CROTOXIN- crotalus durissus terrificus venom injection, solution
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Disclaimer: This homeopathic product has not been evaluated by the Food and Drug Administration for safety or efficacy. FDA is not aware of scientific evidence to support homeopathy as effective.

DESCRIPTION

Crotoxin preparations are made from the venom of the South Amercian rattlesnake, *Crotalus durissus.* They are homeopathic formulations that include sterile injectables for intravenous and subcutaneous use.

Active ingredient: Crotoxin.

INDICATIONS

According to Clarkes's Materia Medica, Crotalus venom preparations are indicated as homeopathic medications for; Amblyopia. Apoplexy. Appendicitis. Bilious fever. Boils. Cancers. Carbuncles. Cerebro-spinal meningitis. Chancre. Ciliary neuralgia. Convulsions. Delirium tremens. Dementia. Diphtheria. Dysmenorrhoea. Dyspepsia. Ears, discharges from. Ecchymosis. Epilepsy. Eyes, affections of. Erysipelas, Face, eruption on ; distortion of. Haematuria. Hemorrhagic diathesis. Headache. Heart, affections of. Herpes. Hydrophobia. Intestinal haemorrhage. Jaundice. Keratitis. Liver, disorders of. Lungs, affections of, Mastitis. Measles. Milk-leg. Meningitis. Ovaries, affections of. Ozaena, Palpitation. Peritonitis. Perityphlitis. Phlebitis. Psoriasis palmaris. Purpura. Pyaemia. Remittent fever. Rheumatism. Scarlatina. Sleeplessness. Small-Pox. Stings. Sunstroke. Syphilis. Tetanus. Thirst. Tongue, inflammation of ; cancer of. Ulcers. Urticaria. Vaccination, effects of. Varicosis. Varicocele. Vomiting, bilious. White-leg. Whooping-cough. Yellow Fever.

Clinical experience shows that Crotoxin also provide relief from some forms of pain.

PHARMACOLOGIC CATEGORY

Anti-cancer.

PHARMACOLOGY

The principal active components in the venoms are cytotoxins. Crotoxin (CT) is a pre-synaptic bi-partite beta-neurotoxin with phospholipase A2 (PLA2) activity. Evaluation by the Developmental Therapeutics Program of the National Cancer Institute (NSC 624244) for cytotoxicity in vitro against a panel of human tumour cell lines showed enhanced cytotoxicity towards melanoma, CNS and non-small cell lung cancer lines. Toxin-phospholipid interaction and subsequent accumulation of products of phospholipid hydrolysis in the membrane may alter membrane packing, disrupt lipid domains and affect protein conformation resulting in effects like inhibition of type II Ca^{2+} channels or alteration of transmembrane signaling pathways. Current thought suggests that CT binds to the upregulated Crocalbin that is upregulated in malignant cells. The Nicotinic Acetylcholine receptor is also upregulated in tumor cell lines and have been clearly identified as having a role in proliferation especially in lung cancer and CNS tumors – tumor populations with high sensitivity to CT. The released PLA2 produces arachidonic acid at the membrane surface that activates protein kinase C (PKC) and possibly other tyrosine kinases. PKC in turn phosphorylates endogenous caspases and the activated caspases initiate the process of programmed cell death.

PHARMACODYNAMICS

Studies in animal models were performed using murine and human tumour cell lines. The responses are summarized in Table 1.

Table 1: Activity of Crotoxin in animal models

Tumour or inoculum	Implant or innoculum	Host	GI (%)	ILS (%)	CR (%)
MET-A [a]	1 x 106 cells, i.p.	BDF1mice	-	33	-
	1 x 105 cells, i.p.	BDF1mice	-	indefinite	>90
B-16 melanoma	2 x 106 cells, i.p.	C-57 B1 mice	-	25	-
	2 x 105 cells, i.p.	C-57 B1 mice	-	62	-
Lewis lung carcinoma[b]	fragments	BDF1mice	83	-	12
Walker 256 carcinosarcoma [c]	1 x 106 cells, i.p.	Sprague-Dawley rats	-	67	20
MX-1 (human ductal carcinoma)[d]	fragments	SCID mice	68	-	-
	fragments	Nude rats	89	-	-
EO-1 (human oligodendroglioma)[a]	2 x 106 cells, s.c.	SCID mice	65	-	-
	1 x 106 cells, s.c.	SCID mice	75	-	-
	1 x 105 cells, i.c.	SCID mice	-	40	-

GI: growth inhibition, ILS; increased life span, CR; complete response

Prior data showed that CT-induced cytotoxic effects appeared to be highly selective toward cell lines expressing an upregulated density of Epidermal-like Growth Factor receptors, though CT appeared to unexpectedly promote EGFR phosphorylation. Enhanced EGFR activity in cancer cells and tumors is associated with increased growth, survival and angiogenesis of tumors. CT stimulated phosphorylation of the EGFR is a cellular response to the induction of apoptosis.

Secondary Pharmacology

CT belongs to the group of the beta-toxins, characterized by the presence of phospholipase A_2 activity associated with at least one subunit and its high specific toxicity towards neuromuscular transmission. It can produce flaccid paralysis and death due to paralysis of respiratory muscles. Artificial respiration can keep the animals alive and, provided that the dose is not higher than 1.5 LD_{50}, is followed by recovery.

A consistent report of pain relief in patients being treated with CT led to a study to investigate analgesia induced by CT and the effects of atropine and Naloxone on antinociceptive actions of CT in mice and rats. The results showed that CT administered by parenteral injection exhibited a dose-dependent analgesic action in mice using hot plate test and acetic acid-writhing test. The peak effect of CT analgesia was seen 3 h after its' administration (in contrast to its pharmacokinetics). CT had significant analgesic action in rat tail-flick test. In the mouse acetic acid-writhing test, intra-cerebral ventricle administration of CT also produced marked analgesic effects. Atropine at 0.5 mg/kg (im) or 10 mg/kg (ip) or Naloxone at 3 mg/kg (ip) failed to block the analgesic effects of Crotoxin. In animal models of neuropathic pain induced by rat sciatic nerve transaction, it was revealed that CT has prolonged activity persisting for up to 64 days. It was found that the analgesia was mediated by activation of central muscarinic receptors and partially, by activation of alpha-adrenoceptors and 5-HT receptors.

An important and attractive characteristic of CT in addition to being of considerable importance in CT's clinical and therapeutic application is the ability of the host to become resistant to its neurotoxic activity. Mice injected daily with progressively increasing doses of CT develop tolerance to the lethal action of this toxin. Treated mice (LD_{50} = 0.09mg/Kg) tolerated daily doses of CT 20- to 35-fold higher than the original LD_{50} without the characteristic signs of toxicity.

CONTRAINDICATIONS

Crotoxin is intended to be used as a monotherapy. Clinical experience suggests that concurrent use of radio- or chemotherapy may inhibit the activity of the drug. As the known receptors include those sensitive to nicotine is it is likely that nicotine will inhibit the activity of Crotoxin.

PRECAUTIONS

Dose escalation is an important part of the treatment regime in order to avoid adverse events.

Pregnancy: No significant data has been collected on the use of Crotoxin during pregnancy. No animal reproduction studies have been conducted to assess the effects of Crotoxin on the developing fetus.

Nursing mothers: It is not known whether this drug is distributed into breast milk. Crotoxin is not orally bioavailable therefore it is unlikely to have any detrimental effects

Pediatric use: There is no information on the use of Crotoxin in children and young adults.

INTERACTIONS

No detailed reports of drug interactions have been reported.

ADVERSE REACTIONS

The majority of reported adverse effects to Crotoxin are associated with the neurotoxic components of the venom.

In clinical studies with Crotoxin, subjects reported the following dose related adverse effects; anxiety, increased blood pressure, diplopia, nystagmus and strabismus.

SERIOUS ADVERSE REACTIONS

With the injection of Crotoxin, allergic reactions, sometimes severe (anaphylactic), have been reported. Anaphylaxis is manifest usually within 30 minutes of administration. The use of antihistamines was found to alleviate the allergic reaction. Co-administration of antihistamines is advised.

FORMATS

CT is supplied in a solution of 0.9% saline for injection, at a concentration of 0.4mg/ml for injection with 0.01% benzalkonium chloride as a preservative. Dilution of this solution with saline for injection (SFI) can be useful for the initiation of therapy by subcutaneous injection in addition to tolerising the patient and their immune system to the drug. By intravenous (i.v.) administration the drug is diluted for administration thought a drip. Always follow your health practitioner's instructions.

DOSAGE AND ADMINISTRATION

The maximum reported tolerable dose of Crotoxin by intramuscular bolus injection is 0.18 mg/m^2. Injection site reactions are common though will subside in 2-3 weeks with continued use. With dose escalation procedures dose of 0.5 mg/m^2 have been attained.

A maximum tolerable dose for Crotoxin by intravenous administration when combined with a dose escalation procedure has not been determined. Doses of 0.32 mg/m^2 have been attained without serious adverse effects. Bolus or infusion processes may be employed with dose escalation procedures.

STORAGE

Crotoxin should be stored refrigerated (2-10°C / 38-50 °F).

STABILITY

For maximum shelf life, the product should be stored at 4°C when not in use though shipping at ambient temperatures (10-30 °C) is not expected to significantly affect the product. Crotoxin, stored as directed, is potent for a period in excess of 60 months.

SAFETY INFORMATION

Neurological: CT exerts analgesic activity presumed to be in the CNS despite the suggestion in pharmacokinetics studies that CT does not accumulate in the brain. Intracerebroventricular administration of CT to mice and rats yielded analgesic responses and was not toxic to the exposed nerve cells at the doses tested. In other studies CT appeared to increase the anxiety level in rodents. It is assumed that CT exerts these effects through peripheral pathways.

Cardiovascular: CT has no direct effects on the heart *in-vivo*. Rat hearts showed no response to the

presence of CT though it is the most resistant species to this neurotoxin. In Guinea pigs, which have a high sensitivity to CT, no effect was noted in the heart unless protein was removed from the perfusion solution. At this point the contractile force of the heart was reduced by approximately 25%, reported to be a consequence of non-specific binding that occurred in the absence of albumin. No impact (NOEL) on heartbeat was noted at doses of 0.25mg/kg. Studies on the cardiovascular effects in anaesthetized dogs demonstrated that CT induced a transient and minor reduction (20%) in blood pressure on administration. With the administration of sequential doses of CT, the animal became resistant to the vascular effects of CT.

Respiration: CT is characterized by its ability to induce respiratory paralysis at high doses through blockade at the neuromuscular junction of the phrenic nerve. However, no respiratory effects were observed with the use of CT at 0.25 mg/Kg in dogs (maximum human dose is <0.015 mg/Kg). This would demonstrate a clear dose relationship between a NOEL and overt respiratory failure. In cases where respiratory paralysis was induced, the animal's survival could be assured with the use of artificial ventilation. Crotoxin's direct effects on the phrenic diaphragm has been well-established and provided the mechanism by which crotoxin interferes with respiration. Furthermore, it has been established that resistance to the toxic effects of Crotoxin are located at this site and resistance can be induced using low doses of Crotoxin.

Renal: The effects of Crotalus durissus terrificus venom and CT were studied on glomerular filtration rate (GFR), urinary flow (UF), perfusion pressure (PP) and percentage sodium tubular transport (%TNa+). The infusion of Crotalus durissus terrificus venom (10 microg/ml) and CT (10 microg/ml) increased GFR (control = 0.78 +/- 0.07, venom = 1.1 +/- 0.07, CT = 2.0 +/- 0.05 ml g(-1) min(-1), P<0.05) and UF (control = 0.20 +/- 0.02, venom = 0.32 +/- 0.03, CT = 0.70 +/- 0.05 ml g(-1) min(-1), P<0.05), and decreased %TNa+ (control = 75.0 +/- 2.3, venom = 62.9 +/- 1.0, CT = 69.0 +/- 1.0 ml g(-1) min(-1), P<0.05). The infusion of crude venom tended to reduce PP, although the effect was not significant, whereas with CT PP remained stable during the 100 min of perfusion. The kidneys perfused with crude venom and CT showed abundant protein material in the urinary space and tubules. It was concluded that Crotalus durissus terrificus venom and CT, its major component, caused acute nephrotoxicity in the isolated rat kidney. The current experiments demonstrated a direct effect of venom and CT on the perfused isolated kidney.
All in-vivo pharmacological studies have shown the toxic effects of CT to be reversible.

Acute effects of CT in Primates

The i.v. motor effects of CT were also assessed in *Cebus* monkeys. Similar effects of CT intoxication (0.1mg/Kg, i.v.) were noted in monkeys as in dogs with the additional observation of ptosis of the eyelids and of the jaw, paralysis of the neck muscles in addition to the generalized loss of muscle strength. They also became aphonic, could not swallow and presented diaphragmatic respiration. Consciousness was preserved as in dogs.

Chronic rodent toxicity studies with Crotoxin

Intravenous studies: The aim of the study was to evaluate the toxicity of Crotoxin after repeated intravenous administration in the mouse. CT was administered intravenously at a dose of either 0.015, 0.03 or 0.04 mg/kg for a duration of 29 days. Ten (10) males and ten female mice (CD-1) were used per dose. There was no mortality during the study. Varying degrees of flaccid paralysis in the rear limbs and occasionally in the front limbs were observed in the 0.04 mg/kg/day group only. Symptoms disappeared by the time mice had to receive the next injection. No toxicity signs were observed in other dose groups. Activity levels were normal. Moderate dyspnea pareses and weight loss (10%) were observed for the first week. Animals injected with 0.03 mg/kg showed only a weight loss of 5% during the first 3 days and no changes were found in animals injected with 0.015 mg/kg (NOEL). No changes were detected in hematologic or clinical chemistry parameters. Gross or microscopic pathology examinations showed no organ abnormalities. It was concluded that doses of 0.015 and 0.03 mg/kg showed no drug-related signs. Doses of 0.04 mg/kg produced findings of flaccid paralysis, which subsided within 24 hours. These symptoms were only observed during the first two weeks of the study.

Intramuscular studies: Mice injected daily with CT at 0.075 mg/kg via intramuscular for 30 days did not show signs of toxicity, except for a weight loss of less than 3% in the first week. Pathology studies showed several degrees of myotoxic degeneration at the sites of injection accompanied by basophilic myotubules, indicating regeneration. Full regeneration of myotoxic lesions occurred within 3 weeks.

Studies on the chronic toxicity of CT usually showed that the typical clinical signs of neurotoxicity, as well as pathologic and biochemical evidences associated to CT induced myotoxic effects in mice tend to decrease and/or disappear during the second or third week of treatment.

Pharmacokinetics

Studies on organ distribution of ^{125}I-CT show that 1h after the intravenous injection into mice only 5% remains in plasma, while major amounts are found in liver (16%), kidneys (8.26%) and the carcass (48%). About 9% is excreted with the urine in three hours. Two hours after injection the liver contain 12% of the amount injected, kidney 3% and the carcass 22%. After 26 h only 12% of the radioactivity injected remains, mainly in liver.

Disposition of CT administered i.v. at the doses of 0.045 and 0.06 mg/kg was also followed by double-sandwich ELISA amplified by avidin-peroxidase. Plasma concentrations of CT fell rapidly in the first hour and then slowly up to a level below the detection limit of the assay 18h after injection. Disposition was consistent with a two-compartment open model with half-lives of 6.4 ± 0.4 min for the α-phase and 4.8-5.2 h for the β-phase. The AUC was 12 ± 1.9 $\mu g.min.ml^{-1}$.

Following the intramuscular injection of CT at 0.1 mg/kg, there is a rapid absorption of toxin from the injection site, reaching a peak (50-60 ng/ml) 10-15 min after injection. The half-lives of the α and β phases were 23 ± 4 min and 4.7 ± 0.3 h, respectively. However, the AUC was 7.0 ±1.0 $\mu g\ min.ml^{-1}$, i.e., smaller than that obtained for i.v. injection of a smaller dose. This may indicate that absorption from the injection site is rapid but incomplete, the toxin bound to muscular tissue being slowly released.

Serum kinetics of CT were investigated in BALB/c mice after a single 10 micrograms s.c. dose of venom obtained from Crotalus durissus terrificus. CT levels were 254 +/- 141 ng/ml serum (X +/- S.E.) 15 min after venom injection, 3.9 +/- 0.5 ng/ml serum at 30 min and undetectable thereafter.

Reproduction and Teratogenicity

The effects of Crotoxin in reproduction have notyet been completed. As Crotoxin is intended to treat cancer it would suggest that the drug could adversely affect rapidly dividing cells and active contraception should be practiced with CT's use.

Local Tolerance

CT causes injection site reactions when injected s.c. or i.m. due to its myotoxic action. In humans, these reactions subside with repeated administration over the course of 2-3 weeks.

CLINICAL EXPERIENCE

Pure CT and CT/Cardiotoxin formulations have been through 6 clinical investigations. The results of five of these studies have been published in scientific journals. The results from each condensed into table 1 and briefly summarized below. While CT is the principle active agent of interest the drug has been employed alone and in combination with Cardiotoxin, there is no strong evidence that Cardiotoxin has such activity *in-vivo* despite its' published and unpublished potent anti-tumour effect in-vitro. With that said, both products have been employed in human Phase I studies as listed in Table 2 and 3.

Table 2: Human Clinical Oncology Studies.

Product	Indication	No. of patients	Duration	Route	Drug ADR
CT plus Cardiotoxin	Cancer	15	30 days	i.m.	2 anaphylactic reactions
CT plus Cardiotoxin	Cancer	5	8 weeks	i.t. & p.t.	Not available
CT	Cancer	23	30 days, longest 117 days	i.m.	1 anaphylactic reaction
CT	Cancer	6	Longest 75 days	i.v.	None reported
CT	Cancer	6	54 days	i.v.	grade 1 to 2 drug-related events of anorexia, diplopia and

					nystagmus.
CT	Cancer	6	35 days	i.v.	None reported

i.m.; intramuscular, i.t.: intratumour, p.t.; peri-tumour, i.v.; intravenous

A phase I study was performed to evaluate the maximum tolerated dose (MTD), safety profile and pharmacokinetic data with CT plus Cardiotoxin (Costa et al., 1997). Fifteen patients with refractory malignancies were entered after providing written informed consent. CT plus Cardiotoxin 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 drug-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.

Table 3: Cancer types observed in treated Subjects and disease response.

Reference	Product	Dose	Cancer types involved
Plata et al. 9 patients	CT	0.3mg/ m^2 (0.009mg/Kg) i.m & i.t Daily	Breast (2 **CR**), Pancreas (2 PR), liposarcoma (1 PR), Mesothelioma (1 PR), Fusocellular sarcoma (1 PR), Glioma (1 PR), Orbital adenocarcinoma (1 PR)
Costa et al. 1997 15 patients	Crotoxin + Cardiotoxin	0.0025-0.023 mg/Kg i.m. Daily	NSCLC (1 PR, 1 PD), Breast (2 SD, 1PD), Laryngeal (1 SD), Cervical (1 SD, 1 PD), Ovarian (1 SD), Stomach (PD), Pancreas (1 PD), Gall bladder (1 PD), Colon (1 PD), Rectum (1 SD), Maxilla (1 PD)
Costa et al. 1998 5 patients	Crotoxin + Cardiotoxin	0.014mg/Kg i.m. Weekly	Breast (2 **CR**, 1PR), Squamous cell (1 **CR**), Chordoma (1 PR)
Hawkins 6 patients	CT	0.03mg/m^2 (0.0009mg/Kg) i.m. Weekly	Colon cancer (4 PD), Pancreatic (2 PD)
Cura et al. 23 patients	CT	0.03-0.22 mg/m^2 (0.0009- 0.007mg/Kg) i.m. Daily	Breast (1 **CR**, 2 PD), Rectal (1 PR), larynx (1 PR), Thyroid (1 PR), Fibrosarcoma (1 SD), Bladder (1 PD), Cervix (2 PD), Gastrointestinal (6 PD), NSCLC (3 PD), Head & Neck (1 PD), Fallopian (x1), Ewing sarcoma (1 PD), Liposarcoma (1 PD)
Medioni et al 6 patients	CT	0.04-0.32 mg/m^2 i.v. over 2 hr, 5 of 7 days per week.	Colorectal Lieberkuhnian adenocarcinoma (PD), epithelioid mesothelioma (PD), non-small-cell lung cancer (PD), carcinoma of the unknown primary site (PD), invasive ductal carcinoma (PD) and ovarian papillary adenocarcinoma (PD).
Delgado et al 6 patients	CT	0.04-0.32 mg/m^2 i.v. pump, 7 days per week.	Nasal squamous cell carcinoma (SD), glioblastomas (PD), endometrial adenocarcinoma (PD), NSCLC (PD) and prostatic carcinoma (SD)
TOTAL	70 subjects		7 SD, 13 PR, 8 CR

From December 1996 to August 1997, five patients with histologic confirmed local advanced cancer (breast cancer 3, squamous cell cancer of the hand 1, chordoma 1) were treated at Bernardo Houssay Hospital, Oncology Unit with CT plus cardiotoxin, at the full dose of 0.014 mg/kg once a week, inoculated peritumorally and distributed into four different injections around the tumor, for no less than eight courses. All patients gave written informed consent according to local ethics committee requirements (Costa et al., 1998).

A complete response was observed in 3 pts (breast cancer 2, skin carcinoma of the hand 1), and a partial response was registered for the other two pts. The patient with local-advanced breast cancer (carcinoma en cuirasse) who was inoculated intra-and-peritumoral with CT plus Cardiotoxin for 6 weekly courses (0.014 mg/kg/week) with the drug had a >80% reduction in tumor. A 133 days follow-up demonstrated not only an objective complete response of the primary tumor mass, but the disappearance of supraclavicular tumor mass as well a significant reduction in lymphangitis.

No toxicities were observed, except for a mild local pain at the site of the injection. It was concluded that weekly CT plus cardiotoxin given by subcutaneous, peritumoral route is an active treatment for advanced skin metastatic tumors that is well tolerated and safe.

Phase I clinical trials of CT (Cura et al., 2002) was performed at the Hospital “General San Martìn” (Universidad Nacional de Rosario), Paranà, Entre Rìos. According to the protocol approved by the National Administration of Foods, Medicines and Medical Technology (A.N.M.A.T, Argentina), twenty six patients with solid tumours refractory to conventional therapy were admitted after signing a written informed consent. Twenty three patients were evaluated after the administration of CT as a daily intramuscular injection for 30 consecutive days (1-3 cycles) at doses from 0.03 to 0.22 $mg.m^{-2}$. No drug-related deaths occurred in this study. Reversible (non-limiting) neuromuscular toxicity (Grades I to II) with diplopia and palpebral ptosis resulting from self-limited paresis of the external ocular muscles due to neuromuscular toxicity was the most characteristic side effect observed starting at the dose of 0.18 mg/m^2.

Strabysmus and/or nystagmus appeared at 0.22 mg/m^2. Patients complained by the discomfort during nystagmus episodes and no further increase in dose were considered. At the doses employed, neuromuscular toxicity did not impair the function of intrinsic ocular musculature and never extended to other muscular groups. No impairment of pharyngeal or laryngeal muscles was observed. Dysphonia, dysphagia, disartria or changes in FVC, suggesting drug related involvement of respiratory muscles were consistently absent (Cura et al., 2002). Neuromuscular toxicity did not require any dose adjustment and disappeared after one to three weeks even continuing the treatment. Except for transient increases (Grades I-III) in the levels of aminotransferases and creatinine kinase attributable to CT myotoxicity (Table III.1.3.1.5.2d), no other drug-related limiting toxicities were observed.

A Phase I clinical study was recently completed by the Sponsor at the George Pompidou Hospital (Paris, France) under EudraCT: 2009-010622-19. Titled as an “Open Label Phase I Clinical Trial of Crotoxin in Patients with Advanced Cancer using an Intravenous Route of Administration,” designed to be conducted in two cohorts, cohort 1 was conducted with six patients (one male and five females) with advanced solid tumours and no further therapeutic options. The primary objectives of the cohort 1 study was to; 1. Confirm that human subjects can be made tolerant to i.v. CT; 2. Assess the safety and tolerability of CT administered i.v. to Stage IV cancer patients using intra-patient dose escalation procedure; 3.Define Maximum Tolerated Dose (MTD) associated with intra-patient dose escalation. The secondary objectives of the study were to determine the frequency and titer of anti-CT antibodies, document any objective anti-tumour responses and assess if CT could affect analgesic activity: evaluated using questionnaire filled by patients during daily infusions.

Cohort dose escalation was planned once per week, with weekends off. Crotoxin was administered to subjects as out-patients daily by i.v. administration at the hospital over a 2-hour period by saline drip (Medioni et al, 2017). Patients received Polaramine 10 mg i.v. (antihistamine) before CT administration and Ranitidine 50mg (anti-emetic) intravenously prior to treatment to minimize the potential for anaphylaxis. The escalation schedule extended o0ver 54 days, with dose increased from 0.04 to 0.32 mg/m^2. The patients were monitored daily at the clinic for the duration of the infusion (2 hours) and observed for 30 min following the infusion for adverse reactions. A total of 15 patients were screened and 6 were enrolled. Patient diagnosis and dose levels is summarized in Table 4.

Table 4. Patient diagnosis and dose levels in Cohort 1

Patient number	Diagnosis	Last dose received	Day last dose received
001	COLORECTAL LIEBERKUHNIEN ADENOCARCINOMA diagnosed 17Jul2007	Dose 8: 0.32 mg/m^2/day (2 days)	58
002	EPITHELIOID MESOTHELIOMA diagnosed 4 Jul2008	Dose 8: 0.32 mg/m^2/day (3 days)	53
003	NON-SMALL-CELL LUNG CANCER diagnosed 23Apr2004	Dose 8: 0.32 mg/m^2/day (5 days)	54
004	CARCINOMA OF UNKNOWN PRIMARY SITE diagnosed 16Nov2010	Dose 7: 0.28 mg/m^2/day (4 days)	46
005	INVASIVE DUCTAL CARCINOMA diagnosed 20Nov2009	Dose 5: 0.20 mg/m^2/day (4 days)	33

006	OVARIAN PAPILLARY ADENOCARCINOMA diagnosed 21Nov2006	Dose 7: 0.28 mg/m^2/day (7 days)	54

Patients were treated with 8 cycles of Crotoxin administered i.v. over 2h daily, 5 days out of 7, with weekly intra-patient dose escalation. Dose escalation started at 0.04 mg/m^2/day and the last dose was 0.32 mg/m^2/day. Dose escalation extended over a period of 54 days (8 weeks).

For Serious Adverse Events (SAEs) unrelated to the study drug there were none in Patient 001, Patient 003 & Patient 004 (Medioni et al., 2018). Patient 002 experienced Anemia at dose level 6 (0.24 mg/m^2/day) at day 37, grade 3, unrelated, reported as SAE, not reported as (Sudden Unexpected Serious Adverse Reaction (SUSAR), study drug was interrupted for 1 day. Patient 005 experience Hypoxia at dose level 5 (0.20 mg/m^2/day) at day 29, grade 3, unrelated, reported as SAE, not reported as SUSAR. The study drug dose was not changed though treatment and hospitalization was required. Also, Sepsis grade 4 and confusion grade 2 at dose level 5 (0.20 mg/m^2/day) at day 35, unrelated, reported as SAE, not reported as SUSAR, patient died 2 days after stopping the study. Patient 006 also experienced Hypoxia at dose level at dose level 3 (0.12 mg/m^2/day) at day 17, grade 2, unrelated, reported as SAE, not reported as SUSAR study drug dose not changed though treatment and hospitalization was required. Fever was reported at dose level 6 (0.24 mg/m^2/day) at day 43, grade 1, unrelated, reported as SAE, not reported as SUSAR, study drug dose was interrupted for 1 day requiring treatment and hospitalization.

Table 5. Summary of Cohort 1 Adverse Event during Study

Subject no	Unrelated to study drug		Related to study drug		Total
	AE	SAE	AE	SAE	
001	9	0	4	0	13
002	11	1	0	0	12
003	8	0	0	0	8
004	25	0	1	0	26
005	22	3	0	0	25
006	15	2	1	0	18

There were no reported SAEs or SUSARs related to the study drug (Table 5). Adverse Events (AEs) related to the study drug were not reported in Subjects 002, 003 & 005. Diplopia and nystagmus were the most common. For those patients for whom pain was reported appeared to experience relief when CT was administered. The analgesic effect in all subjects appeared to dissipate during the weekends when no drug was being administered. The reported analgesic activity of Crotoxin in the Cura et al study (2002) was confirmed. All six patients presented Progressive Disease potentially due to the slow dose escalation protocol. It was established that intra-patient dose escalation of Crotoxin was safer than simple bolus administration.

Part 2 of the Phase I study was recently completed at the University Hospital Pitié-Salpêtrière (Paris, France). It followed design of Medioni et al (2017) in intra-patient dose escalation study to treat six patients (5 males and 1 female) with advanced solid tumours and no further treatment options: 1 nasal squamous cell carcinoma, 2 glioblastomas, 1 endometrial adenocarcinoma, 1 NSCLC and 1 prostatic carcinoma (Gil-Delgado et al., 2018). Lightweight Rythmic™ pump capable of continuous delivery (no weekend breaks) allowed patients to stay at home. Dose escalation from 0.08 to 0.64 mg/m^2/day over 35 days and was carried on Mondays, Wednesdays and Fridays, followed by 2h observation at the clinic. Two of 6 patients developed possibly drug-related G1 diplopia and 1/6 pts increased ASAT/ALAT. One patient recruited with pre-existent diarrhoea syndrome with uncontrolled G2 hypomagnesaemia, G3 hypokalaemia and G2 anaemia, developed complete arrhythmia with asymptomatic atrial fibrillation that resolved with amiodarone. Patient was hospitalised for observation and the event was classified as a possible study drug-related SAE as well as related to the digestive syndrome and tubulopathy resulting from previous chemotherapy (nivolumab and platinum salts). It was concluded CRTX dose escalation is safe but too slow, doses achieved too low and too late for advanced pts. However, stable disease was observed in 2/6 patients and no DLT or MTD were reached.

Overall, of the 70 subjects recorded as having been treated with CT or CT plus Cardiotoxin; 8 have had complete remissions, 13 had partial responses and 8 had stable disease. These outcomes were achieved

in the absence of optimized treatment programs.

CLINICAL REFERENCES

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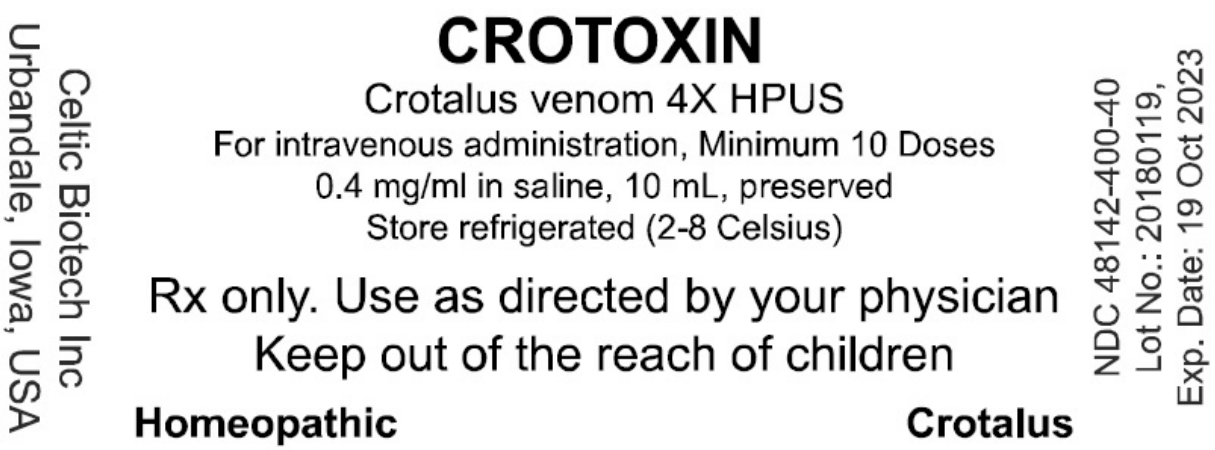
PRINCIPAL DISPLAY PANEL - NDC: 48142-400-40 - 10 mL Vial Label



CROTOXIN

crotalus durissus terrificus venom injection, solution

Product Information			
Product Type	HUMAN PRESCRIPTION DRUG	**Item Code (Source)**	NDC:48142-400
Route of Administration	INTRAVENOUS		

Active Ingredient/Active Moiety		
Ingredient Name	**Basis of Strength**	**Strength**
CROTALUS DURISSUS TERRIFICUS VENOM (UNII: 2XF6I0446G) (CROTALUS DURISSUS TERRIFICUS VENOM - UNII:2XF6I0446G)	CROTALUS DURISSUS TERRIFICUS VENOM	4 [hp_X] in 1 mL

Inactive Ingredients	
Ingredient Name	**Strength**
SODIUM CHLORIDE (UNII: 451W47IQ8X)	9 mg in 1 mL
BENZALKONIUM CHLORIDE (UNII: F5UM2KM3W7)	0.1 mg in 1 mL

Packaging

#	Item Code	Package Description	Marketing Start Date	Marketing End Date
1	NDC:48142-400-40	10 mL in 1 VIAL, MULTI-DOSE; Type 0: Not a Combination Product	04/30/2018	

Marketing Information

Marketing Category	Application Number or Monograph Citation	Marketing Start Date	Marketing End Date
Unapproved homeopathic		04/30/2018	

Labeler - Celtic Biotech Iowa, Inc. (079574151)

Establishment

Name	Address	ID/FEI	Business Operations
Complete Pharmacy and Medical Solutions		004417520	ANALYSIS(48142-400) , API MANUFACTURE(48142-400) , HUMAN DRUG COMPOUNDING OUTSOURCING FACILITY(48142-400) , MANUFACTURE(48142-400)

Revised: 3/2018 Celtic Biotech Iowa, Inc.