Abstract
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The peripheral action of tetrodotoxin – a translational research study

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Clinical study – peripheral action

Tetrodotoxin

- Tetrodotoxin (TTX), brand name Halneuron™, is a small molecule that blocks voltage-gated sodium channels on neurons.
- It exerts its analgesic effect by inhibiting the initiation and conduction of impulses in the peripheral nervous system.
- Phase 3 clinical trials are ongoing or planned to evaluate the analgesic effect of TTX in cancer related pain and chemotherapy induced neuropathic pain.

Study Design

This is a single center study of the safety and tolerability of TTX in healthy volunteers designated TTX-CINP-201PK. It consisted of 2 parts: a randomized, double-blind, placebo-controlled, parallel group, dose-comparison using a lyophilized formulation (Cohorts 1-4) and a randomized, open-label, crossover-design comparison of a lyophilized and liquid TTX formulations (Cohort 5).

Dosing Cohorts

Cohort	n (TTX : placebo)	Drug Dose (TTX/Placebo)	Dosing Schedule		Cumulative Dose
			Day 1	Day 2	
1	7[a]:2	15 µg once a day	AM	AM	30 µg
2	7[b]:2	15 µg twice a day	AM, PM	AM, PM	60 µg
3	6:2	30 µg once a day	AM	AM	60 µg
4	6:2	30 µg twice a day	AM, PM	AM, PM	120 µg

[a] One subject withdrew consent after enrollment.
[b] Study drug withheld for one subject. Assessments continued.

Cohort	n (TTX lyophilized: TTX liquid)	Drug Dose (TTX)	Dosing Schedule		Cumulative Dose
			Day 1	Day 3	
5	5:5	30 µg	AM lyophilized	AM liquid	60 µg
			AM liquid	AM lyophilized	

Safety assessments

- Adverse event observations, physical exams, neurological exams
- Neuro assessments (grip, tandem gait, heel raises, hand rapid-alternating movements, finger-nose testing, heel-shin maneuvers)
- Vital signs, ECG, spirometry
- Labs (chemistry panel, complete blood count, urinalysis)

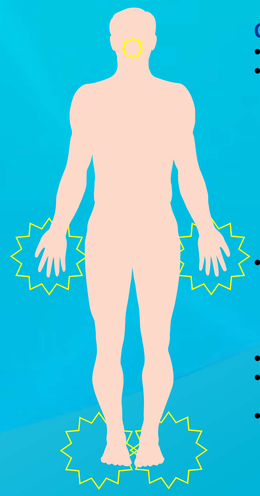
Results

Clinical Reponses

- 8 of 8 placebo recipients did well. No study drug related adverse events (AEs), changes in neuro exam, or any safety assessments.
- 35 of 36 active recipients did reasonably well. Mild to moderate AEs (if any), subtle neurological findings (if any).
- 1 of 36 active recipients had a strong reaction.

Adverse Events

Cohort	Major adverse events	Major neurological deficit
1	Perioral tingling (PT)	None
2	Perioral tingling (PT)	Vibratory sense (Vib), gait
3	PT, finger tingling	Vib, finger-nose maneuver
4	PT, hand / feet / head tingling	Vib, finger-nose maneuver
5	PT, hand / feet / head tingling	None



Case study

- 44 year old Hispanic woman, 59.7 kg.
- Day 1 Dose 1 (15 mcg). Unremarkable early course.
  - 4 hr neuro: Grip strength decreased 24 kg to 8 kg; tandem gait time prolonged 34 to 65 seconds, calf raises reduced 63 to 29.
  - Neuro exam:
    - Mildly decreased vibration, sharp-dull, & hot-cold sensation
    - Slower finger-nose, rapid alternating motion, & heel-shin maneuvers
- Day 1 Dose 2 (15 mcg):
  - AEs: perioral tingling
  - Sensory & motor deficits progressed within 3-4 hrs; decrease neuro function to all sensory & motor assessments.
- Sensorium preserved.
- Gradual improvement through Day 2 & 3 with no sequelae.
- Effects were specific to PNS. Sensorium, lung function, ECG, vital signs, and labs were not significantly affected.

Selected other AEs from other clinical trials and pufferfish poisoning: ataxia, nystagmus, dizziness.

Total of all adverse events and reactions suggest no central effect. All can be accounted for as actions on the peripheral nervous system or muscular system.

Further investigate by quantitating TTX in tissues in a rat model.

Rat study – quantitation in tissues

Methods & Materials

Treatment groups

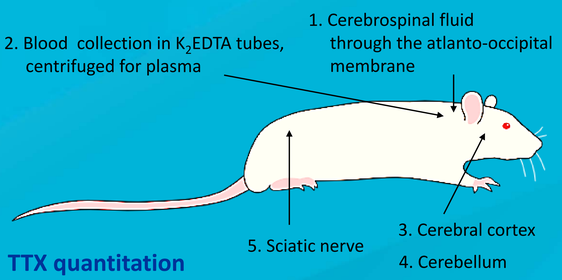
TTX and placebo were administered subcutaneously once in adult male Sprague-Dawley rats

Treatment	Dose	Treatment duration	Route	n
TTX	8 µg/kg	60 min	SC	8
TTX	4 µg/kg	60 min	SC	8
TTX	2.6 µg/kg	60 min	SC	8
Placebo	1 mL/kg	60 min	SC	8

TTX dosages used were effective in a rat model of chemotherapy-induced peripheral neuropathy (CIPN) in a previous study.

Tissue collection

Rats were anaesthetised with isoflurane/oxygen, followed by i.p. urethane. The anaesthesia level was regularly examined and maintained at a deep level. The following tissues were collected.



TTX quantitation

The cerebral cortex, cerebellum and sciatic nerve samples were homogenized with deionized water for analysis. All samples (CSF, plasma, cerebral cortex, cerebellum and sciatic nerve) were extracted by weak cation exchange solid phase extraction and eluted with 5% formic acid in methanol : deionized water. The eluant was dried and reconstituted in 5% HFBA in deionized water for LC/MS/MS analysis.

Acknowledgement

The preclinical study was supported in part by the National Research Council of Canada Industrial Research Assistance Program.

The efficacy of TTX for chemotherapy-induced neuropathic pain and cancer related pain is being further investigated in phase III clinical trials.

Results

Mean TTX concentration

Treatment	Plasma, ng/mL (%CV)	Sciatic nerve, ng/g (%CV)	CSF, ng/mL (%CV)	Cerebellum, ng/g (%CV)	Cerebral cortex, ng/g (%CV)
8 µg/kg TTX	5.19 (38.2)	3.16 (13.9)	<LLOQ (-)	<LLOQ (-)	<LLOQ (-)
4 µg/kg TTX	1.84 (8.9)	1.55 (11.8)	<LLOQ (-)	<LLOQ (-)	<LLOQ (-)
2.6 µg/kg TTX	0.91 (9.6)	1.75 (18.3)	<LLOQ (-)	<LLOQ (-)	<LLOQ (-)
Placebo	<LLOQ (-)	<LLOQ (-)	<LLOQ (-)	<LLOQ (-)	<LLOQ (-)

LLOQ – lower limit of quantitation, CV – confidence interval

- No TTX was observed in the placebo control across all matrices.
- TTX was observed in plasma.

TTX did not cross the blood-brain barrier between the CNS (cerebral cortex, cerebellum, CSF) and the circulatory system, at the dose range effective in alleviating mechanical allodynia in CIPN models.

The presence of TTX in the PNS supports the theory of blockage of peripheral nerve impulses, leading to the blockage of pain signals.

Summary and Conclusions

- TTX does not produce any central effect as it does not cross the blood-brain barrier.
- TTX can cross the blood-nerve barrier. This is consistent with a peripheral action - a reduction of pain signals transmitted along peripheral nerves.
- Proprioceptive sensory deprivation to the cerebellum, likely at the level of the dorsal root ganglia, rather than a direct effect on the cerebellum may produce AEs that mimic a cerebellar syndrome in some humans. Motor weakness from blockage of muscle sodium channels contributes additionally to neurological findings.

Disclosures

This study was funded by WEX Pharmaceuticals Inc. JR was the principal investigator of the clinical trial. VL was the medical monitor of the clinical trial. JR was an employee of Comprehensive Clinical Development, Inc during the conduct of the clinical trial.
VL is currently an employee of PAREXEL International, Waltham, MA, USA, but all work relating to the study was performed under contract to Comprehensive Clinical Development, while in private practice at Puget Sound Neurology. DW, MK and WK are employees of WEX. HW, FYZ and DS are employees of Cerebrasol who performed the preclinical study on contract.