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Pharmacokinetic studies of 13-*cis*-retinoic acid in pediatric patients with neuroblastoma following bone marrow transplantation

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Abstract A phase I clinical trial of 13-*cis*-retinoic acid (*cis*-RA) was undertaken to determine the maximally tolerated dose (MTD) and pharmacokinetics (PK) of *cis*-RA following bone marrow transplantation (BMT) in children with high-risk neuroblastoma. Mean peak serum levels of *cis*-RA in 31 pediatric patients ranged from 4.9 to 8.9 μM following doses of 100–200 mg/m^2 per day, divided into two doses every 12 h administered orally. The PK of *cis*-RA obeyed a single-compartment model following first-order absorption in the majority of patients. A linear increase in the mean peak serum levels and area under the time-concentration curve (AUC) with increasing dose was observed. The average half-lives of absorption and elimination were 1.0 and 5.8 h, respectively. At the MTD of 160 mg/m^2 per day, the mean *cis*-RA peak serum concentration was 7.2 ± 5.3 μM. AUC values were not altered significantly during a 2-week course of treatment or over a long period of multiple courses. Levels of *trans*-retinoic acid, a metabolite of *cis*-RA, remained low but were similar on days 1 and 14, whereas the 4-oxo-13-*cis*-RA metabolite had increased in 64% of patients by day 14. Peak serum *cis*-RA concentrations correlated with clinical toxicity as grade 3 to 4 toxicity was seen in 44% of patient-courses (8/18) with peak serum levels ≥ 10 μM, but only 13% (12/96) with peak serum levels < 10 μM. These results show that *cis*-RA given at 160 mg/m^2 to children achieved serum concentrations known to be effective against neuroblastoma in vitro, and the PK for *cis*-RA differs from that reported for *trans*-retinoic acid in children.

Key words Neuroblastoma · 13-*cis*-Retinoic acid · Pharmacokinetics · Pediatrics · Bone marrow transplantation

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Introduction

Retinoids can modulate cellular proliferation and differentiation of a variety of cell types in vitro as well as in vivo [1–3]. The synthetic retinoid 13-*cis*-retinoic acid (*cis*-RA), or isotretinoin, a stereoisomer of the naturally occurring all-*trans*-retinoic acid (*trans*-RA), has shown activity in skin papillomas and in the prevention of secondary carcinomas in patients with prior head and neck squamous cell carcinomas [4, 5]. *Cis*-RA is derived from *trans*-RA by modification of the terminal carboxyl group.

Tumor recurrence is a major problem for children with high-risk neuroblastoma despite intensive chemotherapy and radiotherapy supported by bone marrow transplantation (BMT) [6, 7]. Human neuroblastoma cell lines respond to both *trans*-RA and *cis*-RA in vitro with neurite outgrowth, cell cycle arrest, and decreased

expression of the N-*myc* oncogene [8–11]. In addition, in clinical studies *cis*-RA has shown activity against neuroblastoma in patients [12–14]. Thus, *cis*-RA could be useful in delaying or preventing tumor recurrence if given to patients after BMT.

Responses to *cis*-RA, namely growth arrest and differentiation, have been observed in neuroblastoma cell lines cultured from recurrent tumors following chemoradiotherapy [6, 7], suggesting that resistance to cytotoxic chemotherapy does not lead to resistance to *cis*-RA. Most neuroblastoma cell lines, with or without N-*myc* amplification, show expression of the RA receptors α and γ and induction of the β-retinoic acid receptor after exposure to RA [15].

Studies in vitro aimed at modeling clinical therapy with *cis*-RA have shown that clinically achievable levels of *cis*-RA can produce sustained growth arrest of neuroblastoma cells [7, 9]. An RA-sensitive neuroblastoma cell line was exposed to 5 μM *cis*-RA for 2 weeks, then fresh medium for 2 weeks, followed by retreatment with *cis*-RA for 2 weeks, with the levels of *cis*-RA confirmed by HPLC. Complete inhibition of cell growth and morphological differentiation for 120 days after beginning the *cis*-RA treatment (78 days after the last exposure to *cis*-RA), and downregulation of N-*myc* was observed [9]. Eight additional RA-sensitive human neuroblastoma cell lines were treated for 7 days with 5 μM *cis*-RA and showed sustained growth inhibition over 28 days.

The pharmacokinetics (PK) of *cis*-RA in adults has been studied in healthy human volunteers [14, 16], patients with various dermatologic disorders [17, 18], and patients with epithelial malignancies [17, 19, 20]. These studies showed that the drug concentrations in plasma fit a one- or two-compartment open model. However, the PK of *cis*-RA in children has not been reported. This study was therefore designed to describe the PK of *cis*-RA after oral administration to pediatric patients aged 2–12 years (median 4 years) following BMT for the treatment of high-risk neuroblastoma.

Materials and Methods

Materials

Cis-RA (Accutane) was kindly provided by Dr. Judith Prestifilippo of Hoffman La Roche, N.J. The internal standard $R_0$11–5036 for HPLC was kindly provided by Dr. P.F. Sorter of Hoffman La Roche. Methanol and inorganic chemicals for buffers were of analytical grade.

Patient characteristics

The patient population for this phase I study of oral *cis*-RA consisted of patients diagnosed with high-risk neuroblastoma, aged 2–12 years (median 4 years), at a median of 3 months (range 1–10) following autologous ($n = 49$) or allogeneic ($n = 2$) BMT utilizing several different pre-BMT regimens of intensive chemotherapy or chemoradiotherapy. Details of the clinical results of this phase I trial have been presented elsewhere [7, 20]. PK studies were carried out in 31 (17 male and 14 female) of the 51 evaluable pediatric patients. Informed consent was obtained from the patient and/or parents. The study was approved by the individual hospitals' Human Investigations Committee (IRB) at each institution where the patients were entered on study, and was carried out under FDA IND #36,781.

The treatment schema

Cis-RA was administered orally to pediatric neuroblastoma patients after BMT as outpatients, at increasing dose levels. The doses were 100, 125, 160, or 200 mg/m^2 per day (dose levels 1 to 4) administered in two equally divided doses every 12 h for 2 weeks (day 1–14), followed by a 2-week rest period (days 15–28), to four groups of patients [7, 20]. A minimum of three patients were entered at each level. The numbers of patients entered at dose levels 1, 2, 3, and 4 were 16, 10, 15 and 6, respectively. One intrapatient 25% dose escalation was allowed after a minimum of two courses at the initial dose without dose-limiting toxicity (DLT). A maximum of 12 courses was given according to the protocol, or until disease progression or DLT occurred [7, 20]. However, five patients continued therapy beyond 12 courses at the parents' and/or referring physician's preference for a total of 13–17 courses.

Sample collection and extraction of *cis*-RA from serum

Blood samples for PK studies were obtained on days 1 and 14 of the first course at pretreatment (0 h), and 2, 4, 6 and 8 h after taking *cis*-RA. On day 1, these samples were drawn after the second dose was administered. Since, this study was primarily designed as a clinical trial given as an outpatient regimen to facilitate patient participation, the blood samples could only be collected for up to 8 h during regular clinic hours. Samples were obtained for PK studies based on venous access and parents ability to comply with clinic visit requirements. Blood samples for PK studies were also obtained, if possible, when the *cis*-RA dosage was decreased or escalated in subsequent courses. These blood samples were protected from light by covering all of the tubes immediately after collection with aluminum foil and by centrifuging and handling in dim yellow light. The serum was separated and placed in amber-colored vials and frozen in darkness at $-70\,^{\circ}$C until HPLC analysis was performed [7].

The extraction solvent consisted of analytical grade methanol (100%), containing the antioxidant butylated hydroxytoluene (BHT, 0.1 mg/ml). This method effectively removes the proteins that cause interference in HPLC analysis. Serum samples were thawed to room temperature and aliquots of 0.2 ml were pipetted directly into 0.8-ml extraction solvent (1:4 parts) contained in 4-ml amber centrifuge tubes and 25 ng (50 µl from a stock of 0.5 µg/ml) of $R_0$11–5036 as internal standard [7, 16, 20] was added to control the variances introduced by sample handling and analysis. The mixture was immediately vortexed and allowed to stand in an ice-bath (0 °C) for 5 min. These tubes were then centrifuged at 680 *g* for 10 min in a refrigerated clinical centrifuge. The clear supernatant (methanol phase) was removed from the precipitated proteins and 0.05–0.2 ml was then used for the HPLC assay. The extraction efficiency using this extraction procedure was 75.9%. As *cis*-RA is highly photosensitive [2, 3], all of the samples were handled in yellow or very dim indirect light in order to prevent isomerization. The standard solutions were stable ($\geq 95\%$) at $-20\,^{\circ}$C for 3 months.

HPLC assay of *cis*-RA

For the HPLC assay a Waters Associates HPLC (Milford, Mass.) system was used, which consisted of model 510 pump, U6 K

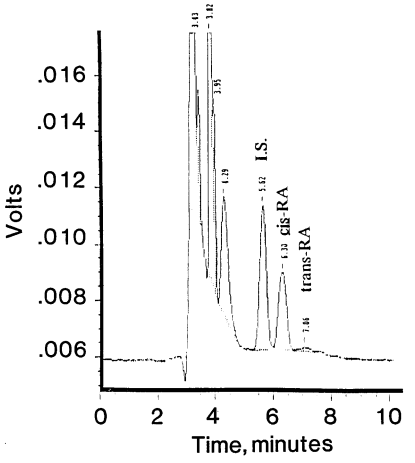

Fig. 1 A typical HPLC chromatogram of a blood sample serum extract showing the separation of internal standard R_0 11–5036, *cis*-RA and *trans-RA* peaks, which eluted at 5.6, 6.3 and 7.1 min, respectively. This separation was accomplished under isocratic conditions as described in Materials and methods

injector, Lambda-max model 481 UV/VIS detector and μC-18 Waters reverse phase column (30 cm × 4.6 mm ID). A guard column filled with inert Corasil Type II (37–50 μm bulk packing material) was used to protect the column. HPLC system control, data collection, integration and quantitation were performed using the MAXIMA 820 software version 3.10 on an IBM PS/2 model 70/386 computer [7, 20].

The HPLC assay utilized for *cis*-RA was a modification of a previous assay [21], which has been described previously by the authors [20] and is similar to other HPLC assays available for the analysis of *cis*-RA from extracted serum [21–26]. The mobile phase for the HPLC assay was methanol/water 80:20, and *cis*-RA was eluted isocratically with this HPLC solvent for 20 min at a flow rate of 1.0 ml/min. The variable wavelength detector was set at 350 nm/0.01 V OD (lambda max of *cis*-RA). The *cis*-RA peak eluted at 6.3 min, and *trans*-RA eluted at 7.1 min and both were separated from internal standard $R_0$11–5036 which eluted at 5.6 min (Fig. 1). The polar metabolites (such as oxo-derivatives) eluted early between 3 and 4.5 min and compared well with similar HPLC elution profiles [21]. A concomitant shift in the retention times of all compounds was seen occasionally, which was corrected after the HPLC column was cleaned daily by washing with 100% methanol. The calibration curve was linear in the range of 15–600 ng ($R^2 = 0.9857$) with the lower limit of quantitation being 15 ng in the injected volume. The intraday variations for this assay ranged from 2.8% to 9.6% coefficient of variation (%CV) and the interday variation was 4.6%CV. The *cis*-RA concentrations in plasma and serum obtained at the same time in pediatric patients showed near identical drug concentrations with a%CV less than 3%.

Pharmacokinetic analysis of *cis*-RA data

The concentrations of *cis*-RA were obtained from the quantitation of HPLC assay results and a mean concentration value was estimated for each dose level. Analyses were performed on individual sets of data using one- and two-compartment open model(s) with oral absorption. Curve fitting was performed using the computer software package ADAPT II [27]. The area under the time-concentration curve (AUC), area under the moment curve (AUMC) and mean residence time (MRT) were calculated using the trapezoidal method. The AUC was calculated up to the last sampling time and then extrapolated to infinity using the last concentration and terminal rate constant of elimination. Since a 12-h sampling could not be performed, we could not calculate the AUC for this dosing interval.

The apparent total body clearance* (TBCl) was calculated using the equation TBCl = dose/AUC, and the volume of distribution (Vd) was calculated using the equation Vd = dose/(AUC × terminal rate constant of elimination) [28]. Because of the limited number of time-points evaluated, it was difficult to fit the data sets from a few patients to a PK model accurately. In these cases, the average of the terminal elimination rate constants from patients within the same dose level with complete data sets was used to calculate the AUCs. These estimations were performed in a similar manner to that reported previously for *trans*-RA [29]. The terminal half-life of the drug was calculated using the terminal elimination rate constants.

Toxicity

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria. The DLT was defined as grade 3 toxicity not returning to acceptable (less than grade 2) levels prior to initiation of the next cycle of therapy, or any grade 4 toxicity. The maximally tolerated dose (MTD) was defined as the dose immediately below the dose at which at least 50% of the patients treated experience DLT, with a minimum of three patients treated at each dose level. Toxicity for each level was evaluated by combining patients who entered or were escalated/de-escalated to a given dose. Patients were monitored weekly during therapy by physical examination, complete blood count with differential, serum albumin, total protein, transaminases, alkaline phosphatase, lactate dehydrogenase, cholesterol, uric acid, total bilirubin, glucose, and triglycerides [7]. In addition, serum calcium, phosphorus, blood urea nitrogen, creatinine, creatinine clearance, and urinalysis were required at study entry. The study was amended to require serum calcium and phosphorus on days 0, 7, and 14 of each course after hypercalcemia was observed in several patients [7, 20]. Because of DLT in six of nine evaluable patients treated at 200 mg/m^2 per day divided into two equal doses, the MTD was determined to be 160 mg/m^2 per day [7, 20].

Statistical analyses

To determine the relationship between increasing dose and achieved peak concentrations and AUC, the Pearson correlation coefficient was used and its significance level was determined using Student's *t*-test. Nonparametric tests were used for AUC comparisons. The AUC on day 1 was compared with the AUC on day 14 using the Wilcoxon paired-sample test. The AUC comparison in one patient, where day 1 and day 14 AUCs were not paired, was done as two independent groups, using the Mann-Whitney *U*-test. The relationship between peak serum concentrations and the number of toxicity occurrences was analyzed using Fisher's exact probability test. A value of $P \leq 0.05$ was considered as significant in all of these tests.

Results

Pharmacokinetics of *cis*-RA

Figure 1 shows a typical HPLC chromatogram of a serum sample using isocratic elution. The internal

*TBCl was only apparent total body clearance because we did not know the actual fraction absorbed (absolute bioavailability, F) of the drug

Table 1 Serum *cis*-RA concentration profiles (μM) over time at different dose levels (N = number of data points available for that time point at that dose)

	Time				
	0	2	4	6	9
Dose 100 mg/m^2 per day					
N	18	16	19	16	1
Mean	2.78	4.56	4.86	4.02	2.72
SD	1.72	2.78	3.58	2.47	–
Dose 125 mg/m^2 per day					
N	28	10	30	6	3
Mean	4.20	4.20	5.65	4.62	3.64
SD	2.69	1.68	2.92	2.78	1.74
Dose 160 mg/m^2 per day					
N	35	15	35	13	9
Mean	4.05	5.98	7.19	6.55	6.82
SD	2.72	4.83	5.27	3.16	3.13
Dose 200 mg/m^2 per day					
N	8	5	12	4	2
Mean	3.93	4.70	4.02	8.93	7.91
SD	2.26	2.88	1.49	10.14	7.79

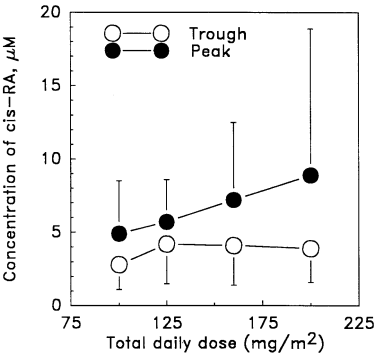

Fig. 2 Linear relationship between the average peak serum concentrations and increasing total daily dose of *cis*-RA ($r = 0.998$; $P = 0.001$); 100 mg/m^2 per day, 5 patients, 20 courses; 125 mg/m^2 per day, 10 patients, 35 courses; 160 mg/m^2 per day, 16 patients, 45 courses; 200 mg/m^2 per day, 7 patients, 15 courses). Values are means $\pm$ SD

standard, *cis*-RA and *trans*-RA peaks are clearly separated and eluted at 5.6, 6.3 and 7.1 min, respectively. The polar metabolites eluted earlier, between 3 and 4.5 min, under these elution conditions and no interference was observed between these peaks and the peak of *cis*-RA or *trans*-RA.

PK analysis of *cis*-RA was performed in 31 of 51 eligible patients (17 male and 14 female) entered on this study. A typical serum *cis*-RA concentration-time profile is described in Materials and methods. The average peak serum concentrations of *cis*-RA were $4.9 \pm 3.6\ \mu M$ for the first dose level (100 mg/m^2 per day) and $8.9 \pm 10.0\ \mu M$ for the fourth dose level (200 mg/m^2 per day). The average PK data of *cis*-RA are shown in Table 1. A dose of 160 mg/m^2 per day was designated as the MTD based on clinical DLTs. This dose level achieved a mean peak serum level of $7.2 \pm 5.3\ \mu M$, and a trough level of 4.1 ± 2.7 μM (Fig. 2). Although there were significant interpatient and intrapatient variabilities in the data, there was a linear increase ($r = 0.998$; $P = 0.001$) in the mean peak serum concentration of *cis*-RA with corresponding escalating dose levels (50 to 100 mg/m^{2}per dose every 12 h; Fig. 2, Table 2). Although there was a marginally significant increase in trough levels between dose levels 100 and 125 mg/m^2 per day, following that, trough levels reached a plateau and did not significantly increase with escalating doses (Fig. 2, Table 2).

A one-compartment open model with first-order rate of absorption was selected to describe the PK of *cis*-RA in pediatric patients. The average half-life of absorption was 1.0 ± 0.5 h and the average half-life of elimination was 5.8 ± 2.0 h (Table 2). The MRT using non-compartmental analysis, ranged from 6.8 to 10.2 h at four consecutive dose levels of *cis*-RA and increased with increasing dose (Table 3.) The mean time to achieve serum peak levels ranged from 3.7 to 4.1 h after oral drug administration (Table 2).

There was a linear increase ($r = 0.983$, $p = 0.016$) in the AUC of *cis*-RA with corresponding escalating dose levels from 50 to 100 mg/m^2 day every 12 h (Fig. 3, Table 3). Even though there was a linear increase in AUC in the dose range evaluated, it appears likely that at higher doses the AUC may have reached a plateau (Fig. 3). Even though there were large variations in the peak serum concentrations of *cis*-RA, the AUCs were more consistent from patient to patient (Fig. 3). No significant alterations in AUC between day 1 and day 14 were observed over seven courses of treatment in a single patient, where PK sampling was performed over 203 days of therapy (Fig. 4; $P > 0.2$, nonparametric Mann-Whitney U-test for independent samples). The most likely reason for the large variability seen in peak levels could be variations in absorption of the drug following oral intake. Large variations in peak levels have also been reported by various investigators in studies conducted in adults.

A comparison of the AUC levels on days 1 and 14 was performed in eight patients for a total of 15 courses. Although there were some variations in the AUC, there was no statistically significant change in the AUC over the 14 days of treatment ($P > 0.5$, nonparametric Wilcoxon test for paired samples; Fig. 5). Similarly, a comparison between peak concentrations of *cis*-RA achieved on day 1 and day 14 in the same patients showed no significant difference ($P > 0.5$, nonparametric Wilcoxon test for paired samples, data not shown).

No significant differences were observed between male and female pediatric patients using a two-tailed unpaired t-test (Table 4). At all dose levels the mean AUC by gender did not yield significant differences nor

Table 2 Pharmacokinetic (PK) parameters of *cis*-RA at all dose levels. Values are means ± SD

Dose (mg/m^2/day)	Number of patients/ courses	Peak level (μM)	Time to peak (h)	Trough level (μM)	Absorption half-life (h)	Elimination half-life (h)
100	5/20	4.9 ± 3.6	3.7 ± 1.5	2.8 ± 1.7	0.9 ± 0.7	3.9 ± 2.1
125	10/35	5.7 ± 2.9	3.8 ± 0.9	4.2 ± 2.7	0.9 ± 1.4	7.2 ± 6.9
160	16/45	7.2 ± 5.3	4.1 ± 1.3	4.1 ± 2.7	1.7 ± 1.4	4.1 ± 1.4
200	7/15	8.9 ± 10.0	4.0 ± 1.4	3.9 ± 2.3	0.4 ± 0.4	7.8 ± 8.4

Table 3 Pharmacokinetic (PK) parameters of *cis*-RA at all dose levels. The number of patients/courses differs from Table 2 because of the limited number of sampling time-points; AUCs could not be calculated for all the patients (*TBCl* total body clearance, *Vd* volume of distribution, *MRT* mean residence time based on noncompartmental analysis [= area under the moment curve/area under the concentration-time curve]). Values are means ± SD

Dose (mg/m^2/day)	Number of patients/ courses evaluated	$AUC_{0\rightarrow\infty}$ (μM h)	Apparent TBCl ($1/m^2$/h)	Apparent Vd ($1/m^2$)	MRT (h)
100	4/17	46.18 ± 17.60	4.28 ± 2.22	22.9 ± 15.9	6.8 ± 2.1
125	8/25	58.96 ± 25.90	7.45 ± 5.17	27.8 ± 19.3	7.8 ± 3.1
160	16/24	76.98 ± 39.06	5.33 ± 4.82	31.0 ± 27.6	7.7 ± 2.2
200	4/04	86.05 ± 45.57	5.49 ± 2.26	36.1 ± 31.6	10.2 ± 7.5

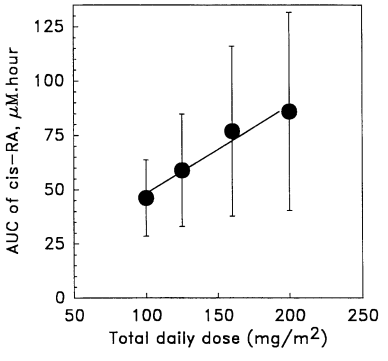

Fig. 3 Linear relationship between the area under the concentration time curve (AUC) and increasing total daily dose of *cis*-RA ($r = 0.983$, $P = 0.016$; 100 mg/m^2 per day, 4 patients, 17 courses; 125 mg/m^2 per day, 8 patients, 25 courses; 160 mg/m^2 per day, 16 patients, 24 courses; 200 mg/m^2 per day, 4 patients, 4 courses). Values are means ± SD

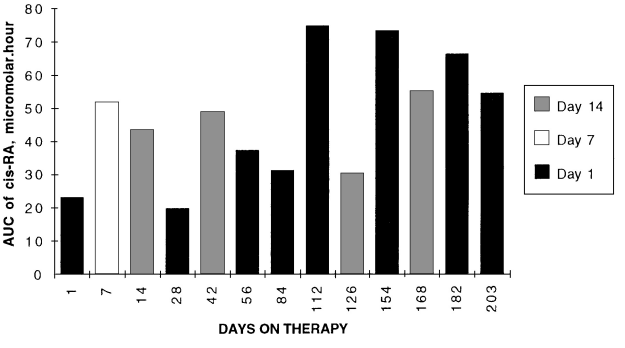

Fig. 4 Area under the time concentration curve (AUC) for *cis*-RA over 203 days (seven courses) of therapy in a representative patient. No statistically significant changes in AUC were detected ($P > 0.2$) between day 1 and day 14 AUCs. Samples were collected on days 1, 7 and 14, first course, then days 1 and 14 on subsequent courses. In all these courses, the patient received 100 mg/m^2 per day (dose level 1)

any trends. At two dose levels the male patients appeared to have elevated mean AUC values and the oposite was seen at the remaining two dose levels. Thus, we conclude that gender does not influence the average AUC of *cis*-RA. With the limited patient age distribution in this study the effect of age on PK parameters could not be discerned.

Trans-RA was detected at trace concentrations in approximately 50% of the patients' specimens. As the half-life of *trans*-RA is much shorter than the half-life of *cis*-RA, only trace concentrations of *trans*-RA were detected and PK analyses of the *trans*-RA derived from *cis*-RA were not possible in this group of patients. Low concentrations of *trans*-RA were also observed on day 14 and the levels did not change over the 14-day treatment period. The major metabolite of *cis*-RA, 4-oxo-13-*cis*-RA was not quantitated; however, based on the peak height ratios, the levels could be compared between days 1 and 14. Comparing the levels of 4-oxo-13-*cis*-RA on day 1 and day 14, an increase was observed in 64% of the patients and the remaining patients either had no change or a slight decrease in levels of this metabolite.

Toxicity

A correlation was noted between the incidence of grade 3/4 clinical toxicity and peak serum levels of *cis*-RA achieved during individual courses. Of 114 PK data sets/18 showed peak serum concentrations $\geq 10\ \mu M$. Of these 18, 8 courses (44%) were associated with grade

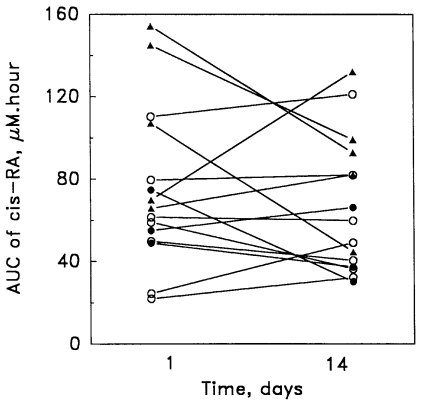

Fig. 5 Comparison of the AUC of *cis*-RA between day 1 and day 14 at various dose levels (● 100 mg/m^2 per day, one patient, three courses; ○ 125 mg/m^2 per day, two patients, seven courses; 160 mg/m^2 per day, five patients, five courses). Overall, or at each dose level separately, there was no significant change in AUC from day 1 to day 14 ($P > 0.5$)

Table 4 AUC of *cis*-RA at different dose levels in relation to gender

Dose (mg/m^2/day)	Gender	Patients/ Courses	AUCP (mean ± SD)	*P*-value
100	M	1/13	46.87 ± 18.11	0.78
	F	3/4	43.93 ± 18.22	
125	M	3/3	37.47 ± 10.91	0.13
	F	4/22	61.89 ± 26.08	
160	M	8/10	85.84 ± 33.73	0.36
	F	7/14	70.64 ± 42.53	
200	M	2/2	54.76 ± 18.43	0.21[a]
	F	2/2	117.33 ± 44.44	

[a] Owing to the limited number of data points, this statistic is not valid

3/4 clinical toxicity (skin, liver and hypercalcemia). Among the remaining 96 courses where peak serum concentrations of *cis*-RA were less than 10 μ*M*, only 12 courses (13%) showed grade 3/4 clinical toxicity. The difference in these toxicity profiles based on peak serum *cis*-RA concentrations was highly significant ($P = 0.003$) using Fisher's exact probability test. A similar relationship was not observed using AUC as the correlating factor [7]. PK data from four patients who had a dose reduction due to toxicities showed a concomitant decrease in the serum peak levels and AUC following the dose reduction.

Discussion

This study determined the PK of *cis*-RA in pediatric patients with neuroblastoma following BMT. The PK of *cis*-RA in adults has previously been studied in healthy human volunteers [14, 16, 32], patients with dermatologic disorders [17, 18], and patients with cancer [17, 19, 23]. The PK of *trans*-RA has been studied in pediatric patients [28], but no study has been reported with *cis*-RA in young children with malignancies. Previous studies in adults [15–19] have been performed with single daily doses given chronically, which may be more toxic than administering the drug on an intermittent dosing schedule, allowing patients time to recover from drug toxicity [7]. Administering the drug in two divided doses may also decrease toxicity by decreasing the peak serum level. This intermittent schedule has been shown to be effective in inducing differentiation and long-term growth arrest in neuroblastoma cell lines in vitro [9], and achieves sustained responses in neuroblastoma patients [7].

The intermittent dosing schedule of *cis*-RA has been shown to result in acceptable toxicity in children following BMT, with an MTD of 160 mg/m^2 per day [7]. The DLTs are a combination of skin, hepatic, hematopoietic, gastrointestinal and metabolic (hypercalcemia) toxicities [7, 20]. Peak serum concentrations of *cis*-RA $\geq 10\ \mu M$ correlated with an increased incidence of grade 3 to 4 toxicities. A similar correlation was not observed with AUC. This suggests that decreasing the dose for patients with serum *cis*-RA levels $> 10\ \mu M$ would minimize toxicity, since we observed that reducing dose levels reduced the peak serum concentrations predictably and further toxicity was not encountered. Thus, peak serum levels should be monitored during *cis*-RA therapy. The relationship of peak serum concentrations of *cis*-RA to toxicity is being further evaluated in a Children's Cancer Group trial (CCG-3891). One limitation in this study design was that the patients were not hospitalized and samples could be obtained only during regular clinic hours, which limited the number of time-points at which samples could be obtained.

Outpatient administration was chosen to maximize patient participation and to develop a treatment regimen feasible for future studies. Compounding this was the fact that administration of the drug was every 12 h. These two factors prevented prolonged determinations of the terminal half-life, which has been reported in other studies [15–19]. To prove that this difference in half-life is due to a significant difference in drug disposition between adults and children, additional PK data are required.

The peak serum levels of *cis*-RA and the corresponding AUCs showed a linear increase with increasing dose of the drug. This contrasts with the PK data on *trans*-RA [33], which do not demonstrate any relationship between AUC and increasing dose. AUC at the highest dose level tends to plateau despite increasing the dose. During a full 14-day dosing period with *cis*-RA, there was no significant difference in the overall AUC on day 14 compared with the AUC on day 1, which is in agreement with previous reports for *cis*-RA in adults [17]. This observation is distinctly different

from the PK of *trans*-RA in children and adults, where a fivefold decrease has been reported in AUC from day 1 to day 14 [28–30].

In this study, the terminal half-life of *cis*-RA ranged from 3.9 to 7.8 h, which is much longer than the 45 min reported for *trans*-RA [28], and differs from a previous study which showed the terminal half-life of *cis*-RA to be 17 h in adults [34]. This difference in the half-life of *cis*-RA among various studies is most likely dependant on the adequacy of the sampling times and how well the curve fittings were performed. The differences in PK between *trans*-RA and *cis*-RA described previously by various investigators are from studies in adult patients (28–30, 33, 34).

This study describes the PK of *cis*-RA for the first time in pediatric patients and it is the first opportunity to compare the PK of *cis*-RA with those of *trans*-RA in children, reported by Smith et al. [28]. It is possible that *cis*-RA acts as a prodrug for *trans*-RA which is the active form as there are no nuclear receptors yet known for *cis*-RA. The PK of *cis*-RA, as a prodrug, may not necessarily translate into higher concentrations of more active isomer(s) because of the faster elimination characteristics of the *trans*-RA. However, if *cis*-RA can provide sustained intracellular levels of the active isomer over a prolonged period of time, it may be therapeutically beneficial, and has indeed achieved responses in neuroblastoma patients [12–14]. We observed low concentrations of *trans*-RA on day 1 and day 14, and unlike the reported information regarding *trans*-RA administration in children [28], the levels of *trans*-RA did not change over the 14-day period. *Trans*-RA is an enzymatic and light-induced metabolite of *cis*-RA [16, 35], with a very rapid half-life of elimination [28–30, 33] thus, treatment with *cis*-RA may provide a therapeutic advantage.

As *trans*-RA is a highly active retinoid, isomerization of *cis*-RA to *trans*-RA may contribute to the pharmacological effect of *cis*-RA. A comparison of our findings with the previously reported study of *trans*-RA in children [28], demonstrates that *cis*-RA has a longer half-life, achieves higher mean peak serum levels and thus achieves a far greater AUC than *trans*-RA. At the MTD dose of 60 mg/m^2 per day in pediatric patients, *trans*-RA achieves a peak plasma concentration of 0.62 μM, which is 12-fold lower than the mean peak serum levels of *cis*-RA (7.2 ± 5.3 μM) at the MTD of 160 mg/m^2 per day [28]. Also, unlike *trans*-RA, during chronic administration of *cis*-RA the PK profile does not change significantly during the course of therapy. The markedly different PK parameters of *cis*-RA, when compared to *trans*-RA, suggest that this drug could have a therapeutic advantage over *trans*-RA, especially in cases where resistance to *trans*-RA is suspected due to the rapid elimination and the inability to maintain drug levels during prolonged therapy. A Children's Cancer Group phase III randomized trial (CCG 3891) utilizing the MTD value for *cis*-RA from this study is in progress to examine the efficacy of the drug in children with high-risk neuroblastoma.

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