

Phase I Trial and Pharmacokinetics of Fenretinide in Children with Neuroblastoma¹

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ABSTRACT

Purpose: Fenretinide (4HPR), a synthetic retinoid, induces apoptosis in neuroblastoma cells. A Phase I study in children with neuroblastoma was designed to determine maximum tolerated dose, toxicity, and pharmacokinetics.

Experimental Design: Fifty-four patients received oral 4HPR, once daily, for 28 days, followed by a 7-day interruption, for up to 6 courses. The starting dose was 100 mg/m²/day. At least 3 patients were entered at each escalating 4HPR dose level. Pharmacokinetic sampling was performed on days 1 and 28 of the first course.

Results: Fifty-four patients, of whom 53 were evaluable, received doses between 100 and 4000 mg/m²/day for a total of 168 courses. Additional dose escalation was precluded by capsule number intake. A total of 34 of 53 evaluable patients showed manageable, reversible toxicities, which were not dose related. One dose-limiting toxicity (nyctopia grade 3) occurred after the 1000 mg/m²/day dose. Twelve patients showed grade 2 toxicity: skin xerosis (6 cases); nyctopia (3 cases); hepatic toxicity (1 case); diarrhea (1 case); and headache (1 case). Stable disease was observed in 41 patients for a median period of 23 months (range 2–35+). After first administration, average 4HPR peak plasma levels ranged

from 0.6 to 6 μM (after 100 and 4000 mg/m²/day, respectively) and increased 2-fold (to 1.3 and 12.9 μM, respectively) after the 28-day treatment. 4HPR half-life increased from 17 h after the first administration to 25 h after the 28th administration. Incidence of grade 2–3 toxicity was 0 of 12 (0%), 7 of 22 (31%), and 4 of 8 (50%) with peak 4HPR concentrations <3 μM, 3–10 μM, and >10 μM, respectively. After repeated treatment, retinol levels decreased from 20 to 10% of pretreatment levels after all of the doses.

Conclusions: In children, 4HPR administration up to 4000 mg/m²/day over 28 days, followed by a 7-day interruption, results in manageable toxicity and in drug plasma concentrations comparable with those that induce apoptosis in neuroblastoma cell lines.

INTRODUCTION

Neuroblastoma, a neoplasm of the sympathetic nervous system, is the second most common malignant solid tumor in childhood. Prognosis for patients with this neoplasm has improved because of advancements in medical care, but the overall 5-year survival is still <60% (1). Age and stage at diagnosis are the most significant prognostic factors. Outcome in children older than 1 year with metastatic disease is uniformly poor. In the past, the use of more intensive chemotherapy produced a significant increase in the percentage of patients achieving complete tumor response (2, 3). However, despite the use of supra-lethal doses of chemoradiotherapy with bone marrow rescue, the incidence of fatal relapses was still high, and long-term survival was only 25%. Little improvement was obtained by intensifying chemotherapy in the induction and consolidation phases or by adding other treatment modalities such as cytokines, monoclonal antibodies, and radioactive metabolic drugs (3–5). Despite the improvements, innovative therapy is still needed because eradication of residual disease, which is responsible for relapse, is not achieved by the majority of neuroblastoma patients.

Retinoids with differentiating or apoptotic-inducing effects have been extensively studied *in vitro* in neuroblastoma cell lines, and their activity in patients with neuroblastoma is under intense clinical investigation. ATRA³ associated with IFN-α2a has shown clinical activity in refractory neuroblastoma (6), and a Phase I trial of 9cisRA has recently been concluded in children with various refractory tumors, including neuroblastoma (7). Interestingly, 13cisRA showed minimal activity in refractory

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³The abbreviations used are: ATRA, all-*trans*-retinoic acid; 9cisRA, 9-*cis*-retinoic acid; 13cisRA, 13-*cis*-retinoic acid; 4HPR, fenretinide; HZV, herpes zoster virus; MIBG, metaiodobenzylguanidine; MTD, maximal-tolerated dose; DLT, dose-limiting toxicity; AUC, area under the curve; PR, partial response; CR, complete response.

neuroblastoma (6), but it significantly increased survival when administered to patients with minimal residual disease (8).

4HPR is a synthetic retinoid, which has been shown to cause a decrease in the incidence of carcinogen-induced epithelial tumors in animal models, and has low toxicity as compared with other retinoids (9). *In vitro* studies have demonstrated that 4HPR, at concentrations ranging from 1 to 10 μM , suppresses the growth of malignant cells of various histotypes, including neuroblastoma, and that this effect is associated with the induction of apoptosis (10–13). At present, clinical studies of 4HPR have been limited to adults. In a tertiary prevention study in women with prior breast cancer (14), a 200-mg daily dose was administered for 5 years, and the most common side effects were diminished dark adaptation (19% of cases) and dermatological disorders (19%; Ref. 15). Because diminished dark adaptation or nyctalopia is associated with a decrease in plasma retinol levels (16, 17), drug discontinuation at the end of each month was introduced in clinical trials using 4HPR to minimize this side effect.

The high efficacy of 4HPR in inducing apoptosis in neuroblastoma cells and its good safety profile in humans prompted us to study the effects of this retinoid in children with neuroblastoma. Because we think that long-term administration of 4HPR might be effective in preventing relapse in patients with minimal residual disease, we designed a Phase I study including children with advanced nonprogressive neuroblastoma to assess toxicity of 4HPR prolonged administration. The aims of the study were to determine the MTD, the toxicity, and the pharmacokinetics of 4HPR in children. Other aims of the trial were to study the effects of 4HPR on retinol plasma levels in children and to collect preliminary data on the clinical activity of 4HPR in neuroblastoma.

PATIENTS AND METHODS

Patient's Eligibility. Patients with stage 3 or 4 neuroblastoma older than 12 months at diagnosis were eligible for the study if they were at high risk of developing progressive disease or further relapse defined as: (a) resistant disease; (b) partial remission at the end of treatment, including high-dose chemoradiotherapy with hematopoietic stem cell rescue; or (c) stage 4 disease in second or further complete remission. Other eligibility criteria were: neutrophil count $> 500/\text{mm}^3$; platelet count $> 50,000/\text{mm}^3$; and serum transaminases, bilirubin, and creatinine below twice the normal values. Exclusion criteria were: any severe organ dysfunction; life expectancy < 4 weeks; known HIV, hepatitis C virus, or hepatitis B virus infection; or treatment with other experimental drugs during the 30 days before the study. The study was approved by the ethical committees of the two institutions where patients were enrolled (Istituto Gaslini, Genova, Italy, and Istituto Nazionale Tumori, Milan, Italy). Written informed consent was obtained from the patients' parents.

Study Design and Analysis. 4HPR was administered p.o. once a day for 28 consecutive days. Patients received subsequent courses of 4HPR at 1-week intervals provided that no DLT or progressive disease had developed. A maximum of six courses were given according to the protocol. Because the oral dose of 4HPR in adults with breast cancer was 200 mg/day

(14), the starting dose in our pediatric population was 100 mg/ m^2 , and it was increased by 100 mg/ m^2 at each dose level up to 700 mg/ m^2 . When no toxic effects were observed, the dose was increased by 30% at each dose level. A minimum of 3 patients treated for at least one course without DLT was required before entering any new patients to the following dose level. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (18). Required monitoring included weekly physical examination, complete blood cell count, and complete blood chemistry. To grade the severity of nyctalopia, patients and their parents were asked specifically about: dark adaptation; sight in dim light; and impairment in playful activities attributable to visual disturbances before treatment, weekly, and after each cycle of 4HPR treatment. Nyctalopia was scored as grade 1 when either dark adaptation or sight in dim light were reduced, grade 2 when both side effects were present, and grade 3 for impairment in playful activities. Ophthalmological evaluation (visual acuity, color vision, dark adaptation, electroretinogram, and dilated funduscopy) was performed in patients with grade 3 nyctalopia.

DLT was defined either as grade 3 toxicity not returning to less than grade 2 before the next course of therapy or grade 4 toxicity. In this case, the drug was temporarily discontinued until resolution of toxicity, after which the patient was again started on 4HPR at the lower dose level. If the same toxicity recurred despite dose reduction, the patient had to discontinue treatment. MTD was defined as the dose below which at least 50% of patients experienced severe or limiting toxicity.

Tumor response was assessed according to the International Response Criteria for Neuroblastoma (19). Bilateral bone marrow aspirates and biopsies were required before study entry. Patients with evidence of marrow tumor cells before therapy were monitored monthly. Disease status was also assessed by physical examination, computed tomography and/or ultrasonography scans, urine catecholamine measurement every month, and either metaiodobenzylguanidine scintigraphy or bone scan every 3–6 months. The duration of response was calculated starting from entry into the study.

Drug Formulation and Administration. The drug was provided in 50- and 100-mg capsules by Janssen-Cilag Pharmaceutical Division (Schaffhausen, Switzerland). It was administered p.o. at breakfast for 28 days. When the capsules were too large to be swallowed, the inner oil content (0.7 ml = 100 mg) of each capsule was withdrawn by a syringe (20 ml, 18G \times 1½ needle) and, immediately afterward, was administered in a dairy beverage.

Pharmacokinetics. Whenever possible, depending on venous access and patient availability, blood samples for pharmacokinetic evaluation were obtained after the first and last dose of 4HPR administered in the first course. Here, we report the peak levels (C_{max}), the AUCs, and the half-life ($t_{1/2}$) of 4HPR, as well as the effect of 4HPR on retinol levels. The results of other analyses, including the results on metabolites, are reported in detail in a separate article.⁴ Samples that were drawn through a venous catheter were collected in heparinized tubes immediately before and 1, 3, 4, 6, 9, 12, 16, 24, and 48 h

⁴ F. Formelli, manuscript in preparation.

Table 1 Patient's characteristics at enrollment in the trial

No. of enrolled patients	54
No. of assessable patients	53
No. of assessable courses	168
Median age (yr)	9
Range	2–22
Male/female	34/20
Stage	
3	4
4	50
Previous treatment	
Chemotherapy	54/54
MGT + PBSC ^a	37/54
MIBG therapy or RT	12/54
Status at entry	
First partial remission	20
Second or additional partial remission	20
Second or additional complete remission	5
Resistant disease	9

^a MGT + PBSC, megatherapy plus peripheral blood stem cell rescue; RT, radiotherapy.

after drug administration. Samples were protected from light by wrapping the collection tubes in aluminum foil. Plasma was rapidly separated from blood by centrifugation and stored at -20°C until analysis, which was carried out within 1 month. Aliquots of 0.2 ml of plasma were added to 0.4 ml of CH_3CN containing butylated hydroxytoluene (125 $\mu\text{g}/\text{ml}$) as an antioxidant. Plasma concentrations of 4HPR and of retinol were then measured by high-performance liquid chromatography, as described previously (16, 17). 4HPR, to be used as standard for high-performance liquid chromatography analysis, was supplied by the R. W. Johnson Pharmaceutical Research Institute (Spring House, PA), whereas the reference standard for retinol was obtained from Sigma (St. Louis, MO). The AUC from 0 to 24 h ($\text{AUC}_{0-24\text{ h}}$) was calculated by the trapezoidal method (20). Half-life was calculated by dividing $\ln 2$ by the elimination rate constants (β). The β values were calculated by linear regression of the observed blood \ln concentrations (at least 3 values) to the last measured plasma concentrations (at 48 h). Pharmacokinetic parameters are reported as mean values \pm SD.

Statistical Analysis. Comparison of half-life after the first and the 28th administration was performed by Wilcoxon's test for paired samples in patients for whom half-life had been evaluated after both administrations. The association between the frequencies of toxicity and 4HPR peak levels was analyzed by Fisher's exact test.

RESULTS

Patient's Characteristics. Between September 1995 and March 2001, 54 patients (34 males, 20 females) were enrolled into the trial, and their characteristics are shown in Table 1. The majority of patients (50 of 54) had stage 4 disease at diagnosis. All patients had previously received intensive chemotherapy; 37 of them had received megatherapy plus peripheral blood stem cell rescue as consolidation treatment and 12 of them had received radiometabolic treatment with ^{131}I -metaiodobenzylguanidine or radiotherapy. Furthermore, 20 patients were in partial remission after the end of first line therapy and 20 after second line treatment; 5 patients were in second or further

complete remission. Each patient had stopped all therapy at least 3 months earlier, and disease extension was fully evaluated at the time of study entry. Nine patients were being treated for tumor progression occurring during first line therapy. Two patients treated in second PR and 1 in second CR had been treated with 13cisRA in the first line protocol.

Dose Escalation, Toxicity, and Outcome. Doses, number of patients, number of evaluable courses, and toxicity after each dose level are reported in Table 2. Taken together, 53 patients were assessable for a total of 168 evaluable administered courses. Some patients received more than the courses reported in Table 2 (as shown in Table 3), but because we were unable to collect full toxicity data, we report data only on the fully evaluable courses. Three patients continued therapy beyond the six courses of the protocol on the referring physician's decision for a total of eight courses. Four patients at the dose level of 1700, 2300, 3000, and 4000 mg/m^2 , respectively, discontinued treatment on parents' decision after three (1 case) and four courses. Three patients < 4 years of age at entry into the study received the drug that had been removed from the capsule, 1 at the 200 mg/m^2 and 2 at the 1000 mg/m^2 level. Additional attempts to administer higher doses of the drug with this modality was discouraged because of the extremely unpleasant taste. Rounding off of the dose was necessary only in few patients, only in the first three dose levels, and it resulted in a difference no greater than 15%. The doses were rounded up to the nearest multiple of 50 for a few days and then the excess administered dose was corrected by rounding down the following doses. At the dose level of 400 mg/m^2 , 1 patient refused treatment after the first day and was excluded from the study. More than 3 patients entered at several dose levels (Table 2) because of the enrollment of new patients before previous patients had concluded the 28-day course of 4HPR. At the 1000 mg/m^2 level, 6 patients were enrolled because of the occurrence of one case of grade 3 nyctalopia among the first 3 enrolled patients. The highest dose administered was 4000 $\text{mg}/\text{m}^2/\text{day}$, and no severe toxicity was observed. The patients' compliance at this dose was extremely poor because of the high number of capsules that had to be taken. This induced us to stop the study at the dose of 4000 $\text{mg}/\text{m}^2/\text{day}$, and MTD was not reached.

Toxic effects were observed in 34 of 53 patients. The highest grades of toxicity reported were grade 2–3 and were observed in only 13 patients. The most commonly observed toxicities were skin xerosis and nyctalopia. Cutaneous, ungual, or mucosal toxicities were observed during treatment in 15 of 53 patients (28%) and consisted mainly of dry skin and dry lips. Eleven of 53 patients (21%) had nyctalopia. Ten patients at different dose levels experienced mild (grade 1–2) nyctalopia, and only 1 patient at the 1000 $\text{mg}/\text{m}^2/\text{day}$ dose level suffered grade 3 nyctalopia, which was confirmed by ophthalmological evaluation. This severe adverse reaction appeared at the end of the first course, and it did not completely regress during the 7-day drug discontinuation. Afterward, the patient received the lower dose (700 mg/m^2) for the remaining five courses with no additional toxicity. There were no cases of pseudotumor cerebri, but 2 patients had grade 2 and grade 1 headache at the 300 and 400 $\text{mg}/\text{m}^2/\text{day}$ dose levels, respectively. One patient developed freckles, 1 presented HZV infection, and 1 had cholelithiasis. At the highest dose (4000 $\text{mg}/\text{m}^2/\text{day}$), 1 patient experienced re-

Table 2 Toxicity of 4HPR in neuroblastoma patients

Dose level (mg/m ² /day)	No. of patients	No. of evaluative courses	Type	Toxicity			
				No. of patients and grade			
				n.a. ^a	1	2	3
100	6	12	Nyctalopia		1		
			Skin rash		1		
200	5	18					
300	4	10	Headache			1	
400	3	10	Nyctalopia			1	
500	6	14	Headache		1		
			Nyctalopia		2		
			Skin xerosis			2	
600	4	25	Ungual dystrophy		1		
			Nyctalopia		1		
			Cholelithiasis	1			
700	3	14	Freckles	1			
			HZV infection	1			
			Nyctalopia		1		
1000	6	21	Nyctalopia		1	2	1
			Skin xerosis		2		
1300	3	12	Nyctalopia		1		
			Skin xerosis		1		
			Ungual dystrophy		1		
1700	3	9	Skin xerosis		1	1	
			Conjunctivitis		1		
2300	3	6	Nyctalopia		1 ^b		
3000	3	8	Skin xerosis		1 ^b		
			Skin xerosis			3	
4000	4	9	Hepatic toxicity			1	
			Diarrhea		2	1	

^a n.a., not applicable.

^b The same patient complained of nyctalopia and skin xerosis.

versible grade 2 hepatic toxicity consisting in elevated transaminases levels, and 3 patients developed diarrhea. All adverse reactions rapidly resolved after discontinuing treatment.

Tumor Response. Although measurement of tumor response was not a primary end point of this Phase I study, disease status was monitored monthly, and we analyzed the tumor response in all patients who completed at least one course of therapy. Tables 1 and 3 list the characteristics of the patients that were studied.

Twelve patients showed early progression of the disease during or after the first course of treatment with 4HPR (Table 3). The disease remained stable in 24 patients for a period ranging from 2 to 17 months (median 5), but thereafter, they died of disease progression. The remaining 17 patients are alive without progression from 11 to 37 months (median 14) after beginning 4HPR. Among the 53 patients who were assessable for response a correlation between status at entry and outcome was found: 8 of 9 patients (88%) treated with 4HPR for progressive disease underwent additional progression and died. In the group of 44 patients who were in PR (39) or in second or additional CR, early progression was observed in 19 (49%; $P < 0.05$). Sixteen patients showed disease stabilization lasting more than 12 months, 9 of whom had been treated with doses of 1000 mg/m²/day or higher. Five patients, all but 1, treated with doses above 1000 mg/m² day showed a clearing of some neoplastic lesions, including bone marrow in 3 cases and bone and bone marrow in 2 cases.

Pharmacokinetics. Pharmacokinetic analyses were performed on 49 of 53 assessable patients on the first day

(100 mg/m²/day, 6 patients; 200 mg/m²/day, 5 patients; 300 mg/m²/day, 4 patients; 400 mg/m²/day, 3 patients; 500 mg/m²/day, 5 patients; 600 mg/m²/day, 4 patients; 700 mg/m²/day, 2 patients; 1000 mg/m²/day, 6 patients; 1300 mg/m²/day, 3 patients; 1700 mg/m²/day, 3 patients; 2300 mg/m²/day, 1 patient; 3000 mg/m²/day, 3 patients; and 4000 mg/m²/day, 4 patients). Forty-two patients also had pharmacokinetic analyses performed on the 28th day of the first course. The peak concentrations (C_{max}) of 4HPR were achieved within a median of 4 h (range, 3–16 h) and 5 h (range, 3–20 h) after the first and 28th drug administration, respectively (data not shown). Fig. 1 reports the mean peak levels and the AUC_{S_{0–24 h}} of 4HPR after the first and the 28th administration. No association was found between age and peak levels (data not shown). Values of the patients who experienced grade 2–3 toxicity are reported separately from those of patients who experienced no or mild (grade 1) toxicity. The peak levels and the AUC_{S_{0–24 h}} at day 28 were higher than those found at day 1, with an average 2-fold increase. Similarly, trough concentrations increased by approximately three times after 28 daily treatments (data not shown). After the 28th administration, the correlation between the dose that was administered, and the peak plasma concentrations was linear in patients who experienced some form of adverse reaction, whereas in patients with no toxicity such a correlation was evident only up to the 2300 mg/m² doses. The same behavior was observed for the correlation between doses and AUC_{S_{0–24 h}}. At the doses where toxicity occurred, the mean peak levels and the AUC_{S_{0–24 h}} of patients who experienced some toxicity were always higher than those where no toxicity was observed. The

Table 3 Characteristics of patients at study entry and response to therapy

No.	Sex, age (yr)	Stage	Primary tumor site	Time (mo) from diagnosis to 4HPR therapy	Disease status at study entry	Sites of neoplasia at study entry				4HPR therapy daily dose/no courses	Tumor response/duration (mo)
						Primary site	Metastatic sites	¹²³ I-MIBG positivity	VMA-HVA ^a urinary		
1	M, 7	4	Adrenal	9	PD	Yes	BM	PT, BM	E	100/1	PD/1
2	M, 6	4	Adrenal	23	PD	No	BM,B	BM	E	100/1	PD/1
3	F, 14	4	RPG	53	PD	Yes	BM	PT,BM	E	100/4	SD/6
4	M, 7	4	RPG	20	PR	Yes	BM	PT	N	100/2	SD/2
5	F, 4	4	Adrenal	10	PR	Yes	LN,B	PT,LN,B	N	100/1	PD/1
6	F, 4	4	RPG	17	PR	Yes	B,BM	PT,B	E	100/3	SD/5
7	M, 6	4	Adrenal	48	II-PR	No	LN,B,BM	LN,B,BM	E	200/5	SD/5
8	M, 14	4	Thorax	24	II-PR	Yes	B	B	E	200/5	SD/5
9	M, 2	4	Adrenal	4	PD	Yes	B,H,L	ND	E	200/1	PD/1
10	F, 3	3	RPG	25	II-PR	Yes	–	PT	N	200/4	SD/4
11	M, 5	4	Adrenal	57	III-PR	No	B,BM	B	E	200/3	SD/3
12	F, 3	4	Adrenal	29	II-PR	No	B	B	N	300/1	PD/1
13	M, 6	4	Adrenal	24	II-PR	No	BM	–	N	300/2	PD/2
14	M, 5	4	Adrenal	13	II-PR	No	B,BM	B	E	300/5	SD/5
15	M, 6	4	RPG	24	PR	No	B,BM	B	E	300/2	SD/2
16	F, 2	4	Adrenal	60	II-PR	No	B,BM	B	E	400/1	PD/1
17	M, 4	3	RPG	19	II-PR	Yes	–	–	N	400/4	SD/3
18	M, 3	4	Adrenal	12	PR	Yes	B,BM	P,BM	N	400/NE	NE
19	F, 17	4	Thorax	36	II-PR	No	B,BM	B,BM	E	400/5	SD/5
20	M, 3	4	RPG	48	II-CR	No	–	–	N	500/6	SD/15
21	F, 3	4	RPG	36	II-CR	No	–	–	N	500/3	SD/4
22	M, 3	4	RPG	11	PD	Yes	B,BM	PT,B	E	500/1	PD/1
23	F, 4	4	RPG	55	II-PD	No	B	B	E	500/1	SD/3
24	M, 4	4	RPG	33	II-PR	No	B,BM	B,BM	E	500/1	PD/1
25	M, 16	4	RPG	60	III-CR	No	LN,B	LN	N	500/6	SD/37+
26	F, 5	4	RPG	12	PR	Yes	BM	PT,BM	N	600/8	SD/33+
27	F, 4	4	RPG	8	PR	Yes	–	PT	N	600/8	SD/35+
28	M, 7	4	Adrenal	31	II-PR	No	LN,BM,B	BM	N	600/3	SD/4
29	M, 4	4	Abdominal	12	PR	Yes	B,BM	B	N	600/6	SD/17
30	M, 5	3	Adrenal	36	II-PR	Yes	–	PT	N	700/8	SD/32+
31	M,10	4	Adrenal	24	PD	Yes	B	PT,B	E	700/1	PD/1
32	M, 5	4	Adrenal	12	PR	Yes	B,BM	B,BM	N	700/6	SD/17
33	M, 3	4	RPG	24	II-PR	Yes	B	B	N	1000/6	SD/30+
34	M, 8	4	Adrenal	48	II-PR	No	B,BM	B,BM	N	1000/2	SD/2
35	M, 4	4	RPG	12	PR	Yes	–	PT	N	1000/4	SD/9
36	M, 2	4	Adrenal	8	PD	Yes	B,BM	B,BM,PT	E	1000/1	PD/1
37	M, 2	4	Adrenal	9	PR	No	B	B	N	1000/6	SD/17
38	F, 3	4	Thorax	51	II-PR	Yes	B,BM	B,BM,LN	E	1000/4	SD/5
39	F, 13	4	RPG	11	PR	Yes	LN,H	PT,LN	N	1300/2	SD/6
40	F, 5	4	RPG	29	II-PR	No	LN	LN	N	1300/6	SD/22
41	M, 3	4	RPG	41	II-PR	Yes	LN	LN	N	1300/6	SD/27+
42	M, 8	4	RPG	8	PR	Yes	B,BM	PT,B,BM	E	1700/6	SD/21+
43	M, 4	4	Adrenal	9	PR	Yes	BM	B,PT	N	1700/4	SD/21+
44	F, 4	4	Adrenal	32	II-PR	No	B,BM	B,BM	N	1700/1	PD/1
45	M, 4	4	Adrenal	12	PR	No	B,BM	B	N	2300/3	SD/19
46	F, 2	3	RPG	22	PD	Yes	H,LN	PT,LN,H	E	2300/1	SD/2
47	F, 4	4	Adrenal	12	PR	Yes	B,BM	PT,BM,B	N	2300/2	SD/5
48	F, 2	4	Adrenal	48	II-CR	No	–	–	N	3000/6	SD/17+
49	F, 4	4	Adrenal	22	II-CR	No	–	–	N	3000/6	SD/13
50	M, 4	4	Adrenal	12	PR	No	BM,TP	BM,TP	E	3000/4	SD/12
51	M, 6	4	RPG	9	PR	No	BM	–	N	4000/6	SD/11
52	M, 10	4	RPG	20	II-PR	Yes	H,B	PT,H,B	E	4000/6	SD/9+
53	M, 8	4	RPG	20	PR	Yes	B,BM	PT,B,BM	E	4000/4	SD/11+
54	F, 4	4	Adrenal	9	PR	No	BM	–	N	4000/1	SD/2+

^a VMA, vanilmandelic acid; HVA, homovanillic acid; II, second; III, third; RPG, retroperitoneal ganglia; PT, primary tumor; BM, bone marrow; B, bone; H, hepatic; LN, lymph-nodes; L, lung; PD, progressive disease; SD, stable disease; PR, partial response; MR, minor response; CR, complete remission; NE, not evaluable; ND, not done; E, elevated; N, normal.

average peak levels reached after repeated administrations of 4000 mg/m² were 12.9 ± 6.26 μM, and they were 17.7 ± 4.38 and 8.1 ± 2.41 μM in patients with and without toxicity, respectively. In the same patients, trough levels were on average

9.9 ± 6.75 μM, and they were 15.0 ± 5.13 and 4.9 ± 2.84 μM in patients with and without toxicity, respectively (data not shown).

The half-life of 4HPR after the first and 28th administration

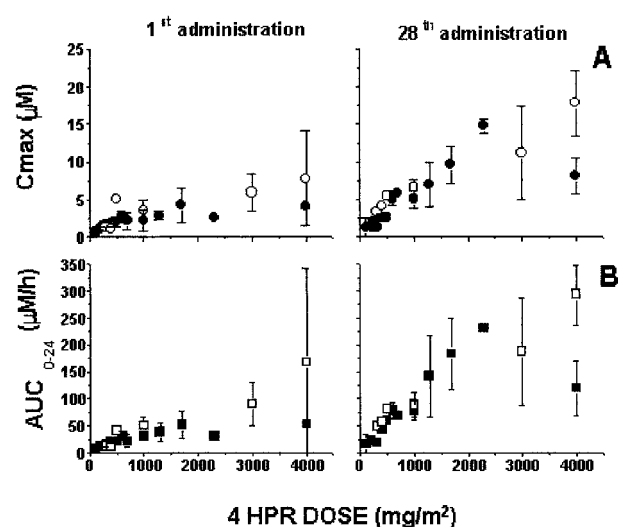


Fig. 1 Means and SD of 4HPR peak concentrations (Cmax) in A and AUC_{0-24 h} in B after the first and 28th administration of the first course in patients with grade 2-3 toxicity (open symbols) and in patients with grade 1 or without toxicity (closed symbols).

Table 4 Half-life ($t_{1/2}$) of 4HPR after the first and the 28th administration^a

Dose (mg/m ²)	Patient no.	4HPR administration	
		First (h)	28 th (h)
100	2	9.3	23.3
	3	18.3	21.0
	7	12.8	30.1
200	10	16.9	28.9
	11	16.5	31.5
	12	14.4	23.1
300	13	15.4	46.2
	27	13.9	36.5
	28	15.8	16.9
1700	42	14.4	20.4
	44	20.4	18.7
	49	28.9	33.0
4000	51	11.2	9.0
	52	11.6	16.1
	54	12.8	21.0
$\bar{x} \pm SD$		16.9 \pm 7.3	25.1 \pm 9.1 ^b

^a Evaluated up to 48 h.

^b $P \leq 0.01$, Wilcoxon test on paired samples, compared with first dose.

is reported in Table 4. In comparing the values, only patients for whom half-life had been evaluated after both administrations were taken into consideration. 4HPR half-life was not influenced by the doses. The mean half-life after the first dose was 17 h, whereas the values after 28 daily treatments were significantly higher, and they were on average 25 h.

The incidence of grade 2 and 3 toxicity was analyzed in relation with 4HPR peak plasma levels. We chose 3 µM as a discriminating value for peak plasma levels because no toxicity was observed in patients with peak levels below 3 µM (0 of 12). Because 10 µM peak levels had been used for similar analysis

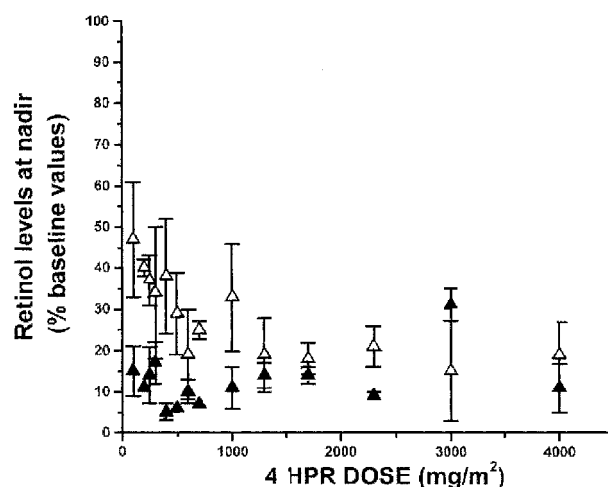


Fig. 2 Retinol levels at nadir expressed as percentage of baseline values after the first (open symbols) and 28th (closed symbols) administration of the first course.

with another retinoid (21), this value was also taken into consideration. The incidence of toxicity in patients with 3-10 µM peak levels was lower (7 of 12, *i.e.*, 31%) than in patients with peak levels >10 µM (4 of 8, *i.e.*, 50%), although the difference was not statistically significant. After these results, comparison was performed between patients with peak levels < 3 µM and those with peak levels \geq 3 µM. The difference was statistically significant ($P = 0.012$), indicating that the probability of grade 2-3 toxicity is significantly higher in patients with 4HPR peak levels > 3 µM.

Effect on Retinol Levels. The effect that the various doses of 4HPR had on pretreatment retinol plasma levels was also assessed. 4HPR caused significant decreases in retinol levels, which reached the lowest values (nadir values) at a median time of 20 h (range, 7-36 h; data not shown). No correlation was found between the onset of adverse reactions and retinol levels at nadir. Therefore, data regarding patients with and without toxicity have been pooled in Fig. 2. After the first administration, the mean retinol levels at nadir dropped to 47% of pretreatment values after the lowest dose (100 mg/m²/day) and to 20% at doses ranging from 1300 to 4000 mg/m²/day. The lack of additional reduction at doses higher than 1300 mg/m²/day may be attributable to the fact that the lowest attainable retinol levels, *i.e.*, <100 ng/ml, had already been reached with this dose. After the 28th administration, retinol levels had dropped from 20 to 10% of pretreatment levels after all of the tested doses.

DISCUSSION

This study demonstrates that 4HPR administered in 28-day courses, followed by a 7-day drug discontinuation, is well tolerated in children with neuroblastoma, up to a dose of 4000 mg/m²/day. The excessive amount of capsules or oil content intake precluded additional dose escalation, and thus the MTD was not reached. Despite this limitation, the highest dose we tested led to average drug plasma levels of 12.9 µM, which are

comparable with those that are effective in suppressing the *in vitro* growth of neuroblastoma cells (10–13).

None of the patients discontinued the drug because of toxicity. Cutaneous toxicity and nyctalopia were the most common side effects, *i.e.*, a toxicity profile similar to what has been observed in adult patients (15). The side effects, which rapidly reversed during the 7-day drug interval, were observed at most dose levels and do not appear to be dose related. Cutaneous toxicity consisted mainly of dry skin and dry lips, which were easily managed with topical emollients. Nyctalopia occurred in 23% of the children, an incidence comparable with what has been observed in adults (15). However, we must point out that the patient with grade 3 nyctalopia was the only one who complained of this side effect, whereas in the remaining 9 cases, nyctalopia was found only after an accurate anamnesis. This side effect occurred at most dose levels, and its severity did not increase as the doses increased. This could be attributable to the fact that during repeated administrations plasma retinol levels were reduced to 10–20% of pretreatment levels, regardless of the dose.

During 4HPR treatment, we observed one case of HZV infection. However, we do not know if this event was associated with 4HPR treatment or with immunodepression caused by previous treatment. In adults, 4HPR increased the functional activity of peripheral blood mononuclear cells (22), but we cannot rule out that 4HPR had a direct effect on latently infected cells because *in vitro* and *in vivo* studies have shown that retinoids can increase viral infections (23). Interestingly, a trend to a higher number of HZV infections was also noted in the breast cancer prevention trial (14), although the difference was not statistically significant (7 cases in the 4HPR arm *versus* 2 cases in the control arm, unpublished data). At the dose level of 4000 mg/m²/day, 3 patients had diarrhea, which may have been attributable either to 4HPR toxicity or to the oil content of the capsules. 4HPR did not influence lipid profile, ionogram, or blood cell count, and only 1 case of elevated transaminases levels was observed at 4000 mg/m²/day. The lack of influence of 4HPR on these parameters is important because liver tests, blood cell count, and calcium are frequently affected by other retinoids. The toxicity profile of 4HPR seems to be different from that of other retinoids used in children. Neurotoxicity was the DLT of ATRA (24) and 9cisRA (7), whereas the DLT of 13cisRA consisted of hypercalcemia (21). Furthermore, the toxicity of these retinoids was associated with peak plasma levels lower than those of 4HPR: 13cisRA peak levels >10 μM were correlated with 50% grade 3–4 toxicities (21), and the DLT of ATRA and 9cisRA occurred at ~1 μM peak levels (7, 24).

The half-life of 4HPR was not influenced by the dose. Its mean value was 17 h, after the first administration, and it increased to 25 h after repeated treatments. The elimination of 4HPR in children does not seem to differ from what was observed in adults whose 4HPR half-life was 27 h after 5 years of treatment (17).

Repeated daily oral administration of 4HPR for 28 consecutive days resulted in a 2-fold increase in peak drug plasma concentrations and AUCs compared with those observed after one single administration. This pharmacokinetic behavior is different from what is observed with ATRA (25) and 9cisRA (7), the plasma levels of which decrease after chronic adminis-

tration. It is also different from that of 13cisRA, which has peak levels after multiple dosing that are similar to those on day 1 (21, 26). As doses increased, 4HPR peak levels and AUCs increased, and the increase was linear up to the 4000 mg/m² dose in patients who experienced toxicity. Impairment of drug absorption seemed to occur in some patients when doses higher than 2300 mg/m²/day were administered, and this may account for the lack of toxicity in these patients. Although no DLT was observed, an association was found between plasma peak levels and toxic effects. After repeated treatments, plasma peak concentrations < 3 μM seem to be safe because no toxicity occurred at these concentrations, whereas the frequency of grade 2–3 toxicity was significantly higher in patients with peak levels > 3 μM, and it increased from 31% in patients with peak levels 3–10 μM to 50% in those with peak levels > 10 μM.

On the basis of our results, it would seem that oral administration of 4HPR once daily for 28 days followed by a 7-day drug discontinuation is better tolerated than administration in three divided doses/day for 7 days every 21 days (27). With this schedule of treatment, the MTD was 2475 mg/m²/day; one grade 4 hepatic and five grade 3 gastroenteric toxicities occurred in 6 of 50 patients, and 3 cases of pseudotumor cerebri were observed at 600, 800, and 3300 mg/m²/day, respectively (27). The steady-state 4HPR plasma levels reported by Villablanca *et al.* (27) after a MTD of 2475 mg/m²/day were comparable with the mean steady state concentrations observed in our study (data not shown) after a similar dose (2300 mg/m²) given once daily (11.5 *versus* 9.6 μM in our study). The higher toxicity of 4HPR observed after three divided doses/day is probably attributable to the fact that threshold-toxic drug levels last longer with this schedule. At present, no data are available on the impact of the two different dose schedules on tumor response.

Although we performed this Phase I study mainly on children with nonprogressive disease to assess the pharmacokinetics and toxicity of prolonged treatment with 4HPR in the pediatric age, we tried some speculations about the efficacy of the drug. The results show that 4HPR is useless for treating patients in a massive disease setting. Similar results in advanced neuroblastoma were found with 13cisRA (21). On the contrary, patients who were treated with 4HPR in a PR phase showed prolonged stabilization of the disease, and some of them showed regression of some lesions. Favorable effects occurred more frequently in patients who received doses of 4HPR of 1000 mg/m²/day or higher and whose plasma levels were above 5 μM after repeated treatment.

In conclusion, only 1 case of DLT was observed in 53 pediatric patients treated with doses of 4HPR up to 4000 mg/m²/day, and drug plasma concentrations equivalent to those that are effective *in vitro* against neuroblastoma cells were achieved with only mild toxicity. In view of this remarkable finding, when compared with other retinoids, the toxicity of 4HPR does not seem to preclude its future use in children. 4HPR acts as a biological modifier involving several distinct pathways (28), and its clinical effects may not be easily measurable in a Phase I study. However, the prolonged stabilization of disease that was observed in some patients is encouraging and suggests that a Phase II study testing 4HPR as possible maintenance therapy for the control of minimal disease in neuroblastoma would be warranted.

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