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Short Communication

30-day intravenous administration of VRCTC-310-ONCO in rabbits

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Abstract

VRCTC-310-ONCO, an agent based on the snake phospholipase A_2 (crotoxin), is currently under clinical development. After phase I study in patients by intramuscular administration, the interest of intravenous (IV) dosing arose. To evaluate IV administration of VRCTC-310-ONCO in rabbits, ten animals were subjected to surgical implant of fixed jugular catheter, by which they received daily IV doses of 0.03 mg/kg body weight of VRCTC-310-ONCO for 30 days ($n = 8$) or saline ($n = 2$). The procedure was well tolerated in all rabbits. One of the animals died after the sixth dose of VRCTC-310-ONCO with CNS involvement; two additional rabbits required dose-reduction. All other rabbits achieved 30 days of treatment and were sacrificed. All rabbits (even controls) developed lymphocytosis and mild anaemia, without changes in blood neutrophils. No changes were found in serum transaminases (GOT and GPT), cholesterol, triglycerides, and γ-glutamyl transpeptidase. At necropsy, chronic granulation tissue was found surrounding the implant in all rabbits. VRCTC-310-ONCO-treated rabbits presented generalised and marked swelling of hepatocytes, with areas of cytoplasmic vacuolisation. No abnormalities were found in kidney, heart, lung, spleen, adrenal gland, uterus, testes and ovary. Additional studies with IV route for VRCTC-310-ONCO, including humans, are required to define its toxicity in the clinical setting.

Keywords: Crotoxin; Phospholipase A_2; Safety; Preclinical study; Intravenous route

1. Introduction

VRCTC-310-ONCO (formerly VRCTC-310) is a novel antineoplastic agent based on phospholipase A_2 [1] purified from *Crotalus durissus terrificus* in equimolar association with cardiotoxin from *Naja Naja atra* [2]. In vitro and in vivo studies during preclinical evaluation have shown VRCTC-310-ONCO to induce a dose-related cytotoxicity in several human cancer cell lines [1,2]. In addition, in vivo effects of crotoxin on the endocrine and immune function are currently under evaluation [3]. Those in vivo studies and toxicity testing have been performed with intramuscular administration [4]. Accordingly, phase I study has evaluated safety of VRCTC-310-ONCO by intramuscular injection [5], showing it to be safe enough as to be further studied in phase II. However, intramuscular route is quite painful and, in addition, pharmacokinetic determinations are difficult. Therefore, the aim of this study has been to evaluate the feasibility of long-term intravenous (IV) administration and its safety in rabbits.

2. Material and methods

2.1. Animals

New-Zealand-Californian rabbits ($n = 10$, five male, weighting 2150–3600 g) were housed individually, with balanced dried food and water ad libitum. After adaptation to the Animal Facilities of the Veterinary School, they were prepared by surgical implantation of Implantofix™ siliconated perfusion catheter (paediatric size 1.4 B, Braun, Chasseneuil, France; 270 mm long) in the jugular vein.

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2.2. Drug under evaluation

VRCTC-310-ONCO, an equimolar mixture of crotoxin and cardiotoxin, was provided by Onco-Venom Research SA (Panamá, Panamá), in 5 ml vials, each containing 2 mg/ml of each crotoxin and cardiotoxin. Toxins were purified in Sephadex column as described [2].

2.3. Dosing

Beginning 2 weeks after implantation, VRCTC-310-ONCO was administered daily, at 0.030 mg/kg of body weight as IV bolus, in eight of the rabbits, while two were used as control and received IV injection of a similar volume of saline. The dose was previously determined as the maximum tolerated dose by the intramuscular route in humans, in a phase I study [5].

2.4. Follow-up and biochemical tests

Blood samples were obtained before beginning VRCTC-310 administration, and at days 9, 17 and 30 thereafter. Routine clinical chemistry tests were performed in serum by means of a Technicon RA-500 autoanalyzer (Bayer Diagnostics, München, Germany) with commercial reagents from Boehringer (Mannheim, Germany). Haematological tests were done in a K-1000 system (Sysmex, Long Grove, IL, USA) with Cell-Pack reagents (Sysmex). Before collection of the samples, rabbits were weighted. This study was performed in compliance with current regulation for animal studies in Argentina, as well as with Directive 86/609/EEC (European Convention for the protection of vertebrate animals used for experimental and other scientific purposes, 1986) and the guiding principles in the use of animals in toxicology.

2.5. Histopathology

At the end of the follow-up, rabbits were sacrificed and subjected to necropsy, with macroscopic and microscopic observation after haematoxylin–eosin staining.

2.6. Statistics

The comparison between values before treatment and at follow-up was done with repeated-measures Analysis of Variance (ANOVA) or Kruskal–Wallis test, depending on the kind of data (normal distribution or not), with a significance level of 0.05.

3. Results

3.1. Clinical tolerance

The surgical procedure was well tolerated in most rabbits. All survived acutely to surgery. On administration, one of the rabbits died after 6 days of dosing, 4 h after its daily dose, with typical manifestations of meningeal involvement (photophobia, tremor and opisthotonos). Two additional rabbits presented also transient signs of CNS involvement for 3 days in the first week of treatment, which disappeared after reduction of the dose to 0.015 and 0.008 mg/kg of body weight, respectively. These two animals completed treatment with the reduced dose.

Ponderal evolution was similar in both VRCTC-310-ONCO treated rabbits and controls (Table 1); nevertheless, two out of the seven rabbits that achieved the end of treatment period did not increase their body weight.

3.2. Laboratory data

Table 1 presents the haemoglobin and leukocyte counts at different times of the study. In the basal study (immediately before the first injection of VRCTC-310-ONCO), all rabbits (either treated or controls) presented similar values of leukocyte count as well as percentages of neutrophils and lymphocytes. After surgery, a 20% decrease of haemoglobin and lymphocytosis (roughly duplicating the basal lymphocyte abso-

Table 1
Haematological parameters of rabbits subjected to an IV daily dose of VRCTC-310-ONCO for 30 days

Group	Day	Weight (g)	Haemoglobin (g/dl)	Leukocytes (cells per mm^3)	Neutrophils (%)	Lymphocytes (%)
VRCTC-310-ONCO	0	3975 ± 775	12.28 ± 0.26	6760 ± 655	38.20 ± 6.92	58.60 ± 6.65
	9	4000 ± 700	11.25 ± 0.40	8817 ± 464	19.33 ± 1.33	76.83 ± 1.08
	17	4000 ± 700	10.19 ± 0.35	9543 ± 592	20.43 ± 0.43	75.57 ± 0.57
	30	4125 ± 825	10.30 ± 0.35	10157 ± 590	20 ± 0.62	76.57 ± 0.72
Control	0	3157 ± 187	12.58 ± 0.25	7343 ± 598	37 ± 6	60 ± 6
	9	3164 ± 180	11.95 ± 0.15	9150 ± 50	20 ± 0	77 ± 1
	17	3214 ± 193	11.20 ± 0.20	8800 ± 400	18 ± 0	79 ± 1
	30	3329 ± 172	10.80 ± 0.80	8400 ± 900	19 ± 0.50	75 ± 1

lute count) was found in all animals, including controls. A proportional decrease in neutrophils was found in both treated and control rabbits, with no difference among groups.

There were no significant differences between basal and under-treatment values of serum transaminases (GOT and GPT), cholesterol, triglycerides, and γ-glutamyl transpeptidase (data not shown).

3.3. Necropsy

Rabbits were sacrificed after 30 days of treatment and subjected to macroscopic examination and histopathologic study. Chronic granulation tissue was found surrounding the implant in all rabbits. VRCTC-310-ONCO-treated rabbits presented generalised and marked swelling of hepatocytes, with areas of cytoplasmic vacuolisation. No abnormalities were found in kidney, heart, lung, spleen, adrenal gland, uterus, testes and ovary.

4. Discussion

In this study, the feasibility of IV administration of VRCTC-310-ONCO has been demonstrated in rabbits. A limited number of studies have addressed the use of fixed catheters for IV infusion in rabbits, which seems to be a useful tool to reduce acute stress and the risk of accidents during handling of the animals. This approach has already been used in rabbits, with satisfactory results, allowing IV administration of chemicals for long periods [6–8].

As in those studies, surgical implantation of the catheter proved to be a useful approach to VRCTC-310-ONCO study. IV administration of VRCTC-310-ONCO at the maximum tolerated dose for human (when administered by intramuscular route) was safe for most rabbits: only one of them died and two required dose-reduction.

For most antineoplastic agents, bone marrow toxicity is the most common dose-limiting factor [9]. No toxicity for bone marrow was found in the rabbits treated with VRCTC-310-ONCO, which is in agreement with previous reports [4] and, if confirmed in clinical trials, may become an interesting property. At baseline, hematological values were normal and agreed with published reference figures [10]. Early changes (decrease of haemoglobin and lymphocytosis with parallel decrease of neutrophils at the 7th day) probably reflected blood loss and chronic adaptation to the implant, since both were also present in control rabbits.

The two rabbits that required dose-reduction presented neurotoxicity. The main active principle in VRCTC-310-ONCO is crotoxin, a neurotoxic phospholipase A_2. Previous studies have determined crotoxin LD_{50} to be 0.8 mg/kg b.w. by acute IP injection [11], death resulting from acute neuromuscular dysfunction [12]. The finding of CNS toxicity is also consistent with previous reports in rats [13]. The product used herein (the association crotoxin + cardiotoxin) has previously shown to decrease neurotoxicity [2]. The LD_{50} of this mixture in Sprague–Dawley rats, via intramuscular administration, is 43 ± 6 mg/kg b.w., which implies a decrease in toxicity of CT of about 20-fold (Onco-Venom Research, unpublished data). If VRCTC-310-ONCO is to be studied by IV injection in humans, neurotoxicity will have to be closely monitored, since it may become a relevant side effect.

In addition to clinical and laboratory evaluation, necropsy was performed in all animals. Only abnormalities restricted to the liver were found in the microscopic examination of the tissues. This finding is consistent with the previous report that, in vitro, crotoxin induces several alterations on rat liver mitochondria, including swelling [14]. Additional studies in humans are required to determine whether this toxic effect has clinical implications

These results, the partial elucidation of its mechanism of action [15] and the encouraging findings after intralesional administration [16] suggest that IV route deserves further study, including formal phase I in humans.

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References

[1] R.E. Corin, L.J. Viskatis, J.C. Vidal, M.A. Etcheverry, Cytotoxicity of crotoxin on murine erythroleukemia cells in vitro, Investigational New Drugs 11 (1993) 11–15.
[2] R.A. Newman, J.C. Vidal, L.J. Viskatis, J. Johnson, M.A. Etcheverry, VRCTC-310—a novel compound of purified animal toxins separates antitumor efficacy from neurotoxicity, Investigational New Drugs 11 (1993) 151–159.
[3] A. Chisari, E. Spinedi, M.J. Voirol, A. Giovambattista, R.C. Gaillard, A phospholipase A2-related snake venom (from *Crotalus durissus terrificus*) stimulates neuroendocrine and immune functions: determination of different sites of action, Endocrinology 139 (1998) 617–625.
[4] L.J. DeTolla, K.C. Stump, R. Russell, L.J. Viskatis, J.C. Vidal, R.A. Newman, M.A. Etcheverry, Toxicity of the novel animal-derived anticancer agent, VRCTC-310: acute and subchronic studies in beagle dogs, Toxicology 99 (1995) 31–46.
[5] L.A. Costa, H.A. Miles, R.A. Diez, C.E. Araujo, C.M. Coni Molina, J.C. Cervellino, Phase I study of VRCTC-310, a purified phospholipase A2 purified from snake venom, in patients with

refractory cancer: safety and pharmacokinetic data, Anti-Cancer Drugs 8 (1997) 829–834.
[6] J.R. Hageman, K. McCulloch, P. Gora, E.K. Olsen, L. Pachman, C.E. Hunt, Intralipid alterations in pulmonary prostaglandin metabolism and gas exchange, Critical Care Medicine 11 (1983) 794–798.
[7] L.M. Perry-Clark, L.D. Meunier, Vascular access ports for chronic serial infusion and blood sampling in New Zealand white rabbits, Laboratory Animal Sciences 41 (1991) 495–497.
[8] D. Zovko, S. Loff, A. Dzakovic, B. Kranzlin, H.P. Hohl, S. Grun, K.L. Waag, Long-term total parenteral nutrition and cholecystostomy tube in a rabbit model surgical procedure: management and preliminary results, Research in Experimental Medicine (Berlin) 196 (1996) 235–242.
[9] P. Calabresi, B.A. Chabner, Chemotherapy of neoplastic diseases. Introduction, in: J.G. Hardman, L.E. Linbird, P.B. Molinoff, R.W. Ruddon, A. Goodman Gilman (Eds.), Goodman and Gilmans The Pharmacological Basis of Therapeutics, Ninth ed., McGraw-Hill, New York, 1996, pp. 1225–1232.
[10] S.T. Wolford, R.A. Schroer, F.X. Gohs, P.P. Gallo, M. Brodeck, H.B. Falk, R. Ruhren, Reference range data base for serum chemistry and hematology values in laboratory animals, Journal of Toxicology and Environmental Health 18 (1986) 161–188.
[11] O. Vital Brazil, J.P. Franceschi, E. Waisbich, Pharmacology of crystalline crotoxin. I. Toxicity, Memorias do Instituto Butantan 33 (1966) 973–980.
[12] O. Vital Brazil, Pharmacology of crystalline crotoxin. II. Neuromuscular blocking action, Memorias do Instituto Butantan 33 (1966) 981–992.
[13] E.G. Moreira, N. Nascimento, G.J. Rosa, J.R. Rogero, V.S. Vassilieff, Crotoxin-induced behavioral effects in rats, Brazilian Journal of Medical and Biological Research 29 (1996) 629–632.
[14] R.H. Valente, J.C. Novello, S. Marangoni, B. Oliveira, L. Pereira-da-Silva, D.V. Macedo, Mitochondrial swelling and oxygen consumption during respiratory state 4 induced by phospholipase A2 isoforms isolated from the South American rattlesnake (*Crotalus durissus terrificus*) venom, Toxicon 36 (1998) 901–913.
[15] N.J. Donato, C.A. Martin, M. Pérez, R.A. Newman, J.C. Vidal, M. Etcheverry, Regulation of epidermal growth factor receptor activity by crotoxin, a snake venom phospholipase A2 toxin. A novel growth inhibitory mechanism, Biochemical Pharmacology 51 (1996) 1535–1543.
[16] L.A. Costa, H. Miles, C.E. Araujo, S. González, V.G. Villarrubia, Tumor regression of advanced carcinomas following intra- and/or peri-tumoral inoculation with VRCTC-310 in humans: preliminary report of two cases, Immunopharmacology and Immunotoxicology 20 (1998) 15–25.