Dose Range-Finding Studies of High Dose Formulations of Buprenorphine in Cats

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Background

Buprenorphine has been widely used in cats for perioperative analgesia, providing analgesia of several hours duration with few unwanted side effects[1-3]. An improved buprenorphine formulation could provide at least 24 hours of analgesia with a single, low- volume injection. The pharmacokinetics and antinociceptive effects of a number of dosages and concentrations of different formulations of aqueous buprenorphine were evaluated using a thermal model of nociception.

Methods

Study Design: Five different aqueous formulations (Table 1) were included in five separate crossover or serial studies (studies I-V, Table 2). Studies included eight (8) variations in buprenorphine formulation, dose and/or the presence of a preservative, tested in groups of 3-6 cats. Doses of 0.02, 0.06, 0.12 and 0.24 mg buprenorphine HCl/kg BW were given by SC injection, in volumes ranging from 0.067 mL/kg to 0.4 mL/kg (Table 2).

Table 1: Formulation identifies (IDs)

Formulation ID	Purpose of Formulation	Buprenorphine Conc. (mg/mL)	Preservatives Present?
A	Standard [a]	0.3	No
B	High Dose, Unpreserved[b]	0.6	No
C	High Dose, Unpreserved[b]	1.2	No
D	High Dose, Preserved[b]	1.2	Yes[c]
E	Negative Control[d]	0	No

[a]Buprenex®; Reckitt Benckiser Heathcare (U.K.) Ltd., Hull, U.K.).
[b]Supplied by Abbott Animal Health
[c]Preserved with methylparaben (2.3 mg/ml) and propylparaben (0.3 mglml) (pH=5.2).
[d]0.5% dextrose; Hospira Inc, Illinois, USA

Table 2: Individual studies performed

Study	Aim	Dose (mg/kg)	Formulation ID[a]	Formulation Conc. Bup. (mg/mL)	Dose Volume (mL/kg)	N	Group[b]
I	Standard dose	0.02	A	0.3	0.067	3	AG6
	& high dose	0.12	A	0.3	0.4	3	AG2
II	Confirm	0.12	A	0.3	0.4	3	AG2
	higher dose						
III	High dose and	0.12	B	0.6	0.2	3	AG7
	concentration	0.12	C	1.2	0.1	3	AG3
IV	Addition of	0.12	D	1.2	0.1	6	AG3
	preservative						
V	Confirmatory:	0	E	0	0.1	6	AG1
	- Negative	0.06	D	1.2	0.05	6	AG4
	control	0.24	D	1.2	0.2	6	AG5
	- Higher &						
	lower dosage						

[a]Formulation ID's, see Table 1
[b]Group Total n's: AG1=6; AG2=6; AG3=9; AG4=6; AG5=6; AG6=3; AG7=3

Animals: Twelve (12) adult, normal, healthy domestic short hair research cats, were used: 6M/6F, bodyweight: mean±SD (range) 6.0±1.1 (4.7-8.3) kg. Six of the cats were used in all five studies and the remaining six (6) cats were used in study V only.

Thermal nociceptive threshold testing: A small probe containing a heating element and a temperature sensor were held against the shaved thorax using an elasticated band. A small lightly inflated bladder was inserted behind the probe to ensure consistent contact between probe and skin (Thermal Threhold Testing System Model TT1, Topcat Metrology Ltd, Ely, UK)[4]. During testing the probe was connected to the control unit with light ribbon cable. When activated, the probe heated at 0.6°C/sec with an automatic cut-off at 55°C if not stopped earlier. To record thermal threshold (TT), the starting skin temperature was recorded and then the heater was activated; heating was switched off when the cat reacted, and the probe temperature recorded as TT. The temperature probe was calibrated prior to each study. Baseline TT were recorded as the mean of five tests taken at 15 minute intervals prior to drug administration. Following buprenorphine administration, TT was measured at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, 24, 30, 36, 48, 60 & 72 hours.

Blood sampling: Blood samples were collected by direct jugular venepuncture prior to buprenorphine administration and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 20, 24, 36, 48 and 72 hours following dosing. Plasma was analysed for buprenorphine and norbuprenorphine concentrations by LC/MS/MS. The Lower Limit of Quantitation (LLOQ) was 0.5 ng/mL buprenorphine or norbuprenorphine.

Clinical observations: Cats were observed for any abnormal clinical signs or behaviour. Attitude, general condition, mydriasis, and euphoria recorded before and at 1, 4, 8, 12, 24, 36 & 48 hours after dosing.

Data analysis: Data from groups (AG 1-7) treated with the same dosage and formulation were combined for the purposes of statistical and pharmacokinetic analysis. Further analysis examined the effect of preservative and dose concentration in animals receiving 0.12 mg buprenorphine/kg (AG 2, 3 and 7).

Pharmacokinetic analysis: Data from individual cats were used to derive peak concentration (Cmax) and time to peak concentration (Tmax). The Area under the concentration time curve (AUC) was calculated using the linear trapezoidal rule to the final concentration time point. The elimination rate constant (K(el)) was estimated using data from the maximum concentration. The AUC from the final concentration-time points to infinity (C/K(el)) was extrapolated using K(el).
In order to allow for incomplete bioavailability following SC injection, estimates for clearance (Clp) and apparent volume of distribution (Vdbeta) are expressed as values Clp/F and Vdbeta/F - where F is the bioavailability. The ratio of AUC norbuprenorphine to AUC buprenorphine was calculated, when possible.

Statistical analysis: Thermal Threshold data were analysed using one and two-way repeated measures (RM) ANOVA to compare AGs and assess changes over time. Post hoc analysis was with Dunnett's or Tukey's multiple comparisons tests as appropriate. P<0.05 was considered significant.

Results

Thermal Threshold

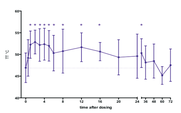
Pretreatment skin temperature was 35.1±0.8°C in all groups, and did not change significantly during any of the studies. Baseline TT in the control group (5% dextrose, AG1) was 47.6±4.1°C and did not change significantly throughout the duration of testing. In all groups treated with any dose or formulation of buprenorphine, TT increased above baseline at some point post treatment (Table 3). Groups of n=6 or more showed statistically significant increases in TT (Table 3). At a dose of 0.12 mg/kg (combined n=18), TT was significantly higher than baseline at most time points from 1-30 hours post treatment (Figure 1). There were no statistically significant differences between any of the treatment groups, although the duration of effect appeared shorter after 0.02 mg/kg than with any of the higher doses (Figure 2). Neither the dose (Figure 2), the concentration (Figure 3), nor the addition of preservative (Figure 4) significantly affected TT.

Table 3: Timing of statistically-significant antinociceptive effects

Group (N)	Treatment: Dose (mg/kg)	Treatment: Conc. Bup. (mg/mL)	Baseline TT (°C) Mean ±SD	Time points post treatment: TT significantly higher than baseline[a]
AG2 (6)	0.12	0.03	46.9±2.4	1-5 hours after treatment
AG3 (9)	0.12	1.2	47.4±4.5	2 hours
AG4 (6)	0.06	1.2	45.4±2.8	6-16 hours
AG5 (6)	0.24	1.2	46.7±1.2	2 &12 hours
AG6 (3)	0.02	0.03	44.8.±0.8	ND[b]
AG7 (3)	0.12	0.6	45.7±0.5	ND

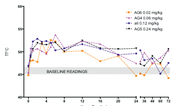
[a]RM ANOVA. P<0.05
[b]ND=Not determined; insufficient group size for statistical evaluation

Figure 1: Mean±SD thermal threshold (TT) in cats (n=18) after SC injection of 0.12 mg/kg buprenorphine



------- is calculated baseline TT
* denotes significant difference from 0.

Figure 2: Mean thermal threshold (TT) in cats after SC injection of buprenorphine: Effect of dose



Figures 3 and 4: Mean thermal threshold (TT) in cats after SC injection of 0.12 mg/kg buprenorphine

Figure 3: Effect of concentration

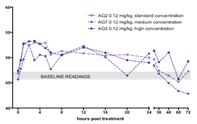
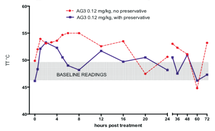


Figure 4: Effect of preservative

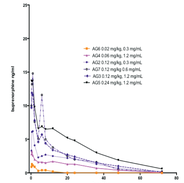


Footnotes Figures 2-4: Shaded box is baseline TT from all groups for visualization
No statistical difference between groups.

Kinetics

Plasma buprenorphine concentrations are presented in Figure 5 and pharmacokinetic data in Table 4. Tmax ranged between 0.25 and 2 hours in all dose groups. Cmax was generally dose-dependent, ranging from 1.01-1.72 ng/mL for the 0.02 mg/kg dose group to 5.3-22.3 ng/mL for the 0.24 mg/kg dose group. Mean AUC was also dose-dependent, ranging from 6.43±3.89 at 0.02 mg/kg to 265.56±53.58 at 0.24 mg/kg. Elimination half-life could not be determined in the 0.02 mg/kg dose group due to limitations of the assay. For doses of 0.06-0.24 mg/kg, there were no significant differences between groups for clearance, apparent volume of distribution or elimination half-life. Estimates of the ratio of norbuprenorphine/buprenorphine AUCs, determined in groups dosed with 0.12 mg/kg or greater, ranged between 0.17 and 1.01 (mean±SD 0.42±0.27). At the 0.12 mg/kg dose, the pharmacokinetics of buprenorphine were not affected by the formulation concentration (Formulation A, B or C) or the addition of a preservative (Formulation C or D).

Figure 5. Plasma buprenorphine concentrations versus time after dose measured in 33 cats after SC injection



[a]F = bioavailability after SC dose compared with IV dose.

Table 4A: Buprenorphine kinetics by dose and group

Group Dose (mg/kg) (Formulation ID*)	Cmax (ng/mL) Mean (Range)	Tmax (hours) Mean (Range)	AUC ng/mL.hr Mean (SD)	k(el) (hr-1) Mean (Range)	Ratio Norbup/ Bup Mean (Range)
AG6 0.02 (A)	1.3 (1.0-5.7)	0.42 (0.25-0.5)	6.43 (3.90)	0.37570 (0.1300- 0.5144)	na[a]
AG4 0.06 (D)	3.4 (1.4-4.9)	0.7 (0.25-2)	61.8 (13.2)	0.0324 (0.0222-0.0431)	na
AG2 0.12 (A)	6.5 (5.7-7.7)	0.75 (0.5-1)	115.6 (32.1)	0.03394 (0.0221-0.0471)	0.45 (0.17-0.34)
AG7 0.12 (B)	14.9 (6.6-29.5)	0.83 (0.5-1)	160.74 (59.8)	0.04851 (0.0348-0.0688)	0.32 (0.21-0.53)
AG3 0.12 (C)	20.8 (4.5-51.4)	0.67 (0.5-1)	163.55 (64.4)	0.04152 (0.0310-0.05063)	0.5 (0.21-0.99)
AG3 0.12 (D)	10.3 (6.1-22.6)	0.7 (0.5-1)	139.6 (11.7)	0.0371 (0.0204-0.0584)	na
AG5 0.24 (D)	15.6 (5.3-22.4)	0.63 (0.25-1)	265.07 (53.6)	0.04206 (0.0302-0.0497)	0.40 (0.18-0.61)

*See Table 1 for Formulation ID's
[a]na; not quantified due to very low or non-detectable norbuprenorphine concentrations (LLOQ=0.5 ng/mL)

Table 4B: Buprenorphine kinetics (Mean (SD) by dose

Dose mg/kg (N)	AUC kg/mL.hr	Clp/F mL/kg/min	Vdbeta/F* l/mL	T1/2el hours
0.02 (3)	6.43 (3.89)	Not determined	Not determined	2.71 (2.28)
0.06 (6)	61.80 (13.18)	17.07(5.06)	0.58 (0.31)	22.44 (5.56)
0.12 (18)	139.10 (35.52)	15.30 (4.03)	0.59 (0.21)	19.77 (6.55)
0.24 (6)	265.56 (53.58)	15.62 (3.13)	0.66 (0.20)	17.18(3.43)

*F = bioavailability after SC dose compared with IV dose.

PK-PD Relationship

The range of normal TT was calculated as the mean ±2SD of all data collected in the control group. Mean normal TT±SD was 47.8±1.2°C (95%CI 46.9-48.7°C); an analgesic (antinociceptive) effect was therefore defined as TT >50.2°C. The time point after dosing in each study group where the TT exceeded this value, and the time to offset, are shown in Table 5, along with the associated plasma buprenorphine concentrations. Examining all animals together, the mean time of the peak concentration was 0.73±0.38 hours (range 0.25-2.0 hours), and the mean first time of peak effect was 2.06±2.81 hours (range 0.5-12 hours). After exclusion of data from those cats where the buprenorphine concentration was below the LLOQ, the mean 'offset plasma concentration for analgesia' (estimated ED50 plasma analgesic concentration) was 2.3±2.0 ng/mL.

Table 5: Onset and offset of antinociception in individual animals

Dose (mg/kg)	Group (AG) Formulation ID*	Cat	Time on (hr)[a]	Time off (hr)[a]	Associated buprenorphine plasma concentration (ng/ml) On	Off	Time of peak concentration (hr)
0.02	AG6 A	4	3	6	11	0.77	1
		5	0.5	12	1.17	ng[c]	0.5
		6	4	12	ng	0.52	1
0.06	AG4 D	9	0.5	72	4.5	ng	0.25
		10	3	30	2.35	0.6	0.25
		2	2	72	1.2	ng	0.5
		5	0.5	16	4.1	1.85	2
		11	0.5	20	3.3	1.6	0.5
		12	12	36	1.5	1.1	0.75
0.12	AG2 A	1	1	24	6.68	3.01	1
		2	3	30	3.51	2.51	0.5
		3	1	24	6.37	1.33	1
		7	2	24	4.4	2.1	1
		8	0.5	5	4.3	1.55	0.5
	AG3 C	9	1	36	6.8	0.7	0.5
		1	1	36	4.51	1.76	1
		2	0.5	48	31.4	0.78	0.5
	AG3 D	3	0.5	48	6.45	0.98	0.5
		1	1	48	6.16	0.9	1
		2	2	36	1.97	2.17	0.5
		3	0.5	48	8.75	0.98	1
		4	0.5	48	7.41	0.87	0.5
	AG7 B	5	1	48	6.1	1.16	1
		6	1	20	11.3	2.62	0.5
		4	1	4	29.5	6.83	1
		5	0.5	36	8.7	1.4	0.5
		6	5	30	6.49	1.75	1
0.24	AG5 D	8	2	24	4.8	5.2	1
		11	3	24	9.25	5.6	0.25
		1	12	16	8.9	8.3	1
		6	1	20	15.1	5.5	0.25
		7	0.5	30	10	3.5	1
		9	0.5	72	21.3	ng	0.25

*See Table 1 for Formulation ID's
[a]Antinociception taken as TT > control TT±2SD: 50.2°C .

Behaviour and Adverse Effects

In all groups treated with buprenorphine, mydriasis developed in most animals and lasted a few hours. The extent and duration of mydriasis was not associated with dose or the increase in TT. No other side effects were noted; cats remained bright, alert and responsive with no obvious behavioural effects. There were no adverse effects that might be considered life-threatening.

Summary and Conclusions

Doses of 0.12 and 0.24 mg/kg buprenorphine in aqueous formulations given SC appear to provide up to 24 hours antinociception in this laboratory animal model of analgesia with no side effects other than mydriasis. The concentration of the formulation appears not to be critical to the antinociceptive effects. Addition of preservatives to a high dose buprenorphine formulation had no impact on its antinociceptive properties or side effect profile. High dose formulations of buprenorphine have potential for clinical use, providing prolonged analgesia by a single SC injection in a minimal dose volume.

Conflict of Interest Disclosure Statement

Dr. Luangdilok is an employee of Abbott, holds Abbott stock and has Abbott stock options. Abbott participated in the study design, research, data collection, analysis and interpretation of data. This research and analysis was funded by Abbott Laboratories. Drs. PM Taylor and JW Sear act as independent consultants in veterinary pharmacology and pharmacokinetics to Abbott Laboratories.

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