Ultra-Low-Dose Naltrexone Plus Morphine Blocks Thermal Hyperalgesia and Attenuates Mechanical Hypersensitivity in a Neuropathic Pain Model

Pain Therapeutics, Inc.

Todd W. Vanderah[1] and Lindsay H. Burns[2]

[1]University of Arizona, Tucson, AZ; [2]Pain Therapeutics, Inc., South San Francisco, CA.

ABSTRACT

Neuropathic pain is a pathological and chronic condition characterized by hyperalgesia (enhanced perception of painful stimuli) and allodynia (innocuous stimuli perceived as painful). Current therapies for neuropathic pain are limited by side effects and insufficient efficacy. Ultra-low-dose opioid antagonists can enhance opioid analgesia and prevent tolerance in rodent nociceptive pain assays. The current work examined morphine plus ultra-low-dose naltrexone (NTX) in the rat L5/L6 spinal nerve ligation (SNL) model of neuropathic pain. After the development of mechanical and thermal hypersensitivities, rats received intrathecal twice daily injections of vehicle, morphine (10µg) or morphine (10µg) + NTX at two different ratios of morphine to NTX for 7 days. An additional group received morphine + NTX at the higher NTX dose once daily for 7 days. Animals were tested every day 30 min. after the a.m. injection. All groups receiving morphine + NTX showed significant anti-hyperalgesia and attenuated tolerance to this effect compared to morphine alone. Most significantly, morphine plus the higher NTX dose administered twice daily resulted in no anti-hyperalgesic tolerance over this 7-day period. While spinal morphine alone had no effect on mechanical hypersensitivity, the combination of spinal morphine + NTX resulted in a significant anti-allodynic effect on Day 1 with trends of activity on Days 2 and 3 of administration. In conclusion, these studies demonstrate that intrathecal morphine plus ultra-low-dose NTX is significantly more efficacious in preventing hyperalgesia than morphine alone and does not result in tolerance over the 7-day period of testing. In addition, the combination of morphine + NTX resulted in significant, although transient, anti-allodynic activity. These findings suggest that excitatory signaling opioid receptors, shown to occur in opioid tolerance, may also be present in neuropathic pain. Thus, while morphine is typically insufficient or ineffective in neuropathic pain, its combination with ultra-low-dose NTX may provide novel efficacy for this indication.

INTRODUCTION

The present work is the first investigation of an opiate combined with an ultra-low-dose opioid antagonist in a model of neuropathic pain. Ultra-low-dose opioid antagonists have been shown to enhance opioid analgesia and alleviate tolerance and withdrawal, including reversing the hyperalgesia associated with tolerance and withdrawal in rodents (e.g. Crain & Shen, 1995; Powell et al, 2002). Increased spinal dynorphin and activation of descending pain facilitating pathways of the rostral ventral medulla (RVM) has been demonstrated to underlie hyperalgesia in analgesic tolerance (Vanderah et al., 2000 & 2001) and in neuropathic pain (Porreca et al., 2001; Wang et al, 2001), although the underlying pathology of neuropathic pain is poorly understood. Ultra-low-dose opioid antagonists have been shown to suppress an aberrant excitatory signaling of opioid receptors that occurs in tolerance (Crain & Shen 1995; Wang et al, 2003) but that is not known to occur in neuropathic pain.

METHODS

Experimental Design

Male Sprague-Dawley 200g rats underwent intrathecal (i.th.) catheterization and were tested for baseline thermal and tactile sensitivity. Rats then underwent SNL surgery, a tight ligation of L5 and L6 spinal nerves, and were tested for thermal and tactile hypersensitivities 5 and 7 days later. Starting 8 days post-SNL, rats received i.th. injections for 7 days and were tested 20-30 min. after the a.m. dose for anti-allodynic and anti-hyperalgesic effects. Treatment groups are below:

Treatment Group	# of Rats	Morphine Dose	NTX Dose	Dose Frequency
Vehicle	12	0	0	Twice daily
NTX alone	6	0	0.33 ng	Twice daily
Morphine alone	6	10 µg	0	Twice daily
1:100K	6	10 µg	0.1 ng	Twice daily
1:33K	6	10 µg	0.33 ng	Twice daily
1:33K once daily	6	10 µ	0.33 ng	*Once* daily

Measurement of Anti-hyperalgesia

Paw withdrawal latency to a thermal nociceptive stimulus was used to assess hyperalgesia. Rats were contained in Plexiglas compartments and a radiant heat source aimed at the plantar surface of the paw through a glass plate. The withdrawal latency is measured by a motion detector that halts both lamp and timer when the paw is withdrawn. Hyperalgesia was indicated by a decrease in latency from a baseline of ~16 s. Percent anti-hyperalgesia, using a maximum of 100% for each animal, and percent anti-nociception were calculated as follows:

$$\%\ \text{Anti-hyperalgesia} = \frac{\text{(Treatment-induced latency - SNL baseline)}}{\text{(Pre-injury baseline - SNL baseline)}}$$

$$\%\ \text{Anti-nociception} = \frac{\text{(Treatment-induced latency - Pre-injury baseline)}}{\text{(Pre-injury baseline)}}$$

Measurement of Anti-allodynia

Allodynia or tactile hypersensitivity (i.e. sensitivity to non-noxious mechanical stimuli) was measured by the withdrawal threshold of the injured paw in response to probing with a series of Von Frey filaments with bending strengths from 0.41 to 15 g. Flinching at the lowest bending force indicates full-blown allodynia. Mean withdrawal threshold was determined by sequentially increasing and decreasing the stimulus strength and analyzing responses using a Dixon non-parametric test. Percent anti-allodynia was calculated as follows:

$$\%\ \text{Anti-allodynia} = \frac{\text{(Treatment-induced threshold - SNL baseline)}}{\text{(Pre-injury baseline - SNL baseline)}}$$

RESULTS

Treatment Effects on Hyperalgesia:

Neither vehicle nor NTX alone had any effect on hyperalgesia or allodynia. All groups receiving morphine + NTX showed significant anti-hyperalgesia compared to vehicle or morphine alone for the week of testing ($p<0.001$ for AUCs, Fig.1). The % anti-hyperalgesia (Fig.2) and % anti-nociception (Fig.3) of each morphine + NTX group were also significantly greater than those of morphine alone or vehicle ($p<0.001$).

Fig. 1

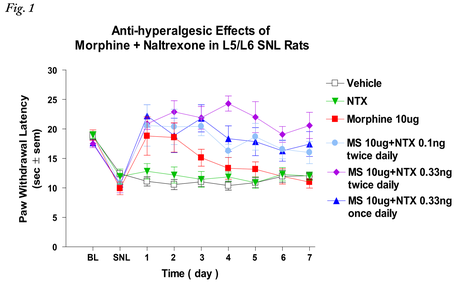

Fig. 1. The anti-hyperalgesic effects of morphine (MS) alone or in combination with NTX in the L5/L6 SNL model. Compound administration started on Day 1 (7 days post-SNL) and continued through Day 7. All morphine + NTX groups produced significantly greater analgesia or anti-hyperalgesia than MS or vehicle ($p<0.001$ for AUCs).

Fig. 2

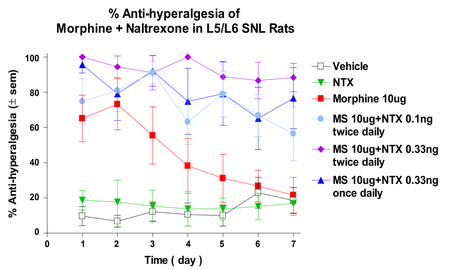

Fig. 2. The % anti-hyperalgesic effects of morphine (MS) alone or in combination with NTX in the L5/L6 SNL model. All morphine + NTX groups produced significant anti-hyperalgesia or analgesia compared to vehicle ($p<0.001$ for AUCs).

Fig. 3

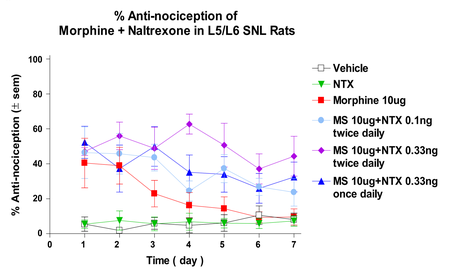

Fig. 3. The % anti-nociception of morphine (MS) alone or in combination with NTX in the L5/L6 SNL model. All morphine + NTX groups produced significant anti-nocicpetion compared to vehicle ($p<0.001$ for AUCs).

Morphine + NTX at 1:100,000:

Although morphine alone resulted in 65 and 73% anti-hyperalgesia on Days 1 and 2, respectively, with return to baseline by Day 5, morphine + NTX (0.1ng or 1:100,000) resulted in 75, 81, 91, 63, 79, 67 and 56% anti-hyperalgesia on Days 1 - 7, respectively (Fig.2). Many individual animals in this group showed 100% anti-hyperalgesia plus anti-nociception, i.e. paw withdrawal latencies above pre-injury baselines, and the % anti-nociception for this group was significantly greater than for morphine alone or vehicle (Fig.3). Morphine alone resulted in tolerance to the anti-hyperalgesia by Day 4 (38% activity); whereas, morphine + NTX at this dose decreased hyperalgesia by over 70% through Day 5 and by over 50% on Days 6 and 7.

Importantly, it is well known that i.th. morphine alone has no anti-allodynic effect (similar to what is reported clinically), yet morphine + NTX at this dose resulted in statistically significant anti-allodynia on Day 1 ($p=0.02$) with trends ($p=0.15$) toward efficacy on Days 2 and 3 (Figs. 4 & 5).

Morphine + NTX at 1:33,000:

The combination of morphine + NTX (0.33ng or 1:33,000) twice daily resulted in 100, 94, 91, 100, 89, 87 and 88% anti-hyperalgesia on Days 1 - 7, respectively (Fig.2), as well as anti-nociception on all days (Fig. 3). No tolerance was seen with morphine + NTX at this dose given twice daily. In addition, this treatment produced an anti-allodynic effect on Day 1 ($p=0.02$) with trends ($p=0.06$) toward anti-allodynic efficacy on Days 2 and 3 (Figs. 4 & 5).

The combination of morphine + NTX (0.33ng or 1:33,000) once daily resulted in 96, 79, 92, 75, 79, 65 and 77% anti-hyperalgesia on Days 1 - 7, respectively (Fig. 2), as well as anti-nociception in several animals on all days (Fig. 3). No significant tolerance was seen from Day 1 - 5. The slightly decreased effect on Day 6 could reflect a decrease in potency, but the 77% anti-hyperalgesia observed on Day 7 suggests a lack of analgesic tolerance overall for this group. The once daily treatment also resulted in anti-allodynia on Day 1 ($p=0.02$) with trends ($p=0.06$) of anti-allodynia on Days 2 and 3.

Fig. 4

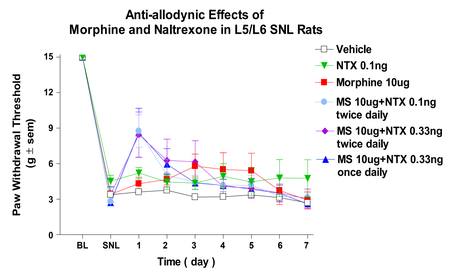

Fig. 4. The anti-allodynic effects of morphine (MS) alone or in combination with NTX in the L5/L6 SNL model. Compound administration started on Day 1 (7 days post-SNL) and continued through Day 7. All morphine + NTX groups produced a significant effect on Day 1 ($p=0.02$).

Fig. 5

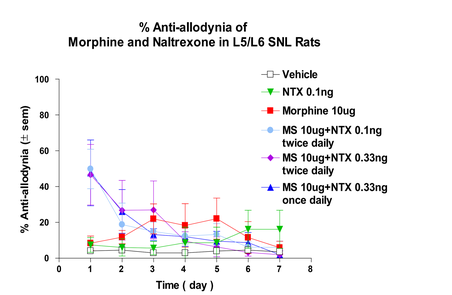

Fig. 5. The % anti-allodynia of morphine (MS) alone or in combination with NTX in the L5/L6 SNL model. All morphine + NTX groups produced a significant anti-allodynic effect on Day 1 ($p<0.001$).

DISCUSSION

While opioids remain the primary drugs of choice for moderate to severe pain, their use in chronic pain is hampered by analgesic tolerance. Ultra-low-dose opioid antagonists combined with opiates prevent analgesic tolerance as well as reverse paradoxical hyperalgesia caused by low doses of opiates (Crain & Shen, 2001). Neuropathic pain and chronic opioid administration result in hyperalgesia, and these two syndromes also share some biological correlates (Vanderah et al., 2000 & 2001). However, neuropathic pain is very often intractable, usually of greater severity, and most often treated with antidepressants or anticonvulsants. Although prolonged opioid treatment for neuropathic pain has been ineffective, the present study shows encouraging results with morphine + ultra-low-dose naltrexone in an animal model of neuropathic pain.

Morphine in combination with ultra-low-dose NTX was significantly more efficacious and did not cause tolerance to the anti-hyperalgesic effect compared to morphine alone. In addition, although morphine alone is completely ineffective against allodynia in the SNL model when given intrathecally to avoid sedative effects, intrathecal morphine + ultra-low-dose NTX elicited significant anti-allodynic activity. From a mechanistic perspective, the efficacy of morphine + ultra-low-dose NTX in this model suggests that neuropathic pain and the hyperalgesia of morphine tolerance or of low-dose opiates share a common mechanism, perhaps excitatory signaling opioid receptors. Morphine + ultra-low-dose NTX not only attenuates or prevents the development of analgesic tolerance but also provides a significant anti-hypersensitivity to mechanical and thermal stimuli. The increased and prolonged efficacy compared to morphine alone, along with the transient anti-allodynic effect demonstrated here, suggests that this combination therapeutic holds promise as an improved treatment for neuropathic pain.

REFERENCES

Crain KF & Shen SM (1995). Ultra-low concentrations of naloxone selectively antagonize excitatory effects of morphine on sensory neurons, thereby increasing its antinociceptive potency and attenuating tolerance/dependence during chronic cotreatment. *PNAS* **92**:10540-10544.

Crain SM & Shen KF (2001). Acute thermal hyperalgesia elicited by low-dose morphine in normal mice is blocked by ultra-low-dose naltrexone, unmasking potent opioid analgesia. *Brain Res* **888**:75-82.

Porreca F, Burgess SE, Gardell LR, Vanderah TW, Malan TP, Lappi DA, Lai J (2001). Inhibition of neuropathic pain by selective ablation of brainstem medullary cells expressing the mu-opioid receptor. *J Neurosci* **21**: 5281-5288.

Powell KJ, Abul-Husn NS, Jhamandas A, Olmstead MC, Beninger RJ, Jhamandas K. (2002). Paradoxical effects of the opioid antagonist naltrexone on morphine analgesia, tolerance, and reward in rats. *JPET* **300**:588-596.

Vanderah TW, Gardell LR, Burgess SE, Ibrahim M, Dogrul A, Zhong C-M, Zhang E-N, Malan TP, Ossipov MH, Lai J, Porreca F (2000). Dynorphin promotes abnormal pain and spinal opioid antinociceptive tolerance. *J Neurosci* **20**: 7074-7079.

Vanderah TW, Ossipov MH, Lai J, Malan TP, Porreca F (2001). Mechanisms of opioid-induced pain and antinociceptive tolerance: descending facilitation and spinal dynorphin. *Pain* **92**: 5-9.

Wang H-Y, Burns LH, Friedman E (2003). Chronic morphine induces mu opioid receptor - G protein coupling switch that is prevented by ultra-low-dose naloxone. *SFN abstracts* 645.18.

Wang Z, Gardell LR, Ossipov MH, Vanderah TW, Brennan MB, Hochgeschwender U, Hruby VJ, Malan TP, Lai J, Porreca F (2001). Pronociceptive actions of dynorphin maintain chronic neuropathic pain. *J Neurosci* **21**: 1779-1786.