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SHORT COMMUNICATION

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Phase I clinical and pharmacokinetic study of oral 9-aminocamptothecin (NSC-603071)

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Abstract *Purpose*: 9-Aminocamptothecin (9-AC) is a topoisomerase I inhibitor with high antitumor activity but poor solubility in conventional vehicles. The purpose of this study was to evaluate the toxicities and pharmacokinetics of a colloidal dispersion (CD) formulation of 9-AC when administered orally on a 5 days per week every 2 weeks schedule. *Method*: This formulation, which was developed for intravenous administration, was orally administered in 20 ml orange juice. A group of 16 cancer patients were treated at doses of 0.2–0.68 mg/m^2 daily. *Results*: Grade 1–2 nausea ($n = 9$) was common, usually occurring during the last 2 days of dosing. No objective responses or cumulative toxicities were observed. Pharmacokinetic analysis of total 9-AC showed highly variable apparent oral 9-AC clearance and half-life. There was marked interpatient variability at each dose level in the 9-AC AUC and C_{max}, and these parameters showed a poor correlation with dose ($r^2 = 0.07$ and 0.38, respectively). *Conclusions*: We conclude that this formulation is not suitable for further clinical development because of poor bioavailability and highly variable and/or saturable absorption or elimination. Another formulation developed for oral administration is under study elsewhere.

Key words 9-Aminocamptothecin · Oral · Pharmacokinetic · Phase I · Topoisomerase inhibitor · CD formulation

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Introduction

9-Aminocamptothecin (9-AC, NSC 603071) is an analogue of the topoisomerase I inhibitor, camptothecin [16], a natural product obtained from the Chinese plant *Camptotheca acuminata* [15]. Camptothecins bind to the DNA-topoisomerase I complex and prevent the resealing of DNA [2, 5]. The resultant accumulation of DNA-enzyme complex results in blockage of RNA polymerase activity [1] and resealing of the topoisomerase I-mediated single-strand breaks. 9-AC, given intramuscularly or subcutaneously, has also demonstrated high antitumor activity against breast, lung and melanoma xenografts, hepatic metastases of primary colon tumors, and intraperitoneally implanted leukemias and sarcomas [7–10, 16]. In some advanced human tumor models like LoVo and HT29, oral administration of 9-AC has resulted in better antitumor activity than subcutaneous administration [8]. As in other camptothecin analogues, an intact E-ring is necessary for activity and the formation of a carboxylate reduces its activity. The closed lactone opens at physiologic pH to form a carboxylate, and no other metabolites have been identified to date [13]. Based on preclinical studies, it has been recommended that repeated courses of 48–72-hour infusions provide optimal antitumor activity. In these studies, 9-AC has demonstrated excellent activity provided lactone concentrations of at least 10 n*M* are maintained for about 48 h with short periods of recovery between courses [10, 12].

Since 9-AC is very insoluble in conventional vehicles, the initial phase I studies with 9-AC were conducted with the dimethyl acetamide (DMA) formulation developed by the NCI. This formulation consists of DMA and a diluent containing 51% polyethylene glycol 400 and 49% 0.01 *M* phosphoric acid. Subsequently, Pharmacia (now Pharmacia-Upjohn, Kalamazoo, Mich.) developed a more convenient colloidal dispersion (CD) formulation consisting of two lyophilized semisynthetic phospholipids. The CD formulation is reconstituted with 20% dextrose/0.9% sodium chloride injection diluent. We conducted a phase I clinical trial using the CD formulation of 9-AC given orally daily for 5 consecutive days every 2 weeks.

The selection of the starting dose for our phase I study of oral administration of the 9-AC CD formulation was based upon results obtained from 5-day oral 9-AC CD administration to dogs (P.K. Narang, Pharmacia-Upjohn, personal communication [3, 6]). In that study conducted by Pharmacia, dogs were given oral doses of 9-AC at 0, 0.5, 1.0, and 2.0 $\mu g/m^2$ per day for 5 days. Five male and five female dogs were studied at each dose level and pharmacokinetic samples were collected up to 2 h after the first and last administration of 9-AC. After the third dose, predose and 1-h samples were collected. The 1.0 $\mu g/m^2$ per day dose caused a 30–40% decrease in the white blood cell (WBC) count and a 25–35% decrease in platelets and was considered the toxic dose low (TDL).

Material and methods

Patients

A group of 16 cancer patients (four colon, four non-small-cell lung, two ovarian, one small-cell lung, one pancreatic, one bile duct, one unknown primary, one breast and one rectal cancer) with adequate organ function (ANC $\geq 2000/\mu l$, platelets $\geq 100\,000/\mu l$, total bilirubin ≤ 1.5 mg/dl, creatinine ≤ 1.6 mg/dl, SGOT not more than three times the institutional upper limit of normal) were enrolled in this study. The median age was 61 years (range 32–72 years). Seven patients were male and nine patients were female. The median Karnofsky performance status was 90% (range 70–100%). Patients with prior gastrectomy or total ileocolectomy were excluded from protocol entry.

9-AC was administered daily for 5 days (one course) repeated every other week. It was administered at 0.33 mg/m^2 per day to one patient; however, this patient received course two through four at 0.2 mg/m^2 per day (for the reason stated above). It was given at 0.2 mg/m^2 per day to six patients and at doses of 0.3, 0.45, and 0.68 mg/m^2 per day to three patients at each dose level. The dose levels chosen were based on toxicity escalations of 100% of the prior dose level until two episodes of reproducible grade 1 toxicity were observed, followed by escalations of 50% of the prior dose level until any one episode of grade 2 toxicity was observed, followed by 25% dose increments. Toxicity assessments were done weekly and response assessments were performed every four cycles of treatment. The median number of chemotherapy cycles given was four (range one to five).

9-AC CD was reconstituted in special diluent provided by Pharmacia-Upjohn through the NCI's Cancer Therapy Evaluation Program (CTEP) consisting of 20% dextrose/0.9% sodium chloride supplied as a sterile solution in 50- or 100-ml flint glass vials. The diluent was presterilized in steam with a pH between 3.5 and 4.5. Both drug and diluent were warmed to room temperature before reconstitution to 20 $\mu g/ml$ final concentration. The reconstituted solution was stored under refrigeration and protected from light and the appropriate dose administered in 20 ml orange juice within 24 h of reconstitution. In our patients, we encouraged dose administration between 9 a.m. and noon. Patients were requested to fast for 1 h before and after dose administration.

Pharmacokinetic analysis

All patients were fully assessable for toxic effects and 9-AC pharmacokinetics. Detailed 9-AC sampling was conducted on day 1 cycle one at all dose levels studied. Intrapatient variation in 9-AC kinetics were not assessed. The detailed sampling times included baseline, and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 24 h after 9-AC administration. Responses and toxic effects were evaluated according to NCI Common Toxicity Criteria. Assays for the levels of total 9-AC were performed by the method of Takimoto et al. [14] using high-performance liquid chromatography.

C_{max} of 9-AC and time to reach C_{max} were visually estimated from plots of plasma 9-AC concentrations versus time. Other pharmacokinetic parameters were determined by standard noncompartmental analysis [4] using Win Nonlin (version 3.1, Statistical Consultants, Lexington, Ky.). The terminal elimination half-life ($t_{1/2}$) was calculated by dividing the slope obtained by log-linear regression of the terminal portion of the plasma concentration-time profile (λ_z) by 0.693. AUC_{0-24} was extrapolated to $AUC_{0-\infty}$ by the addition of $C_{24}/(\lambda_z)^2$ to AUC_{0-24}, where C_{24} is the plasma 9-AC concentration at 24 h. Systemic oral clearance (Cl_{sys}/F) was calculated by dividing the dose by $AUC_{0-\infty}$, where F is oral bioavailability.

Results and discussion

The pharmacokinetic parameters obtained after oral 9-AC administration are listed in Table 1. The apparent oral 9-AC clearance and half-life were highly variable. There was marked interpatient variability at each dose level in the 9-AC AUC_{0-24} and C_{max} and these parameters showed a poor correlation with dose (Fig. 1A,B). The AUC_{0-24} obtained from our study (ranging from 134 ± 108.7 to 353.6 ± 409 ng h/ml) were significantly lower than AUCs obtained using comparable intravenous dosing (ranging from 1513.7 ± 399.3 to 2479.3 ng h/ml) [14].

Table 1 Pharmacokinetic parameters of total 9-AC (*n* number of patients, C_{max} maximal plasma concentration, AUC_{0-24} area under the curve over 24 h, $t_{1/2}$ terminal elimination half-life, Cls/F apparent oral clearance)

Dose (*n*) (mg/m^2/day)	C_{max} (ng/ml)	AUC_{0-24} (ng h/ml)	$t_{1/2}$ (h)	Cl_s/F (l/h)
0.2(6)	9.6 ± 4.7	70 ± 66	9.1 ± 5.5	4.9 ± 4.6
0.3(3)	20.1 ± 10.8	239 ± 243	8.5 ± 7.8	4.9 ± 5.3
0.33(1)	11.1	192	24.3	2.0
0.45(3)	19.6 ± 4.5	163 ± 109	7.5 ± 4.6	2.8 ± 2.7
0.68(3)	24.98	185 ± 147	9.5 ± 5.3	7.5 ± 5.2

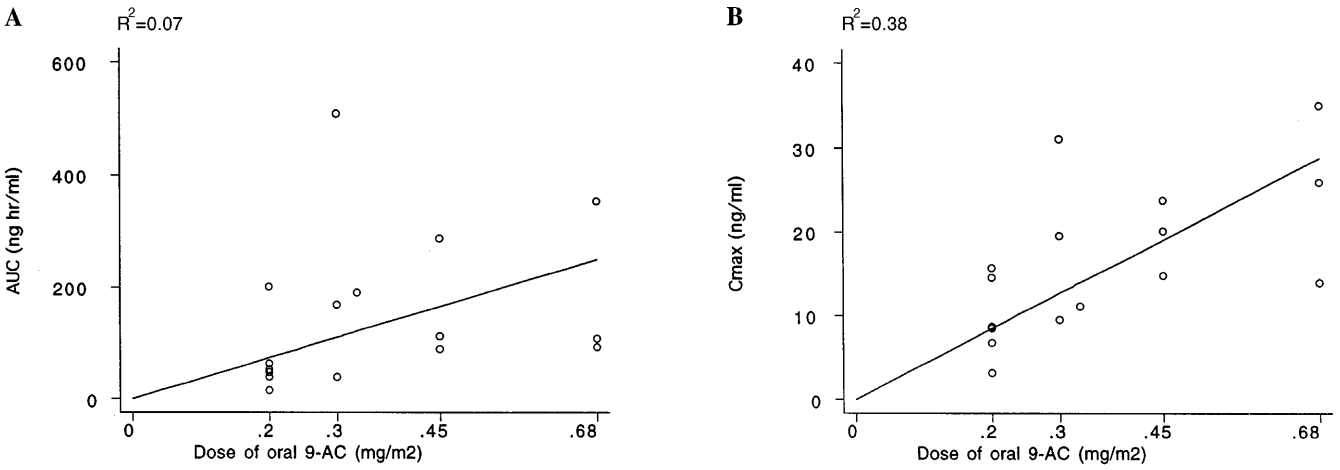

Fig. 1A Relationship between AUC_{0-24h} for oral 9-AC CD at doses ranging from 0.2 to 0.68 mg/m^2 per day. **B** Relationshipbetween C_{max} for oral 9-AC CD at doses ranging from 0.2 to 0.68 mg/m^2 per day

$AUC_{0-\infty}$ values were also determined in this study, and these indicated significant interpatient variability at each dose level (data not shown). The toxicities associated with administration of the oral 9-AC CD formulation were primarily gastrointestinal and systemic in nature. At the 0.2 mg/m^2 dose level, one patient had grade 3 diarrhea which was dose limiting and resulted in cohort expansion to six patients. This patient, however, had a baseline of four to ten small liquid bowel movements per day and was not taking any antidiarrheal medication. Fatigue and anorexia were the most common side effects noted, with most events usually grade 2 or less in severity. One patient dosed at 0.68 mg/m^2 had grade 3 fatigue which quickly resolved to grade 1–2 during the first two cycles of 9-AC treatment after which the fatigue returned at the time of documented progression of disease. Grade 1–2 nausea and/or vomiting ($n = 13$) was common, usually occurring on the last 2 days of dosing. Other significant dose-related toxicities are shown in Table 2. One patient with metastatic rectal cancer and a pleural effusion (baseline WBC 3600/μl) developed grade 2 leukopenia after one cycle of 9-AC. The patient remained leukopenic (WBC 2300/μl) for 8 weeks despite discontinuation of 9-AC; however, the WBC count recovered after administration of filgrastim. A bone marrow examination revealed a hypocellular marrow consistent with a chemotherapy effect and no observed metastases. An important feature of oral 9-AC administration was mild nausea which occurred at all dose levels particularly towards the last 2 days of dosing. The sample size and nausea events per dose cohort were too small to estimate the nature of this effect with precision. No objective responses or cumulative toxicities were observed to date.

We conclude that this formulation is not suitable for further clinical development because of wide interpatient variability and low AUC values despite dose escalation which would suggest but does not confirm either saturable absorption and/or rapid elimination. Speculative reasons for wide interpatient variability in 9-AC kinetics are several and may include aspects of the CD formulation itself, interindividual differences in 9-AC metabolism and differential formation of hitherto unidentified metabolites of 9-AC resulting from enzyme polymorphisms. A formal bioavailability study was not conducted as part of this phase I trial since we had not

Table 2 Worst drug-related toxicities in cycle one of treatment

Dose (ng/m^2/day)	Number of patients	Leukopenia/ neutropenia		Anemia		Diarrhea		Hyperbili-rubinemia		Metabolic[a]		Thrombocytopenia	
		Grade		Grade		Grade		Grade		Grade		Grade	
		1	2	1	2	1	2	1	2	1	2	1	2
0.2	6	2	1	1	–	1	–	–	1	3	2	–	–
0.3	3	1	1	–	–	–	–	1	–	1	–	1	–
0.33	1	–	–	–	–	–	–	–	–	–	1	–	–
0.45	3	–	1	2	1	1	–	–	–	1	–	1	–
0.68	3	1	1	3	–	–	1	–	–	1	1	–	–

[a] Including hyponatremia, hypocalcemia, asymptomatic alteration in serum creatinine, and asymptomatic liver function (transaminases, alkaline phosphatase) abnormalities

reached our maximal tolerated dose. For similar reasons, intrapatient variability in pharmacokinetics was not measured as this part of the study was to be conducted at the recommended phase II dose. It is conceivable that if we continued dose escalation, we may have reached a toxic dose with acceptable AUCs; however, given the high interpatient pharmacokinetic variability, we believe that this intravenous formulation is not suitable for oral use.

Given the wide interpatient variability of 9-AC, declining AUC values with little evidence of toxicity in real-time as patients were being enrolled in this study, we felt it was ethical to stop further enrollment until the results of our analysis were confirmed. A recent modification of 9-AC as gelatin capsules has undergone preliminary pharmacokinetic testing [11]. In a human oral/intravenous crossover study using a single oral dose of 1.5 mg/m^2 and a single 5-min intravenous infusion of 1.0 mg/m^2, 9-AC lactone oral bioavailability ranged between 27% and 49%. Thus, future development of oral 9-AC may be accomplished with this alternative formulation.

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