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Phase I study of VRCTC-310, a purified phospholipase A_2 purified from snake venom, in patients with refractory cancer: safety and pharmacokinetic data

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A phase I study was performed to evaluate the maximum tolerated dose (MTD), safety profile and pharmacokinetic data with VRCTC-310, a natural product derived from purified snake venom fractions, with phospholipase A_2 activity and inhibitory effects against human and murine tumor cell lines. Fifteen patients with refractory malignancies were entered after providing written informed consent. VRCTC-310 was administered as an intramuscular injection daily for 30 consecutive days. Doses were escalated from 0.0025 to 0.023 mg/kg. Toxicities included local pain at the injection site, eosinophilia, reversible diplopia and palpebral ptosis. Dose escalation was stopped at 0.023 mg/kg, when two patients had developed anaphylactoid reactions. Both cases had high VRCTC-310-specific IgG by EIA. MTD was 0.017 mg/kg and the recommended dose for phase II studies is 0.017 mg/kg. Stabilization was found in six patients.

Key words: Anticancer therapy, phase I, pharmacokinetics, phospholipase, snake venom.

Introduction

VRCTC-310 is a natural product derived from purified snake venom fractions, which has shown significant *in vivo* activity against several murine and human experimental tumors, including Lewis lung carcinoma.[1] It is composed of crotoxin (CT), a cytostatic phospholipase A_2 isolated from the venom of the South American rattlesnake *Crotalus durissus terrificus*,[2] and cardiotoxin (CD), a basic membrane disruptive peptide isolated from the venom of the south Chinese cobra *Naja Naja atra*.[3] VRCTC-310 is composed of three peptide fractions with M_r of 7.0, 9.5 and 14.5 kDa, respectively. Evaluation of *in vivo* cytotoxicity by the Developmental Therapeutics Program of the National Cancer Institute against a panel of human tumor cell lines has demonstrated that, compared to the separate CT and CD components, VRCTC-310 exhibited an enhanced cytotoxicity against and selectivity towards human cell lines derived from melanoma as well as CNS and lung tumors.[1] The COMPARE analysis of this pattern of specificity is unlike that of any other known agent for which a mechanism of cell killing has been determined, thus suggesting a unique mechanism of action.[1,4] Both CT and CD are membrane-active substances, and the possibility of achieving cytotoxicity in malignant cells with a phospholipase A_2-based compound by means of specific membrane-receptor binding and subsequent CD-activated enzymatic hydrolysis of membrane phospholipids presents a novel approach to anticancer therapy. When compared to isolated CT and CD,[3,5] VRCTC-310 shows unique pharmacological properties reflecting the synergism between both components. At a specific molar ratio of 3(CD):1(CT), as in VRCTC-310, CD increases the cytotoxic activity of CT and decreases CT-associated neurotoxicity by about 4- to 5-fold,[1] thus extending the therapeutic index of the compound.

The goals of this study were to define the dose-limiting effects of VCRTC-310, and to describe the maximum tolerated dose (MTD), safety profile and pharmacokinetic data after intramuscular injection.

Materials and methods

Patients

Only patients with solid tumors refractory to conventional therapy or for which no effective therapy

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existed were candidates for this study. Eligibility criteria included: histologically confirmed advanced cancer, age older than 18 years, Eastern Cooperative Oncology Group (ECOG) performance status below 2 (ambulatory and capable of self care), life expectancy enabling the completion of at least two 30 day courses of therapy, no major surgery within 14 days or radiotherapy and/or chemotherapy within 28 days, adequate hematopoietic (absolute neutrophil count above 1500/ml and platelet count above 100 000/ml), hepatic (total bilirubin level below 1.5 mg/dl) and renal (creatinine concentration below 1.5 mg/dl, creatinine clearance above 60 ml/min) functions. Exclusion criteria included active uncontrolled infection, severe allergies or hypersensitivity to VRCTC-310, pregnancy, lactation and any other co-existing medical problems severe enough to prevent full compliance with the study.

All patients gave informed written consent before treatment. Objectively measurable disease was not required. According to federal regulations in Argentina, the protocol was approved by the federal Food, Drugs and Medical Technology Administration (ANMAT, for *Administración Nacional de Medicamentos, Alimentos y Tecnología Médica*, Argentina's national drugs administration), after receiving approval by the Institutional Review Board of the Hospital 'Bernardo A Houssay' and by an independent Ethical Committee.

Treatment

VRCTC-310 was supplied by Ventech Research (Boston, MA) in ampoules containing 1 ml of a 1 mg/ml solution. The starting dose of VRCTC-310 was 0.0025 mg/kg/day administered as an intramuscular daily injection for 30 consecutive days.

Dose escalation proceeded according to Fibonacci's schema from 0.0025 up to 0.023 mg/kg, as listed in Table 1. Individual patient dose escalations were permitted every 30 days.

At least three patients were entered at each VRCTC-310 dose level. Subsequent courses were administered without an interval. Dose escalations were done with increments ranging from 25 to 50%. As after completion of one cycle some patients agreed to continue in the trial with a higher dose, a total of 23 complete cycles were evaluated, corresponding to 15 different subjects. For pharmacokinetic calculations, all cycles are individualized and results presented on the basis of the dose the patient received. On the other hand, for toxicity, results have been recorded and presented for each patient as an individual.

Table 1. Distribution of patients according to VRCTC-310 escalating doses

Level	Dose (mg/kg/day)	Patient nos	Remarks
1	0.0025	1; 2; 3	
2	0.0050	4; 5; 6; 1	Patient no. 6 died due to cancer progression
3	0.0075	7; 8; 9	
4	0.0100	10; 4; 3;	
5	0.0130	7; 11; 12; 3; 10	Patient no. 11 refused therapy for second cycle; patient no. 10 developed grade III allergy reaction
6	0.0170	13; 14; 15	Patient 13 developed grade III allergy reaction
7	0.0230	12; 7	

Evaluation

Toxicities were evaluated according to the common toxicity criteria.[6] Medical records including antecedents, physical examination, ECG and routine laboratory studies were performed before treatment. Chemistries, renal and liver function tests, and urinalysis were performed weekly. Complete blood cell counts and differential leukocyte counts were also obtained.

Pharmacokinetics of VRCTC-310

Eleven serum samples were obtained from each patient by means of venous catheters placed in their arm. Blood was sampled immediately before up to 24 h after the first injection of VRCTC-310. Serum was allowed to separate and frozen at −70°C up to the moment of analysis. The program PKCALC[7] was used to determine the area under the concentration × time curve (AUC) and other pharmacokinetic parameters.

Assay of CT in human serum

A sandwich enzyme immunoassay (EIA) was developed to measure CT concentration in human serum. In brief, these were: horse anti-VRCTC-310 (Instituto Malbrán, Buenos Aires, Argentina) was used for capture, diluted 1:100 and rabbit anti-VRCTC-310 serum (titer 1/10000, prepared in the Facultad de Medicina Veterinaria, UNLP, La Plata, Argentina) for detection, followed by anti-rabbit avidin-biotin-alka-

line phosphatase system (Vectastain Elite ABC; Vector, Burlingame, CA). Reading was done at 405 nm with a Statfax spectrophotometer. The sensitivity of the assay was 100 pg/ml.

Results

Patients and toxicity

The main objective of the study was to determine the toxicities and appropriate VRCTC-310 dose for subsequent trials of untreated or minimally pretreated patients. Between February and September 1996, 15 patients were enrolled: five patients had gastrointestinal cancer, three patients had breast cancer, two patients had lung cancer, two patients had head and neck cancer, two patients had cervical cancer, and one patient had ovarian cancer. Table 2 summarizes their main clinical data.

A total of 23 complete cycles were evaluated in these 15 patients. No deaths attributable to VRCTC-310 occured during this study. No significant toxicities were found up to 0.013 mg/kg; the most common was local pain at the injection site (11 out of 15 patients), eosinophilia (seven out of 15 patients) and spontaneously reversible diplopia and/or mild palpebral ptosis (six out of 15). Two patients developed anaphylactoid reactions requiring epinephrin and antihistamines (after 10 and 14 days of daily injection,

Table 2. Characteristics of the patients

Patient	Age	Sex	Performance status	Tumor location	Stage
1	62	M	2	stomach	IV
2	64	F	1	cervix	IV
3	49	F	1	breast	IV
4	49	M	1	lung	IV
5	64	M	1	pancreas	IV
6	48	M	1	lung	III
7	61	M	1	gall bladder	IV
8	50	M	1	larynx	IV
9	58	M	1	colon	IV
10	62	F	1	breast	IV
11	48	F	1	breast	IV
12	50	M	2	rectum	IV
13	43	F	1	cervix	IIIb
14	61	F	1	ovary	III
15	49	M	1	maxilla	III

Table 3. Toxicity on 23 cycles of VRCTC-310

Patient	Local pain	Anaphylaxis	Diplopia	Palpebral ptosis	Blurred vision	Eosinophilia	Emesis	Photophobia
1			14 days 0.0025 mg/kg					
2	×							
3	×				10 days 0.0025 mg/kg			
4			9 days 0.005 mg/kg	1 day		×		0.005 mg/kg
5	×		12 days 0.005 mg/kg		3 days	0.005 mg/kg		
6	×					×		
7			23 days 0.0075 mg/kg		30 days 0.0075 mg/kg	×		
8	×		21 days 0.0075 mg/kg	10 days	30 days 0.0075 mg/kg	×		
9	×		13 days 0.0075 mg/kg		13 days 0.0075 mg/kg			
10	×	grade 3 0.013 mg/kg	1 day 0.01 mg/kg	7 days	7 days 0.01 mg/kg	×		
11	×							
12			14 days 0.013 mg/kg		18 days 0.013 mg/kg		×	18 days 0.017 mg/kg
13	×	grade 3 0.017 mg/kg		14 days 0.017 mg/kg	18 days 0.017 mg/kg	×	×	
14	×				30 days 0.017 mg/kg	×	×	
15	×		10 days 0.017 mg/kg		8 days 0.017 mg/kg			

respectively); one was receiving her second cycle (at 0.013 mg/kg) and the other was in her first cycle (at 0.017 mg/kg). Both cases had high VRCTC-310-specific IgG (by EIA). When the second anaphylactic reaction was detected, two patients were in the middle of their cycle at 0.023 mg/kg. At this level, dose escalation was stopped, although patients were allowed to end their cycles. None of the patients developed neutropenia or thrombocytopenia (Table 3).

In the protocol, the MTD of VRCTC-310 had been defined as one dose level below the dose which resulted in limiting toxicity in two out of the three patients included in that level. Since anaphylaxis is a severe and eventually lethal condition, the main researcher of the trial decided to stop it when a second case was detected. Since 0.023 mg/kg was the highest dose safely tested (in only two patients), MTD is considered to be higher than 0.023 mg/kg,

This study was not designed to assess clinical efficacy. Nevertheless some striking results were observed: all patients experienced subjective benefit from the treatment. In six cases (nos 3, 4, 10, 12, 13 and 14 in Table 2) disease did not progress in the course of the study. Remission higher than 50% was found in patient no. 4, who entered the trial in April 1996. This patient is still alive and continues in remission, under treatment with VRCTC-310 at 0.01 mg/kg (the dose he received in his second cycle in the trial, after completion of a first cycle at 0.005 mg/kg). Currently (July 1997) patient nos 3, 4, 8, 10, 13 and 14 are alive, with more than 1 year's survival after ending the trial.

Pharmacokinetics of VRCTC-310

Table 4 summarizes the main pharmacokinetic parameters of the patients. Striking differences in every parameter were observed between patients, even within the same dose level. AUC seemed to increase with the dose of VRCTC-310 up to 0.017 mg/kg.

As a trend, in most patients the peak serum concentration was found between 15 and 120 min after injection. Patient no. 19 showed the highest value of this series, with 100 ng/ml after receiving VRCTC-310 at 0.017 mg/kg; however, all other patients were well below this figure (Table 5). However, in most patients a second peak was also found. Figure 1 depicts a typical concentration × time profile, corresponding to patient cycle no. 9 after receiving VRCTC-310 at 0.0075 mg/kg. This second peak was usually lower than the first one; however, in two cases (cycles

Table 4. Peak plasma values of CT, measured by EIA, after intramuscular injection in 23 cycles, corresponding to 15 cancer patients

Cycle	Dose (mg/kg)	Plasma peak value (ng/ml)	Peak time (min)
1	0.0025	0.7	15
2	0.0025	0.8	60
3	0.0025	0.6	15
4	0.005	2.0	15
5	0.005	0.25	60
6	0.005	5.0	120
7	0.0075	6.0	15
8	0.0075	4.0	15
9	0.005	0.9	60
10	0.0075	5.0	15
11	0.01	1.7	15
12	0.01	15.0	15
13	0.01	1.0	5
14	0.013	9.0	30
15	0.013	0.8	45
16	0.013	3.0	15
17	0.013	2.2	15
18	0.017	9.0	30
19	0.017	100.0	240
20	0.017	4.5	45
21	0.023	0.9	30
22	0.023	1.0	30
23	0.013	1.6	45

Table 5. Pharmacokinetic data of patients included

Dose level (mg/kg)	AUC (pg.min/ml)	C_{max} (pg/ml)	t_{max} (min)	Half-life (min)
0.0025	16875	600	30	36
	68500	800	60	5
	132750	800	240	12
0.0050	14875	250	60	–[a]
	2063220	5000	120	311
	380000	6000	15	5
	3651300	5000	15	19
0.0075	2357000	4000	15	115
	160000	900	60	308
	43000	1700	15	39
0.0100	511050	15000	15	7
	36275	1000	5	24
	750000	9000	30	124
0.0130	52700	2000	15	9
	3336000	3500	120	58
	43450	1200	1	78
	4666900	5000	1440	7
	115100	1600	30	40
0.0170	1180000	9000	30	67
	112287000	100000	240	1628
	202125	4500	45	27
0.0230	1451250	1800	1440	16
	127680	1000	30	76

[a]The program showed a negative value.

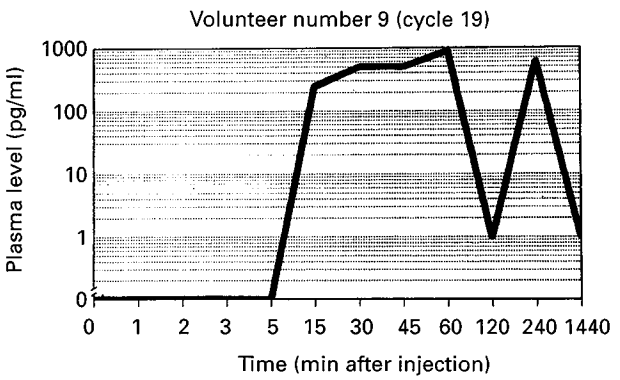

Figure 1. Biphasic pattern of the CT (the active component of VRCTC-310) serum level, determined by EIA, after intramuscular injection of 0.075 mg/kg (0.275 mg/m^2) in a patient suffering from disseminated colon cancer. Time in the abscissa corresponds to sampling time (in min) after injection. Note the biphasic distribution of plasma level: the first peak (900 pg/ml = 0.9 ng/ml) was obtained 1 h after injection and the second 4 h after injection.

nos 17 and 22) the second peak was the highest and it was reached at a very late time (in the 24 h sample). Due to this bimodal profile of concentration × time data, AUC values must be seen with great care (see Discussion) since they may include an overestimation of CT concentration in serum.

This bimodal profile also hindered proper estimation of elimination half-life. Attemps done with arbitrary selection of points after the first peak but before the second resulted in values below 30 min in 10 cycles, with a median of 38 min in the whole sample.

No samples were taken from cerebrospinal fluid, thus VRCTC-310 passage to CNS could not be determined.

Discussion

In this study, the safety profile of VRCTC-310 in humans showed anaphylactic reactions to be the only severe effect, up to 0.023 mg/kg. Pharmacokinetic analysis showed significant variability among patients, probably reflecting differences in bioavailability.

Toxicity is one of the most limiting effects of all antineoplastic drugs, either haematological or non-haematological. Interestingly, VRCTC-310 at the tested dose did not induce leukopenia, thrombocytopenia or anemia, suggesting that at this dose level it is devoid of hematological toxicity. This is consistent with the preclinical study: in none of the species tested could hematological toxicity be found.[1] The only blood cell abnormality was discrete eosinophilia in half of the patients, probably reflecting the induction of immune reactivity against the peptide components of VRCTC-310.

Among non-hematological effects, the only significant toxicity of VRCTC-310 was local pain and mild neurotoxicity. Local pain is a common effect of many antineoplastic drugs; however, in this case it does not seem to be associated with local irritation but with other local effects of VRCTC-310, perhaps phospholipase activity. Pain induced by VRCTC-310 began roughly between 1 and 2 h after injection, while irritative pain is usually an immediate effect. Pain with VRCTC-310 had already been found in preclinical studies in dogs and, consistent with those data, it was spontaneously reversible after several days. However, in order to decrease patient discomfort, dexamethasone was administered as a single 8 mg intramuscular dose before the first dose of VRCTC-310. This treatment abrogated local pain and allowed us to increase the dose up to 0.023 mg/kg. However, since only one dose of dexamethasone was given, immune sensitization against VRCTC-310 could not be abrogated.

As to neurotoxicity, it was only mild and does not seem to constitute significant problems for VRCTC-310 administration at a dose up to 0.023 mg/kg. While some other antineoplastic agents, such as Vinca alkaloids and paclitaxel, do also induce neurotoxicity, their profile is quite different, including sensitive and autonomic nerves. VRCTC-310 affected neuromuscular junctions, resulting in only mild and self-limited ptosis, with or without diplopia. This effect is also consistent with preclinical data[8] and did not require any dose adjustment in this study. While still unclear, the mechanism seems to be an impaired acetylcholine release from nerve endings.[8] However, for phase II studies we suggest to exclude primary or paraneoplastic myasthenia.

The most serious effect, which resulted in ending the protocol, was an anaphylactic reaction. It is an expected effect of VRCTC-310, since the active component is an heterologous protein (snake phospholipase). While this effect did only achieve severity in two cases, half of the patients presented indirect evidence of sensitization, such as eosinophilia. The immunological origin of this reaction is further supported by the detection of specific anti-VRCTC-310 IgG in serum of the affected patients. A similar risk for anaphylactic reactions has been described for another heterologous protein, L-asparinase.[9] For the next studies we propose to evaluate a desensitization approach, in order to avoid or limit this side effect.

Strikingly, no other limiting toxicities were found, suggesting that VRCTC-310 deserves further study for

evaluation of anticancer activity. Preliminary observations suggest that at least some patients took benefit from this treatment: six patients, out of the 15 included, reached stabilization of the disease after treatment and five of them are still alive. Interestingly, the inhibitory concentrations found in preclinical evaluation were several fold higher than peak serum concentration in the patients, suggesting that an indirect mechanism may also be involved; future studies will test this hypothesis.

On the other hand, only limited information is available concerning the pharmacokinetics of VRCTC-310.[5] In this study, intramuscular injection of VRCTC-310 to patients produced highly variable concentration × time profiles at any dose level tested, probably reflecting strong differences in bioavailability by this route. Although with large differences, the half-life of CT in our patients had a median value of less than 1 h. Although our results seem to be to some extent similar, the findings are difficult to compare with preclinical pharmacokinetic data of CT, since only short periods of sampling have been used in mice and rats (Department of Development, Ventech).

Interestingly, most patients presented a bimodal concentration × time profile after a single injection, which is quite unusual. The reason for this pattern is at present unclear, but several possibilities must be taken into account. The easiest is to suppose that either the horse or the rabbit serum (or both) used to capture and detect CT in the EIA recognize this protein, as well as its degradation products. Both sera are polyclonal and very probably bind, with different affinities, to more than one epitope in CT. Although we have no direct evidence of how VRCTC-310 is being eliminated, proteolytic cleavage is likely a putative candidate. Many proteins are taken up by different cell types and subjected to proteolysis, fragments thereafter being released out of the cell. A picture like this would be consistent with the bimodal pattern we found. The availability of monoclonal antibody-based EIA and i.v. administration of VRCTC-310 could help to evaluate this possibility.

Another very interesting explanation refers to the way in by which VRCTC-310 reaches venous blood. Most hydrosoluble substances of low molecular weight readily diffuse out of the muscular interstitium through the capillary endothelial fenestrations. CT, the only component of VRCTC-310 we measured, is a peptide with a M_r of 23.5 kDa and thus does not seem a very good candidate to diffuse easily. The alternate way is the lymphatic route by which substances arrive in the general circulation by the thoracic duct, after traveling by lymphatic vessels. Lymphatic flow is more slow than blood, so if some proportion of CT reaches circulation through capillaries and the rest by lymphatics, a bimodal pattern of serum concentration × time is to be expected. Again, the availability of i.v. administration is required to rule out this possibility. It is noteworthy to realize that significant distribution of VRCTC-310 by the lymphatic route could eventually contribute to its biological effect.

Conclusion

We conclude that the MTD of VRCTC-310 in this study is 0.023 mg/kg or higher. Dose-limiting toxicity was an anaphylactoid reaction with concomitant anti-VRCTC-310 IgG. The recommended dose for phase II studies is 0.017 mg/kg.

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