Second-generation κ -opioid agonist as a peripherally-restricted analgesic

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Abstract

Today, μ -opioids are prescribed for peripheral pain, but they come with the risk of many toxicities including gastrointestinal issues, sedation, and addiction. The compound of study, referred to as JT09, has shown peripheral pain alleviation with high selectivity of κ -opioid receptors compared to μ - and δ -opioid receptors; this prevents the drug from having any effect on the central nervous system as it cannot cross the blood brain barrier. JT09 has been incorporated into ProNeura rods, subdural implants that continually administers the drug for up to 12 months. This technology may render the need for oral and intravenous administration unnecessary, as well as decrease the number of addictive drugs available to the public. Rats were implanted with the ProNeura rods and blood samples were taken at various time intervals. Results are expected to show effectiveness of JT09 as a peripheral pain reliever in the ProNeura platform and not cause symptoms like other medications that cross the blood-brain barrier. Future directions include pharmacokinetics studies, as well as immunohistochemistry studies to observe the location, action, and relation of κ -opioid receptors (KORs) with myocardial infarction (MI) size and its influence on other antigens.

Introduction

The most common reason patients seek medical attention is because of chronic pain¹, and over-prescription of μ -opioids has led to a major public health crisis. However, second-generation κ -opioids have been shown to treat chronic peripheral pain without crossing the blood-brain barrier (BBB) and causing the toxicities mediated by opioid receptors of the central nervous system (CNS).² Three receptors mediate opioid analgesic effects: δ , μ , and κ . κ -opioid agonists (KOAs), like JT09, have yet to exhibit any of the negative effects of other opioid agonists, and have been shown to be the most efficacious of the opioid receptor agonists in visceral pain models. Studies have supported JT09 as an orally-active peripherally-restricted KOA that does not promote the negative side effects of current opioid analgesics.²

ProNeura is a technology that has the potential for continuous subcutaneous drug delivery on an outpatient basis or time periods of six to twelve months. JT09 is uniformly distributed throughout the rod, an ethylene vinyl acetate copolymer (EVA) matrix. This allows for controlled release of the compound as there is no reservoir and no risk of drug dumping.³ This technology is expected to be a good option for the administration of JT09, preventing the need for constant and repetitive intravenous or oral administration.

Methodology

Pain and Behavioral Assessments

Male Sprague Dawley rats (Harlan) were individually housed in a 12-hour light/dark cycle. Food and water was provided ad libitum.

Application of the proprietary JT Pharmaceutical 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 (**Figure 1**).

Pain assessments included acetic acid-induced rat writhing assay and the hot plate assay. Behavioral assessments included self-administration test, conditioned place preference test, locomotor test, and forced swim assay.

ProNeura Administration

The ProNeura rods containing JT09, supplied by Titan Pharmaceuticals, were inserted into the dorsal skin in the neck area on three male and three female rats.⁴ Blood samples were taken from the jugular vein cannulation of the rats at various time points, seen in **Table 1**.

Fig. 2. Acetic acid Writhing Assay

Fig. 3. Hot Plate Assay

Fig. 4. Self-Administration Test

Fig. 5. Conditioned Place Preference Test

Fig. 6. Locomotor Test

Fig. 7. Forced Swim Assay

Results

Pain and Behavioral Assessments

Fig. 8. Screen of lead compounds in the acetic acid-induced writhing assay. Results are shown as the mean $\pm$ 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, $p < 0.05$.

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 $\pm$ 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 operant 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. 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. 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 each trial (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 $\pm$ SEM.

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, $F(3,30)=11.7$, $p<0.0001$), 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 [$F(1,10)=94.7$, $p<0.0001$] and time [$F(3,30)=41.8$, $p<0.0001$] were also significant. Data are expressed as mean $\pm$ SEM with $n = 8$.

ProNeura Administration

Fig. 14. The ProNeura™ implant allows for the continuous release of drug into the patient's body through the process of dissolution. This results in a stable level of medication in the blood, avoiding peaks and troughs as seen in oral dosing. The ProNeura™ drug delivery platform allows for long-term treatment (3-12 months) in an outpatient setting.

Fig. 15. Release characteristics of JT09-containing ProNeura™ implants in rats following subcutaneous (SC) implantation. Serum drug levels were analyzed using HPLC mass spectrometry.

Conclusion

The compound of study, JT09, is as efficacious as morphine in alleviating peripheral pain, while failing to produce undesired CNS-mediated activity such as sedation, dysphoria, tolerance, and addiction. There were no gross abnormalities in a necropsy after completing a 14-day multiple dose study.¹ JT09 is both orally active and acts as a peripherally restricted κ -opioid agonist, thus fitting the criteria of a new pain medication.

Efficacy of the compound in the ProNeura platform also allows for a new method of administration for chronic pain management. From studies in models of myocardial infarction, JT09 may also be a candidate for prevention and treatment of cardiovascular disease and events.

Future Directions

Pharmacokinetics studies

JT09 has shown to be orally available in rats; however, the gut environment of humans is much harsher and may jeopardize its bioavailability. Pharmacokinetics studies will be to determine if JT09 will have the potential of being administered orally in humans and how to optimize delivery. In vivo protocol will be performed to isolate the peptide in serum for HPLC analysis. Previous studies with maximum tolerated dose have given no evidence of toxicity from JT09.

Immunohistochemistry

Immunohistochemistry was used to assess whether κ -opioid receptors are co-localized on macrophages, fibroblasts, or endothelial cell in the setting of myocardial infarctions. Immunohistochemistry studies have shown that the kappa opioid receptor is present and active in response to myocardial infarctions. Continued research will be executed to test the application of the compound to treat cardiovascular disease.

Fig. 16. The kappa opioid receptor is upregulated by ischemia, shown by the receptors being present around the ischemic tissue.

References

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