W5242

Isolation, Characterization, and Synthesis of N-(4-Hydroxy-3-Methylene-Butanoic Acid) Nornalbuphine, a Previously Unidentified Nalbuphine Metabolite

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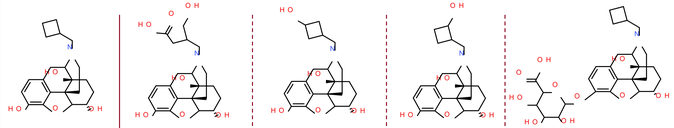
BACKGROUND

Pruritus or itch is associated with a number of disorders or conditions including the majority of patients undergoing dialysis. Nalbuphine HCl ER (Extended Release) oral tablets are currently under development as a treatment for chronic pruritus at Trevi Therapeutics. Nalbuphine is metabolized in the liver predominantly to hydroxylated nalbuphine and glucuronide conjugates. The 3'-hydroxy, 4'-hydroxy, and the glucuronide conjugates have been previously reported in the literature. Exploratory work indicated the formation of a previously unidentified metabolite (M1) in human plasma and urine. Here, we sought to definitively identify this metabolite, to chemically synthesize and confirm its structure, and to perform a comparison of metabolic plasma profiles across multiple species.



Nalbuphine API
Exact Mass : 357.1940
Formula : $C_{21}H_{27}NO_4$

M1
Exact Mass : 405.1788
Formula : $C_{21}H_{27}NO_7$

M3
Exact Mass : 373.1889
Formula : $C_{21}H_{27}NO_5$

M4
Exact Mass : 373.1889
Formula : $C_{21}H_{27}NO_5$

M5
Exact Mass : 533.2261
Formula : $C_{27}H_{35}NO_{10}$

METHODS

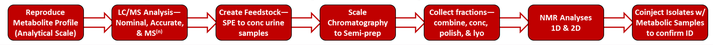
Isolation and Characterization: Urine samples from subjects treated with Nalbuphine ER were subjected to solid phase extraction (SPE) and eluents were evaporated to dryness. Reconstituted SPE eluents were injected on a semi-preparative liquid chromatography (LC; Agilent Zorbax SB-C8) column, and fractions corresponding to the metabolites were collected and then lyophylized. Polished metabolite fractions were analyzed using liquid chromatography/mass spectrometry (LC-MS; Thermo Orbitrap XL, resolving power of 60,000) and nuclear magnetic resonance spectroscopy (NMR; Varian 500 MHz).

Synthesis: Nalbuphine metabolite M1 (N-[4-hydroxy-3-methylene-butanoic acid]-nornalbuphine) was prepared by a concise sequence of reactions involving N-alkylation of noroxymorphone, followed by stereoselective reduction of the C6 carbonyl. The starting material, noroxymorphone hydrochloride, was obtained by palladium-catalyzed N-deallylation of commercially available naloxone.

Cross-Species Plasma Bioanalysis: Plasma samples were obtained from rats and dogs administered oral nalbuphine for 28 days and compared to plasma obtained from human subjects treated with Nalbuphine ER. Samples were assayed using reversed-phase HPLC (Agilent 1100) with tandem mass spectrometric detection (LC-MS/MS; ABSciex, API4000 triple quadrupole). Analytes and the reference standards were extracted from plasma by protein precipitation with acetonitrile. Under these conditions M1, 3'-hydroxy, and 4'-hydroxy metabolite reference materials were separated chromatographically into two distinct diastereomers and are referred to as M1a/M1b, etc.

RESULTS

Isolation and Characterization: Preliminary studies indicated that the unidentified metabolite (M1), the two monohydroxylated metabolites (M3 & M4) and nalbuphine 3-β glucuronide (M5) appear to be the main nalbuphine metabolites upon oral nalbuphine administration in human plasma and urine. Accurate mass spectra were obtained on all metabolites of interest in urine samples. Using a 5 ppm search, molecular formulae for these metabolites corresponded to the addition of three oxygen atoms (M1), a single oxygen atom (M3 & M4), or a glucuronide (M5). M1 has been previously described at "trihydroxy" metabolite, but with isolation and further characterization of the isolate using ^{1}H, COSY, HSQC, and HMBC NMR, it is clear the resonances of the cyclobutane ring are significantly different from the analogous resonances in nalbuphine. The dramatic changes observed for CH2(3') are consistent with a methylene adjacent to a heteroatom. In the HMBC spectrum, correlations from CH2(5') to a carbon resonance at ~180 ppm are consistent with the presence of a carbonyl moiety. An M1 structure in which the cyclobutane ring is metabolized to 4-hydroxy-3-methylbutanoic acid was consistent with NMR and MS observations.



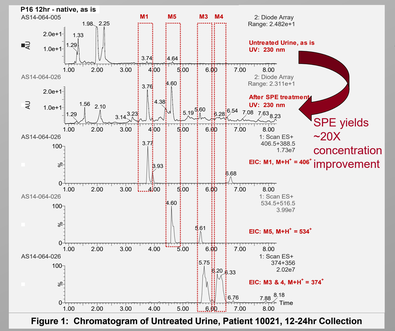


Figure 1: Chromatogram of Untreated Urine, Patient 10021, 12-24hr Collection

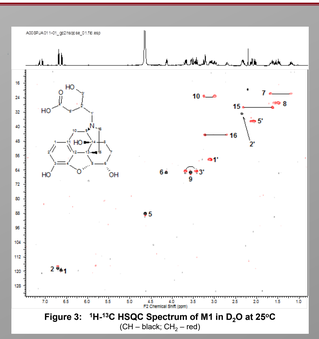


Figure 3: ^{1}H-^{13}C HSQC Spectrum of M1 in D_2O at 25°C
(CH – black; CH_2 – red)

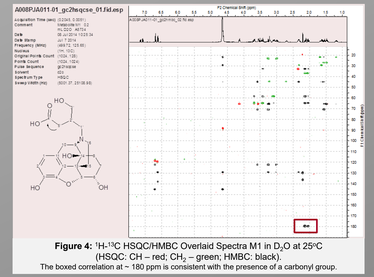


Figure 4: ^{1}H-^{13}C HSQC/HMBC Overlaid Spectra M1 in D_2O at 25°C
(HSQC: CH – red; CH_2 – green; HMBC: black).
The boxed correlation at ~ 180 ppm is consistent with the presence of a carbonyl group.

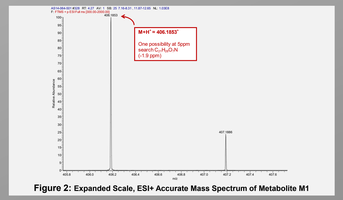


Figure 2: Expanded Scale, ESI+ Accurate Mass Spectrum of Metabolite M1

Accurate Mass Results

	Measured m/z	Theoretical m/z	Accuracy (ppm)	Formula	Comment
API	358.2009$^+$	358.2013$^+$	-1.0	$C_{21}H_{28}NO_4^+$	only possibility
M1	406.1853$^+$	406.1860$^+$	-1.9	$C_{21}H_{28}NO_7^+$	only possibility
M3	374.1958$^+$	374.1962$^+$	-1.0	$C_{21}H_{28}NO_5^+$	only possibility
M4	374.1958$^+$	374.1962$^+$	-1.0	$C_{21}H_{28}NO_5^+$	only possibility
M5	534.2317$^+$	534.2334$^+$	-3.2	$C_{27}H_{36}NO_{10}^+$	most likely of 2 possibilities

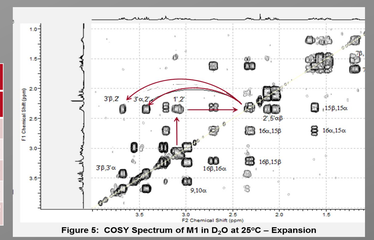


Figure 5: COSY Spectrum of M1 in D_2O at 25°C – Expansion

Synthesis: Noroxymorphone was alkylated with commercially available 4-(bromomethyl)-2(5*H*)-furanone to give a key butenolide intermediate. The butenolide then underwent hydrogenation, followed by carbonyl reduction to yield a side chain-lactonized form of M1 that underwent hydrolytic lactone-ring opening upon exposure to mildly alkaline conditions. Metabolite M1 was purified by preparative reversed phase chromatography and fully characterized. The 3'-hydroxy and 4'hydroxy nalbuphine metabolites were also prepared by similar sequences involving various cyclobutane-based alkylating agents.

RESULTS

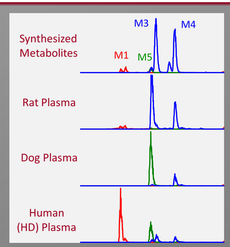
Cross-Species Plasma Bioanalysis: Cross-species comparison of human, rat, and dog plasma indicated the presence of metabolites M3, M4, and M5 in all three species. Metabolite M1 was formed in the rat but was not detected in the dog. There were notable species differences in the stereoselective metabolism of nalbuphine to M1 and 4'-hydroxy metabolites. Both M1a and M1b were formed in the rat at nearly equal concentrations whereas M1a predominated in human. In human plasma 4'-hydroxy-b was predominant whereas the 4'-hydroxy-a predominated in rat and dog plasma.

MS Relative Response Factors

Nalbuphine	M1	M3	M4	M5
100%	12%	111%	94%	8%

Relative Nalbuphine Metabolite Levels

Species	M1a	M1b	M3a	M3b	M4b	M5
Rat	+	+	+	+	+	+
Dog			+		+	+++++
Human (HD)	+++	+	+	+	+	++



CONCLUSIONS

A previously unidentified nalbuphine metabolite, M1, has been successfully characterized. The metabolism of the nalbuphine cyclobutane ring to a 4-hydroxy-3-methylbutanoic acid moiety was confirmed by comparison to a synthesized M1 authentic standard. Metabolite M1 appears to be of importance in human plasma. Cross-species comparison of plasma and urine confirmed the formation of M1, 3'-hydroxy, 4'-hydroxy, and the 3-β glucuronide conjugate across the rat, dog, and human indicating that these metabolites, including M1, are not unique to humans.

REFERENCES

Hawi, Amale, et al. "A proof-of-concept study with pharmacokinetics demonstrating anti-pruritic activity of oral nalbuphine in hemodialysis patients with uremic pruritus." *JOURNAL OF INVESTIGATIVE DERMATOLOGY*. Vol. 134. 75 VARICK ST, 9TH FLR, NEW YORK, NY 10013-1917 USA: NATURE PUBLISHING GROUP, 2014.

Narita, Ichiei I (03/01/2008). "Uremic pruritus in chronic hemodialysis patients". Journal of nephrology (1121-8428), 21 (2), p. 161.

Cohen, Sheila E., et al. "Nalbuphine is better than naloxone for treatment of side effects after epidural morphine." *Anesthesia & Analgesia* 75.5 (1992): 747-752.

Wang, Hong-Jaan, et al. "New finding of nalbuphine metabolites in men: NMR spectroscopy and UPLC–MS/MS spectrometry assays in a pilot human study." *Metabolomics* 10.4 (2014): 709-718.