Prophylactic Tetrodotoxin Treatment of a Rat Model of Oxaliplatin-Induced Peripheral Neuropathy

Poster
TU583

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Tetrodotoxin

- Tetrodotoxin (TTX), trademark Halneuron®, is a small molecule that blocks voltage-gated sodium channels on peripheral neurons.
- It exerts its analgesic effect by inhibiting the initiation and conduction of impulses in the peripheral nervous system, especially ectopic discharges.
- 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

There has been an urgent need for an effective treatment for Chemotherapy Induced Neuropathic Pain with a desire to prevent neuropathy, enabling optimum chemotherapy delivery. In this study, we investigated the effects of prophylactic dosing with TTX on the development of oxaliplatin- induced neuropathic pain in rats. Mechanical allodynia and neuropathic pain state, was assessed using a series of graduated Von Frey hairs.

Methods & Materials

Treatment groups

Description	Dose	Schedule	Route	n
Sterile normal saline	1mL/kg	QD x 18 days	SC	13
TTX for injection	6 µg/kg	QD x 18 days	SC	13
TTX for injection	8 µg/kg	QD x 18 days	SC	13
Duloxetine	15 mg/kg	QD x 18 days	PO	13

- TTX (two dose concentrations, 6 µg/kg and 8 µg/kg), normal saline and duloxetine (15 mg/kg) were dosed concurrent with twice a week oxaliplatin until chemotherapy induced peripheral neuropathy (CIPN) develops in the normal saline group and then up to 5 days after oxaliplatin treatment stops.
- Significant mechanical allodynia appeared after 3 oxaliplatin injections in the normal saline treated group. Oxaliplatin injections were stopped after a final boost injection on day 13.
- TTX for injection and normal saline were administered subcutaneously, once a day for 18 days, which is 5 days after oxaliplatin treatment stopped. Duloxetine suspension was orally administered once daily for 18 days.

Methods & Materials (cont'd)

Oxaliplatin-induced neuropathy

- Oxaliplatin was administered intravenously through the tail vein at 4 mg/kg, twice a week for 4 injections.
- 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).

Schedules and timelines

Study day	TTX, duloxetine and normal saline administration *	Oxaliplatin administration *	Von Frey assessment ‡
Day 1	✓		✓
Day 2	✓		✓
Day 3	✓	✓	✓
Day 4	✓		✓
Day 5	✓		✓
Day 6	✓	✓	✓
Day 7	✓		✓
Day 8	✓		✓
Day 9	✓		✓
Day 10	✓	✓	✓
Day 11	✓		✓
Day 12	✓		✓
Day 13	✓	✓	✓
Day 14	✓		✓
Day 15	✓		✓
Day 16	✓		✓
Day 17	✓		✓
Day 18	✓		✓
Day 20			✓
Day 23			✓
Day 27			✓

* TTX, duloxetine, and normal saline were administered at 0 hours, followed 1 hour later by oxaliplatin injection. ‡ Von Frey assessments were performed 2 hours after TTX, duloxetine, or normal saline, which is equivalent to 1 hour after oxaliplatin.

Results

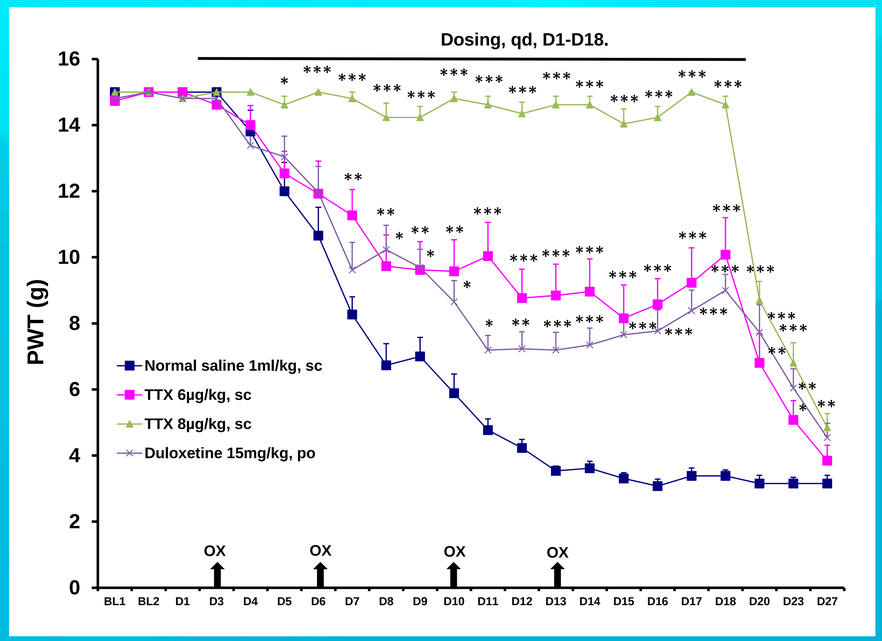


Figure 1. Effect of TTX and Duloxetine on the development of oxaliplatin- induced neuropathic pain. Each value represents the mean ± 1 standard error of the mean. PWT expressed in g as assessed with graduated von Frey hairs at 2h after TTX, duloxetine or normal saline injection where administered. OX: Oxaliplatin 4 mg/kg, IV.
*, **, ***: P < 0.05, 0.01 and 0.001, respectively, compared to the normal saline (negative control) group at the same time points (one-way ANOVA); n =13 for each group.

Normal saline alone had no effect on the development of oxaliplatin-induced neuropathic pain / CIPN in rats, indicated by the decrease in PWT from 15 g to 6 g during the first two injections of oxaliplatin, to 4 g after the third and fourth injections of oxaliplatin, and to 3 - 4g throughout the rest of the observation period up to day 27.

Duloxetine partly suppressed the development of CIPN in rats. PWT decreased from 15 g to 7 g over the period covered by the first three injections of oxaliplatin and remained at a similar level during the fourth injection. However, 3 days after the final dose of oxaliplatin, PWT gradually increased to 9 g. After cessation of duloxetine treatment, PWT decreased to a level similar to that in the normal saline group.

TTX at 6 µg/kg suppressed the development of CIPN in rats. PWT decrease from 15 g to 10 g after the first two injections of oxaliplatin, remained at a level above 8 g over the period of the third and fourth injections of oxaliplatin, then increased to 10 g three days after the final injection of oxaliplatin. TTX thus countered neuropathic pain symptoms. 9 days after cessation of TTX treatment (on day 27), PWT decreased again to a level similar to that observed in the normal saline group.

TTX at 8 µg/kg robustly suppressed the development of CIPN. PWT remained at a level around 15 g throughout the entire period of all 4 injections of oxaliplatin and for the duration of the TTX treatment period. 9 days after cessation of TTX treatment (on day 27), PWT gradually decreased, but remained significantly higher statistically than that observed in the normal saline group.

Summary and Conclusions

- The effect of TTX in suppressing oxaliplatin induced neuropathy / CIPN is dose-related.
- TTX at 8 µg/kg completely prevented the reduction in PWT. PWTs were significantly higher than that of duloxetine at 15 mg/kg from day 6 to day 18.
- TTX at 6 µg/kg reduced the changes in PWT. PWTs were significantly higher than that of duloxetine at 15 mg/kg on day 11.
- These results suggest that the TTX prevented the development of oxaliplatin induced neuropathic pain / CIPN in rats, and this effect was dose-dependent.
- TTX was superior to the positive control.
- TTX maybe useful in preventing the development of neuropathic pain associated with chemotherapy.

The efficacy of Halneuron® (TTX) in the treatment of 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.