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Pharmacokinetics of continuous-infusion amsacrine and teniposide for the treatment of relapsed childhood acute nonlymphocytic leukemia*

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Summary. The systemic disposition of both amsacrine and teniposide was determined in children receiving treatment for resistant acute nonlymphocytic leukemia. As part of a phase I–II study, amsacrine and teniposide were given as continuous 72-h i.v. infusions at doses of 75–150 and 150–250 mg m^{-2} day^{-1}, respectively. Plasma samples obtained during steady state were analyzed for drug concentrations by high-performance liquid chromatography assays specific for each compound. Clearance and systemic exposure values for both amsacrine and teniposide were calculated for 14 patients, and data were available for teniposide alone in an additional 14 subjects. Interpatient variability in clearance was substantial for each drug, producing overlapping systemic exposure across dose levels. No evidence of dose-dependent drug clearance was evident. Clearance values for teniposide given in combination with amsacrine were similar to previous values obtained when teniposide was given in an identical manner but as a single agent. In all, 80% of patients experienced some degree of mucositis after chemotherapy administration. Severe mucositis (Pediatric Oncology Group grades 3–4) occurred in 18% of cases, all of whom showed teniposide steady-state plasma concentrations above the median population value (11.9 μg/ml; $P < 0.0001$). A comparison of the results of the present study on teniposide combined with amsacrine with those previously obtained for single-agent teniposide suggest that amsacrine produced little additive gastrointestinal toxicity. The evaluation of anticancer drug pharmacokinetics in individual patients during combination chemotherapy regimens helps to determine the relative importance of each agent when toxicity patterns are similar.

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Introduction

Potential complementary effects of teniposide and amsacrine on the topoisomerase II activity of leukemic cells provide a rationale for testing this drug combination in patients [14]. A dose-ranging study of these two agents given as simultaneous continuous infusions has recently demonstrated their efficacy in relapsed or refractory childhood acute leukemia [8]. This report describes the pharmacokinetics of amsacrine and teniposide given together in pediatric patients.

Pharmacokinetic variability of anticancer drugs in pediatric patients has been shown to be substantial [4] and is related to clinical response for some agents [3]. For example, a phase I investigation of teniposide demonstrated a relationship between its pharmacokinetic parameters (e.g., steady-state plasma concentration, clearance) and its efficacy or toxicity [12]. Similar data, albeit limited, have been reported for amsacrine [5, 9] and are consistent with in vitro studies that show a close association of intracellular amsacrine concentrations with those of the extracellular environment [1]. Thus, when interpatient pharmacokinetic variability is substantial, the therapeutic range is narrow, and toxicities are potentially overlapping, phase I–II studies of combination chemotherapy such as that reported herein may be optimally conducted by concomitant pharmacokinetic evaluations rather than by relying only on dose as a measure of systemic exposure.

Several previous pharmacokinetic studies of amsacrine in adult [5, 7, 9, 16] and pediatric patients [11] are limited because of non-specific assay methodology [5, 16] or suboptimal extraction methods that measured neither free nor total drug concentration, but rather a component that was freely exchangeable with organic solvents that did not precipitate proteins [7, 10]. One study characterized amsacrine disposition using a sensitive, specific high-performance liquid chromatography (HPLC) assay for total drug concentration following short i.v. infusions of single-agent amsacrine [9]. The present study extends those findings by characterizing amsacrine given in a combination regimen with high-dose teniposide as a continuous infusion to children.

Table 1. Individual dosing regimens and pharmacokinetic data

Patient	Teniposide ($n = 28$)			Amsacrine ($n = 14$)		
	Dose (mg m^{-2} day^{-1})	C_{pss} (μg/ml)	Cl_s (ml min^{-1} m^{-2})	Dose (mg m^{-2} day^{-1})	C_{pss} (ng/ml)	Cl_s (ml min^{-1} m^{-2})
1	200	21.6	6.4	100	1,215	57
2	200	11.3	12.3	100	351	197
3	200	7.7	18	100	399	174
4	200	5.1	27.5	100	288	241
5	200	7.1	19.6	100	618	112
6	200	6	23.3	100	1,590	44
Mean ($n = 6$)		9.8	17.9		744	143
SD		6.2	7.6		535	88
7	250	18.8	9.2	100	425	163
8	250	10.1	17.2	100	949	73
9	250	18.2	9.6	100	1,595	44
10	250	19.9	8.7	100	633	110
Mean ($n = 4$)		16.8	11.2		901	98
SD		4.5	4		511	51
11	250	13	13.4	150	278	375
12	250	12.4	14	150	657	159
13	250	7.9	22	150	680	153
14	250	10.4	16.8	150	1,430	73
Mean ($n = 4$)		10.9	16.6		761	190
SD		2.3	3.9		482	129
15	150	12.2	13.6	100	NA	NA
16	150	5.3	19.7	100	NA	NA
17	150	7.7	13.6	100	NA	NA
18	200	4.4	31.6	100	NA	NA
19	200	15.2	9.1	100	NA	NA
20	250	11.7	14.8	125	NA	NA
21	250	13.9	12.5	125	NA	NA
22	250	6.8	25.5	125	NA	NA
23	250	10.3	16.9	150	NA	NA
24	250	18.5	9.4	150	NA	NA
25	250	22.4	7.7	150	NA	NA
26	250	12.8	13.6	150	NA	NA
27	250	25.9	6.7	150	NA	NA
28	250	17.2	10.1	150	NA	NA
Mean (all patients)		12.6	15.1		793	141
SD		5.8	6.5		479	91

NA, Not available

Patients and methods

Patients and therapy. All patients in this study presented with treatment-resistant acute nonlymphocytic leukemia and were enrolled at St. Jude Children's Research Hospital after informed consent had been obtained. These patients are a subset of a larger group enrolled in a previously reported phase I–II study of amsacrine and teniposide [8]. The subjects' average age was 8.5 years (range, 1.5–17 years), and 68% of them were boys. All had previously received at least one of the two study drugs as a single agent prior to their enrollment in the present study. Laboratory values for renal and liver function were within normal limits prior to therapy (serum creatinine, <1.3 mg/dl; blood urea nitrogen (BUN), <25 mg/dl; total bilirubin, <1.5 mg/dl; (ALT), <50 IU/l (AST), <47 IU/l).

The treatment protocol consisted of simultaneous 72-h continuous infusions of amsacrine and teniposide through separate intravenous sites. Initial doses given were 150 mg m^{-2} day^{-1} for teniposide and 100 mg m^{-2} day^{-1} for amsacrine. Teniposide doses were escalated in increments of 50 mg m^{-2} day^{-1} (i.e., from 200 to 250 mg m^{-2} day^{-1}) and amsacrine doses were escalated in increments of 25 mg m^{-2} day^{-1} in subsequent patients. There was no dose escalation for individual subjects. Pharmacokinetic studies for both amsacrine and teniposide were done at doses of 200 and 250 mg/m^2 teniposide and 100 mg/m^2 amsacrine and then at the maximal delivered doses of teniposide (250 mg m^{-2} day^{-1}) and amsacrine (150 mg m^{-2} day^{-1}; Table 1). Additional pharmacokinetic studies of teniposide alone were done at doses of 150, 200, and 250 mg m^{-2} day^{-1} as given in combination with amsacrine at doses of 100, 125, and 150 mg m^{-2} day^{-1}, respectively.

Pharmacokinetic studies. Total plasma amsacrine was recovered from heparinized blood samples using a liquid-extraction method previously described by Jurlina and Paxton [6]. PD114606 (an acridine derivative supplied by Parke-Davis, Ann Arbor, Mich.) was added to samples prior to extraction as the internal standard. Quantitation of amsacrine and PD114606 in extracted samples was achieved by HPLC. The sample was eluted with a mobile phase consisting of acetonitrile-water-triethylamine phosphate (39.6:59.4:1 by vol.; final pH, 5.1) at a flow rate of 1.12 ml/min. A 5-μm μBondapack phenyl column (250 × 4.6 mm; Waters Associates, Milford, Mass.) was used to separate peaks of interest using UV detection at 254 nm. As performed in our laboratory, this method has a coefficient of variation of <10% (between days) and 91%–101% accuracy for amsacrine concentrations ranging from 200 to 1,400 ng/ml.

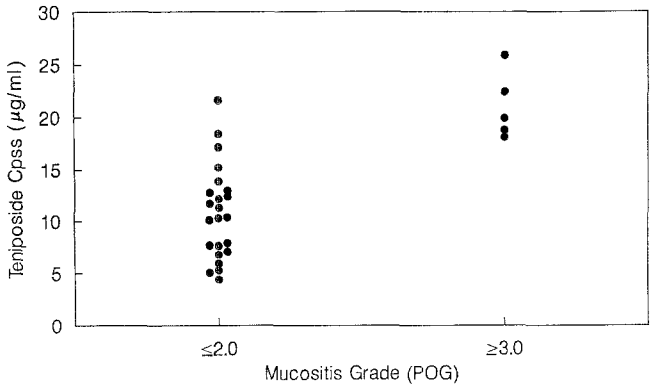

Fig. 1. Grade of mucositis and the average steady-state teniposide plasma concentration (*Teniposide* C_{pss}) for 28 patients receiving 72-h continuous infusions of teniposide and amsacrine (P <0.0001). The median value for the entire group was 11.9 μg/ml

Teniposide plasma concentrations were determined using an HPLC assay that separates 4-hydroxy-teniposide, the parent drug, and its picro derivative with a lower limit of sensitivity of 0.2 μg/ml as previously determined in our laboratory [12, 15]. Blood samples for the measurement of amsacrine and teniposide concentrations were obtained at 24, 48, and 72 h after the start of the continuous infusions. Systemic drug clearance (Cl_s) for each subject was estimated from the mean of steady-state plasma concentrations (C_{pss}) obtained at 48 and/or 72 h ($Cl_s = K_o/C_{pss}$, where K_o = zero-order drug-infusion rate).

Assessment of mucositis was conducted daily for 21 days after chemotherapy administration and grading was done using criteria of the Pediatric Oncology Group by one of the present authors (J. M.), who had no knowledge of the pharmacokinetic results. These criteria delineate grade 3–4 toxicity from lower grades according to the inability of the patient to eat due to oral ulcerations.

Statistical analysis. The relationships between ordinal data (e.g., mucositis) and pharmacokinetic data were evaluated using the Mann-Whitney test. Other comparisons of pharmacokinetic parameters were analyzed by two sample t-tests.

Results

The pharmacokinetics of both amsacrine and teniposide were determined in 14 patients. An additional 14 subjects received amsacrine at similar doses; however, only teniposide pharmacokinetics were studied due to the limited sample volume (Table 1). Teniposide concentrations were determined in both 48- and 72-h samples in >90% of patients. The C_{pss} values averaged 13.1 ± 6 and 12.9 ± 5.9 μg/ml, respectively. Individual concentrations varied within 20% of each other. In a small proportion of patients, sufficient sample was obtained to quantitate amsacrine at both 48- and 72-h time points; however, the C_{pss} values for most subjects were based on one determination assayed in duplicate.

Systemic clearance of teniposide (ml min^{-1} m^{-2}) varied over a 5-fold range among these patients, producing overlapping systemic exposure (C_{pss}) at the different dose levels evaluated, although the average teniposide C_{pss} value rose proportionally with dose. Similarly, variable amsacrine clearance resulted in large differences in systemic exposure among patients (Table 1), with overlapping being observed at the different dose levels. No relationship was found between clearance values for teniposide and those for amsacrine (correlation coefficient, 0.19; $P = 0.64$) or between doses of either drug and the respective clearance values.

The combination of teniposide and amsacrine at these doses and levels of exposure produced substantial treatment-related thrombocytopenia (platelets, $<50 \times 10^9$/l) and neutropenia (absolute neutrophil count, $<0.5 \times 10^9$/l) in all patients. Mucositis occurred in 80% of the patients studied and in 18% of these it was considered to be severe (grade 3–4 according to criteria of the Pediatric Oncology Group). The incidence of mucositis in all patients treated and in those enrolled in the pharmacokinetic study was similar and significantly related to teniposide C_{pss} values (P <0.0001). Figure 1 shows the relationship between systemic exposure to teniposide and the degree of mucositis. Steady-state plasma concentrations of >16 μg/ml frequently produced mucositis. This relationship between teniposide concentration and severe mucositis (grade 3 or greater) was independent of the simultaneous amsacrine concentrations.

Discussion

The pharmacokinetics of amsacrine previously reported for 13 adult patients with acute nonlymphoblastic leukemia [9] are similar to the results found in the present study. The mean clearance values found for the adult patients, who were given 75–90 mg/m^2 i.v. over 1 h, was 179 ml min^{-1} m^{-2} and varied over a 3-fold range. The mean clearance reported for the pediatric population in the present study was 141 ml min^{-1} m^{-2}, but the latter was more variable (8-fold). The clearance of amsacrine appeared to be independent of dose up to a level of 150 mg m^{-2} day^{-1} and did not seem to be altered by teniposide administration.

A relationship between systemic exposure to amsacrine and myelosuppression has been described by several investigators [5, 9] but was based on limited data. Unfortunately, the extensive marrow aplasia observed in all subjects in the present trial precluded investigation of this pharmacodynamic relationship.

The pharmacokinetics of teniposide (without simultaneous amsacrine administration) have been previously reported by our laboratory and others [2, 12, 13]. The average teniposide clearance values found in the present study (15.1 ml m^{-2} min^{-1}) is similar to that previously reported for the single agent given as a 72-h continuous infusion at a dose of 100–250 mg m^{-2} day^{-1} [12]. Also, the previously reported relationship between gastrointestinal toxicity and systemic exposure to teniposide [12] persisted in the present study despite concurrent amsacrine administration. Severe mucositis (grade 3–4) occurred only in patients showing teniposide C_{pss} values above the median found for this population.

Wide interpatient variability in the systemic pharmacokinetics of each drug was demonstrated in the present study, despite the normal indices of renal and hepatic function shown by all subjects. A measure of systemic exposure (e.g., clearance or steady-state concentration) rather than drug dose may correlate more precisely with adverse

effects and potentially therapeutic effects, especially in trials using multiple agents. For example, patient 13 received 250 mg/m^2 teniposide daily, yet had a steady-state drug concentration of only 7.9 μg/ml because of a substantially higher than average clearance value (22 ml min^{-1} m^{-2}). In contrast, patient 1 achieved a steady-state concentration of 21.6 μg/ml, representing a high probability for the development of severe mucositis, despite the teniposide dose's being 20% lower.

No correlation was found between the clearances of the two drugs. Teniposide clearance in patient 6 (23.3 ml min^{-1} m^{-2}) was almost twice the population mean, whereas amsacrine clearance (44 ml min^{-1} m^{-2}) was substantially lower than average. Systematic evaluation of pharmacokinetic and pharmacodynamic relationships in this manner may provide a more objective method of therapy escalation, thus improving the definition of a therapeutic index for the combination.

Our results demonstrate that the interpatient variability of amsacrine and teniposide pharmacokinetics yields a 5- to 8-fold difference in steady-state concentrations in patients receiving the same dose (adjusted for body size). Prospective phase I–II studies using a strategy of escalating the systemic exposure (AUC) to anticancer drugs instead of the dose appear to be warranted and are under way [13].

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