Nalbuphine attenuates itch in the substance P-induced mouse model

A. Hawi[1], R. Hunter[2], L. Morford[2] and T. Sciascia[1],
[1]TREVI Therapeutics, Inc., New Haven, CT USA [2] Covance Laboratories, Greenfield, IN USA

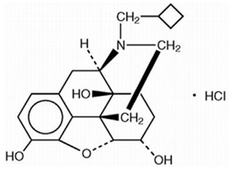
Abstract

Nalbuphine is a mixed mu antagonist/kappa agonist opioid. The effect of nalbuphine on substance-P (SubP) induced scratching was studied in the mouse model. This model is relevant to antihistamine-resistant pruritus and is observed in patients with various dermatopathologies.

Studies were conducted in male C57BL/6 mice treated subcutaneously (SC) dosed with vehicle (phosphate buffered saline, PBS) only or nalbuphine (10-30 mg/kg). Mice received either PBS or SubP (250nM in 0.050 mL) injected intradermally into the rostral part of the back and video recorded. Itching was scored by counting the number of scratches over 30 minutes following SubP (or vehicle) challenge.

Following SubP administration in the untreated mice, itching began within 3-5 minutes from the pruritogen administration with the highest itch intensity in the first 30 minutes post SubP. Following nalbuphine administration, a significant reduction in itch (p<0.001) was noted with a 43% reduction in itch at 10 mg/kg dose and 51% at the 30 mg/kg dose.

Ambulation was not suppressed in mice injected with nalbuphine doses up to 30 mg/kg indicating that attenuation of scratching was not due to decreased locomotor activity. These data suggest that nalbuphine holds promise as a potential anti-pruritic treatment.



μ-antagonist and κ-agonist

- Binding and functional activity studied in cloned mouse μ, δ, and κ opioid receptors expressed in human embryonic kidney (HEK) cell lines and the cloned human opioid receptors expressed in Chinese Hamster Ovary (CHO) cells.
- In the presence of an internal pool of β-endorphin, nalbuphine can act as an antagonist in inhibiting opioid μ-receptor activation.
- Nalbuphine is also a partial agonist at opioid κ-receptors with a high binding affinity of 6 nM comparable to that of the dynorphin endogenous opioid κ- receptor (5 nM).

Affinity (Ki), Potency (IC50) and Activity (% inhibition of forskolin stimulated adenylyl cyclase activity) in cells expressing individual mouse or human opioid receptors

		NALBUPHINE			MORPHINE			
Cell System	[3H] ligand	Ki (nM)	Forskolin-Stimulated Activity IC50 (nM)	Forskolin-Stimulated Activity Max Inh.(%)	Ki (nM)	Forskolin Stimulated Activity IC50 (nM)	Forskolin Stimulated Activity Max Inh (%)	Reference
HEK-μ[1]	DAMGO	--	8.4 ± 1.6	69 ± 4	2.2 ± 0.5	17 ± 8	48 ± 4	Gharagozlou et al, 2002
HEK-δ[1]	DP-DPE	242.5 ± 103	2039 ± 554	71 ± 7	68.5 ± 19.5	1101 ± 614	39 ± 4	Gharagozlou et al, 2003
HEK-κ[1]	U-69593	233 ±33	1677 ± 917	58 ± 9	26 ±3	213 ± 137	55 ± 5	Gharagozlou et al, 2006
CHO-μ[2]	DAMGO	0.700 ± 0.30	--	--	--	--	--	Toll et al, 1998
CHO- δ[2]	DPDPE	153 ± 38	--	--	--	--	--	Toll et al, 1998

Opioid Hypothesis: *Opioid Receptors Implicated in Itch Sensation*

- Specialized neuronal pathways mediating the sensation of itch may work through neuroanatomical interactions with pain mediating pathways at various CNS levels (Schmelz, 2005).
- Pain and itch sensation may result from neurophysiological interactions influenced by the interplay of mu and kappa opiate receptor biology (Schmelz, 2005).
- Mu and kappa opioid receptors are found on peripheral nerves and keratinocytes. Skin-peripheral nerve interactions may be involved in itch sensation process (Steinhoff, 2006).
- Nalbuphine administered intravenously is an effective agent in treatment of morphine induced pruritus.

Nalbuphine reduces Morphine-Induced Itch in Human

Opioid μ-receptor agonist induced pruritus syndrome likely has two separate mechanisms of action.

- A non-opioid receptor-mediated release of histamine from mast cells via a direct activation of guanine nucleotide-binding proteins ("G proteins").
  - Can be effectively treated with standard doses of a histamine antagonist.
- Opioid μ-receptor mediated pruritus.
  - Does not respond to histamine antagonist medications
  - Does respond to opioid μ-receptor antagonist class of medications

Nalbuphine possibly exerts its effect by antagonizing morphine opioid μ-receptor mediated pruritus. (Wang, 1998)

Nalbuphine effective at attenuating morphine-induced itch following IV administration at doses ranging between on average between 15 and 100 mg/day (p<0.01)

Cohen, 1992; Wang, 1998 & Liao, 2011

Would nalbuphine be effective in treating peripheral mediated itch?

Substance-P Itch Mouse Model

Substance-P (SubP) induced scratching behavior is a standard animal itch model widely used in preclinical studies.

- Peripheral stimulation by SubP mimics characteristics of itch-related scratches in humans.
- Scratching is not inhibited by histamine H1 receptor antagonist and elicits responses even in mast cell-deficient mice.
- Thus model is relevant to antihistamine-resistant pruritus, a condition observed in patients with many diseases such as atopic dermatitis, chronic cholestasis and prurigo nodularis.

Study Design

- Scratching behavior study design following methods previously described by for the substance P mouse itch model (Kuraishi, 1995).
- Model established using nalfurafine as a positive comparative control (PCC) and vehicle-only (untreated) group.
  - Nalfurafine shown to attenuate SubP itch at 10 ug/kg SC in mouse model (Umeuchi, 2003).
- Nalbuphine tested at doses with no effect on spontaneous locomotor activity to ensure that potential effect is not confounded by sedation.

Conduct of Experiment

Day -1
- Male C57BL/6 mice (~20-25g) were shaved at the injection sites
- Animals randomized to treatment groups (5-15 animals per group)

Pre-Dose on Study Day
- Mice individually housed in plexiglass cages with dedicated video camera
- Acclimate animals to cage
- Monitor by video recording scratching at baseline for 30 min

Test article dosing
- Pre-treat mice with vehicle or test drug 30 minutes prior to challenge
- Subcutaneous (SC) Injection in left side of back/shoulder
- Monitor by video & record scratching/behavior for 30 to 60 min post challenge

Challenge
- Administer SubP (250 ng/0.05 mL) or Vehicle (0.05 vmL) intradermally (ID)
- ID injection to the right side of the rostral back (upper right shoulder area)
- Monitor by video & record scratching to right injection site for 30-60 min post-challenge

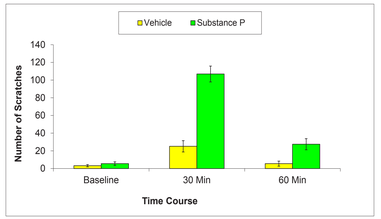
Scoring of Scratching

Review videos
- Two independent scorers
- Scorers blinded to dosing scheme
- Scorers trained prior to study

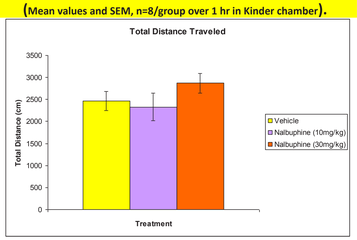
Scratching count
- Count number of scratches to right shoulder with hind paws
- Continuous scratching over one second counted as one event
- Monitor other behavior (e.g. licking or biting) for information only

Data Analysis
- Data collected and analyzed in 30 minute bins
- Report mean values and SEM
- Two way repeated measures ANOVA with a Fisher LSD post-hoc test, where p ≤ 0.05 was considered significant

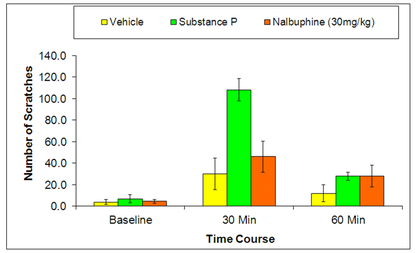
Scratching time course following intradermal administration of SubP (250nM/0.050 mL in PBS) or vehicle only (PBS, 0.05 mL)



Nalbuphine HCl does not affect locomotor activity of mice, as measured by total distance traveled (Mean values and SEM, n=8/group over 1 hr in Kinder chamber).

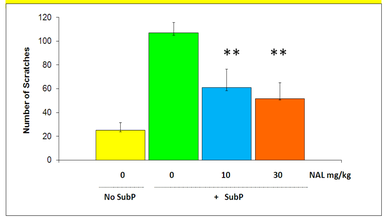


Scratching time course following pre-treatment with Nalbuphine 30 mg/kg (SC) 30 minutes prior to SubP challenge



Experimental set up for monitoring the animals

Effect of Nalbuphine on SubP Scratching in mice



** p<0.001 when compared with vehicle treated group (green bar) by ANOVA

Mean number of scratches 0-30 minutes following ID administration of vehicle (PBS, 0.05 mL) or SubP (250 nM in 0.05 mL PBS) in mice pre-treated SC with either vehicle, PCC or nalbuphine

Group	Treatment	Dose (mg/kg)	SubP	# of animals per group	# Scratch 0-30 min		p Value
					Mean	SEM	
1	VEH/VEH	Vehicle	No	15	25.1	6.40	<0.001
2	VEH/SubP	Vehicle	Yes	20	106.9	8.96	NA
3	PCC/SubP	0.01	Yes	5	62.2	18.15	0.001
4	NAL/SubP	10	Yes	10	60.9	15.52	<0.001
5	NAL/SubP	30	Yes	15	51.7	13.36	<0.001

Mouse Study Conclusions

- SubP itch mouse model was successfully established.
- Viability supported by response to PCC (nalfurafine) that elicited a 42 % reduction in scratching from 107 to 62 at 10 ug/kg SC (P<0.001).
- Itching began within 3-5 minutes from ID administration of the pruritogen and was intense for 30 minutes post-dose.
- Subcutaneous pre-treatment with nalbuphine resulted in a significant reduction in itch (p< 0.001) with ~43% reduction in itch at the 10 mg/kg dose (from 107 to 61 scratches) and 51% at the 30 mg/kg dose (from 107 to 52 scratches).
- Though there was a trend for a dose dependence on itch reduction, there was no statistical difference between the tested nalbuphine doses.

Nalbuphine Anti-pruritic Properties: *Two independent pharmacological mechanisms?*

- Nalbuphine's clinical effectiveness in suppressing itch from epidurally administered morphine suggest a centrally mediated effect via mu opioid antagonistic pharmacologic action. (Wang, 1998).
- Nalbuphine's anti-scratching effect in the intradermal SubP administered mouse model, with nalfurafine as an active control, suggests nalbuphine can be effective in the treatment of peripherally mediated pruritic conditions via kappa opioid agonistic pharmacologic action.
- Thus, nalbuphine's ability to work both centrally and peripherally on itch potentially makes it a good therapy for various pruritic conditions.

Next Steps

TREVI is focused on developing Nalbuphine ER for severe chronic itching conditions. Nalbuphine ER is an oral extended release formulation of an opioid that has a unique dual agonist/antagonist mechanism of action and has been shown in both animal and human clinical trials as being effective in pruritus. The clinical development program will focus on two debilitating chronic itch conditions:

(1) Uremic Pruritus - a severe and persistent form of itching and a common complication of end-stage renal disease .
(2) Prurigo Nodularis - a chronic dermatologic condition characterized by nodular eruptions of unknown etiology where pruritus is a significant clinical complaint.

Trevi expects to initiate the clinical efficacy trials in Uremic Pruritus and Prurigo Nodularis in the first half of 2014.

trevi™ THERAPEUTICS