Peptide-Derived Orally-Active Kappa-Opioid Agonist for Peripheral Pain: Preclinical Results

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ABSTRACT

Kappa-opioid agonists have been particularly efficacious in peripheral pain models but suffer from centrally mediated effects that have limited their development. Derivatives of the tetrapeptide D-Phe-D-Phe-D-Nle-D-Arg-NH_2, such as CR665, exhibit high peripheral to central selectivity in analgesic models when administered IV and benefits patients experiencing visceral or neuropathic pain. However, compounds such as CR665 are not active when administered orally. Application of the JT Pharmaceuticals (JT Pharma) non-natural amino acid technology (Figure 1) to CR665 in Phase I of this project produced derivatives that exhibit peripheral analgesic activity when dosed orally but does not promote CNS-based effects. Lead compound **JT09** engages the kappa-opioid receptor with EC_{50}s in the low nM range while agonist selectivity for kappa over other peripheral opioid receptors (mu or delta) was >11,000-200,000-fold. No antagonistic activity was detected. To assess peripheral and central pain modulation, a rat writhing model of peripheral pain and a hotplate model of CNS-mediated pain were performed. Results indicate that **JT09** acts as efficacious as morphine in alleviating peripheral pain, while failing to produce undesired CNS-mediated activity. In an operant self-administration procedure where rats are required to press a lever to receive an intravenous drug infusion, **JT09** failed to maintain lever responding, indicating no abuse liability. In contrast, highly salient rewards (e.g., sucrose and cocaine) readily maintained operant responding. Additionally, **JT09** did not promote other CNS effects associated with morphine (sedation, dysphoria, tolerance, addiction). Thus, we propose that **JT09** is a candidate for development as an orally available, peripherally-restricted, kappa-opioid agonist for peripheral pain.

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INTRODUCTION

- Pain is the most common symptom that leads people to seek medical intervention in the United States today.
- The most difficult pain to manage successfully is chronic peripheral pain, which includes visceral, thermal, bone, and neuropathic pain, and pain associated with cancer.
- Currently, there are two major types of chronic pain medications in use – opioids and non-opioids – both of which have inherent toxicities (nausea, vomiting, constipation, renal toxicity, depressed breathing, thromboembolytic risk, neurotoxicity, tolerance, addiction etc.).
- At this time, there does not exist an analgesic for the treatment of chronic, peripheral pain that does not have side-effects associated with undesired central activity or inadequate receptor selectivity.
- A properly designed opioid receptor agonist could fulfill this role, with requirements that the compound is orally active and targets a specific peripheral pain receptor without crossing the blood brain barrier (BBB) to elicit toxicities mediated by opioid receptors in the central nervous system (CNS).
- The mediation of opioid analgesic effects occurs through three receptors: mu-, kappa-, and delta.
- Agonists at the mu-receptor are the most used opioid receptor agonists, but suffer from induction of euphoria, addiction, respiratory depression, and GI tract inhibition.
- Kappa opioid receptor agonists (KOAs) exhibit none of these effects and have been shown in visceral pain models to be the most efficacious of the opioid receptor agonists.
- **We believe that using the JT Pharma peptide modification technology, orally active peripheral KOAs can be created that will not cross the BBB, constituting a new type of pain medication.**

MATERIALS AND METHODS

Subjects: Male Sprague Dawley rats (Harlan) were individually housed in a 12 hr light/dark cycle. Food and water was provided ad libitum.
Synthesis of Analogs: Application of the proprietary JT Pharmaceutical (JT Pharma) non-natural amino acid technology to CR665 produced orally active derivatives. The Position 4 D-Arg residue of CR665 was converted to derivatives containing modified D-Arg or D-Lys residues.
Pain Assessments: Acetic acid-induced rat writhing assay; Hot plate assay.
Behavioral Assessments: Self-administration test; Conditioned place preference test; Locomotor Test; Forced swim assay.
Statistical Analysis: For all pain assessments, a Student's t-test was used comparing each compound individually to saline and morphine controls. For all behavioral assessments, all experiments were analyzed with analysis of variance (ANOVA). All results were considered significantly different if $p<0.05$.

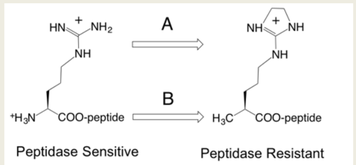

Fig.1. The JT Pharma Peptide Modification Strategy

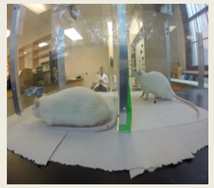

Fig. 2. Acetic Acid Writhing Assay

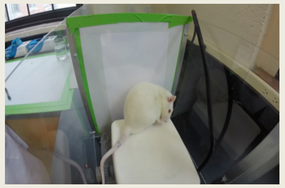

Fig. 3. Hot Plate Assay

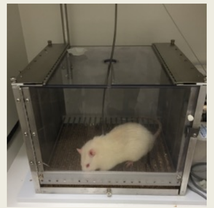

Fig. 4. Self-Administration Test

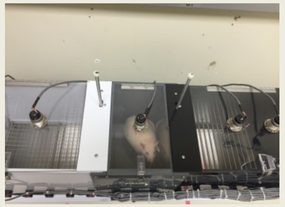

Fig. 5. Conditioned Place Preference Test

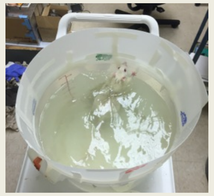

Fig. 6. Forced Swim Assay

Fig. 7. Locomotor Test

RESULTS

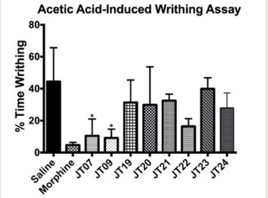

Fig. 8. Screen of lead compounds in the acetic acid-induced writhing assay in rats. Results are shown as the mean ± SEM with n = 2-8 for each compound. Asterisk indicates that the value is not significantly different from the morphine control value by Student's T-Test, $p \not< 0.05$.

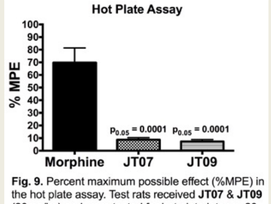

Fig. 9. Percent maximum possible effect (%MPE) in the hot plate assay. Test rats received **JT07** & **JT09** (20mg/kg) and were tested for hot plate latency 20 min later. Control rats received morphine (10mg/kg, i.p.) and were tested for hot plate latency 20 min later. Results are shown as the mean ± SEM with n = 8 for each compound. **JT07** & **JT09** were significantly different from morphine ($p_{0.05}$ = 0.0001 & 0.0001, respectively).

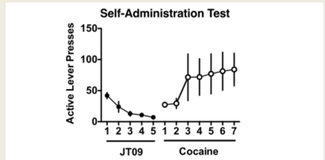

Fig. 10. Mean active lever presses and mean infusions (n=8) two-hour session operant self-administration sessions. **JT09** failed to maintain lever responding in rats over a five-day period [Figure 3]. The number of infusions decreased on all 4 days compared to day one of JT09 administration [$F(4,28)=9.04$, $p<0.0001$, and Dunnett post hoc, $p<0.05$]. Further, to ensure that these rats were not deficient in reward processing, we replaced **JT09** with cocaine using a nose-poke operandi and the number of cocaine active lever presses increased over the 7 days [$F(6,42)=4.6$, $p<0.0012$] with significantly more active lever presses on days 6 and 7 (Dunnett post hoc, $p<0.05$).

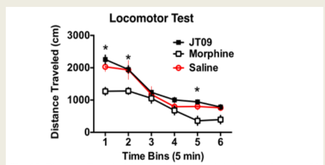

Fig. 12. Distance Traveled time (in centimeters) during activity tests, 30 min after a single dose of **JT09** (20 mg/kg, p.o.), morphine (10 mg/kg, i.p.) or saline (2 mL, p.o.). Saline and **JT09** were statistically indistinguishable in all time bins (Student's t-test, $p<0.05$). There was a significant interaction between morphine and **JT09** [Figure 6, $F(5,70)=7.0$, $p<0.0001$], specifically **JT09** had higher locomotor activity relative to morphine during time bins 1, 2, and 5 (Sidak's multiple comparison, $p<0.05$). Further, the main effect of treatment [$F(1,14)=18.6$, $p<0.0007$] and time [$F(5,70)=84$, $p<0.0001$] were also significant. Data are expressed as mean ± 8.

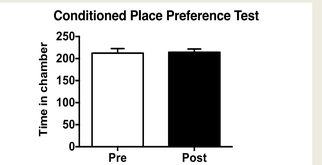

Fig. 11. JT09 (20 mg/kg, p.o.) had no effect on compartment placement in a conditioned place preference procedure. Baseline preferences for each compartment (black bar) were assessed prior to conditioning and did not change following drug treatment ($^*p<0.05$).

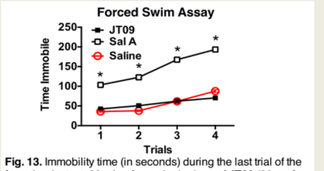

Fig. 13. Immobility time (in seconds) during the last trial of the forced swim test, 30 min after a single dose of **JT09** (20 mg/kg, p.o.), salvinorin A (1 mg/kg, i.p.) or saline (2 mL, p.o.). Saline and **JT09** were statistically indistinguishable in each trial (Student's t-test, $p<0.05$). There was a significant interaction between Sal A and **JT09** [Figure 5, $F(3,30)=117$, $p<0.0001$], specifically rats treated with **JT09** had lower amounts of time spent immobile relative to Sal A during all trials (Sidak's multiple comparison, $p<0.05$). Further, the main effect of treatment [$F(1,10)=947$, $p<0.0001$] and time [$F(3,30)=418$, $p<0.0001$] were also significant. Data are expressed as mean ± SEM with n = 8.

Summary

- **JT09** is as efficacious as morphine in alleviating peripheral pain, while failing to produce undesired CNS-mediated activity.
- **JT09** does not promote other CNS effects associated with morphine (sedation, dysphoria, tolerance, addiction).
- **JT09** did not present gross abnormalities in a necropsy after completing a 14 day multiple dose study.
- **JT09** is both orally active and acts as peripherally restricted KOA, thus fitting the criteria of a new pain medication.
- **JT09** is a candidate for development as orally available, peripherally-restricted, kappa-opioid agonists for peripheral pain.

Acknowledgements

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