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A Mouse Model of Oxaliplatin-induced Neuropathic Pain

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BACKGROUND

Chemotherapy induced neuropathic pain resulting from toxic effects of chemotherpy agents is a burden for cancer patients and causes unwanted side effects. Therapies such as platin based molecules, like oxaliplatin or cisplatin, have been reported commonly induce nerve damage and neuropathic pain acutely and chronically. To understand neuropathic pain resulting from chemotherapy and to develop chemotherapies with better side effect profiles there is a need for suitable animal models which are responsive to commonly used clinical therapies in these conditions.

In this study we explored previously described chronic oxaliplatin induced neuropathic pain model which shows sensitivity to cold/cool stimulus. We used a chronic model with repeated oxaliplatin challenges. In this study we followed mice up to 18 weeks (4.5 months) and evaluated cold/cool allodynia by using two different assays. We also explored pharmacological responses to pregabaline and duloxetine, two widely used therapies for neuropathic pain, in this mouse model.

MATERIALS AND METHODS

Animals. All experiments were performed under approval by the National Animal Experiment Board, Finland. Male C57Bl/6N mice at the age of 8-10 weeks were used for the experiments. Mice were housed at standard temperature (22 ± 1°C) and in a light-controlled environment (lights on from 7 am to 8 pm) with *ad libitum* access to food and water.

Oxaliplatin teatments. Mice were injected with oxaliplatin 3 mg/kg i.v. every 3 days for 9 times, so that last injection was given 27 days after the first injection. Vehicle for oxaliplatin was 5% glucose in saline, which was administered to vehicle treated mice at same time-points. Mice were closely monitored for any acute and chronic effects with oxaliplatin and vehicle treated mice.

Neurologial index. Neurological index test was used to evaluate qualitatively cuprizone related CNS effects during the 6-week exposure to cuprizone. Altogether 33 variables were monitored by scoring each variable based on the change from the normal. Mice were scored according to their phenotype, score 0= normal, score 1=slightly impared, score 2= moderately impaired, score 3= severely impaired.

Tail immersion/flick test. The mouse was removed from its cage and the body was gently wrapped in a tissue. With the tail kept exteriorized, the animal was held horizontally over the water bath. Water temperature was set to 15 ºC when testing the mice. When the animal's tail was motionless and pointing downwards (the handler may gently stroke it to straighten it), the tail is introduced vertically into the water to a depth of ~5 cm. At this time the electronic timer is started using either the computer program Stopwatch or a manual timer. The timer is stopped and the time is recorded when the animal demonstrates one of the following reactions referred to as "Tail Flicks":

1. Tail curves ventrally and suddenly lashes towards the surface of the water (not necessarily breaching it) with a whip-like movement.
2. Tail wiggles from side to side several times with increasing urgency.
3. Tail slowly curves ventrally either stopping in mid-curve or coiling up towards the surface without completing a flick.

To avoid tissue damage, the cut-off time was at 40 seconds, after which the test was halted and the animal replaced in its cage. The value entered for that animal was 40 seconds.

Acetone cooling test. Mice were be placed in individual cylinders on a floor made of a wire grid 20 min prior the baseline or experimentation. The floor stand on an elevated stage allowing easy observation and application of acetone. To produce a cooling sensation, acetone (25 microliters) was applied to the plantar surface of the hindpaw of the animal. For both baseline and post-drug time points, nocifensive behaviours were recorded for a maximum of 60 seconds post-acetone application; two determinations were made, about 10 min apart to allow the initial cooling sensation to disappear. The cumulative time spent either slapping, flinching, lifting and licking the affected hind paw were recorded using a stop watch (timer). Reported data consisted of average time displaying a constellation of nocifensive behaviours over 60 seconds.

All testing was conducted in a blinded manner.

Statisical analysis. All values are presented as mean ± standard deviation (SD) or standard error of mean (SEM), and differences were considered to be statistically significant at the P<0.05 level. Statistical analysis was performed using StatsDirect statistical software. Statistical comparisons were made between cuprizone treated and control mice by using t-test or Mann-Whitney U-test, or one-way ANOVA, when applicable.

RESULTS

	Control									Oxaliplatin												
Parameter	M1	M2	M3	M4	M6	M10	M16	M19	change %	M5	M7	M8	M9	M11	M12	M13	M14	M15	M17	M18	M20	change %
Head Tremor	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Head Twitch	0	0	0	0	0	0	0	0	0 %	0	0	1	0	0	0	0	0	0	0	0	0	12.5%
Head Bobbing	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Head Searching	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Body Tremor	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Body Twitch	1	1	1	1	1	1	0	0	75 %	1	1	1	1	1	1	1	1	1	1	0	1	91.7%
Tail tremor	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Tail twitch	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Straub tail	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Piloerection	0	0	0	0	0	0	1	0	12.5%	0	0	0	0	0	0	0	0	0	1	0	1	16.7%
Shallow respiration	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Flattened body posture	0	0	0	0	0	0	0	0	0 %	0	0	0	0	1	0	1	0	0	0	2	0	25 %
Swollen face	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Ptosis	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Irritability	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Seizure	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Urine staining	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Lacrimation	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Salivation	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Limb Splay	1	0	0	1	1	0	1	1	62.5%	0	1	1	1	1	0	1	0	0	0	1	1	58.3%
Catalepsy	0	0	0	0	0	0	1	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Abnormal Gait	0	0	0	1	0	0	0	0	12.5%	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Tip toe walking	0	0	0	0	0	0	1	0	12.5%	0	0	1	0	0	0	0	0	0	0	0	0	12.5%
Abnormally slow movements	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Excessive grooming	0	0	0	0	0	0	0	0	0 %	0	0	1	0	0	0	0	0	0	0	0	0	12.5%
Circling	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Sniffing	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Chewing	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Excessive locomotor activity	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Dehydration	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Loss of startle response	1	1	1	1	1	1	1	1	100 %	1	1	1	1	1	1	1	0	1	1	1	1	91.7%
Loss of righting reflex	0	0	0	0	0	0	0	0	0 %	0	0	0	0	0	0	0	0	0	0	0	0	0 %
Tail pinch	0	0	1	0	1	1	0	0	37.5%	0	1	0	1	0	1	1	0	0	0	1	0	41.7%

Figure 1. Neurological index at two weeks after the onset of oxaliplatin injectrions. Neurological index was performed at an early time-point after 3 repeated injections with oxaliplatin to qualitatively observe the mice in the study for any major neurological or general behavioral differences. Both groups exhibited mild or no change in majority of the evaluated parameters and no distinct differences between control and oxaliplatin treated mice were observed. Data are presented as the score of each variable and relative proportion of mice showing change in whole group.

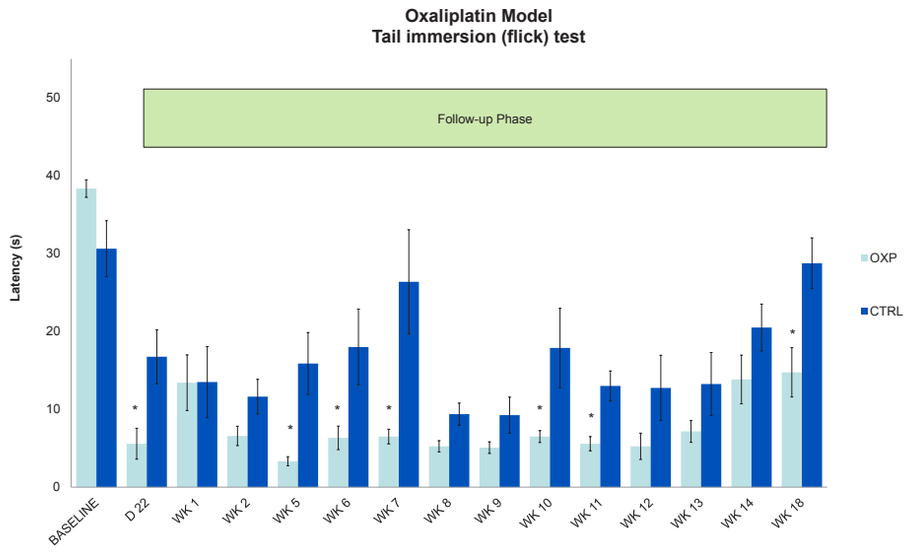

Figure 2. Tail immersion test over time. Significant difference in latency to tail flick response was seen in multiple but not in all time-points. There was also significant variability in responses in control mice between time-points suggesting that assay is not very stable to measure cool allodynia by tail immersion test in the model. Data are presented as mean ± SEM. *p<0.05; n=8-12/group

RESULTS

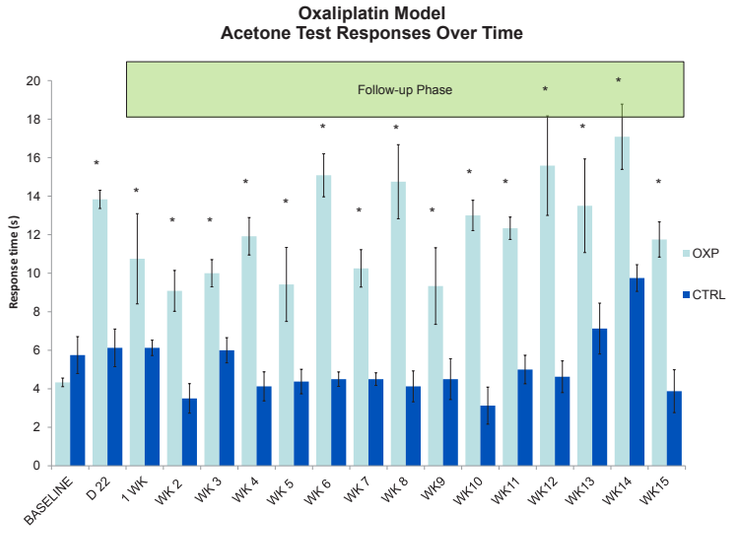

Figure 3. Acetone cooling test over time. A droplet of acetone was applied to the plantar surface of the hind paw and time spent in flicking, shaking or biting the affected hind paw was measured. Significant difference in response times was seen in all time-points between control and oxaliplatin treated mice. Despite the signficant difference there was also significant variability in reponses in both control and oxaliplatin treated mice between time-points. Data are presented as mean ± SEM. *p<0.05, t-test between control and oxaliplatin group; n=8-12/group

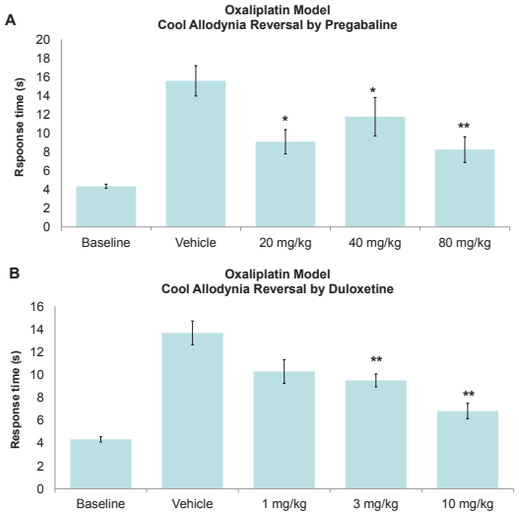

Figure 4. Pharmacological reversal of acetone cooling response in oxaliplatin treated mice. (A) Various doses of pregabaline signficantly decreased response times, suggesting alleviation of cool allodynia with anti-epileptics. (B) Similarly, duloxetine a SNRI anti-depressant, previously shown to have clinical benefit in neuropathic pain, also signficantly reduced the response time in the test. Pregabaline and duloxetine dose responses were performed on week 15 and 18,post induction, respectively. Data are presented as mean ± SEM. *p<0.05; ANOVA; n=8-12/group

CONCLUSIONS

This sudy explored the use of oxaliplatin induced neuropathic pain model in mice. Chronic and repeated exposure to oxaliplatin induced persistent cool allodynia as evidenced by acetone test. Tail immersion test, on the other hand, gave a more variable outcome than cool allodynia responses over time with occasional failure to show statistically signficant difference in tail flick reponse between control and oxaliplatin mice. Furthermore, tail immersion test was found to be more variable also in control mice, suggesting that with repeated testing normal responses may vary significantly.

Pharmacological responses were found in selected acetone cooling test in which both pregabaline and duloxetine alleviated allodynia responses. Data suggets the usefulness of this model to examine therapies against chemotherapy induced neuropathy.

Neurological Index test did not indicate clear side effects with the oxaliplatin treatment, suggesting that there are no other clear neurobehavioral changes resulting from oxaliplatin treatment than cool/cold allodynia.

Future studies will focus on further optimization of the model with modified exposures to oxaliplatin, but also by introducing other measures of allodynia such as mechanical and thermal allodynia tests as well as functional motor readouts.