Efforts to Accelerate Development of Non-Opioid, Non-Addictive Pain Therapeutics Within the NIH HEAL Initiative Preclinical Screening Platform for Pain and Validation Examples

Smriti Iyengar[1], Sarah A. Woller[1], Elizabeth Dugan[2], Katelyn Buban[2], Jennifer Hagedorn[2], Mark A. Varney[2], Taleen Hanania[2] & Mark O. Urban[2]

[1]NINDS, Rockville, MD USA, [2]PsychoGenics Inc., Paramus, NJ USA

Background

The National Institutes of Health Helping to End Addiction Long-term℠ Initiative, or NIH HEAL Initiative℠, Preclinical Screening Platform for Pain (PSPP) program aims to accelerate the discovery and development of new non-opioid, non-addictive pain therapeutics. Toward this goal, PSPP is collaborating with PsychoGenics, Inc. to validate preclinical models and endpoints to enable screening and profiling of assets, including small molecules, biologics, natural products, and devices. Here, we describe the validation of one such effort to optimize the paclitaxel and oxaliplatin models of chemotherapy-induced painful neuropathy in the rat.

Methods

Animals: Adult male and female Sprague Dawley rats (n=10, each sex) were used in these studies. All housing and testing of the animals were in accordance with the Principles of Laboratory Animal Care and the approval of PsychoGenics Inc., Institutional Animal Care and Use Committee in AAALAC-accredited facilities.

Paclitaxel dosing: For the paclitaxel model studies, paclitaxel (Biolyse Pharma) was initially injected at several doses (2 mg/kg, i.p.; 4 mg/kg, i.p.; 2 mg/kg, i.v.) on alternate days (Day 0, 2, 4, 6) to determine the optimal dose and route of administration based on the pharmacokinetic properties of the compound. For the behavioral studies evaluating the effects of paclitaxel on hind paw tactile and cold sensitivity, paclitaxel was injected at a dose of 4 mg/kg, i.p. on four alternate days (Day 0, 2, 4, 6) for a total of 4 injections at a dose volume of 1 ml/kg. Vehicle control rats received the vehicle consisting of 16.7% Ethanol, 16.7% Cremaphor in saline.

Oxaliplatin dosing: For the oxaliplatin model studies evaluating effects of oxaliplatin on hind paw tactile and cold sensitivity, oxaliplatin (Biolyse Pharma) was injected 2 days per week (3 day interval) for 4 consecutive weeks at a dose of 3 mg/kg, i.v. and dose volume of 1 ml/kg. Oxaliplatin was prepared in saline vehicle.

Hind paw tactile sensitivity: Hind paw tactile sensitivity was measured by applying von Frey filaments to the plantar hind paw and determining the 50% Withdrawal Threshold using the "up-down" method (Chaplan et al. 1994 J Neurosci Methods 53:55-63).

Hind paw mechanical priming: To examine the effects of mechanical priming of the hind paws on the development of tactile hypersensitivity, rats received mechanical stimulation of both hind paws during Week 2 as follows: 5 consecutive brushes of the plantar surface of the paw from heel to toe using a medium fiber strength, full size toothbrush. 5 consecutive taps of the plantar surface of the paw with the toothbrush; a repeat of the plantar brushing but with only 3 brushes and 3 consecutive applications of the 15 g von Frey filament to the toe area of both hind paws. This procedure was performed once each day for 5 consecutive days (Simmons et al. 2014 J Neurosci Methods 233:50-53).

Hind paw cold sensitivity: An acetone bubble from a 1 ml syringe (~50 µl) was applied to the plantar surface of the hind paw and a withdrawal response or no response was recorded. The acetone was applied 5 times (once every 5 min) to each hind paw (Choi et al. 1994 Pain 59:369-376).

Hind paw cold priming: To examine the effects of cold priming of the hind paws on the development of cold hypersensitivity, rats received acetone stimulation of both hind paws during Week 2. An acetone bubble from a 1 ml syringe (~50 µl) was applied to the plantar surface of the hind paw. The acetone was applied 5 times (once every 5 min) to each hind paw. This was repeated 3 times for a total of 15 applications for each hind paw (total 30 applications each day). This procedure was performed each day for 5 consecutive days.

This project has been funded in whole or in part with Federal funds from the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Department of Health and Human Services, under Contract No. 75N95019D00026.

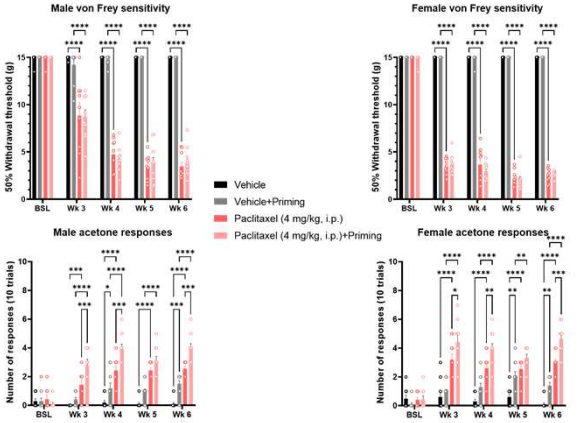
Paclitaxel model

Rat plasma exposures

Dose	Route	Male			Female		
		Cmax (ng/ml)	Day 1 AUC (ng.h/ml)	Day 1-7 AUC (ng.h/ml)	Cmax (ng/ml)	Day 1 AUC (ng.h/ml)	Day 1-7 AUC (ng.h/ml)
2 mg/kg	IV	N/A	2,160	8,640	N/A	4,468	17,872
2 mg/kg	IP	139	568	2,272	275	1,165	4,660
4 mg/kg	IP	204	1,780	7,120	376	2,207	8,828
6 mg/kg	IP	276	2,898	11,592	494	4,241	16,964

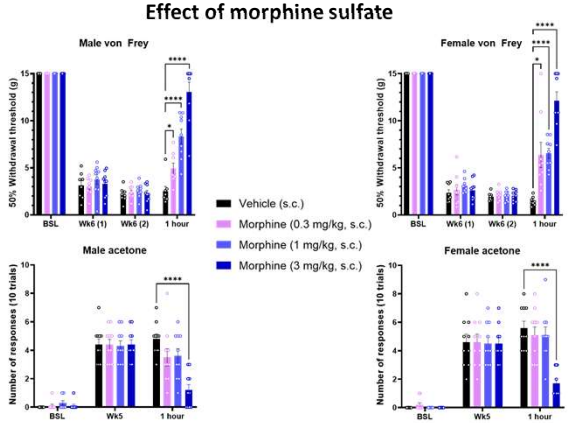
Pharmacokinetics of paclitaxel dosed in rats at 2 mg/kg, i.v., 2 mg/kg, i.p., 4 mg/kg, i.p., and 6 mg/kg, i.p. Rat plasma exposures following dosing of 4 mg/kg, i.p. are consistent with exposures associated with clinical doses of paclitaxel (AUC 6000-8000 ng.h/ml). Rats did not tolerate the 6 mg/kg, i.p. dose

Hind paw tactile and cold hypersensitivity



Hind paw tactile and cold responses in male and female rats treated with paclitaxel (Day 0, 2, 4, 6; Wk 1). Tactile and cold responses for the left and right hind paws did not significantly differ, so the data are represented as the mean of left and right hind paw (tactile) or total number of acetone responses/10 total applications (5 applications left/right hind paw). Data represented as mean±sem; n=10/group; * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 Dunnett's test

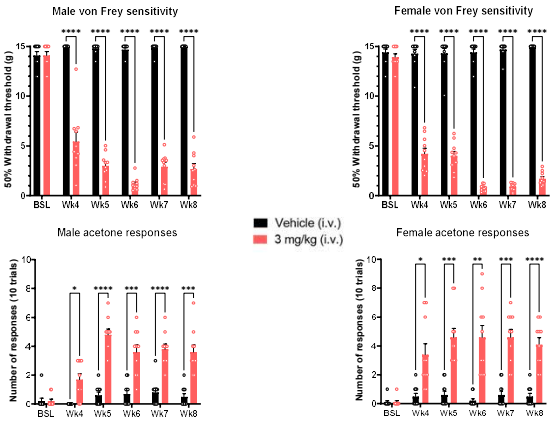
Effect of morphine sulfate



Effect of morphine sulfate on hind paw tactile and cold hypersensitivity in male and female rats treated with paclitaxel (Day 0, 2, 4, 6; Wk 1). Tactile and cold responses for the left and right hind paws did not significantly differ, so the data are represented as the mean of left and right hind paw (tactile) or total number of acetone responses/10 total applications (5 applications left/right hind paw). Data represented as mean±sem; n=10/group; * p<0.05, **** p<0.0001 Dunnett's test

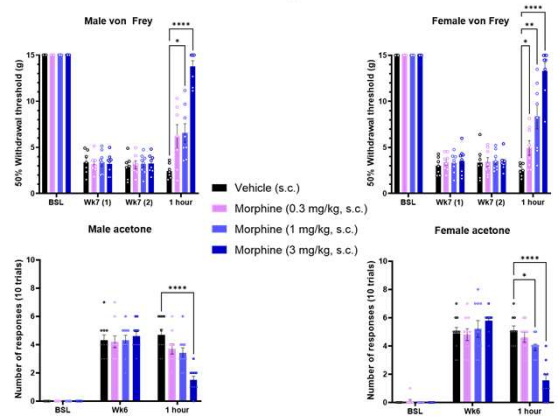
Oxaliplatin model

Hind paw tactile and cold hypersensitivity



Hind paw tactile and cold responses in male and female rats treated with oxaliplatin (2x per week; Wk1-4). Tactile and cold responses for the left and right hind paws did not significantly differ, so the data are represented as the mean of left and right hind paw (tactile) or total number of acetone responses/10 total applications (5 applications left/right hind paw). Data represented as mean±sem; n=10/group; * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 Dunnett's test

Effect of morphine sulfate



Effect of morphine sulfate on hind paw tactile and cold hypersensitivity in male and female rats treated with oxaliplatin (2x per week; Wk1-4). Tactile and cold responses for the left and right hind paws did not significantly differ, so the data are represented as the mean of left and right hind paw (tactile) or total number of acetone responses/10 total applications (5 applications left/right hind paw). Data represented as mean±sem; n=10/group; * p<0.05, ** p<0.01, **** p<0.0001 Dunnett's test

Conclusion

The results from these validation studies demonstrate reproducible bilateral hind paw tactile and cold hypersensitivity in male and female rats in the paclitaxel and oxaliplatin models of chemotherapy-induced painful neuropathy. The optimal dose of paclitaxel used in these studies (4 mg/kg, i.p.) resulted in plasma exposures consistent with clinically effective doses. Tactile and cold hind paw hypersensitivity in the paclitaxel and oxaliplatin models were significantly inhibited by the reference analgesic morphine sulfate, providing pharmacological validation of these pain endpoints.