Development of Stained Release Buprenorphine for use as an Improvroved Analgesic in Toxicology Studies

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Important Update:

In order to remain compliant with the most current regulatory guidelines, we have updated the labeling on our SR formulations from Buprenorphine and Meloxicam SR to Buprenorphine and Meloxicam ER. **As of July 1, 2022, SR preparations mentioned in the attached study are now labeled as ER,** with no changes to the formulation of the medication(s).

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DEVELOPMENT OF SUSTAINED RELEASE BUPRENORPHINE FOR USE AS AN IMPROVED ANALGESIC IN TOXICOLOGY STUDIES: ASSESSMENT OF FORMULATION PHARMACOKINETICS

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Acute toxicology safety testing procedures can involve animal pain and distress. US regulatory agencies and the OECD recently adopted and updated procedures that incorporate the routine use of systemic analgesics to avoid or reduce pain and distress for eye irritation testing procedures. Buprenorphine is recommended as a useful analgesic for such toxicology studies. However, buprenorphine requires a minimum of twice-daily dosing at 12 hour intervals to maintain effective analgesia. A study was therefore conducted to evaluate sustained release formulations to determine their usefulness for once-per day or less frequent dosing. The pharmacokinetics of two sustained release formulations of buprenorphine were compared to buprenorphine in saline using male 2-2.5 kg New Zealand White rabbits. Sustained release formulations were prepared using N-methyl-pyrrolidone (NMP) or Triacetin as the vehicle. Following subcutaneous dosing, blood samples were collected at intervals to four days; plasma buprenorphine was determined using liquid chromatography with mass spectrometry. Both the NMP and Triacetin formulations produced higher plasma buprenorphine concentrations than the saline formulation at time points from 12 hours on. The Triacetin formulation also resulted in higher concentrations at earlier time points, as well as concentrations near or above 0.1 ng/mL—a concentration previously associated with analgesic activity in other species—to 96 hours post-dosing. Body weight and food consumption were recorded, and did not show adverse effects of treatment with sustained release formulations. No abnormal clinical signs or local lesions were observed. These results suggest that the Triacetin sustained release formulation of buprenorphine can significantly reduce the dosing interval required and can be a useful replacement for twice-daily treatments.

Background and literature

Buprenorphine is a useful analgesic in toxicology studies. However, buprenorphine's utility is limited by its pharmacokinetics, which set a need for q12h dosing in rabbits and other laboratory animal species.

We have tested sustained release formulations which offer the potential for once-per-study dosing.

Catbagan DL et al. (2011) *Am J Vet Res* (72:4) 461-6.
Foley PL et al. (2011) *J Am Assoc Lab Anim Sci* (50:2) 198-204.
ICCVAM (2010) *ICCVAM test method evaluation report: Recommendations for routine use of topical anesthetics, systemic analgesics and humane endpoints to avoid or minimize pain and distress in ocular safety testing.*
Nunamaker EA et al. (2013) *J Am Assoc Lab Anim Sci* (52:1) 1-9.
OECD (2012) *Test no. 405: Acute eye irritation/corrosion.*

Materials and methods

Drug formulations:
Buprenorphine HCl–immediate release
NMP-SR buprenorphine–sustained-release formulation (pilot)
SR™-Lab buprenorphine–sustained-release formulation (final)

Subjects: 24 male New Zealand Albino rabbits, approx. 2-2.5 kg (Robinson Services, Clemmons, NC); rabbits were individually housed in suspended stainless steel perforated bottom cages, fed Harlan Teklad diet 2031 and given water ad libitum

Treatment: all treatments were given as subcutaneous injections using a 22 gauge needle, after clipping fur

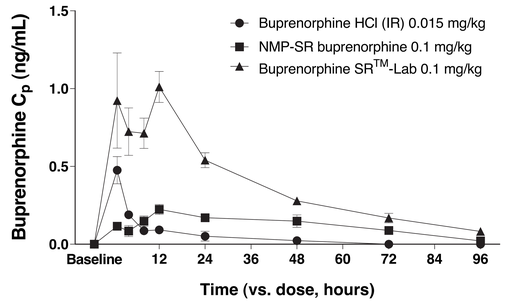
Sample collection: samples of approximately 1 mL were collected from the medial artery of the ear and placed into EDTA tubes; plasma was separated by centrifugation, stored and shipped frozen

Observations: rabbits were weighed, monitored daily for food consumption, and checked twice daily for signs of inflammation, behavioral changes or gross toxicity

Institutional: Procedures were approved by the PSL IACUC and conducted in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals and the USDA Animal Welfare Act. PSL is an AAALAC-accredited laboratory.

Bioanalytical:
Extraction–Internal standard (buprenorphine-D4) and ammonium hydroxide were added to samples and extracted with n-butyl chloride and acetonitrile; organic phases were dried and reconstituted with formic acid in water and acetonitrile
Chromatography–Injections were run using a YMC ODS-AQ column and Shimadzu Prominence LC; peaks were detected using a Sciex API 5000 in positive ion mode (MRM)
Analysis–Peaks were integrated and analyzed using Analyst 1.4.2 software

Results 1: Pharmacokinetics



Buprenorphine plasma concentrations (C_p) were determined for 96 hours following injection. Over this period, the NMP formulation showed consistent buprenorphine availability, while the Triacetin formulation showed variable plasma concentrations. Area under the curve (AUC) values, calculated for the first 12 hours ($AUC_{(0\text{-}12h)}$) and for the entire experiment ($AUC_{(0\text{-}96h)}$), are shown below (ng*h/mL, mean ± SEM).

Formulation	$AUC_{(0\text{-}12h)}$	$AUC_{(0\text{-}96h)}$
Buprenorphine HCl	2.15 ± 0.25	4.18 ± 0.91
NMP-SR buprenorphine	1.57 ± 0.32	11.93 ± 0.90
SR™-Lab buprenorphine	9.25 ± 1.64	36.75 ± 2.88

Results 2: Observations

No skin irritation, behavioral changes or overt signs of toxicity were noted at any time.

Results 3: Body weight and food consumption

Different buprenorphine formulations were associated with similar body weight changes and food consumption; the observed values were consistent with historical observations in this lab.

Formulation	*Food intake (days 1-4, g)*	*Weight change (days 1-4, kg)*
Buprenorphine HCl	134.3 ± 8.8	0.13 ± 0.03
NMP-SR buprenorphine	125.9 ± 17.8	0.09 ± 0.06
SR™-Lab buprenorphine	136.3 ± 5.7	0.12 ± 0.02

Conclusions

We have achieved a sustained buprenorphine $C_p > 0.1$ ng/mL for 96 hours without obvious toxicity; this C_p is important because it is associated with efficacy.

The sustained C_p correlates well with previous findings on these formulations in other species.

Our findings of no overt signs of toxicity following treatment with the SR™-Lab formulation correlate well with previous findings.

The combination of sustained C_p and lack of obvious adverse effects indicates that the SR™-Lab formulation is appropriate for single dosing in toxicology studies requiring analgesic efficacy for multiple days.