In vivo PK, side effect profile, and efficacy of multiple classes of analgesics in rats

Elizabeth Dugan[2] , David Budac[2],Conor McDonnell[2], Mark Urban[2], Sarah A. Woller[1], Smriti Iyengar[1], Taleen Hanania[2], Mark A. Varney[2]

[1]Division of Translational Research, National Institutes of Neurological Disorders and Stroke, National Institutes of Health, Rockville, MD 20852

[2]PsychoGenics Inc., Paramus, NJ, 07652

Background

In collaboration with the NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP), we evaluated clinically used analgesics, including morphine, gabapentin, duloxetine and ketoprofen through the tiered approach established to profile potential novel analgesics. First, pharmacokinetic studies were conducted to guide dosing, select the route of administration, and to determine the time course, supporting subsequent behavioral studies. Next, the modified Irwin and rotarod tests were conducted to evaluate potential neurologic, physiologic, and fine motor effects that may impact outcome measures in the pain models. Following side effect profile assessment, efficacy was evaluated in the plantar incisional pain and L5/L6 spinal nerve ligation (SNL) models. The rat plantar incisional pain model is an established model of acute post-operative pain induced by incision of the skin and the plantaris muscle (Brennan et al. 1996). The model is characterized by transient hind paw tactile allodynia and spontaneous guarding behaviors. SNL is a model of peripheral neuropathic pain resulting from chronic nerve compression in which tactile and cold allodynia are produced (Kim and Chung, 1992).

Methods

Pharmacokinetics: Compounds were dosed in male and female SD rats (n=4/group/sex) for serial plasma collections. Separate cohorts of animals were used for evaluation of brain exposures.

Irwin: The modified Irwin test (Irwin 1968, Mathiasen and Moser, 2018) uses a battery of 39 observational assessments to evaluate neurologic and physiologic effects of a test article in male and female rats (n=4/group/sex).

Rotarod test: Compounds were dosed in male and female SD rats (n=10/group/sex) and animals were evaluated on an accelerating rotarod. The rotarod accelerated from 0-17 RPM over 5 seconds and was then maintained at 17 RPM for an additional 40 seconds. Latency to fall (seconds) was recorded.

Plantar Incision model: Male and female SD rats received a 1 cm incision in the plantar aspect of the hind paw. Animals (n=10/group/sex) were tested 1-day post-op for hind paw hypersensitivity or guarding score, and effects of compounds were determined following dosing. Paw withdrawal thresholds (PWTs) and guarding scores were assessed in separate cohorts. PWTs were determined with von Frey filaments using the “up-down” method (Chaplan et al. 1994 J. Neurosci Methods. 53(1):55-63). A guarding score was recorded for each animal every 5 minutes for 60 minutes. The scores for each animal were added and a final score was recorded (max 39).

Spinal nerve ligation (L5/L6) model: Male and female SD rats received tight ligation of the L5 and L6 spinal nerves. Animals were tested 14 days post-op for hind paw hypersensitivity, and effects of compounds were determined following dosing. Paw withdrawal thresholds were determined with von Frey filaments using the “up-down” method (Chaplan et al. 1994 J. Neurosci Methods. 53(1):55-63). Acetone Evaporation Test on day 21 of SNL surgery: Acetone (~50 µl) was gently applying to the plantar surface of the hind paw and rats are observed for 20 seconds for withdrawal or no withdrawal response.

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.

In vivo Pharmacokinetics

Compound Dose	Plasma Male / Female Drug Levels (µM)				Brain Male / Female Drug Levels (µM)	
Morphine	.5 hour	1 hours	2 hours	8 hours	.25 hour	8 hours
6 mg/kg, PO	5 / 5.8	2.3 / 3.2	.7 / .9	.06 / .07	1.2 / .9	.04 / .03
Gabapentin	.5 hour	2 hours	4 hours	8 hours	2 hours	8 hours
60 mg/kg, PO	66 / 97	101/102	61 / 45	29 /19	75 / 82	28 / 19
Ketoprofen	.5 hour	2 hours	4 hours	8 hours	2 hours	8 hours
6mg/kg, PO	24 / 28	26 / 38	22 / 29	15 / 8.8	.2 / .4	.04 / .03

Duloxetine levels are maintained through 8 hours post-administration

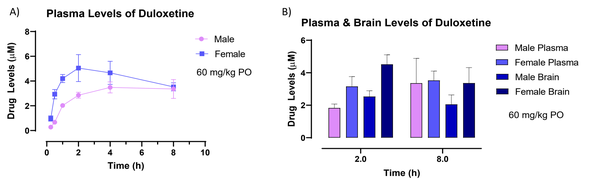

Figure 1: Data points are mean values ± SEM. A) Drug levels in plasma from male and female rats over 8 hours (n= 4 males and n=4 females except n=3 at 2 hours in males and n=2 at 2 hours and n= 3 for 4 and 8 hours in females) B) Drug levels in brain from male and female rats over 8-hours (n=3 for females and males at 2 hours, n=4 for females and males at 8 hours).

Side Effect Profile Assessment

Compound	Doses (mg/kg)	Irwin Observations	Rotarod - Latency to Fall
Morphine	1, 3, 6 and 10	Sedation, ↓ locomotor activity, and unusual behavior (eating bedding) at through 2 hours	Reduced at 30 and 60 minutes
Gabapentin	30, 60, 100 and 300	↓ body position, sedation, ↓ locomotor activity, and ↓ pupil size	Reduced at 1 and 2 hours
Ketoprofen	1, 3, 6 and 10	Well tolerated in male and female rats	No Effect

Duloxetine produced decreased body position, decreased locomotor activity, and sedation

Table 2: Heat map depicting the severity scores of the observed behaviors

Modified Irwin **Severity Score (percentage)**

		Vehicle					10 mg/kg					30 mg/kg					60 mg/kg					100 mg/kg				
Behaviors	**Maximum Score**	BL	1hr	2hr	4hr	6hr	BL	1hr	2hr	4hr	6hr	BL	1hr	2hr	4hr	6hr	BL	1hr	2hr	4hr	6hr	BL	1hr	2hr	4hr	6hr
Body Position	8							6.3	31.3	18.8	25.0		12.5	31.3	31.3	37.5		18.8	25.0	25.0			43.8	62.5	50.0	56.3
Locomotor Activity	8							6.3	18.8	31.3	18.8		18.8	43.8	31.3	43.8		37.5	37.5	50.0	50.0		62.5	87.5	81.3	81.3
Sedation/Excitation	8								12.5	12.5	12.5		12.5	50.0	31.3	62.5		25.0	18.8	43.8	37.5		75.0	68.8	81.3	75.0
Piloerection	32									1.6	3.1			1.6				3.1			4.7					1.6
Exophthalmos	32							7.8	9.4	4.7	3.1		18.8	15.6	10.9	12.5		15.6	14.1	23.4	15.6		26.6	31.3	42.2	37.5
Arching	32								1.6						1.6	1.6				1.6						
Body Tremor	8																							12.5		
Chromodacryorrhea	8																								6.3	
Diarrhea	8																						12.5	12.5		
Increased Defecation	8																	6.3					25.0			
Righting Reflex	8																						6.3			
Reactivity to Touch	24							16.7	25.0	12.5	8.3		14.6		8.3			22.9	16.7	25.0	8.3		16.7	16.7	25.0	12.5
Abdominal Tone	16		6.3		12.5	6.3								6.3	6.3			6.3	6.3		6.3		12.5	15.6	6.3	6.3
Aggressiveness to Handler	16																	6.3	6.3					6.3	12.5	6.3
Pupil Size	8										12.5							12.5	25.0	12.5				25.0		12.5
Visual Placement	24		4.2	4.2					8.3	4.2	6.3		6.3	4.2	8.3	8.3		18.8	4.2	12.5	8.3		10.4	4.2	8.3	8.3

Figure 2: Heat map depicting the severity scores of the observed behaviors. Note: Severity Score = (Sum of Score Across Animals/Maximum Score) *100. Empty cells indicate that a particular behavior was not observed in the 8 animals at the indicated dose and timepoint (thus the severity score would be 0). This table does not indicate the direction of the change (e.g., increase / decrease in a behavior).

Duloxetine does not affect rotarod performance

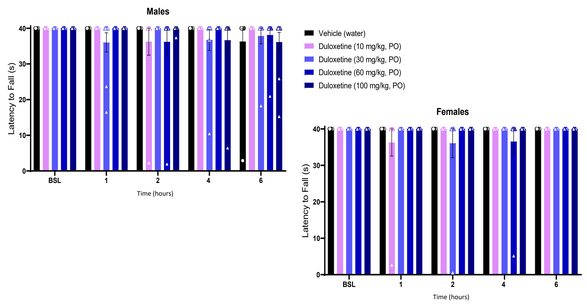

Figure 3. Latencies to fall off the rotarod apparatus following administration of duloxetine for male (left, n=10) and female (right, n=10) rats. Data represents mean ± SEM, with individual data points superimposed.

Efficacy Assessment

Compound (mg/kg)	Plantar Incision								SNL					
	Paw Withdrawal Threshold Male/Female				Guarding Score Male/Female				Paw Withdrawal Threshold Male/Female				Acetone Evaporations Male/Female	
Morphine	1 hour	2 hours	4 hours	6 hours	1 hour	2 hours	4 hours	6 hours	1 hour	2 hours	4 hours	6 hours	1 hour	4 hours
1	p<0.001/p<0.0001	p<0.01/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p<0.001/p<0.0001	p<0.0001/p<0.0001	p<0.05/p<0.01	p<0.05/p>0.05	p<0.0001/p<0.001	p<0.05/p<0.01	p>0.05/p<0.05	p>0.05/p>0.05	p<0.01/p<0.001	p>0.05/p>0.05
3	p<0.0001/p<0.0001	p<0.001/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p<0.0001/p<0.0001	p<0.0001/p<0.01	p<0.01/p<0.01	p<0.05/p>0.05	p<0.001/p<0.01	p<0.001/p<0.01	p<0.01/p<0.05	p>0.05/p>0.05	p<0.0001/p<0.0001	p>0.05/p>0.05
6	p<0.0001/p<0.0001	p<0.0001/p<0.01	p<0.05/p>0.05	p>0.05/p>0.05	p<0.0001/p<0.0001	p<0.0001/p<0.0001	p<0.05/p<0.01	p<0.0001/p>0.05	p<0.0001/p<0.0001	p<0.0001/p<0.001	p<0.05/p<0.01	p>0.05/p>0.05	p<0.0001/p<0.0001	p<0.01/p>0.05
Gabapentin													2 hours	4 hours
10	p<0.01/p<0.001	p<0.001/p<0.01	p<0.01/p<0.05	p<0.05/p<0.01	p<0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p<0.01/p<0.05	p<0.05/p<0.001	p>0.05/p<0.0001	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
30	p<0.0001 /p<0.001	p<0.0001 /p<0.001	p<0.001/p<0.01	p<0.05/p<0.01	p<0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p<0.0001/p>0.05	p<0.01/p<0.001	p<0.05/p<0.01	p<0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
60	p<0.001/p<0.0001	p<0.0001/p<0.0001	p<0.0001 /p<0.001	p<0.001/p<0.01	p<0.01/p>0.05	p<0.0001/p<0.01	p<0.001/p<0.01	p>0.05/p>0.05	p<0.0001/p<0.0001	p<0.0001/p<0.0001	p<0.0001/p<0.01	p<0.001/p<0.01	p<0.01/p<0.0001	p<0.01/p<0.0001
100	p<0.0001/p<0.0001	p<0.0001/p<0.0001	p<0.0001/p<0.0001	p<0.001/p<0.001	p<0.0001/p<0.01	p<0.0001/p<0.001	p<0.0001/p<0.01	p<0.0001/p>0.05	p<0.0001/p<0.0001	p<0.0001/p<0.001	p<0.0001/p<0.001	p<0.001/p<0.01	p<0.01/p<0.01	p<0.05/p<0.001
Ketoprofen													1 hour	3 hours
0.3	p>0.05/p>0.05	p<0.05/p<0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p<0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
1	p>0.05/p>0.05	p>0.05/p<0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p<0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
3	p>0.05/p<0.05	p<0.05/p>0.05	p<0.05/p<0.01	p>0.05/p<0.001	p<0.05/p>0.05	p<0.01/p<0.05	p<0.01/p<0.01	p<0.01/p<0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05
6	p<0.01/p<0.05	p<0.0001/p<0.05	p<0.01/p<0.001	p<0.001/p<0.01	p>0.05/p>0.05	p<0.001/p<0.01	p<0.001/p<0.001	p<0.0001/p<0.01	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05	p>0.05/p>0.05

Duloxetine reduced tactile allodynia and guarding behaviors in the plantar incision model

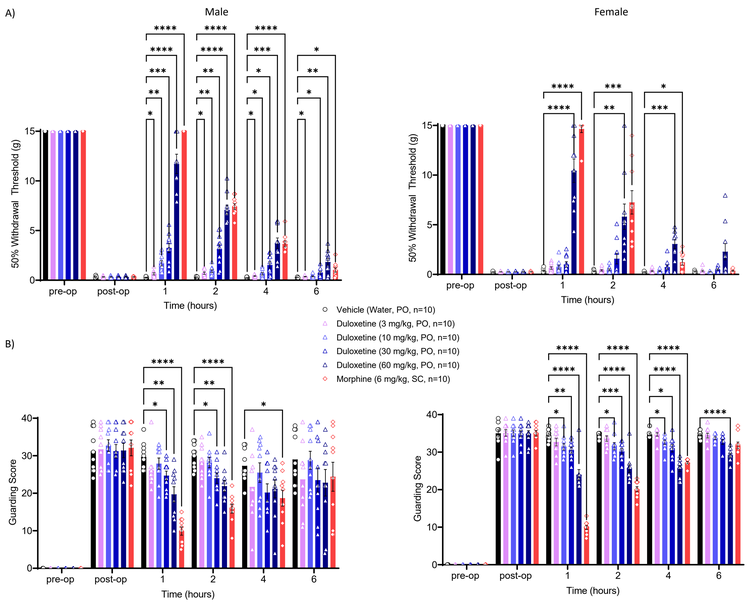

Figure 4: A) Paw withdrawal thresholds (PWTs) and B) Cumulative guarding score for males (left) and females (right) prior to and post-surgery, and post-treatment. Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, **** p<0.0001 .

Duloxetine reduced tactile and cold allodynia in the SNL model

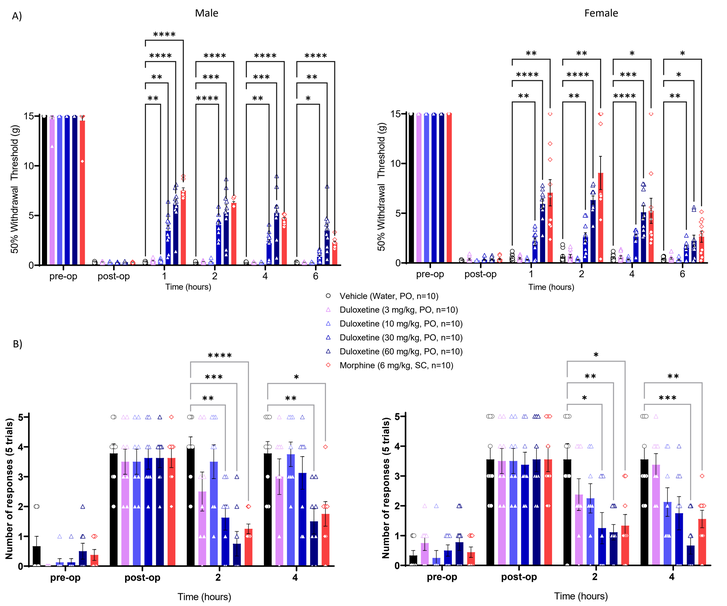

Figure 5.: A) PWT and B) Acetone response in SNL male (left) and female (right). Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions

The results of these studies of clinically used analgesic standards demonstrate the validation of the models and endpoints within the PSPP program and highlight the goal of providing a robust platform to accelerate the discovery and preclinical development of non-opioid, non-addictive treatments for pain.

PSPP is currently accepting assets for evaluation

For eligibility and participation inquiries, contact:

Smriti Iyengar, Ph.D.
Program Director
smriti.iyengar@nih.gov

Sarah Woller, Ph.D.
Scientific Project Manager
sarah.woller@nih.gov

For more information about PSPP, visit (or scan the QR):
https://pspp.ninds.nih.gov/

PsychoGenics
Redefining Drug Discovery Through Innovation

NIH National Institute of Neurological Disorders and Stroke

HEAL
NIH · Helping to End Addiction Long-term
https://heal.nih.gov