An Electrophysiological Examination of the Mechanism of Action of Tetrodotoxin for the Treatment of Chemotherapy-induced Neuropathic Pain in a Rat Model of Oxaliplatin-Induced Peripheral Neuropathy

Poster
PWD325

Donald Wong Ph.D.[1], Walter Korz HCA[1], Haifeng Wei Ph.D.[2], Fei-Yue Zhao Ph.D.[2], David Spanswick Ph.D.[2]

[1] WEX Pharmaceuticals Inc., Vancouver, BC, [2] Cerebrasol Ltd, Montreal, Quebec

Tetrodotoxin

- Tetrodotoxin (TTX), trademark 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 nervous system.
- Clinical trials have been ongoing to evaluate the analgesic effect of TTX in chemotherapy induced neuropathic pain, cancer pain, as well as other neuropathic and nociceptive pain conditions.

Specific Aim

To examine by electrophysiology the effects of tetrodotoxin (TTX) on spontaneous and mechanically-evoked activity from dorsal root (DRG attached) and sciatic nerve (DRG detached) in oxaliplatin-treated rats.

Methods & Materials

Oxaliplatin-induced neuropathy

- Oxaliplatin was administered intravenously through the tail vein at 4 mg/kg, twice a week for up to 4 weeks.
- The development of neuropathic pain, characterised by significant mechanical allodynia, was monitored using a series of graduated von Frey hairs applied to the hind-paw to trigger a withdrawal response (Paw Withdrawal Threshold, PWT).
- Only those rats with significant mechanical allodynia (Paw Withdrawal Threshold ≤ 4.0 g) were selected for further drug testing.

Treatment with TTX or vehicle

Description	Dose	Schedule	Route	n
Vehicle. DRG recording	1mL/kg	QD x 5 days	SC	6
TTX. DRG recording	8 µg/kg	QD x 5 days	SC	6
Vehicle. Sciatic nerve recording	1mL/kg	QD x 5 days	SC	6
TTX. Sciatic nerve recording	8 µg/kg	QD x 5 days	SC	6

- Vehicle or TTX were administered subcutaneously daily for 5 consecutive days.
- Recording of spontaneous and mechanically-evoked activity from dorsal root filaments or sciatic nerve fibres were carried out on day 6, one day after cessation of dosing.

Methods & Materials (cont'd)

Electrophysiology

- Dorsal root recording (DRG attached): Rats were anaesthetised with urethane. The left L5 dorsal root at the area close to where it joins the spinal cord was sectioned and repeatedly teased into fine bundles. Each individual bundle was looped on a unipolar silver electrode for the recording of spontaneous and mechanically-evoked activity. Spontaneous activity was recorded for at least one min. Then 3 von Frey hairs (1, 4 and 10 grams) were tested in sequence applied to the receptive field on the left hind-paw to evoke responses. The neural activity was recorded using standard electrophysiological recording techniques on a PC using Cambridge Electronics Design (CED) Spike 2 software.
- Sciatic nerve fibre recording (DRG excluded): Rats were anesthetised with urethane. The left sciatic nerve was exposed and sectioned at about the greater trochanter level. The nerve was repeatedly teased into small fibre bundles for recording as described above for DRG.

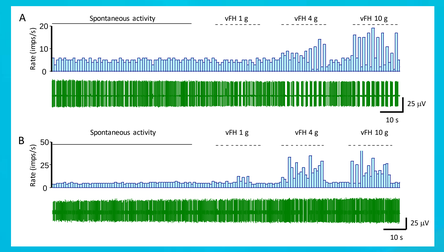


Figure 1. Two representative DRG filaments (A & B) recorded from vehicle-treated rats with chemotherapy-induced neuropathy. Both units showed spontaneous activity and responses to von Frey hair stimulation.

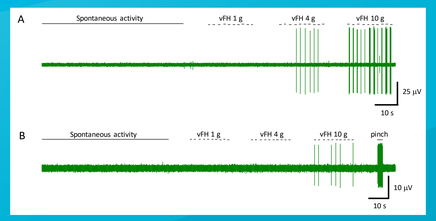
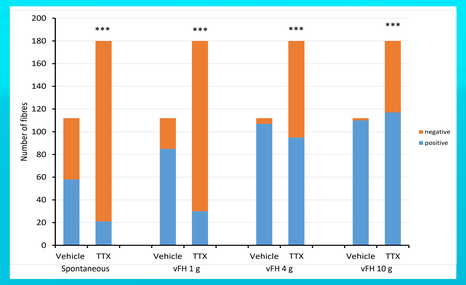


Figure 2. Two representative DRG filaments (A & B) recorded from TTX-treated rats with chemotherapy-induced neuropathy. Both units showed no spontaneous activity and reduced response to von Frey hair stimulation.

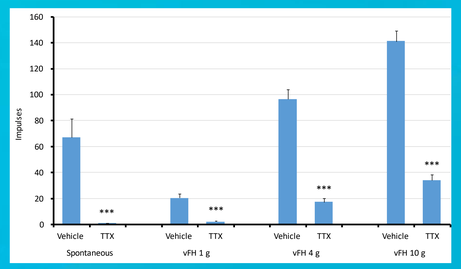
Results

Dorsal root ganglion (DRG)



***: P < 0.001, compared to vehicle group, χ^2 test. Negative and positive: negative (filaments without) and positive (filaments with spontaneous activity or mechanically-evoked responses).

Figure 3. The number of DRG filaments showing spontaneous and mechanically evoked activity is significantly reduced but not abolished by TTX.



*, ***: P < 0.05 and 0.001, compared to vehicle group, unpaired Student's *t*-test. Spontaneous: spontaneous activity (impulses/min); mechanical responses: impulses/10 trials.

Figure 4. Firing rates associated with spontaneous activity and mechanically evoked responses of DRG filaments are reduced by TTX but persistent at lower levels.

Sciatic nerve

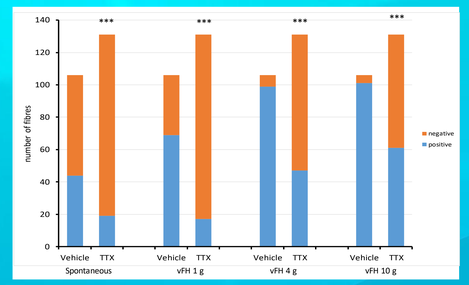


Figure 5. The number of sciatic nerve filaments showing spontaneous and mechanically evoked activity is significantly reduced but not abolished by TTX.

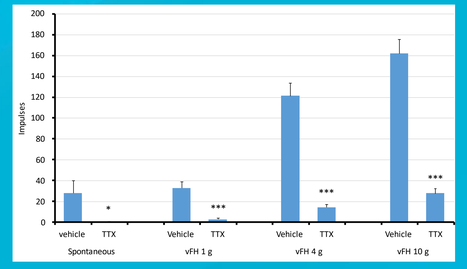
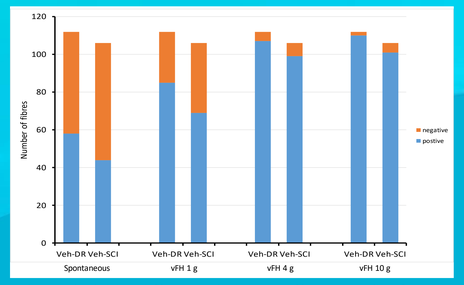


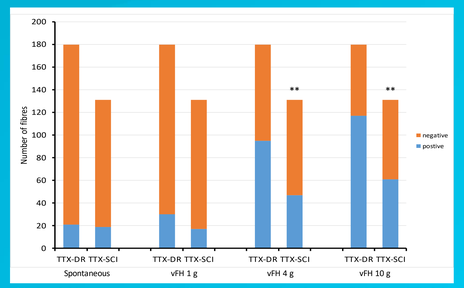
Figure 6. Firing rates associated with spontaneous activity and mechanically evoked responses of sciatic nerve are reduced by TTX but persistent at lower levels.

DRG vs Sciatic nerve



Veh-DR and Veh-Sci: DRG filament and sciatic nerve filament recordings from rats treated with vehicle. vFH: von Frey hair. Negative and positive: negative (filaments without) and positive (filaments with spontaneous activity or mechanically-evoked responses).

Figure 7. Comparison of the numbers of DRG and sciatic nerve filaments with spontaneous activity and mechanically-evoked responses in neuropathic rats treated with vehicle. There was no statistically significant difference between the two groups, either in spontaneous activity or mechanically evoked responses, $\chi 2$ test.



**: P < 0.01, compared to DRG filament recording, $\chi 2$ test. TTX-DR and TTX-Sci: DRG filament and sciatic nerve filament recording in rats treated with TTX. vFH: von Frey hair. Spontaneous: spontaneous activity (impulses/min); mechanical responses: impulses/10 trials. Negative and positive: negative (filaments without) and positive (filaments with spontaneous activity or mechanically-evoked responses).

Figure 8. Comparison of the number of dorsal root and sciatic nerve filaments with spontaneous activity and mechanically-evoked responses in neuropathic rats treated with TTX.

Summary and Conclusions

- The number of DRG filaments and sciatic nerve fibers showing spontaneous and mechanically-evoked activity as well as their firing rate were significantly lower after TTX treatment compared to vehicle, but not completely abolished.
- TTX most likely has a principal site of action at the peripheral nerve level, including the receptive fields, axons. However, the involvement of cell bodies of peripheral nerves (i.e., at the level of DRG) in the efficacy of TTX is also congruent with the study results.
- TTX appears to act as an analgesic, not anesthetic. It may reduce background pain but leave nociception intact.

The efficacy of Halneuron® (TTX) for chemotherapy-induced neuropathic pain is being further investigated in phase II & III clinical trials.

Disclosures

This study was funded by WEX Pharmaceuticals Inc. DW and WK are employees of WEX. HW, FYZ and DS are employees of Cerebrasol who performed the preclinical study on contract. All experiments were conducted with the approval of Animal Use Protocol which followed the guidelines of the Canadian Council on Animal Care.