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

Problem Statement: Pain is the most common reason for seeking medical care, with more than 25 million Americans suffering daily from chronic pain.[1] Opioids have been regarded for decades as among the most effective drugs for the treatment of pain[2]. Their use in the management of acute severe pain and chronic pain related to advanced medical illness is considered the standard of care in most of the world.[2] However, concerns related to effectiveness, safety, and abuse liability dictate the need for a new approach to opioids as a therapeutic product.

Abstract: To ensure that no one suffers from pain without adequate treatment it is imperative to identify methods that would enhance pain relief while reducing the likelihood of addiction and other adverse events when opioids are selected for therapy. MLB-001, a novel opioid analgesic, holds that promise.

Opioids taken at a sufficient analgesic dose can cause adverse effects, including sedation, dizziness, nausea, vomiting, constipation, and respiratory depression.[3] Reducing the opioid dose can ameliorate many of these side effects, however the patient then experiences inadequate pain relief. Tolerance, physical dependence, and addiction are concerns that may also prevent proper prescribing and in turn inadequate pain management.[3] 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[4,5], while quinidine blocks the extensive first-pass metabolism of dextromethorphan and improves its therapeutic effectiveness.[6] 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 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 the potential to attenuate the development of opioid tolerance and likability.

Background: Tolerance, or a decrease in effectiveness over time with the same amount of drug, is a common complication of opioid treatment.[3,7] Tolerance requires an increasing amount of the drug to achieve the same effect. The first indication of tolerance is most commonly a decrease in the duration of analgesia (relief from pain) for a given opioid followed by a decrease in analgesic effect. When tolerance occurs a physician typically makes dose adjustments to increase the potency to once again provide adequate pain relief, this dose adjustment can increase side effects and often leads to increased opioid use.

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.[8] 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.[5] 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.[5] 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.[9] Placebo-controlled clinical studies have also reported that DM can ameliorate opioid withdrawal symptoms in a dose-dependent manner in human subjects.[10] 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. As stated by Zhang[6] for an inhibitor of drug elimination to be of therapeutic benefit, several criteria must be satisfied. First, the inhibition must be specific. Second, the inhibitor should have a low potential for activity and toxicity at the doses used. Finally, the therapeutic need for an inhibitor should be such that therapeutic demands cannot easily be met by a simple dosage adjustment. The Q-DM interaction appears to meet each of these three criteria, 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 due to an effective dose that is considerably lower than that used currently for chronic pain.

Solution: Rodents are commonly used to study mechanisms of pain as studies in humans may be difficult to perform and ethically limited.[11] As pain cannot be directly measured in rodents, many methods that quantify “pain-like” behaviors or nociception have been developed.[11] 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.[12] Tail flick testing was used to assess the analgesic activity of MLB-001 in rats.

Seven experimental groups were evaluated during the study (Table 1). Each group contained seven male rats. For this study the ED_{50} of morphine (25 mg/kg) was evaluated alone and in combination to determine if there was a synergistic or additive effect with dextromethorphan and quinidine.

Table 1: Experimental Groups.

Group #	Treatment	Dose (mg/kg)
1	Vehicle	0
2	Morphine Sulfate (MS)	25 mg/kg
3	Dextromethorphan (DM)	25 mg/kg
4	MS +DM 1:1 (W:W)	25 mg/kg : 25 mg/kg
5	MS/DM/Quinidine Sulfate (Q) [ratio 1:1:0.1]	25 mg/kg : 25 mg/kg : 2.5 mg/kg
6	MS/DM/Q [ratio 1:1:0.5]	25 mg/kg : 25 mg/kg : 12.5mg/kg
7	MS/DM/Q [ratio 1:1:1]	25 mg/kg : 25 mg/kg : 25 mg/kg

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 (Table 2).

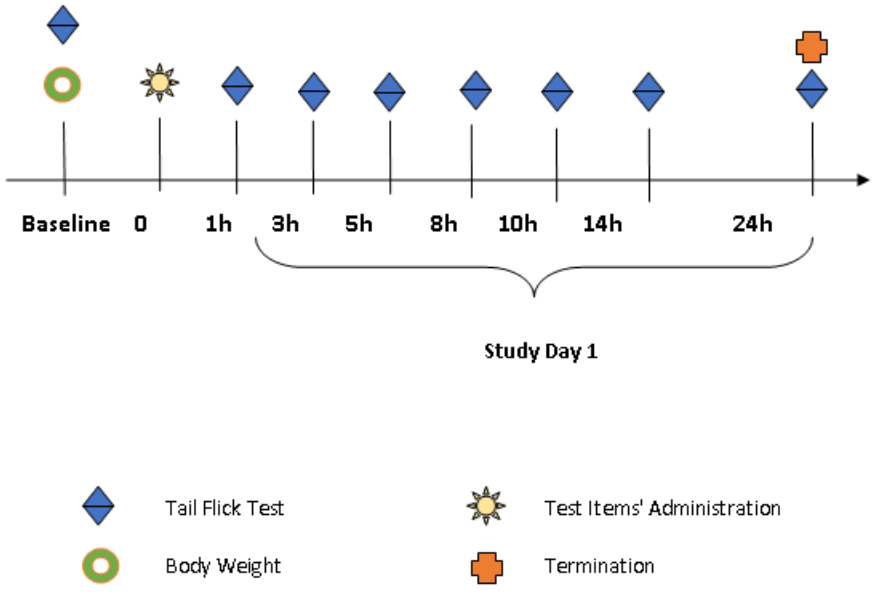
Table 2: Mean group body weight (grams).

Group #	Treatment	Mean	SEM*
1	Vehicle	224.57	3.29
2	Morphine Sulfate (MS)	218.74	3.91
3	Dextromethorphan (DM)	214.79	4.39
4	MS +DM 1:1 (W:W)	228.53	4.42
5	MS/DM/Quinidine Sulfate (Q) [ratio 1:1:0.1]	223.64	2.97
6	MS/DM/Q [ratio 1:1:0.5]	229.00	4.31
7	MS/DM/Q [ratio 1:1:1]	218.29	3.91

* Standard error of the mean

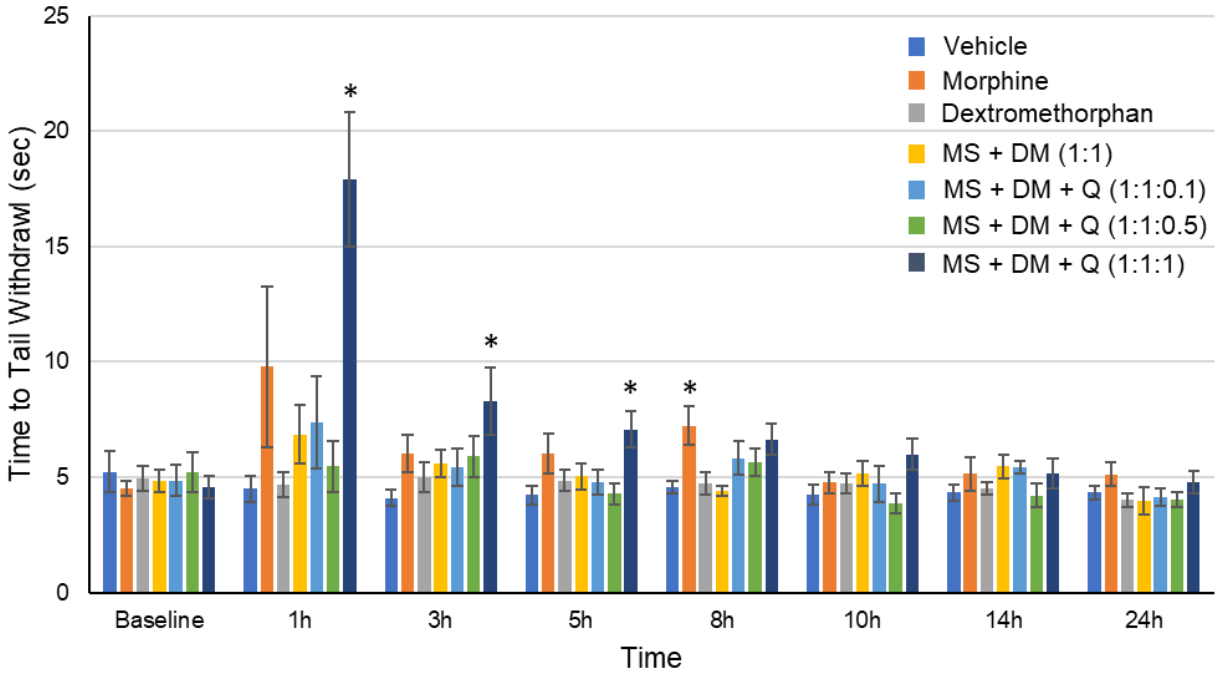
Response to pain was assessed using the Ugo Basile Tail Flick instrument. On study day 0, animals were placed on the Ugo Basile Tail Flick instrument surface and held gently in such a way that beneath their tails there is a photoelectric cell that serves as heat source. The latency time until the animals flick their tails from this heat source (42ºC) is measured in seconds. The maximum exposure to the heat source was 30 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 30 seconds. On study day 1, animals were dosed (treatments, Table 1), and the tail flick test was performed at 1, 3, 5, 8 ,10,14 and 24 hours post dosing (Figure 1).

Figure 1: Schematic depiction of study and treatment



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 measured using the tail flick (sec).



* p<0.05 vs. Vehicle (Group 1); numerical values in appendix, Table 3

The analgesic effect, as determined by the tail flick latency time, of morphine administered at a dose of 25 mg/kg was 9.79 ± 3.48 seconds 1-hour post dosing (Figure 2). This value was not statistically higher than the mean value of the vehicle group (4.49 ± 0.55 seconds) due to the relatively high variability anticipated when dosing a compound at its ED_{50}. The activity of morphine alone decreased at 3-hours post-administration.

The potentially synergistic or additive analgesic effect of morphine in combination with dextromethorphan and quinidine was evaluated. The most effective combination treatment was with Morphine Sulphate: Dextromethorphan: Quinidine (MS:DM:Q) at a ratio of 1:1:1 (each administered at a dose of 25 mg/kg). At 1-hour post compound administration the mean group tail flick latency time was 17.90 ± 2.92 seconds, which was statistically longer than the mean tail flick latency time recorded following treatment with morphine (9.79 ± 3.48 sec.) or dextromethorphan (4.67 ± 0.52 sec.) alone (Figure 2). The effect of this treatment (MS:DM:Q at a ratio of 1:1:1) was still significant ($p<0.05$) when compared to the vehicle treatment group 5-hours post dosing (7.06 ± 0.79 sec. vs. 4.23 ± 0.41 sec. for the vehicle group), Figure 2.

This study showed that 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, thereby reducing the risk of adverse effects. Additional studies designed to determine the duration of pain relief and the tolerance effect with MLB-001 are warranted.

Conclusion: Pain is a universal human experience that in the short term serves to protect an individual from harm, but in the long term can become a debilitating condition that is difficult to treat with the currently available analgesics often lacking efficacy and suffering dose-limiting adverse effects. We have shown

that MLB-001 has more antinociceptive activity than morphine alone, thereby controlling pain at a significantly lower opioid dose. These initial findings, and 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,[5,9,10] suggest that MLB-001 may be a powerful approach for enhancing analgesia in humans while simultaneously decreasing opioid cravings and likeability, and preventing opioid tolerance and dependence.

Citations:

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Appendix

Table 3: Mean± SEM group latency time measured using the tail flick (sec).

Group #	Treatment	Baseline		1h		3h		5h	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	Vehicle	5.23	0.89	4.49	0.55	4.09	0.35	4.23	0.41
2	MS	4.50	0.33	9.79	3.48	6.01	0.81	6.04	0.86
3	DM	4.93	0.53	4.67	0.52	5.00	0.66	4.86	0.47
4	MS +DM 1:1	4.84	0.50	6.84	1.27	5.59	0.60	5.03	0.57
5	MS/DM/Q 1:1:0.1	4.86	0.67	7.36	2.00	5.43	0.80	4.77	0.53
6	MS/DM/Q 1:1:0.5	5.21	0.86	5.46	1.13	5.90	0.89	4.27	0.47
7	MS/DM/Q 1:1:1	4.56	0.48	17.90*	2.92	8.29*	1.45	7.06*	0.79
Group #	**Treatment**	**8h**		**10h**		**14h**		**24h**	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	Vehicle	4.57	0.25	4.24	0.42	4.33	0.37	4.33	0.30
2	MS	7.24*	0.83	4.77	0.47	5.14	0.71	5.13	0.51
3	DM	4.74	0.50	4.71	0.43	4.51	0.29	4.00	0.29
4	MS +DM 1:1	4.41	0.21	5.17	0.54	5.46	0.52	3.99	0.59
5	MS/DM/Q 1:1:0.1	5.83	0.75	4.71	0.80	5.43	0.27	4.13	0.36
6	MS/DM/Q 1:1:0.5	5.66	0.59	3.86	0.45	4.21	0.51	4.04	0.32
7	MS/DM/Q 1:1:1	6.64	0.66	5.99	0.69	5.17	0.65	4.77	0.50