer (Sciex). The transitions for E7070 were selected from *m/z* 383.9 to 319.8 and for M1 from 235 to 170.5. Deuterated internal standards were used for the quantitation. The lower limits of quantification for E7070 and M1 were 0.10 and 0.01 μg/ml in plasma, respectively. In urine 0.05 μg/ml and in feces 0.05 μg/g of both analytes could be quantitated. The upper limits of quantification for E7070 and M1 were 10 μg/ml in urine and 10 μg/g in feces.

Pharmacokinetic analysis

The E7070 and M1 concentrations in plasma were expressed in mmol/l by dividing the concentration in μg/ml by their molecular weights (385.85. and 236.3, respectively). The radioactivity levels in plasma were expressed in mmol/l as the amount of radioactivity expressed in Bq/ml divided by the radioactivity level of the infusion solution in Bq and multiplied by the molarity of the infusion solution (in mmol).

The pharmacokinetic parameters were calculated by applying a non-compartmental analysis using the pharmacokinetic computer program WINNONLIN (Standard Edition version 3.0, 1999; Pharsight, Mountain View, CA). The maximal drug concentration (C_{max}) was derived directly from the experimental data. The first-order rate constant λ_z associated with the terminal elimination portion was calculated by log-linear regression analysis of the concentration versus time curve. The area under the plasma concentration–time curve (AUC_{inf}) was calculated by the linear trapezoidal rule up to the last sampling time point with detectable concentration (C_{last}) with extrapolation to infinity of the terminal elimination phase. The terminal half-life ($t_{1/2}$) was calculated by the equation $\ln 2/\lambda_z$. The apparent clearance (CL) was calculated by dividing the administered dose by the AUC_{inf}. The apparent volume of distribution at steady state (V_{SS}) by multiplying CL by the mean residence time extrapolated to infinity (MRT_{inf}). MRT_{inf} is determined as $MRT_{inf} = (AUMC_{inf}/AUC_{inf}) - (1/2 \times \text{duration of infusion})$, where $AUMC_{inf}$ is the area under the first moment curve with extrapolation to infinity.

The excretion of total radioactivity in urine and feces (in mmol) was calculated as the total amount of excreted radioactivity in Bq divided by the radioactivity of the administered dose in Bq and multiplied by the molarity of the infusion (in mmol). The excretion of E7070 and M1 in urine and feces as a percentage of the administered dose was calculated as the total amount of excreted drug (in mmol) and metabolite (in mmol) divided by the molarity of the infusion (in mmol) × 100.

Statistical analysis

All pharmacokinetic parameters (C_{max}, AUC_{inf}, CL, $t_{1/2}$ and V_{SS}) and the excretion of the total radioactivity, E7070 and M1 in urine and feces are expressed in mean ± SD.

Results

Patients and treatment

Thirteen patients were enrolled in the study. Six patients were included in the first cohort that received 1000 mg of E7070 radiolabeled with [benzene sulfonamide-^{14}C]ER-35744 during the first course. Seven patients were included in the second cohort that received 1000 mg of E7070 radiolabeled with [indole ring-U-^{14}C]ER-35744 during the first course. The patient characteristics are summarized in Table 1.

Pharmacokinetics

The individual plasma concentrations of total radioactivity in both cohorts (determined by the LSC

Table 1. Patient characteristics

Characteristics	Cohort 1[a]	Cohort 2[b]
No. of patients	6	7
Sex		
male	5	4
female	1	3
Age (years)		
mean	56	51
SD	8.8	11.3
WHO PS		
0	0	2
1	5	5
2	1	0
Primary tumor		
lung	1	1
gastric	2	1
colorectal	1	1
pancreas	1	0
melanoma	0	1
ovarian	0	1
carcinoid	0	1
hypopharynx	0	1
primary unknown	1	0
Prior treatment		
prior surgery	4	4
prior radiotherapy	2	3
prior chemotherapy	6	7
prior immunotherapy	0	1

[a]The patients in cohort 1 received [benzene sulfonamide-^{14}C]ER-35744.
[b]The patients in cohort 2 received [indole ring-U-^{14}C]ER-35744.

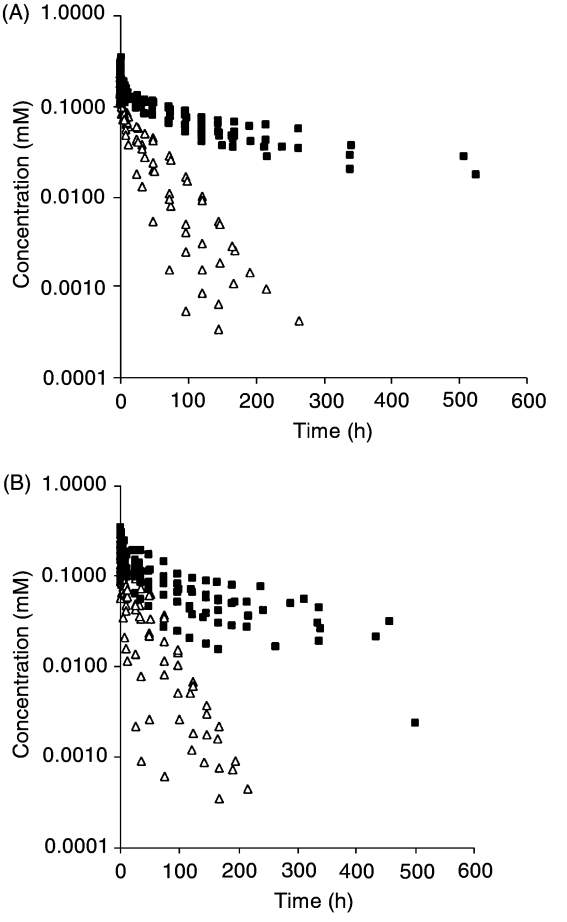

Figure 2. Individual plasma concentrations of total radioactivity (squares) and the contribution of E7070 (triangles) as a function of time after the administration of 1000 mg E7070 containing 3.7 MBq of ^{14}C-labeled E7070 in the first cohort (n=6) (A) and in the second cohort (n=7) (B).

method) Versus the mean of the E7070 (determined by the LC/ESI-MS/MS method) as a function of time are depicted in Figure 2(A, cohort 1 and B, cohort 2). In both cohorts the radioactivity levels (expressed as E7070 equivalents in mmol/l) in plasma are much higher than can be explained by E7070 (and M1) alone. The E7070 concentrations were substantially higher than the M1 concentrations in plasma in both cohorts of patients. The calculated plasma AUC_{inf} of E7070 (3.75 ± 2.02 h · mmol/l) is higher than the AUC_{inf} of M1 (0.14 ± 0.05 h · mmol/l) (Table 2). The radioactivity levels in plasma and red blood cells (as determined by the LSC method) of all patients (n=13) are outlined in Figure 3. The radioactivity concentration in plasma was higher than in red blood cells during and shortly after the infusion of ^{14}C-labeled E7070. Later, the radioactivity concentrations in both compartments are in the same range. The courses of the distribution and elimination phases of radioactivity in plasma and red blood cells are synchronic.

The mean urinary and fecal excretion of radioactivity as a percentage of the administered dose in the first and the second cohort is presented in

Table 2. Pharmacokinetic parameters of E7070 and M1 in plasma after i.v. administration of 1000 mg ^{14}C-labeled E7070 (n=13)

Pharmacokinetic parameter	E7070	M1
C_{max} (mM)		
Mean	0.20	0.0026
SD	0.05	0.0008
$t_{1/2}$ (h)		
mean	19.4	117.2
SD	7.7	47.7
AUC_{INF} (h · mM)		
Mean	3.75	0.14
SD	2.02	0.05
CL (ml/min)		
Mean	17.3	NA
SD	15.6	NA
V_{SS} (l)		
Mean	21.5	NA
SD	4.4	NA

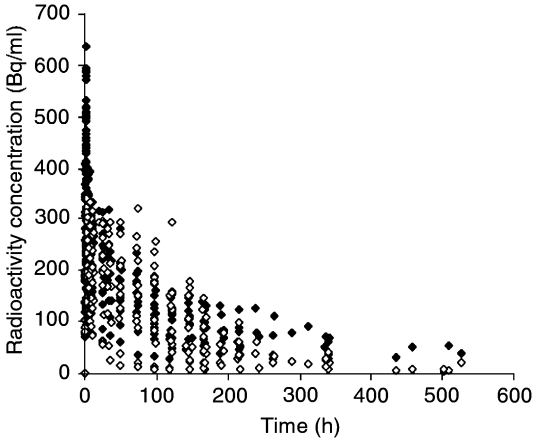

Figure 3. Red blood cell concentrations (open symbols) and plasma concentrations (solid symbols) of radioactivity in all patients (n=13).

Table 3. Percentage of the radioactive dose recovered after i.v. administration of 1000 mg ^{14}C-labeled E7070 (cohort 1 and 2)

Time after E7070 administration (h)	Cohort 1: n=6 (%, mean ± SD)	Cohort 2: n=7 (%, mean ± SD)
(a) In urine		
0–12	14.18 ± 8.2	18.73 ± 12.2
12–24	9.75 ± 4.4	8.97 ± 4.7
24–36	6.89 ± 2.0	6.39 ± 1.5
36–48	4.58 ± 0.8	4.08 ± 0.9
48–60	4.64 ± 0.8	3.65 ± 0.9
60–72	3.73 ± 0.6	2.61 ± 0.9
72–84	3.66 ± 0.9	2.93 ± 1.5
84–96	2.80 ± 1.6	2.04 ± 1.3
96–120	4.25 ± 1.4	3.22 ± 1.8
120–144	3.39 ± 1.2	2.67 ± 1.0
144–168	2.86 ± 1.3	2.08 ± 0.7
168–192	2.00 ± 0.8	1.82 ± 0.9
192–216	1.92 ± 0.9	1.71 ± 0.5
216–240	1.48 ± 0.4	1.33 ± 0.5
240–264	0.80 ± 0.7	1.50 ± 0.9
264–288	1.09 ± 0.2	1.19 ± 0.09
288–312	0.93 ± 0.02	0.98 ± 0.4
312–336	ND	1.05
total urinary excretion	63.7 ± 9.8	61.5 ± 5.5
(b) In feces		
0–48	5.28 ± 3.61	4.46 ± 2.50
48–96	8.94 ± 2.49	6.75 ± 2.33
96–144	5.22 ± 1.54	6.39 ± 2.60
144–192	3.42 ± 2.10	2.72 ± 2.11
192–240	2.23 ± 2.41	0.85 ± 0.51
240–288	0.72 ± 0.61	0.61 ± 0.48
288–336	0.29 ± 0.05	0.34
total fecal excretion	22.7 ± 2.6	21.1 ± 3.1

Table 3. After the iv. infusion of ^{14}C-labeled E7070 the urinary recovery of radioactivity is higher than the fecal recovery in cohort 1 (63.7 ± 9.8 and 22.7 ± 2.6%, respectively) and in cohort 2 (61.5 ± 5.5 and 21.1 ± 3.1%, respectively) over a mean collection period of 11 and 10 days, respectively. The total recovery of radioactivity in urine and feces is 86.4 ± 9.0% (cohort 1) and 82.6 ± 4.6% (cohort 2) of the administered dose. In the first 48 h post dosing only 41% (cohort 1) and 43% (cohort 2) of the administered dose was excreted in urine and feces, which illustrates the slow excretion of E7070 and its metabolites. The cumulative urinary and fecal recovery of total radioactivity, E7070 and M1 are shown in Table 4. The total radioactivity levels are much higher (86.4 and 82.6% in cohort 1 and 2, respectively) than the cumulative recovery of E7070 (2.3 and 2.7% in cohort 1 and 2, respectively) and M1 (5.3 and 5.1%, respectively) in urine and feces. The cumulative recovery of E7070 is almost equal in urine (1.2 ± 0.7 and 1.5 ± 0.8%) and feces (1.1 ± 0.4 and 1.2 ± 0.6%), whereas the cumulative recovery of M1 is higher in feces (3.7 ± 1.6 and 3.5 ± 1.1%) than in urine (1.7 ± 0.6 and 1.7 ± 0.6%) in both cohorts. For the whole patient population (n=13), the cumulative recovery of E7070 and M1 (mean ± SD) is 1.4 ± 0.8% (E7070) and 1.7 ± 0.6% (M1) in urine and 1.1 ± 0.5% (E7070) and 3.6 ± 1.3% (M1) in feces. With reference to the high total radioactivity levels that cannot be explained by E7070 and M1, the detection of metabolites other than M1 was performed in urine. Therefore, a mixed urine sample (100 μl) obtained in six patients (three of the first cohort and three of the second cohort) from 0 to 24 h after the infusion of radiolabeled E7070 was injected on an HPLC system coupled online with a radio-isotope detector. The results are depicted in Figure 4 showing a chromatogram with 15 peaks. E7070 and M1 are represented by the peaks numbered 15 and 3, respectively. The major peak (number 7) represents a metabolite that is formed by glucuronidation after hydroxylation of E7070. This glucuronidated metabolite has a m/z value of 576.3 as identified by mass spectrometry.

In addition, an ascites paracenthesis was performed prior in one patient with malignant ascites to the start of the E7070 infusion and 7 days after the E7070 administration. The total volume of the evacuated ascites post-dosing was 5.5 l containing a radioactivity level of 3% of the administered dose.

Safety

During this mass balance study the toxicities (related to E7070) observed following the first course of ^{14}C-labeled E7070 were generally mild (maximally CTC grade 1–2). The toxicities included anemia (n=1),

Table 4. Urinary and fecal excretion values of total radioactivity, E7070 and M1 following i.v. administration of 1000 mg containing 3.7 MBq ^{14}C-labeled E7070 (cohort 1 and 2)

	Total radioactivity (%, mean ± SD)[a]	E7070 (%, mean ± SD)	M1 (%, mean ± SD)
Cohort 1 (n=6)			
urine	63.7 ± 9.8	1.2 ± 0.7	1.7 ± 0.6
feces	22.7 ± 2.6	1.1 ± 0.4	3.7 ± 1.6
total	86.4 ± 9.0	2.3 ± 0.9	5.3 ± 1.6
Cohort 2 (n=7)			
urine	61.5 ± 5.5	1.5 ± 0.8	1.7 ± 0.6
feces	21.1 ± 3.1	1.2 ± 0.6	3.5 ± 1.2
total	82.6 ± 4.6	2.7 ± 1.2	5.1 ± 0.8

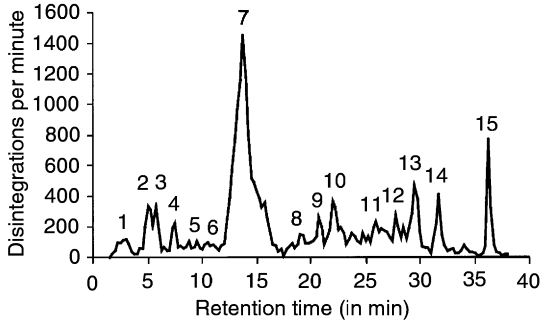

Figure 4. Chromatogram of a urine sample analyzed by a HPLC method with online radioisotope detection. 15=E7070, 3=M1 metabolite, 7=metabolite formed by glucuronidation of E7070.

sinus tachycardia (n=1), nausea (n=1), stomatitis (n=1), diarrhea (n=1), fatigue (n=2) and paresthesias in the face (n=1). All patients continued treatment at a dose of 700 mg/m^2 E7070 infused i.v. in 1 h every 3 weeks.

Discussion

The aim of this mass balance study was to determine quantitatively the (metabolic) disposition of E7070 and its metabolite M1 after i.v. administration. Based on the elimination half-life of E7070 as determined in phase I studies, the anticipated collection time for urine and feces was 7 days.[9,10] However, due to the prolonged retention of radioactivity in plasma, the collection time had to be extended to a maximum of 14 days. The urinary and fecal recovery of total radioactivity (86.4% in cohort 1 and 82.6% in cohort 2) indicates that the duration of urinary and fecal collection was sufficiently long to characterize the excretion of E7070 and its metabolite M1 despite the slow rate of elimination of radioactivity from the central compartment. Following the i.v. administration of ^{14}C-labeled E7070, the major route of elimination of the radioactive tracers is by the kidneys with a mean cumulative urinary recovery of 63.7% (cohort 1) and 61.5% (cohort 2) of the administered dose. We may conclude that the benzene sulfonamide and indole moieties of E7070 show the same elimination pattern and this probably indicates that the majority of metabolites contain an intact sulfonamide link. E7070 is excreted by the renal and gastrointestinal routes to the same extent, but its metabolite M1 is primarily excreted by the gut (Table 4). Only a minority of the excreted radioactivity is represented by E7070 and M1. Further detection of metabolites in urine showed that the high total radioactivity levels are caused by 13 compounds other than E7070 and M1. Glucuronidation appears to be an important metabolic route in humans in contrast to preclinical studies in which this metabolite was not identified. Further research is ongoing to elucidate the chemical structures of all metabolites.

From the measured radioactivity level in ascites (3% of the administered dose) at 1 week post-dosing in one patient we may conclude that E7070 and/or its metabolites can accumulate in third 'spaces'. However, further clinical studies should demonstrate whether this observation has any therapeutic or toxic consequences.

Preclinical pharmacokinetic studies have been performed to elucidate the disposition of E7070 after a single i.v. administration of ^{14}C-labeled E7070 in mice and rats. In contrast to the observations in humans, the mean cumulative fecal excretion of radioactivity (86 and 67%, respectively) was higher than the urinary excretion (13 and 39%, respectively) calculated as a percentage of the administered dose during a collection period of 7 days in rats and 14 days in mice. In urine, the majority of excreted radioactivity represented polar metabolites, and only a minority of radioactivity was excreted as unchanged E7070 and M1. Preliminary indications are that the majority of radioactivity in feces was associated with E7070 and M1.

In human plasma, the radioactivity levels were substantially higher than can be explained by E7070 (and M1 in the first cohort), which indicates that E7070 is extensively metabolized. The M1 concentration is also very low compared to the E7070 concentrations. In combination with the low recovery of M1 in urine and feces, we may conclude that M1 is only a minor metabolite in man. Based on the higher radioactivity concentrations in plasma compared to red blood cells in humans during and shortly after the infusion, E7070 and its metabolites possessing the benzene sulfonamide moiety and the indole moiety reached higher initial concentrations in plasma than in red blood cells (Figure 3). In contrast, the pharmacokinetic studies in rodents showed that the radioactivity concentration in red blood cells was higher than the radioactivity concentration in plasma after administration of ^{14}C-labeled E7070. It remains unclear why the red blood cell:plasma ratio is higher in rodents than in humans.

In conclusion, the results of this mass balance study demonstrate that radiolabeled E7070 given by i.v. infusion is slowly excreted via urinary and fecal routes. E7070 is excreted by the renal and gastrointestinal routes, but M1 is primarily excreted by the gut. The total radioactivity was primarily excreted by the renal route. A high proportion of the radioactivity in human plasma, urine and feces is represented by compounds other than E7070 and M1. These high radioactivity levels are caused by the extensive formation of metabolites other than M1. Thus, E7070 is primarily eliminated by metabolism. Glucuronidation appears a major metabolic pathway. Currently, studies are ongoing to identify the chemical structures and pharmacological activities of the other metabolites.

Acknowledgments

The authors thank the medical and nursing staff of the Antoni van Leeuwenhoek hospital, Paul Jonkergauw and Harry Maessen for the professional support of this study. Michel Hillebrand is kindly acknowledged for his analytical support. We also thank the cancer patients who gave of their valuable time to participate in this study.

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(Received 9 May 2002; accepted 18 June 2002)