Effect of DPI-125 on opioid withdrawal behaviors in rats: a comparison study with methadone and buprenorphine

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Introduction

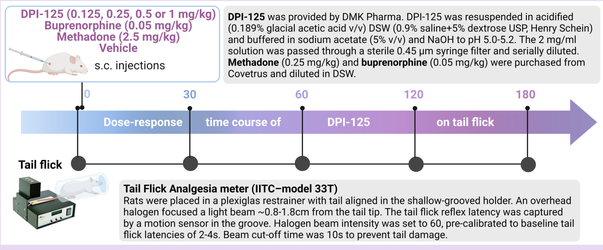
Opioid withdrawal syndrome, which comprises symptoms such as increased pain sensitivity and sickness-like behavior, is a salient concern in patients with opioid use disorder. While medication for opioid use disorder (MOUD) can help reduce opioid use, a major hurdle for patients is the experience of withdrawal when transitioning to stable MOUD dose regimens, reducing the desire to initiate treatment. Experimental and theoretical evidence suggests that DPI-125, a small-molecule triple (mu, delta and kappa) opioid receptor agonist, has safety and efficacy advantages over currently approved MOUD agents in terms of reduced respiratory depression (delta agonism) and reduced likability (kappa agonism). In this study we examine the timing and effectiveness of DPI-125 in reducing common symptoms of opioid withdrawal relative to standard-of-care MOUD treatments, methadone and buprenorphine.

Methods

Subjects: 80 male Sprague Dawley rats (250-325g) were purchased from Envigo. Rats were singly-housed and tested across 3 cohorts:

Cohort#1 [n=20]: DPI-125 **Dose-Tail Flick Response-Time** + **Withdrawal Pilot**
Cohort#2 [n=36]: **Main Withdrawal Experiment** (0.5-1.0mg/kg DPI-125)
Cohort#3[n=24]: **Extension Withdrawal Experiment** (0.125-0.5mg/kg DPI-125)

DPI-125 Dose-Tail Flick Response-Time (Cohort 1 n=20)



DPI-125 was provided by DMK Pharma. DPI-125 was resuspended in acidified (0.189% glacial acetic acid v/v) DSW (0.9% saline+5% dextrose USP, Henry Schein) and buffered in sodium acetate (5% v/v) and NaOH to pH 5.0-5.2. The 2 mg/ml solution was passed through a sterile 0.45 µm syringe filter and serially diluted. **Methadone** (0.25 mg/kg) and **buprenorphine** (0.05 mg/kg) were purchased from Covetrus and diluted in DSW.

Tail Flick Analgesia meter (IITC–model 33T)
Rats were placed in a plexiglas restrainer with tail aligned in the shallow-grooved holder. An overhead halogen focused a light beam ~0.8-1.8cm from the tail tip. The tail flick reflex latency was captured by a motion sensor in the groove. Halogen beam intensity was set to 60, pre-calibrated to baseline tail flick latencies of 2-4s. Beam cut-off time was 10s to prevent tail damage.

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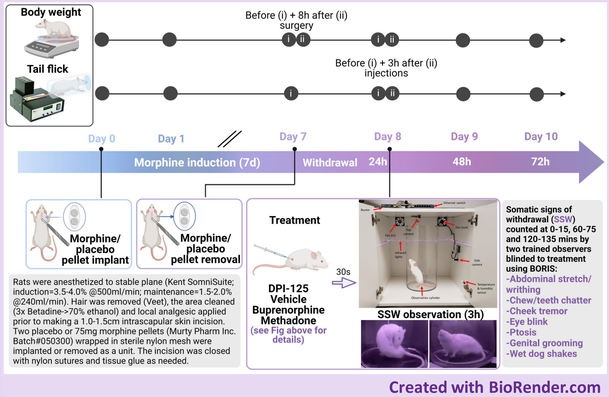
Withdrawal Expts. (Cohort 1 n=20; Cohort 2 n=36; Cohort 3 n=24)



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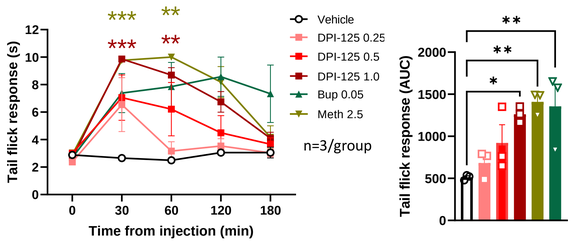
Data Analysis: Time course data for tail flick, body weight and across SSW period were analyzed by two-way ANOVA with treatment (between-subjects) and time (within-subjects) factors. Treatment effects in tail flick AUC and SSW count data were analyzed by one-way ANOVA. Dunnett's multiple comparisons compared vehicle-vehicle or morphine-vehicle controls to the treatment groups, with planned contrasts for DPI-125 against methadone and buprenorphine.

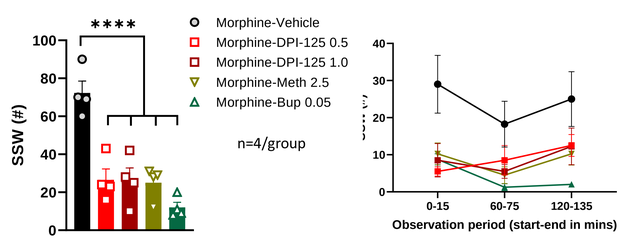
Cohort#1 (Pilot)

Dose-dependent antinociceptive effects of DPI-125



Dose-response-time course of tail flick response (left) showed significant analgesic effects of 1.0mg/kg DPI-125 (**p<0.01, ***p<0.001) comparable to 2.5mg/kg methadone, relative to vehicle after 30 and 60 mins. 0.25 and 0.5 mg/kg DPI-125 showed comparable analgesic effects to 0.05mg/kg buprenorphine at 30 minutes. **AUC analysis (right) of tail flick** showed significant analgesic effects of 1.0mg/kg DPI-125 (p<0.05), comparable to 2.5mg/kg methadone and 0.05mg/kg buprenorphine (p's<0.01) across the 3h period.

DPI-125 reduces morphine-induced spontaneous withdrawal



Total SSW (left) after treatment across all observation periods were significantly reduced by 0.5 and 1.0mg/kg DPI-125, 2.5mg/kg methadone and 0.05mg/kg buprenorphine (all p's < 0.0001). **Analysis by observation period (right)** showed that the effect was consistent across time points, but with greatest effect size for DPI-125 in the first 15 mins.

Cohort#2 (Main Withdrawal Experiment)

Analgesia and adaptation after morphine pellet with weight loss and hyperalgesia in withdrawal

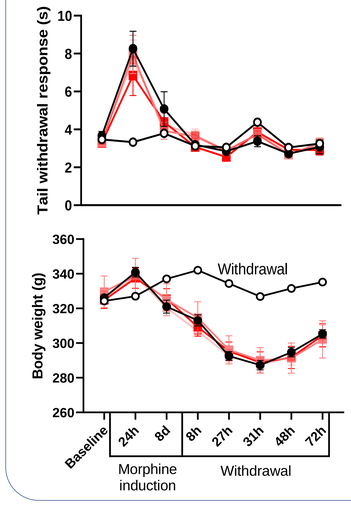
Tail flick response latency (top) after morphine pellet implantation was significantly higher (analgesic) relative to placebo after 24h (all p's <0.05) which showed tolerance by day 8. Methadone and DPI-125 showed significant hyperalgesia relative to placebo controls at 48h and/or 72h after opioid withdrawal (p's < 0.05). **Body weight (bottom)** significantly decreased by 10-15% after morphine pellet removal compared with placebo pellet, with peak loss ~31h into withdrawal.

DPI-125 reduces morphine-induced spontaneous withdrawal

Total SSW (left) after treatment across all observation periods were significantly reduced by 0.5 and 1.0mg/kg DPI-125, 2.5mg/kg methadone and 0.05mg/kg buprenorphine (all p's < 0.0001) to a similar level as placebo-implanted vehicle-treated rats. **Analysis by observation period (right)** showed a significant treatment by observation period interaction in which 0.5mg/kg DPI-125 (p<0.01), 1.0mg/kg DPI-125 (p<0.001), 2.5 mg/kg methadone (p<0.05) had reduced SSWs relative to morphine-vehicle rats (but similar to placebo-vehicle). Buprenorphine showed significantly reduced SSWs (p<0.05) at 120-135min after injection.

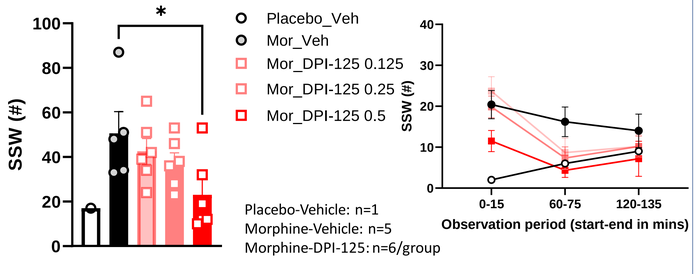
Cohort#3 (Extension Withdrawal Experiment with lower doses of DPI-125)

Analgesia and adaptation after morphine pellet with weight loss and hyperalgesia in withdrawal



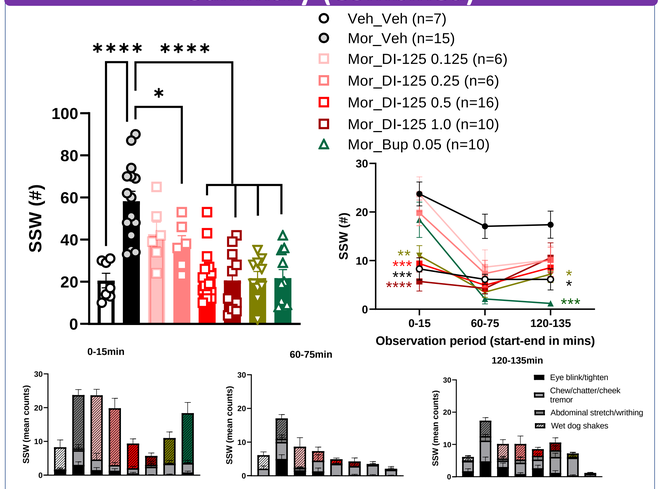
Tail flick response latency (top) after morphine pellet implantation was higher (analgesic) relative to placebo after 24h and showed tolerance by day 8 – a similar pattern to the main study. **Body weight (bottom)** also decreased by 10-15% after morphine pellet removal compared with the placebo pellet control, with peak loss ~31h into withdrawal.

DPI-125 dose-dependently reduces morphine-induced signs of withdrawal



Total SSW (left) after treatment across all observation periods were significantly reduced by 0.5mg/kg DPI-125. ($p < 0.05$). **Analysis by observation period (right)** showed that the effect of 0.5mg/kg DPI-125 was consistent across time points. Although not significant, 0.125 and 0.25 mg/kg doses had reduced means relative to the morphine-vehicle group.

Summary (Combined)



Total SSW (top-left) after treatment across all observation periods were significantly reduced by 0.25mg/kg DPI-125 (p<0.05), 0.5 and 1.0mg/kg DPI-125 (p's<0.0001), 2.5mg/kg methadone (p<0.0001) and 0.05mg/kg buprenorphine (p<0.0001), to a similar level as placebo-implanted vehicle-treated rats. **Analysis by observation period (top-right)** showed a significant treatment by observation period interaction in which 0.5mg/kg DPI-125 (p<0.001), 1.0mg/kg DPI-125 (p<0.0001), 2.5 mg/kg methadone (p<0.01) had reduced SSWs at 0-15 mins, similar to placebo-vehicle. These effects persisted at 60-75 mins after injection however, given the relatively short half-life of DPI-125, did not persist to 120 mins. **Analysis by specific SSW category across the observation periods (bottom 3)** illustrates the shift from active (e.g. wet dog shakes) to pain-related (e.g., ptosis, writhing) across time.

Conclusions

- Using analgesia as a benchmark for mu receptor engagement, 1.0 mg/kg DPI-125 showed comparable antinociceptive properties to 2.5 mg/kg methadone.
- DPI-125 dose-dependently reduced signs of spontaneous withdrawal in morphine-dependent rats, with comparable efficacy to standard-of-care treatments methadone and buprenorphine.
- DPI-125 demonstrated a possibly accelerated time course for reducing signs of withdrawal versus methadone and buprenorphine.

These data suggest the potential utility of DPI-125 in improving outcomes of patients with opioid use disorder.

References

1. *Friard, O. and Gamba, M. (2016) BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol Evol, 7: 1325–1330.*
2. *Tokuyama S., Wakabayashi H. and Ho I.K. (1996) Direct evidence for a role of glutamate in the expression of the opioid withdrawal syndrome Eur. J. Pharmacol. 295:123-129.*
3. *Bobzean SAM, Kokane SS, Butler BD, Perrotti LI. (2019) Sex differences in the expression of morphine withdrawal symptoms and associated activity in the tail of the ventral tegmental area. Neurosci Lett. 705:124-130.*
4. *Yi SP, Kong QH, Li YL, Pan CL, Yu J, Cui BQ, Wang YF, Wang GL, Zhou PL, Wang LL, Gong ZH, Su RB, Shen YH, Yu G, Chang KJ. (2017) The opioid receptor triple agonist DPI-125 produces analgesia with less respiratory depression and reduced abuse liability. Acta Pharmacol Sin. 38(7):977-989.*

Acknowledgments

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- All experiments were conducted at the NYU Rodent Behavior Laboratory in accordance with policies of the NIH Guide for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee (IACUC) of New York University Langone Health (Protocol#PROTO202000101 – PI A. Mar)