Title: Sustained antinociceptive activity of MLB-001 in the rat tail flick model

Problem Statement: Opioids, particularly morphine, are one of the most effective analgesics used to treat postoperative or cancer pain. However, long-term opioid administration eventually reaches a dose ceiling due to the rapid onset of analgesic tolerance coupled with the slow development of tolerance to associated side effects, which eventually limits dose escalations and efficacy.[1] The need for an effective, long term analgesia remains.

Abstract: Tolerance occurs when a patient no longer responds to the same drug dose. The first indication of tolerance is most commonly a decrease in the duration of analgesia (relief from pain) followed by a decrease in analgesic effect. The continued development of tolerance over time requires continually progressive increases in drug doses to maintain pain relief, which increases side effects and often leads to increased opioid use, thereby making its long-term use counterproductive.

MLB-001 is a novel pharmaceutical composition of morphine sulfate (MS), dextromethorphan (DM) and quinidine (Q) that addresses issues of tolerance. Dextromethorphan potentiates the morphine analgesia, permitting pain control at a significantly lower dose of morphine[2,3], while quinidine blocks the extensive first-pass metabolism of dextromethorphan and improves its therapeutic effectiveness.[4] Pain control is maintained with a significantly lower dose of morphine thereby attenuating the development of opioid tolerance and ameliorating associated side effects.

MLB-001 has repeatedly been shown to be more effective at blocking the detection of pain in the rat tail flick model compared to MS alone, or in combination with DM, and has also demonstrated a more durable response.

Background: Insights into the mechanisms underlying the development of tolerance to the analgesic effects of morphine and physical dependence on morphine have indicated the involvement of the N-methyl-D-aspartate (NMDA) receptor. Blockade of this receptor with an antagonist (a non-competitive NMDA receptor channel blocker) reduced or prevented the development of tolerance and dependence.[5] However, the clinical use of most NMDA receptor antagonists is unlikely due to their toxicity. A non-toxic, well-studied, NMDA receptor antagonist, such dextromethorphan (DM), can antagonize the NMDA receptor without toxic side effects.

Dextromethorphan potentiates morphine analgesia and combinations of DM and morphine have been shown to provide pain control at a significantly lower dose of morphine.[3] Further studies have also shown that in contrast to potentiation of analgesia, DM does not enhance the abuse potential of morphine but rather the DM component may inhibit the development of tolerance and dependence to the opioid.[3] Dextromethorphan has also been reported to decrease the self-administration of several drugs of abuse, including morphine, methamphetamine, cocaine, and nicotine. Most drugs of abuse increase extracellular levels of dopamine (DA) in the shell of the nucleus accumbens. Pretreatment with DM was shown to attenuate the effects of chronic morphine on nucleus accumbens DA levels.[6] Placebo-controlled clinical studies have also reported that DM can ameliorate opioid withdrawal symptoms in a dose-dependent manner in human subjects.[7] These results all indicate that treatment that combines DM with opioid analgesics may be a powerful approach for simultaneously decreasing opioid cravings and likeability, preventing opioid tolerance and dependence, and enhancing analgesia in humans.

However, the therapeutic efficacy of dextromethorphan is limited by its extensive first-pass metabolism. Co-administration with quinidine (Q), a potent cytochrome P450 2D6 inhibitor, has been shown to block DM metabolism and improve its therapeutic effectiveness. Therefore, we are developing an optimized composition of morphine, dextromethorphan and quinidine (MLB-001) that has enhanced pain relief at

a lower dose of morphine, an improved side effect profile, and less addictive potential than other opioids.

Solution: Rodents are commonly used to study mechanisms of pain with methods that quantify "pain-like" behaviors or nociception. The tail flick test involves application of a heat stimulus to the tail of mice and rats, and the time taken for the tail to "flick" or twitch is recorded.[8] Tail flick testing was used to assess the analgesic activity of MLB-001 in rats.

A prior study showed that once daily treatment with MS:DM:Q at a ratio of 1:1:1 (each administered at a dose of 25 mg/kg) had significant antinociceptive activity 1, 3 and 5 hours post compound administration. Suggesting that MLB-001 has the potential to increase the analgesic effect at a reduced dose of morphine. The current study was designed to determine the duration of pain relief and the tolerance effect with MLB-001.

Eighteen experimental groups were evaluated during the study (Table 1). Each group contained seven male rats. For this study the ED_{90} of morphine (37 mg/kg) was evaluated alone and in combination to determine if there was a synergistic or additive effect with dextromethorphan and quinidine. Treatments were administered twice daily (BID) in each group for the duration of the study. Naloxone, which is used to diagnose opioid dependence was administered IP at a dose of 10mg/kg after 5, 10, 15 or 30 days of repeated treatment dosing as shown for groups 3-18 in Table 1.

Table 1: Experimental Groups.

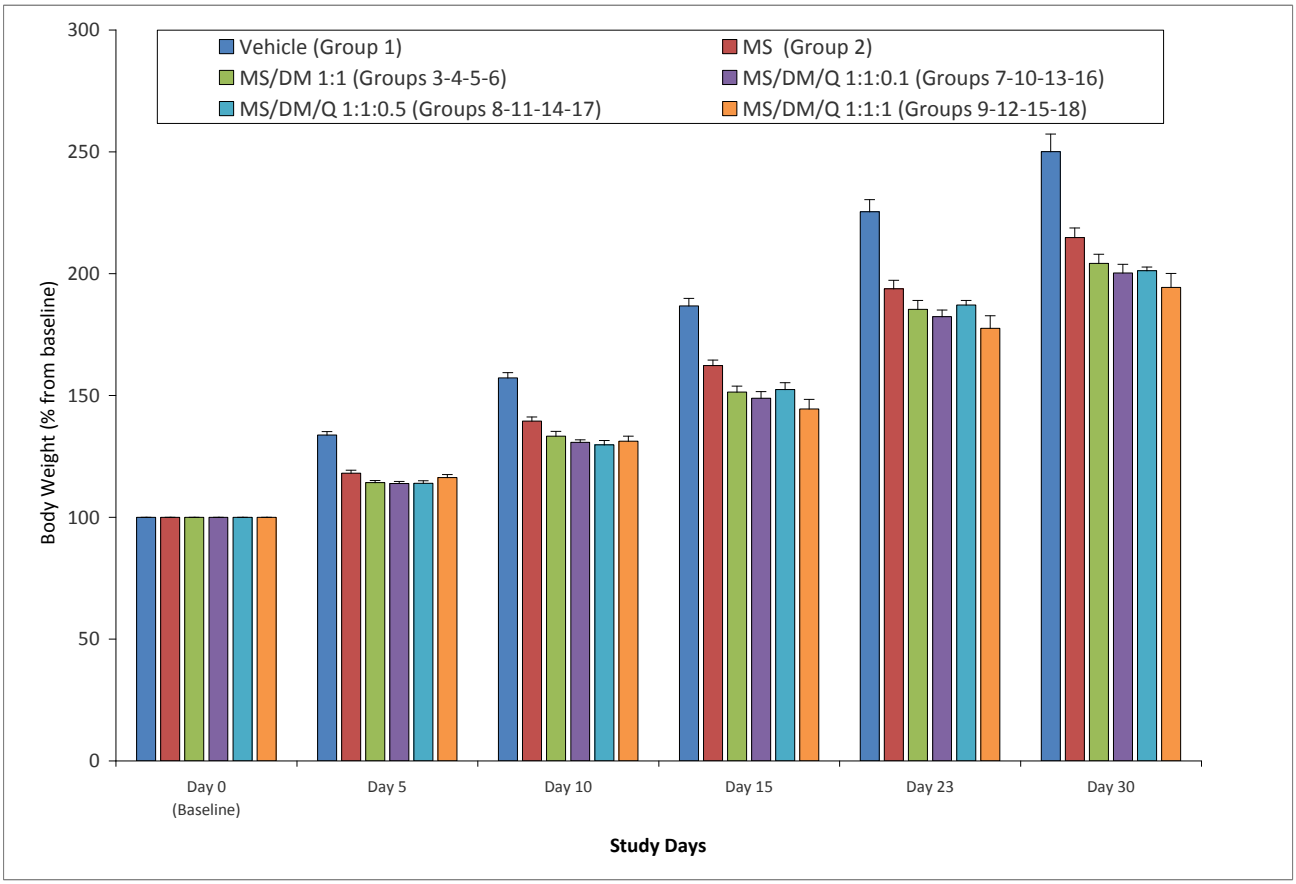
Group #	Treatment	BID Dose (mg/kg)
1	Vehicle	0
2	Morphine Sulfate (MS)	37 mg/kg
3*, 4#, 5^, 6'	MS + Dextromethorphan (DM) 1:1 (W:W)	37 mg/kg : 37 mg/kg
7*, 10#, 13^, 16'	MS/DM/Quinidine Sulfate (Q) [ratio 1:1:0.1]	37 mg/kg : 37 mg/kg : 3.7 mg/kg
8*, 11#, 14^, 17'	MS/DM/Q [ratio 1:1:0.5]	37 mg/kg : 37 mg/kg : 18.5 mg/kg
9*, 12#, 15^, 18'	MS/DM/Q [ratio 1:1:1]	37 mg/kg : 37 mg/kg : 37 mg/kg

* Naloxone challenge day 5
# Naloxone challenge day 10
^ Naloxone challenge day 15
' Naloxone challenge day 30

The challenge with Naloxone was performed after completion of the PM tail flick test. Following the Naloxone challenge the animals were observed for 10 minutes for signs of dependence and the relevant group was terminated on that study day (e.g. 5, 10, 15, and 30). At all-time points signs of opioid dependence were observed in the morphine and combinations groups (Groups 2-18).

Body weights were measured prior to baseline testing on study day 0, to calculate dose concentration and to assure weight variation of animals at the time of treatment initiation did not exceed ± 20% of the mean weight. Body weights were taken again on study days 5, 10, 15, 23 and 30 (Figure 1).

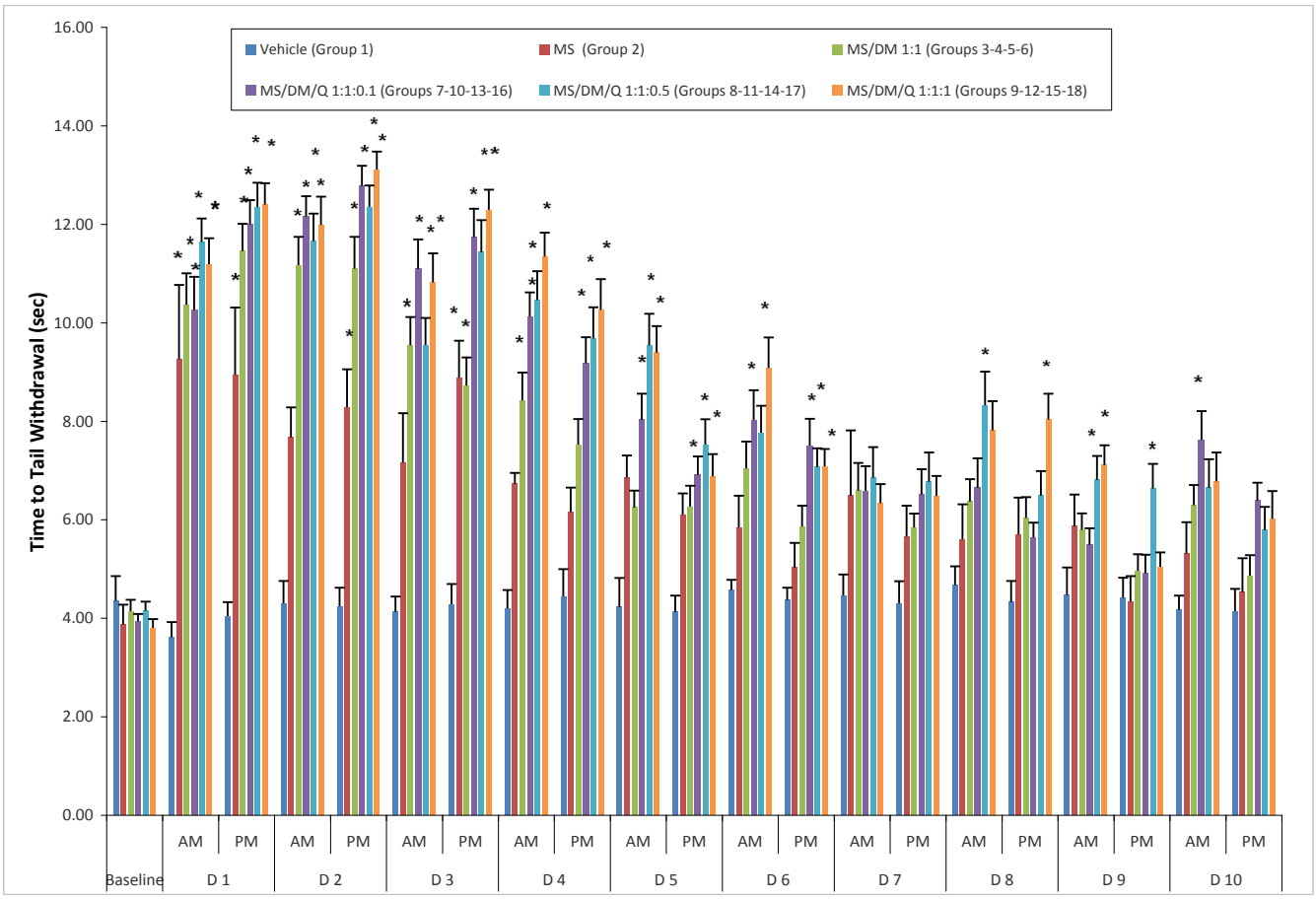
Figure 1: Mean body weight (% from baseline).



Response to pain was assessed using the Ugo Basile Tail Flick instrument. On study day 0, animals were placed on the Ugo Basile Tail Flick instrument surface and held gently in such a way that beneath their tails there is a photoelectric cell that serves as heat source. The latency time until the animals flick their tails from this heat source (35ºC) is measured in seconds. The maximum exposure to the heat source was 14 seconds. If the animal did not flick its tail by this time the animal was removed from the heat source and tail flick time recorded as 14 seconds. The tail flick test was performed twice each day (AM and PM), 60 minutes post treatment administration.

All data are presented as Mean ± SEM. Each treatment group is compared to vehicle (group 1) using one-way ANOVA followed by Tukey post-test. A p value < 0.05 is considered to represent a significant difference.

Figure 2: Mean± SEM group latency time (time to tail withdrawal) days 1-10.



* p<0.05 vs. Vehicle (Group 1)

The mean group tail flick latency time following morphine administration at the ED_{90} dose of 37 mg/kg was 9.26 ± 1.51 seconds 1-hour post AM dosing vs. vehicle 3.61 ± 0.31 seconds on study day 1 (p<0.05, Figure 2). The activity of morphine decreased after 3 days of BID administration. On study day 4, there was no statistical difference between the mean group latency time recorded following vehicle treatment and following MS treatment (Figure 2).

The potentially synergistic or additive analgesic effect of morphine in combination with dextromethorphan and quinidine was evaluated. The two most effective combination treatments were with Morphine Sulphate: Dextromethorphan: Quinidine (MS:DM:Q) at a ratio of 1:1:1 (each administered at a dose of 37 mg/kg; and at a ratio of 1:1:0.5 (MS and DM administered at a dose of 37 mg/kg and Q at dose of 18.5 mg/kg), Figure 2. The effect of these treatments was significant until day 9 (7.11 ± 0.40 sec and 6.81 ± 0.48 sec vs. 4.47 ± 0.56 sec., respectively for the vehicle group (p<0.05, Figure 2). On study day 10 the latency time was no longer statistically different than for the vehicle treated animals 6.78 ± 0.58 sec. and 6.65 ± 0.58 sec respectively vs. 4.17 ± 0.29 sec for the vehicle group (Figure 2). Results from study days 11-30 are located in the appendix, Figures 3 & 4.

This study confirmed previous findings that showed that treatment with MS:DM:Q at a ratio of 1:1:1 has more antinociceptive activity than morphine alone. This study also showed that MS:DM:Q at a ratio of 1:1:0.5 has more antinociceptive activity than morphine alone, when dosed BID. It was further shown that

both MS:DM:Q ratios (1:1:1 and 1:1:0.5) maintained their antinociceptive actively longer (study day 9) than morphine alone (study day 4), indicating that MLB-001 has the potential to address the issue of opioid tolerance. Additional studies designed to show that MLB-001 has less addictive potential than other opioids are warranted.

Conclusion: To ensure that no one suffers from pain without adequate treatment it is imperative to identify methods that enhance pain relief while simultaneously decreasing opioid cravings and likeability, and preventing opioid tolerance and dependence. MLB-001, a novel opioid analgesic, holds that promise. We have shown that MLB-001 not only has more antinociceptive activity than morphine alone, but that it sustains that antinociceptive activity longer, thereby controlling pain at a significantly lower opioid dose for a longer duration. These findings coupled with the scientific literature that supports the hypothesis that the pharmaceutical components of MLB-001 that potentiate the analgesia do not enhance the abuse potential of morphine and may actually inhibit the development of tolerance and dependence,[3,6,7] suggest that MLB-001 has life changing pain relief potential.

Citations:

1. Dumas EO, Pollack GM. Opioid Tolerance Development: A Pharmacokinetic/Pharmacodynamic Perspective. *The AAPS Journal.* 2008;10(4):537.

2. Manning BH, Mao J, Frenk H, Price DD, Mayer DJ. Continuous co-administration of dextromethorphan or MK-801 with morphine: Attenuation of morphine dependence and naloxone-reversible attenuation of morphine tolerance. *Pain.* 1996;67:79-88.

3. Chevlen E. Morphine with dextromethorphan: Conversion from other opioid analgesics. *Journal of Pain and Symptom Management.* 2000;19:42-49.

4. Zhang Y, Britto MR, Valderhaug KL, Wedlund PJ, Smith RA. Dextromethorphan: Enhancing its systemic availability by way of low-dose quinidine-mediated inhibition of cytochrome P4502D6. *Clinical Pharmacology & Therapeutics.* 1992;51:647-655.

5. Mao J, Price DD, Caruso FS, Mayer DJ. Oral administration of dextromethorphan prevents the development of morphine tolerance and dependence in rats. *Pain.* 1996;67:361-368.

6. Steinmiller CL, Maisonneuve IM, Glick SD. Effects of dextromethorphan on dopamine release in the nucleus accumbens: Interactions with morphine. *Pharmacology, biochemistry, and behavior.* Mar 2003;74(4):803-810.

7. Lee S-Y, Chen S-L, Chang Y-H, et al. A Placebo-Controlled Trial of Dextromethorphan as an Adjunct in Opioid-Dependent Patients Undergoing Methadone Maintenance Treatment. *International Journal of Neuropsychopharmacology. 2*015;18(7).

8. D'Amour FES, D. L. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther.* 1941;72:74-79.

Appendix

Figure 3: Mean± SEM group latency time (time to tail withdrawal) days 11-20.

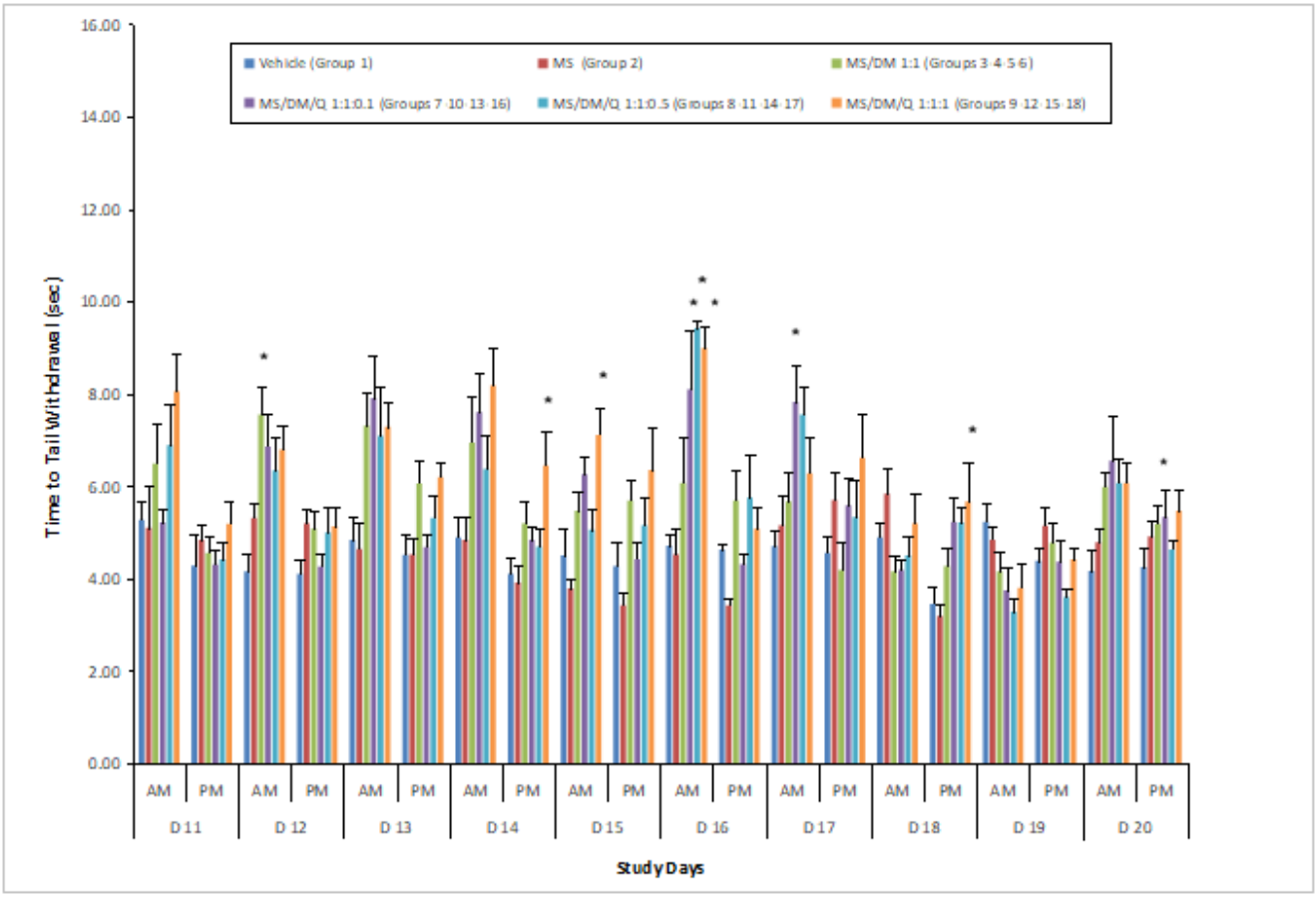


Figure 4: Mean± SEM group latency time (time to tail withdrawal) days 21-30.