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Phase I dose escalation pharmacokinetics of *O*-(chloroacetylcarbamoyl) fumagillol (TNP-470) and its metabolites in AIDS patients with Kaposi's sarcoma

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Abstract The pharmacokinetics of TNP-470 and its major metabolites were investigated in AIDS patients enrolled in a phase I dose escalation trial for the treatment of Kaposi's sarcoma. The patients received TNP-470 by 1-h intravenous infusion in dose cohorts of 10, 20, 30, 40, 50 and 70 mg/m^2. The parent drug and metabolites, MII and MIV, were measured by high-performance liquid chromatography/mass spectrometry (HPLC/MS) in plasma samples collected during and out to 168 h after the beginning of the infusion. Both metabolites were detected in all patients' plasma, while the parent drug was undetectable at time-points as early as 5 min after the end of infusion for some patients. A large interpatient variability of pharmacokinetic parameters among the dosing cohorts was observed for TNP-470, with a mean (±SD) plasma elimination half-life ($t_{1/2}$) of 0.06 ± 0.04 h, plasma clearance (CL) of 1487 ± 1216 l/h and an area under the concentration versus time curve (AUC) of 49.9 ± 35.8 ng/ml · h. Time to maximum plasma concentration (Tmax) typically occurred before the end of the infusion. The predominant plasma metabolite was MII with a $t_{1/2}$ of 1.21 ± 0.43 h, AUC of 1226 ± 2303 l/h and a Tmax occurring between 5 and 15 min after infusion. The reported active metabolite MIV had a $t_{1/2}$ of 0.24 ± 0.13 h, AUC of 24.9 ± 32.6 ng/ml · h and a Tmax occurring between the midpoint of the infusion and 15 min after infusion. The parent drug was undetectable by HPLC/MS/MS in urine samples collected and pooled between 0–6 and 6–24 h from the beginning of drug administration. Metabolite MIV was present in the 0–6-h urine pool of two patients enrolled in the highest dosing cohorts, equivalent to 0.4% of the administered dose. Metabolite MII was present in all 0–6-h samples analyzed and represented 1.12 ± 0.9% of the administered dose. Renal clearance (CL_R) for MII was 140 ± 70 ml/h.

Key words Angiogenesis · Pharmacokinetics · Metabolism · TNP-470 · Kaposi's sarcoma · Mass spectrometry

Introduction

The angioinhibin, *O*-(chloroacetylcarbamoyl) fumagillol (TNP-470), is an analog of the antibiotic fumagillin which is derived from the fungus *Aspergillus fumigatus fresenius*. Fumagillin has been shown to have antiangiogenic activity against endothelial cell proliferation in the presence of saturated levels of basic fibroblast growth factor in vitro at a concentration of 0.5 ng/ml and to suppress tumor-induced vascularization in mice but with severe weight lose. The analog TNP-470 shows a 50-fold increase in antiangiogenic activity over fumagillin without the associated toxicity [1]. Angiogenesis is critical to the growth of tumors. Inhibitors of angiogenesis or angioinhibins have been shown to have the ability to inhibit tumor cell growth in vitro and in murine models in vivo while maintaining an acceptable therapeutic index.

Inhibition of tumor cell growth and/or metastasis by TNP-470 has been demonstrated across a wide range of tumor cell types in vitro and in vivo, including Lewis lung carcinoma, colon adenocarcinoma, fibrosarcoma, schwannomas, neurofibromas, BL6 melanoma, N5706 reticulum cell sarcoma, Walker 256 carcinoma and VX-2 carcinoma [2–7].

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The mechanism by which TNP-470 specifically inhibits the highly proliferative new capillary growth of solid tumors has not been clearly detailed. A study utilizing bovine aortic endothelial cells (BAEC) has indicated that cells are blocked from entering S phase of cell division. Transition from G_0 to S phase induced by basic fibroblast growth factor (bFGF) is inhibited by 50% if TNP-470 is added to the system. Furthermore, S phase is inhibited even if TNP-470 is removed 2–4 h after growth factor stimulation [8]. Human umbilical vein endothelial cells treated with TNP-470 potently inhibit late G_1 mechanisms and prior to S phase [9]. The fumagillin/TNP-470 binding protein, methionine aminopeptidase (MetAP-2), has been identified and is highly conserved between humans and the yeast, *Saccharomyces cerevisiae*. This enzyme may serve as the target for TNP-470 as its expression is greatly induced in dividing cells. The mechanism for TNP-470's specificity toward capillary endothelial cells is under investigation [10].

The in vitro and in vivo metabolism of TNP-470 has been studied in human, rat, dog, and monkey hepatocytes and tissue microsomes, as well as in nonhuman primates [11–13]. TNP-470 is rapidly and extensively metabolized to six metabolites, designated MI through MVI. Of these, only MII, MIII and MIV have been structurally and metabolically characterized. The pathway by which TNP-470 is converted to its metabolites includes a rapid and extensive esterase cleavage of TNP-470 to MIV which is converted to the more polar MII by microsomal epoxide hydrolase, followed by glucuronidation to MIII [11]. The presence of MII and MIV in humans has been recently reported [14–16].

The ability to inhibit and reduce the vascularization of solid tumors in vitro prompted investigators to administer TNP-470 against various malignant tumor diseases in clinical trials [14, 16–19]. One such disease is Kaposi's sarcoma (KS) which is the most prevalent neoplasm associated with HIV infection [20]. The tumors are highly vascularized as evident by there characteristic deep purple color and secretion of known autocrine and paracrine growth factors associated with tumor growth and metastasis [21]. TNP-470 has demonstrated antiangiogenic effects against KS-related spindle cell lines at concentration of 10 pg/ml [22].

Treatment of KS with TNP-470 has been investigated in 39 AIDS patients enrolled in a phase I dose escalation trial conducted by the AIDS Clinical Trial Group (ACTG-215). TNP-470 was administered over a 24-week period in individual dosing cohorts of 10, 20, 30, 40, 50 and 70 mg/m^2. Of the 39 patients, 18% demonstrated a partial tumor response characterized by complete flattening of ≥50% for all previously raised lesions and a ≥50% decrease in diameter of baseline lesions. Tumor response occurred at various dose levels. The median time to partial response was 4 weeks with a median duration of 11 weeks. However, 13% of the patients experienced possible drug-associated adverse events, including neutropenia, hemorrhage and urticaria. The events were observed at doses of 10–50 mg/m^2, while no toxicity was reported in patients treated with 70 mg/m^2 TNP-470 [17].

In the current study, the pharmacokinetics of TNP-470 and its metabolites, MII and MIV, were evaluated in 14 patients enrolled in ACTG-215. Measurement of the parent drug and metabolites in plasma and urine was performed by validated HPLC/MS and HPLC/MS/MS methods which provided enhanced sensitivity and specificity required for the characterization of the rapidly metabolized and eliminated compounds.

Methods and materials

Study design

The drug TNP-470 was administered in a 1-h intravenous infusion once a week in cohorts of 10, 20, 30, 40, 50 and 70 mg/m^2. Four to eight subjects were planned for each cohort with no dose escalation allowed in any patient. Dose escalation occurred after a minimum of four patients had successfully completed 4 weeks of therapy with fewer than 50% of all patients in the cohort experiencing a treatment-related grade 3 or 4 toxicity.

Patients

A total of 39 patients were enrolled at nine ACTG sites within the United States. Inclusion criteria were biopsy-proven AIDS-related KS with five or more measurable lesions and no evidence of pulmonary, symptomatic gastrointestinal or acutely life-threatening KS. Antiretroviral therapy was permitted but not required. Other eligibility requirements included neutrophil counts $>1000/\mu l$, platelet count $>100{,}000/\mu l$, hemoglobin >9.0 g/dl, AST/ALT less than three times the upper normal limit (UNL), creatinine less than 1.5 times UNL, bilirubin <2 mg/dl and APTT/PT $<120\%$ of control. Exclusion criteria included peripheral neuropathy grade 2 or more, history of bleeding disorders or seizures, active opportunistic infection, pregnancy or nursing and a life expectancy of <3 months. Concomitant use of antineoplastics, corticosteroids, anticonvulsants and drugs that may have increased the risk of bleeding was not allowed.

Samples and preparation

At least two patients from each dose cohort had pharmacokinetic samples drawn for evaluation. Initially plasma pharmacokinetic samples were drawn at 0 (predose), 0.5 h (mid-dose) and 1 h (end of infusion), and at 1.25, 1.50, 2, 3, 4, 6, 24, 96 and 168 h. Additional time-points at 1.08, 1.17, and 1.33 h were added to the 30–70 mg/m^2 cohorts when it was observed that TNP-470 was rapidly eliminated in the lower dose cohorts. At each time-point, 10 ml blood was collected into a sodium heparin vacutainer and stored on ice until centrifugation. Plasma was separated within 30 min of sample collection and 300 µl 2% (v/v) sulfuric acid was added to each 3–5 ml of plasma to inactivate degradative enzymes at a pH of 4–5 [23]. Urine samples were pooled from the 0–6-h and 6–24-h study periods and 2% (v/v) sulfuric acid was added. Acidified plasma and urine samples were shipped to the University of Alabama at Birmingham on dry ice and immediately stored at −70 °C until analysis.

Sample analysis

Analysis of plasma samples for TNP-470 and the metabolites MIV and MII was performed by HPLC/MS as previously described [15]. Briefly, the plasma samples were extracted by C-18 reversed-phase solid-phase extraction columns after the addition of a ^{13}C-labeled

tri-deuterated TNP internal standard (^{13}C-TNPd$_3$). Resolution and quantitation was performed by directing the HPLC stream into a heated nebulizer atmospheric chemical ionization interface connected to a mass spectrometer. The [M + NH_4] adduct for each compound was selected in the mass spectrometer quadrupole and ion peak area integration was used for final concentration calculation against a calibration curve generated from authentic standards spiked into acidified drug-free plasma. Quality control samples of authentic standards spiked into acidified drug-free plasma were assayed with patient samples to verify the accuracy of the results. Method accuracy was not less than 87.4% for any compound over the analytical range of the method, with analytical precisions (%CV) of not more than 10%. The limits of quantitation (LOQ) for TNP-470 and MIV were 0.62 ng/ml and 2.5 ng/ml for MII.

Separation and quantitation of the compounds in urine were performed in a similar fashion by diluting 10 µl of the sample into 90 µl of a 100 ng/ml internal standard solution of ^{13}C-TNPd$_3$ in 1:1 (v/v) 2 m*M* NH_4OAC/acetonitrile. The samples were centrifuged at 14,000 rpm for 5 min and 90 µl was manually injected and analyzed under the same HPLC conditions as described. Quantitation of TNP-470 and metabolites in urine was performed by the HPLC interfaced mass spectrometer using triple quadrupole detection rather than a single quadrupole. The [M + NH_4] molecular ion for each compound was selected in the first quadrupole (Q1) and was accelerated into a region filled with an inert gas mixture of argon/nitrogen (Q2). The most abundant fragments generated (daughter ions) were selected in the second quadrupole (Q3) for integration and quantitation. This triple quadrupole technique is commonly known as multiple reaction monitoring (MRM) whereby multiple compounds are measured simultaneously. This MRM technique is very specific, sensitive and fast, even in the presence of endogenous interferences associated with human urine or concomitant drug administration. Analyte concentrations were calculated from calibration curves prepared from authentic standards diluted with acidified drug-free human urine. Quality control samples containing TNP-470, MII and MIV prepared in acidified drug-free human urine at concentrations of 0, 78, 312 and 1250 ng/ml were incorporated into each run to verify the adequacy of the sensitivity and accuracy. The analytical range for all three compounds was 39 to 2500 ng/ml with a regression coefficient (r^2) of >0.99 for a curve fit through the origin. Interday precision (%CV) and accuracy were no more than 11% and not less than 96%, respectively. The LOQ was 39 ng/ml for MII, MIV and TNP-470.

Pharmacokinetics

The pharmacokinetic parameters were calculated using noncompartmental analysis on concentration versus time data generated from the first weekly dose of TNP-470 for each dose cohort. Concentration versus time plots were generated for each patient utilizing all measurable time points (≥LOQ). Regression analysis was performed on all points between the Cmax, Tmax to minimum concentration (Cmin), time to minimum concentration (Tmin) coordinates where the number of points was three or more ($r^2 > 0.87$ for all patients). Elimination half-life ($t_{1/2}$) was calculated as −0.693/K for the natural log of the concentration vs time curve regression line. Area under the concentration curve (AUC) was calculated by the trapezoidal rule for all points between initiation of infusion (zero time-point) and Cmin, Tmin coordinates. Plasma clearance (CL) was calculated as dose/AUC. Renal clearance (CL_R) was calculated as the amount of drug or metabolite excreted during the 0–6-h period divided by the concentration.

Results

Plasma pharmacokinetics

Figure 1 shows the metabolic pathway by which TNP-470 is converted into its three major metabolites, MII, MIII and MIV. The HPLC/MS analysis of plasma samples demonstrated the presence of MII and MIV in every patient at the infusion midpoint and 5–15 min after infusion. However, TNP-470 was not present above the LOQ of the analytical method 5–15 min after infusion in 9 of 14 pharmacokinetic data sets. This was due to the rapid disappearance of TNP-470 from the plasma and the absence of the 5- and 10-min post-infusion time-points for doses ≤20 mg/m^2. Therefore, the elimination half-life was not calculated for TNP-470 in these cohorts. The mean (±SD) $t_{1/2}$ of TNP-470 in all patients at doses ≥30 mg/m^2 was 0.06 ± 0.04 h. Plasma CL and AUC for all patients was 1487 ± 1216 l/h and 49.9 ± 38.5 ng/ml · h. Maximum plasma concentrations (Cmax) ranged from 4.3 to 181.8 ng/ml. Tmax was either at the midpoint or the end of the 1-h infusion. Tmin was between 1.08 and 2 h for doses ≥30 mg/m^2. All patients enrolled in the pharmacokinetic evaluation had measurable plasma MIV concentrations. The $t_{1/2}$ (mean ± SD) for all cohorts was 0.24 ± 0.13 h with an AUC of 24.9 ± 32.6 ng/ml · h. Plasma Cmax values ranged from 1.9 to 98.7 ng/ml. MIV Cmax values were typically five to ten times lower than the corresponding TNP-470 Cmax. Tmax for the MIV metabolite was between the infusion midpoint and 1.25-h time-point. Tmin was predominantly between the 1.5- and 4-h time-point for MIV. For MII, $t_{1/2}$ was 1.21 ± 0.43 h with an AUC of 1226 ± 2303 ng/ml · h, Cmax ranged from 26 to 7196 ng/ml and Tmax occurred between the infusion midpoint and the 1.25-h time-point. Tmin was typically at the 6-h time-point. No MII was detected in the 24-, 96- or 168-h samples. Table 1 summarizes the pharmacokinetic parameters for TNP-470 and its metabolites, MIV and MII. Figure 2 shows the plasma concentration versus time plot for a patient receiving 30 mg/m^2 TNP-470 as a 1-h intravenous infusion.

Urine pharmacokinetics

Urine was collected and pooled during the periods 0–6 h and 6–24 h of the pharmacokinetic study. These samples were assayed by HPLC/MS/MS to investigate the renal elimination of the parent drug and metabolites. None of the samples contained measurable amounts of TNP-470 at an LOQ of 39 ng/ml. Two patients receiving 50 and 70 mg/m^2 TNP-470 had measurable amounts of MIV in their 0–6-h urine sample equal to a CL_R of 14 and 71 ml/h, respectively, representing 0.4% of the administered dose. In contrast, all 0–6-h samples contained measurable amounts of MII. The mean renal clearance for MII was 140 ± 70 ml/h, representing between 0.5% and 1.9% of the total dose administered. The excretion rate of MII ranged from 51 to 379 µg/h and was not dose dependent. Measurable MII renal clearance between 6 and 24 h was negligible, with only two individuals excreting MII equal to 2.2% and 0.05% of the administered dose. Table 2

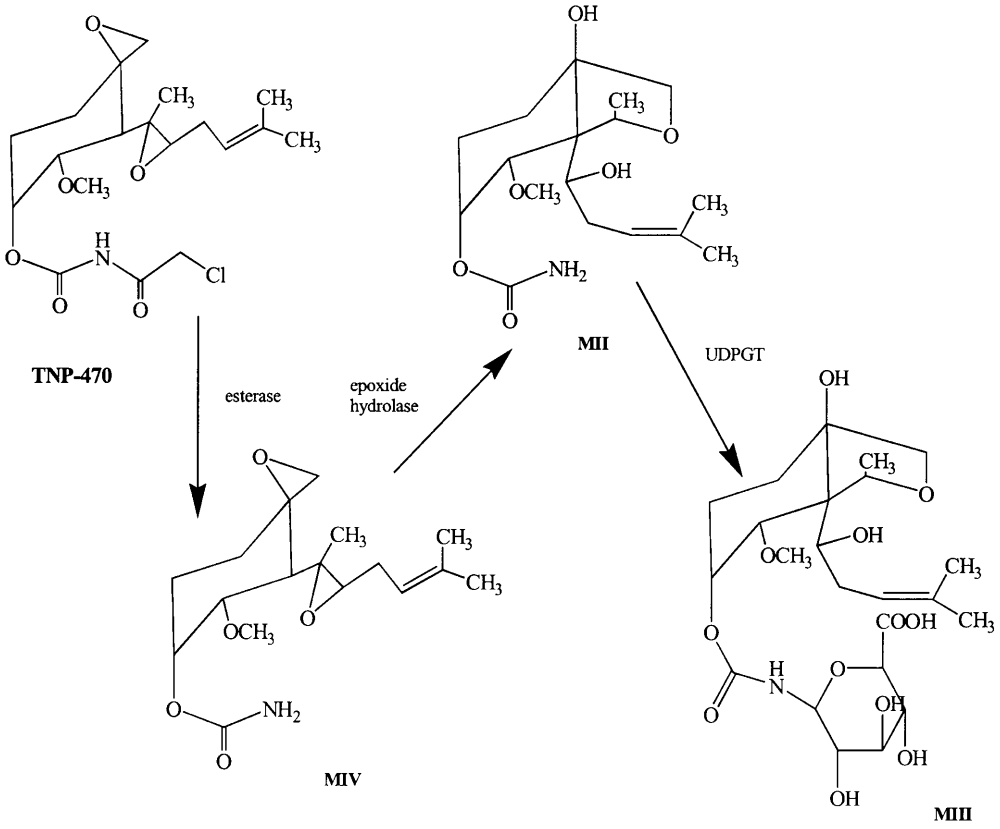

Fig. 1 Metabolic pathway of TNP-470 (edited with permission, Placidi et al. 1999)

summarizes the urinary pharmacokinetic parameters for MII and MIV.

Discussion

A total of 39 AIDS patients from nine ACTG-215 sites with KS were given the angioinhibin TNP-470 in a phase I dose escalation trial. The drug was administered as weekly 1-h intravenous infusions for 24 weeks in escalating dosing cohorts of 10, 20, 30, 40, 50 and 70 mg/m^2. Dose escalation was not allowed within individual patients. The clinical objectives of the trial were to ascertain the possible antitumor effects and determine the maximum tolerated dose when administered on a weekly basis. The clinical outcome suggests that TNP-470 was

Table 1 Plasma pharmacokinetics of TNP-470 and metabolites MIV and MII after 1 h. Values are cohort means ± SD (*n* number of patients evaluated, $t_{1/2}$ elimination half-life, *Cmax* maximum concentration, *Tmax* time to maximum concentration, *AUC* area under the concentration time curve from Cmax:Tmax to Cmin:Tmin, *CL* clearance)

	Dose (mg/m^2)	*n*	$t_{1/2}$ (h)	Cmax (ng/ml)	Tmax (h)	AUC (ng/ml · h)	CL (l/h)
TNP-470	10	2	–	13.5 ± 13.0	0.88 ± 0.64	10.8 ± 9.6	2926 ± 2606
	20	5	–	110.5 ± 73.2	0.75 ± 0.35	34.4 ± 37.1	1424 ± 983
	30	2	0.10 ± 0.01	91.9 ± 26.3	0.75 ± 0.35	63.3 ± 3.0	877 ± 5.1
	40	1	–	163.1	0.52	87.8	774
	50	1	0.02	181.8	1.00	81.4	1197
	70	1	0.03	142	1.00	110.0	1161
	All	12	0.06 ± 0.04[a]	126.9 ± 46.2	0.88 ± 0.25	49.9 ± 38.5	1487 ± 1216
MIV	10	2	0.27 ± 0.15	11.6 ± 13.7	1.13 ± 0.28	11.6 ± 13.9	
	20	5	0.16 ± 0.03	19.2 ± 31.7	0.74 ± 0.41	25.9 ± 49.3	
	30	2	0.35 ± 0.26	54.1 ± 63.0	1.01 ± 0.01	41.9 ± 46.2	
	40	1	0.18	13.0	0.52	11.2	
	50	2	0.30 ± 0.25	22.3 ± 9.33	1.07 ± 0.02	17.9 ± 20.1	
	70	2	0.30 ± 0.06	26.9 ± 16.5	1.09 ± 0.13	31.5 ± 23.1	
	All	14	0.24 ± 0.13	24.2 ± 29.0	0.92 ± 0.32	24.9 ± 32.6	
MII	10	2	1.35 ± 0.21	453 ± 604	0.88 ± 0.64	747 ± 1022	
	20	5	1.09 ± 0.10	257 ± 253	0.91 ± 0.27	482 ± 548	
	30	2	1.34 ± 0.25	290 ± 271	1.21 ± 0.06	421 ± 438	
	40	1	0.84	245	1.08	433	
	50	2	1.78 ± 1.06	908 ± 898	1.03 ± 0.04	1106 ± 1008	
	70	2	0.90 ± 0.04	3884 ± 4685	1.13 ± 0.06	4886 ± 5792	
	All	14	1.21 ± 0.43	900 ± 1858	1.01 ± 0.26	1226 ± 2303	

[a] Calculated from 30–70 mg/m^2 cohorts

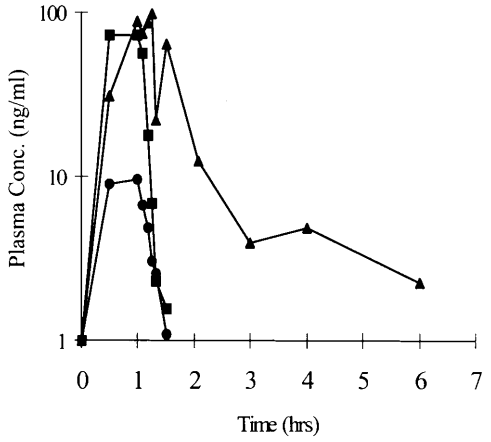

Fig. 2 Time versus concentration curve for a patient receiving 30 mg/m^2 of TNP-470 as a 1-h intravenous infusion (■ TNP-470, ▲ MII, ● MIV)

moderately active and well tolerated [17]. Of the 39 patients, 14 were enrolled in the pharmacokinetic evaluation portion of this study. The pharmacokinetic parameters for TNP-470 and its metabolites MIV and MII demonstrated a wide variability, both on an inter- and intracohort basis.

Rapid degradation of TNP-470 in whole blood prior to acidification to a pH of 4–5 has been reported. The loss of up to 89% TNP-470 (range 9–89% for six individuals) has been observed when whole blood is spiked and stored on ice for 40 min [24]. This could explain the negligible amounts of TNP-470 observed in the 10 and 20 mg/m^2 cohorts as well as the high variability in the pharmacokinetic parameters obtained. The reported degradation of TNP-470 and the internal standard C^{13}-$TNPd_3$ along with variability (>15%) in analyte response factors resulting from residual water in the C-18 solid-phase extract prior to evaporation was not observed. Variance in raw internal standard peak areas performed over four different assays did not exceed 14%. Most of this interassay variance was attributed to mass spectrometer tuning for optimum mass resolution and sensitivity. The variability in response factors for TNP-470, MIV and MII did not exceed 13% for any analyte in four separate analyses. This lack of reported variability was achieved by completely drying the C-18 solid-phase extraction column under 20 mm Hg for 5 min as described previously [15].

Plasma concentrations of TNP-470 rapidly declined below the LOQ of the HPLC/MS method within the time of infusion, with subsequent appearance of MIV and MII at the midpoint of the infusion for doses ≤20 mg/m^2. At doses of ≥30 mg/m^2, TNP-470 was not measurable beyond 30 min after infusion at a concentration of 0.62 ng/ml. The elimination half-life was 0.06 ± 0.04 h with a dose-dependent increase in AUC from 10.8 to 110 ng/ml · h over the 10–70 mg/m^2 escalation trial. The plasma CL of 1487 ± 1216 l/h further supports a rapid metabolism of the parent drug. This rapid metabolism has been also observed in vitro in human, monkey, rat, and dog primary cultured hepatocytes where TNP-470 and MIV decreased to undetectable levels in the extracellular medium within 1–3 h [13]. Noteworthy was the plasma elimination of TNP-470 in three Rhesus monkeys which had received doses of TNP-470 equivalent to 57.6 mg/m^2. The unchanged parent drug was undetectable at 0.05 μ*M* (20 ng/ml) 6 min after an intravenous injection [14]. Similar TNP-470 pharmacokinetics have also been demonstrated in 31 patients with various forms of advanced cancer receiving 177 and 235 mg/m^2 of TNP-470 as a 4-h intravenous infusion on a weekly basis. Tmax occurred at the infusion midpoint and TNP-470 was undetectable (LOQ 0.25 ng/ml) at 60 min after infusion. The elimination half-life for TNP-470 was 3 ± 3 min (0.05 ± 0.05 h) for the 177-mg/m^2 dose and 2 ± 1 min (0.03 ± 0.02 h) for the 235-mg/m^2 dose [16]. These pharmacokinetic parameters in human plasma are consistent with the mechanism by which plasma and tissue esterases extensively hydrolyze TNP-470 to its initial metabolite, MIV. Additional support is derived from the fact that TNP-470 was not detected in any of the 0–6-h urine pools submitted for doses up to 70 mg/m^2.

The TNP-470 metabolite MIV was detected in all patients. Cmax and AUC values varied widely (>50%) among intracohort samples. The AUC values did increase in a dose-dependent fashion as did the parent drug. This observation is in agreement with the tenfold variation seen in MIV levels generated following incubation of 5 μ*M* [^{3}H]TNP-470 with human hepatic microsomes obtained from seven different donors [11]. The MIV $t_{1/2}$ of 0.24 ± 0.13 h is similar to 0.28 ± 0.19 h [14] and 0.15 ± 0.12 h [16] reported previously. Urinary excretion of MIV was limited and occurred in only two patients during the 0–6-h urine collection period, representing approximately 0.4% of the administered dose. These patients were in the higher dosing groups of 50 and 70 mg/m^2 and had Cmax values of 28.9 and

Table 2 Renal pharmacokinetics of metabolites MII and MIV in samples collected during 0–6 h. Values are cohort means ± SD (*n* number of patients evaluated, CL_R clearance, *%* percent of original dose excreted as metabolite, *rate* rate of metabolite excretion)

Metabolite	Dose (mg/m^2)	*n*	CL_R (ml/h)	%	Rate (μg/h)
MII	10	2	188 ± 96	1.9 ± 1.7	60 ± 55
	20	5	168 ± 59	0.8 ± 0.6	51 ± 36
	30	2	124 ± 52	2.3 ± 0.1	193 ± 13
	40	1	167	0.9	98
	50	2	56 ± 60	0.5 ± 0.2	81 ± 27
	70	1	71	1.6	379
	All	13	140 ± 70	1.2 ± 0.9	108 ± 100
MIV	10	0	–	–	–
	20	0	–	–	–
	30	0	–	–	–
	40	0	–	–	–
	50	1	14	0.4	62
	70	1	71	0.4	92
	All	2	42 ± 40	0.4 ± 0	77 ± 21

38.5 ng/ml, respectively, between 0 and 6 h. In accordance with this observation, MIV was present in one of three Rhesus monkeys given 57.6 mg/m^2 of [^{3}H]TNP-470. The total amount of MIV excreted in urine was equal to 0.15% of the administered dose. This animal had the highest MIV plasma level of the three animals tested. In microsomes cultured from human duodenum, jejunum and ileum, as well as stomach and kidney microsomes, it has been shown that MIV is minimally converted to MII [11]. These results suggest a possible saturation of microsomal epoxide hydrolase, the enzyme responsible for conversion of MIV to the polar MII metabolite. The pharmacokinetic evaluation of MII showed that this compound is by far the most abundant and persistent of the three compounds in plasma. However, this metabolite is generally accepted as a nonactive metabolite because of its glucuronidation and excretion in the urine as metabolite MIII [13]. The reported plasma $t_{1/2}$ value of 1.21 $\pm$ 0.43 h is similar to that of the in vivo nonhuman primate $t_{1/2}$ (0.91 $\pm$ 0.37 h) which was based on similar doses [12].

TNP-470 concentrations achieved for doses ranging from 10 to 70 mg/m^2 were much higher than the in vitro minimally effective concentration of 10 pg/ml required for the inhibition of KS-related spindle cell formation. No correlation between pharmacokinetic and clinical pharmacodynamics can be formulated. Of the 14 patients enrolled in the pharmacokinetic portion of ACTG-215, only one patient in the 20-mg/m^2 cohort demonstrated a partial clinical response characterized by ≥50% flattening of all raised baseline lesions at week 2. However, limited tumor response has been reported in other clinical studies [16, 18, 19]. A potentially beneficial modality for TNP-470 is in combination with conventional anticancer drugs. The administration of TNP-470 and other angioinhibins together with conventional cytotoxic anticancer drugs to mice for the treatment of Lewis lung carcinoma has been shown to lead to decreases in primary tumor growth and metastasis. A lesser response was seen for TNP-470 alone and in combination with other angioinhibins [25]. Coincubation of 5 μM [^{3}H]TNP-470 and 250 μM cyclophosphamide with monkey hepatocytes resulted in an increase in TNP-470 and MIV concentration compared to controls. The combination of Taxol and [^{3}H]TNP-470 resulted in a 2.5-fold increase in TNP-470 and MIV concentrations. In both incubation mixtures decreases in MII by as much as 50% compared to controls were found. Cyclophosphamide and Taxol were postulated to modify epoxide hydrolase's ability to convert MIV to MII. It was also stated that MIV has potent antiangiogenic properties, whereas MII is pharmacologically inactive [26].

Synergistic drug-drug interactions between angiogenic inhibitors TNP-470 and MIV with commonly used cytotoxic anticancer agents may represent a clinically valuable treatment regimen in some types of solid tumor diseases. Interaction between controlling neovascularization and metastasis with angioinhibins and tumor killing in place with classical anticancer cytotoxic agents may provide a potentially lethal combination to solid tumors in early disease stages. Clinical trials utilizing these combinations are warranted.

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