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Tetrodotoxin treatment of mechanical allodynia in rats with oxaliplatin and vincristine-induced neuropathy

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Abstract

Tetrodotoxin (TTX) is a potent sodium channel blocker that is being investigated as therapy for cancer pain in late stage clinical trials. In addition to the cancer itself, cancer treatment is also well documented to induce peripheral neuropathies including pain. Patients with chemotherapy induced peripheral neuropathy (CIPN) exhibit symptoms such as pain, tingling, and numbness. In the present study, we examined whether TTX can alleviated CIPN induced by oxaliplatin and vincristine in rat models. Sprague-Dawley rats were administered oxaliplatin (4 mg/kg for up to 5 injections) or vincristine (0.1mg/kg for up to 10 injections). Mechanical allodynia was measured as paw withdrawal threshold (PWT) using von Frey filaments. Rats showing significant mechanical allodynia (PWT ≤ 4g) were used for active agent dosing that included TTX and Duloxetine. TTX (8μg/kg) was administered daily for five days. Controls included vehicle and Duloxetine (30 mg/kg). TTX treatment produced a rapid increase in PWT (reversal of mechanical allodynia) as early as 1 hour. PWT continued to rise over the 5 dosing days and persisted for at least 2 days after the last treatment. TTX tends to act more rapidly and is at least equal to Duloxetine in terms of its overall efficacy and duration of action. Treatment with vehicle did not produce any changes in PWT. In a substudy, the daily TTX dose was split (4μg/kg twice a day, and 2.6μg/kg three times a day) for rats with oxaliplatin induced peripheral neuropathy. Administration of TTX in smaller, more frequent doses achieved more stable therapeutic effect than a single larger dose. These results suggest that TTX has a positive therapeutic effect on CIPN. A dose finding clinical trial has recently been completed in 125 patients with moderate to severe CINP, with a Phase II/III prepared for launch.

Tetrodotoxin

- Tetrodotoxin (TTX) 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.
- Clinical trials have been ongoing to evaluate the analgesic effect of TTX in cancer pain.

Specific Aim

To evaluate the effects of TTX on mechanical allodynia in rats with oxaliplatin-induced or vincristine-induced neuropathy.

Methods & Materials

Oxaliplatin-induced neuropathy

- Oxaliplatin was administered intravenously through the tail vein at 3 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 Withdrawl Threshold ≤ 4.0 g) were selected for further drug testing.

Vincristine-induced neuropathy

- Vincristine was administered intravenously through the tail vein at 0.1 mg/kg 5 times a week for 2 to 3 weeks.
- The development of neuropathic pain was monitored using a series of graduated von Frey hairs applied to the hind-paw to trigger a PWT.
- Only those rats with significant mechanical allodynia (PWT ≤ 4.0 g) were selected for further drug testing.

Treatment with TTX, Duloxetine or placebo

Treatment	Dose	Schedule	Route	n
Placebo	1mL/kg	QD (8am) x 5 days	SC	7
TTX for injection	8 μg/kg	QD (8am) x 5 days	SC	7
TTX for injection	4 μg/kg	BID (8am, 5pm) x 5 days	SC	7
TTX for injection	2.6 μg/kg	TID (8am, 3pm, 10pm) x 5 days	SC	7
Duloxetine	30 mg/kg	QD (8am) x 5 days	PO	7

- TTX for injection and placebo were administered subcutaneously one to three times a day for 5 days. Duloxetine was dosed orally once daily for 5 days.

Endpoint

- Paw Withdrawal Threshold (PWT) to von Frey hairs was assessed pre-dose, 60, and 120 minutes following each TTX dosing as well as on days 7, 10 and 14 following the start of the dosing course.

Results

Oxaliplatin-induced neuropathy

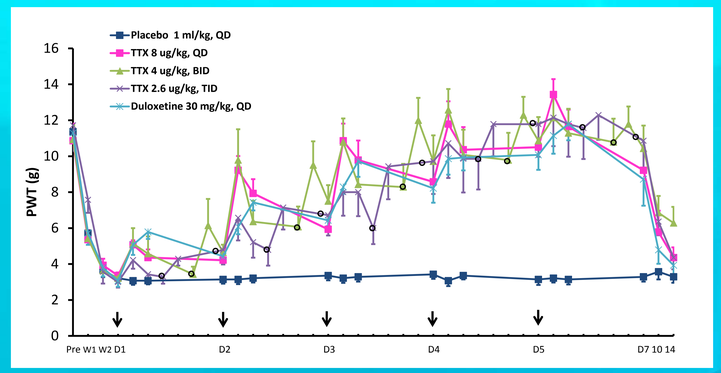

Figure 1. TTX increased PWT in rats with oxaliplatin-induced neuropathy from day 1 to day 7. Greater von Frey filament size (force) was required to elicit paw withdrawal. Peak effect was observed 1h post-dose. Pre: the day before first oxaliplatin injection; W1, W2: 1st and 2nd week after first oxaliplatin injection, respectively; D1, D2, D3, D4, D5, D7, D10, D14: the 1st, 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course; ↓:First dosing on the day (8 am for each group); o: second/ third dosing on the day where applicable (5 pm for TTX 4 μg/kg, BID group; 3 pm and 10 pm for TTX 2.6 μg/kg, TID group). $P < 0.05$ for D1 1h, D2-D7, as well as D1 2h for TTX 8 μg/kg QD, D1 2h for duloxetine, D10 and D11 for 4 μg/kg BID and 2.6 μg/kg TID.

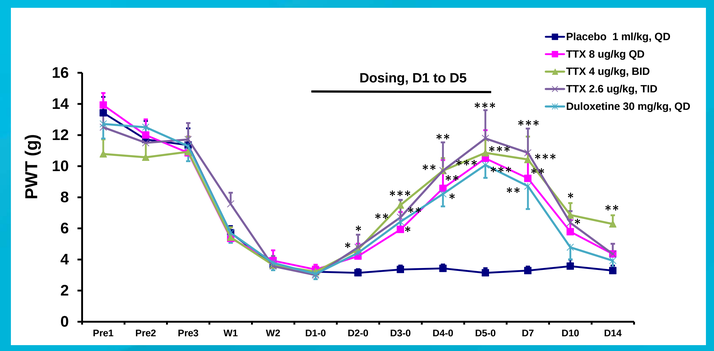

Figure 2. TTX and duloxetine increased baseline PWT in rats with oxaliplatin-induced neuropathy from day 1 to day 7. Greater von Frey filament size (force) was required to elicit paw withdrawal. Pre1, Pre2, Pre3: 1st, 2nd and 3rd days before first oxaliplatin injection; W1, W2: 1st and 2nd week after first oxaliplatin injection, respectively; D1-0: pre-dosing control; D2-0, D3-0, D4-0, D5-0, D7, D10, D14: baseline value for the 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course. *, **, ***: $P < 0.05$, 0.01 and 0.001, respectively, compared to the same time points for placebo group, one-way ANOVA.

Vincristine-induced neuropathy

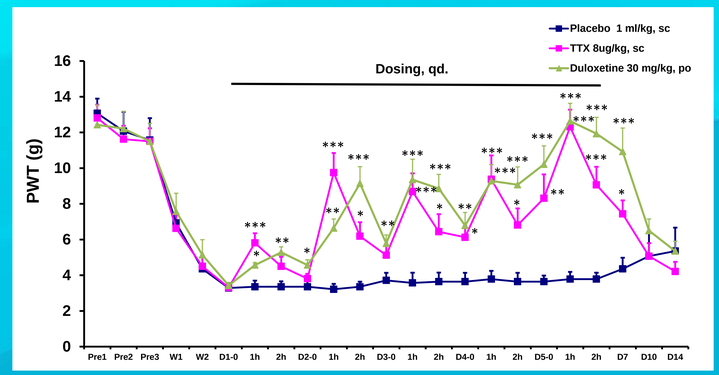

Figure 3. TTX increased PWT in rats with vincristine-induced neuropathy from day 1 to day 7. Greater von Frey filament size (force) was required to elicit paw withdrawal. Peak effect was observed 1h post-dose. Pre1, Pre2, Pre3: 1st, 2nd and 3rd days before first vincristine injection; W1, W2: 1st and 2nd week after first vincristine injection, respectively; D1-0: pre-dosing control; D2-0, D3-0, D4-0, D5-0, D7, D10, D14: baseline value for the 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course; 1 h, 2 h: post-dosing value for 1 hour and 2 hours after each dosing. *, **, ***: $P < 0.05$, 0.01 and 0.001, respectively, compared to the same time points for placebo group, one-way ANOVA.

Summary and Conclusions

- Oxaliplatin-induced neuropathy in rats.
  - TTX administered SC significantly increased PWT from day 1 of dosing.
  - Peak effect at 1 hour after dosing in each dosing day.
  - Remained until at least day 7 in the 5-day dosing course.
  - At 1 and 2 hours post dosing on days 2 and 3, TTX at 8 μg/kg QD, and 4 μg/kg BID produced significantly higher PWT than that of 2.6 μg/kg TID, but no significant difference was observed on later days and at the base-line time points.
- Vincristine-induced neuropathy in rats.
  - TTX administered SC significantly increased PWT from day 1 of dosing.
  - Peak effect at 1 hour after dosing in each dosing day.
  - Remained until at least day 7 in the 5-day dosing course.
- Duloxetine increased PWT in rats with oxaliplatin and vincristine-induced neuropathy.
- TTX provided a more rapid reversal of the PWT decrease than duloxetine and is at least equal to duloxetine in terms of its overall efficacy and duration of action.
- TTX may have therapeutic effects in treating chemotherapy-induced neuropathy.
- Administration of TTX in smaller doses, multiple times may achieve more stable therapeutic effect and generate less adverse side effects than a single larger dose.

The efficacy of TTX for chemotherapy-induced neuropathic pain is being further investigated in a phase III clinical trial.