Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 818 (2005) 233–239
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Simultaneous determination of buprenorphine and its prodrug, buprenorphine propionate, by high-performance liquid chromatography with fluorescence detection: application to pharmacokinetic studies in rabbits☆

Shyun-Yeu Liu[a,*], Kuo-Sheng Liu[b,c], Chun-Hsiung Kuei[b], Jann-Inn Tzeng[c], Shung-Tai Ho[d], Jhi-Joung Wang[c]

[a] *Department of Oral and Maxillofacial Surgery, National Defense Medical Center, Taipei/Chi-Mei Medical Center, No. 901, Chung-Hwa Road, Yung-Kang, Tainan, Taiwan, ROC*
[b] *Department of Chemistry, National Cheng-Kung University, Tainan, Taiwan, ROC*
[c] *Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan, ROC*
[d] *Department of Anesthesiology, Tri-Service General Hospital, Taipei, Taiwan, ROC*

Received 29 October 2004; accepted 4 January 2005
Available online 1 February 2005

Abstract

A rapid, sensitive, precise and accurate high-performance liquid chromatographic assay with fluorescence detection was developed for the simultaneous determination of buprenorphine and buprenorphine propionate in human and animal blood. Buprenorphine propionate was also proven to be a prodrug of buprenorphine. It was comprised of only a one-step extraction procedure with ethyl acetate and normal-phase chromatography on a Betasil Silica column. The recoveries of buprenorphine and buprenorphine propionate were above 84%. Calibration graphs were linear for buprenorphine over the concentration range 2–1500 ng/ml and for buprenorphine propionate over the concentration range 20–1500 ng/ml with a coefficient of variation, both within- and between-day, or less than 10% at any level. The limits of quantitation of buprenorphine and buprenorphine propionate in human or animal blood were 2.0 and 20 ng/ml, respectively, based on a single-to-noise ratio of 3. The method has been successfully applied to pharmacokinetic studies of buprenorphine and buprenorphine propionate in rabbits. The results demonstrated that buprenorphine propionate was rapidly and totally converted to its parent drug, buprenorphine, following intravenous administration. Buprenorphine propionate is a prodrug of buprenorphine.

Keywords: Buprenorphine; Propionate; HPLC; Pharmacokinetic

1. Introduction

Buprenorphine, *N*-cyclopropylmethyl-7α-[1-(s)-hydroxyl-1,2,2-trimethylpropyl]-6,14-endo-ethano-6,7,8,14-tetrahydronororipavine (Fig. 1), is a potent analgesic with a potency of 20–40 times higher than that of morphine [1,2]. It has been widely used in the treatment of acute and chronic pain [1,2], and recently for the treatment of heroin addicts [1,3]. Its main advantages over morphine are a ceiling effect of respiratory depression, low tolerance liability and a lack of significant withdrawal symptoms [1,2]. Buprenorphine is available as an injection for intravenous (IV), intramuscular (IM), intrathecal and epidural administration, and as sublingual tablets [1–3]. The usual recommended doses are 200–600 μg by IV or IM injection every 6–8 h, 30–45 μg intrathecally or 100–300 μg epidurally every 6–12 h, or 400 μg sublingually every 6–8 h [1–3].

☆ work was done in the Chi-Mei Medical Center, Tainan, Taiwan.
* Corresponding author. Tel.: +886 6 2517844; fax: +886 6 2832639.
E-mail address: 400003@mail.chimei.org.tw (S.-Y. Liu).

1570-0232/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2005.01.002

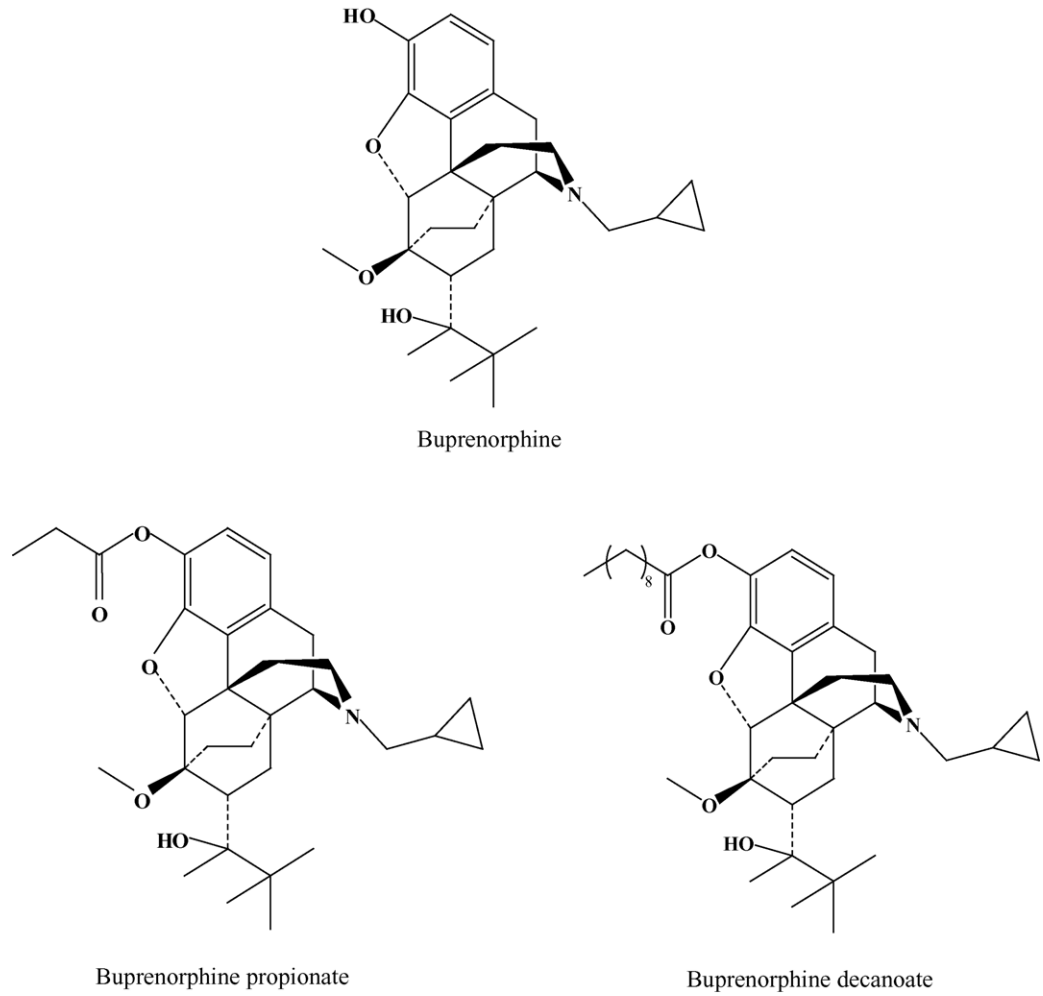

Fig. 1. Chemical structures of buprenorphine, buprenorphine propionate, and buprenorphine decanoate (internal standard).

Recently, an ester of buprenorphine, buprenorphine propionate, was synthesized and studied [4–6]. The ester was found to be more lipophilic than buprenorphine [4]. Previous study also found that buprenorphine propionate when prepared as a depot had a long-lasting analgesic effect, which was 7.5-fold longer than the traditional dosage form of buprenorphine in saline preparation, following IM injection in rats [6]. Since most patients who experience acute pain, such as postoperative pain, posttraumatic pain, and burn pain, often require pain relief in the first 3–5 days after injury, an analgesic with a long-lasting effect may be particularly valuable for this purpose [7]. Therefore, the depot formulation of buprenorphine propionate is worth further evaluation. In this formulation, buprenorphine propionate was synthesized through a prodrug design with esterification [4,5,8–10]. Buprenorphine propionate was suspected to be a prodrug of buprenorphine in in vivo study. However, this was not proven.

In order to evaluate whether buprenorphine propionate was a prodrug or not, an in vivo pharmacokinetic study of buprenorphine propionate was necessary. Also, a sensitive, precise and accurate assay method was required to accomplish this study. In this assay, the sample preparation process should be as simple as possible to avoid any decomposition of the ester during sample preparation [11]. Although many analytic methods were reported to analyze buprenorphine in biological fluids, none of these methods was suitable for the analysis of buprenorphine and buprenorphine propionate simultaneously [12,13]. We, therefore, developed a simple, rapid, sensitive, and selective high-performance liquid chromatography (HPLC) method with a one-step extraction procedure for simultaneous determination of buprenorphine and buprenorphine propionate. This method has been successfully applied to pharmacokinetic studies of buprenorphine and buprenorphine propionate in rabbits following IV administration.

2. Experimental

2.1. HPLC conditions

The HPLC system consisted of a pump (LC-10AD VP, Shimadzu, Kyoto, Japan), an automatic sampler (Model 542,

ESA, Chelmsford, MA, USA), a programmable fluorescence detector (Series 200, Perkin Elmer, Norwalk, CT, USA), and an integrator (Chromatography Data Station V2.1, SISC, Taiwan). A silica gel column (Betasil Slilia, No. 255-700-CPG, 250 mm × 4.6 mm, 5 μm particle size, Keystone Scientific, Bellefonte, PA, USA) was used.

2.2. Chemicals and reagents

Buprenorphine HCl was purchased from the Macfarlan Smith (Edinburgh, UK). Buprenorphine propionate and buprenorphine decanoate (internal standard; Fig. 1) were synthesized by using the method reported previously [4]. In brief, the buprenorphine base was reacted with propionyl chloride or decanoyl chloride (Fluka, Buchs, Switzerland) in the presence of dimethylaminopyridine. Purity (>99%) of buprenorphine propionate and internal standard were assured through elemental analysis, nuclear magnetic resonance, spectroscopy, and gas chromatography with mass detector. All chemicals were of analytical-reagent grade; all solvents were of HPLC grade. All aqueous solutions were prepared using Milli-Q water (Mili-RO60, Millipore, Bedford, USA).

2.3. Sample preparation

In order to prevent the enzymatic hydrolysis of buprenorphine propionate in blood, the blood sample (1 ml) obtained from humans or animals (rats, rabbits, dogs, or pigs) was added immediately into a 10 ml capacity polypropylene (PP) tube which contained 4 ml of chilled ethyl acetate and 25 μl of internal standard (1 μg). Ethyl acetate acted as the extraction solvent. After a 10 s shaking for quenching the hydrolysis reaction, the analytes were then put on a rotary shaker for 30 min at 100 rpm for extraction. After centrifugation at 1880 gm (centrifugal force) for 20 min, the PP tubes were put into a freezer −20 °C for 1 h. After the lower layer (blood) was frozen, the organic layer was poured into another 5 ml PP tube and evaporated to dryness under a stream of filtered dry air. The samples were then reconstituted by 250 μl of the mobile phase of the HPLC system. Aliquots of 200 μl were injected into the HPLC system.

2.4. Chromatography

The assays for buprenorphine propionate and buprenorphine were performed using a mobile phase of 5 mM sodium acetate buffer (pH 3.75) in acetonitrile (2:8, v/v) and a programmable fluorescence detector (excitation 210 nm, emission 338 nm, slit 5 nm at 0–6.2 min for buprenorphine propionate and internal standard; excitation 210 nm, emission 352 nm, slit 5 nm at 6.2–8 min for buprenorphine). A flow-rate of 1.2 ml/min at 25 °C was used and yielded a back-pressure of about 70 bar.

2.5. Calibration graphs

Stock solutions of 15 μg/ml buprenorphine, buprenorphine propionate and internal standard were prepared separately in acetonitile. Calibration standards were prepared by adding 25 μl of internal standard (1 μg) combined with known amounts of buprenorphine (2–1500 ng/ml) or buprenorphine propionate (20–1500 ng/ml) to 0.975 ml of blood. In order to prevent enzymatic hydrolysis of buprenorphine propionate and internal standard, all blood samples (0.975 ml) were added into a chilled ethyl acetate before the testing compounds of buprenorphine, buprenorphine propionate or internal standard were added. The following extraction procedures and HPLC analysis were carried out as described in Section 2.3. Calibration graphs were obtained by plotting drug concentrations against the peak-area ratio of buprenorphine/internal standard or buprenorphine propionate/internal standard. The concentrations of the unknown samples were determined by using the linear regression line of the concentration of the calibration standard [11,14].

2.6. Repeatability, precision and accuracy

The repeatability of the method was estimated by comparing the linear regression slopes and correlation coefficients of the calibration curves from human blood samples. After institutional review board approval, fresh human blood samples which donated by normal healthy volunteers at each testing day were obtained from the blood bank of Chi-Mei Medical Center. Precision and accuracy were determined on spiked human samples at eight concentrations of either buprenorphine or buprenorphine propionate with respect to a calibration graph prepared every day. The precision of the method was expressed as the within- and between-day coefficient of variation (%). The accuracy of the method was determined by calculating the percentages of mean deviations from known concentrations [11,14]. All samples were freshly prepared and processed daily, including preparing the standard solution from the same stock solutions of buprenorphine and buprenorphine propionate (Table 1).

2.7. Recovery

The extraction recoveries of buprenorphine or buprenorphine propionate in human blood were determined at all levels of the calibration graphs by comparing the data obtained by the direct injection of standard concentrations of either buprenorphine or buprenorphine propionate into the HPLC system with those obtained after the whole extraction procedure [11,14].

2.8. Pharmacokinetic studies

Following the guidelines of the American Association for the Accreditation of Laboratory Animal Care, six male

Table 1
Precision and accuracy of buprenorphine determined by the HPLC method

Known concentration (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Accuracy (% mean deviation)
Within-day (n = 10)			
2.00	1.86 ± 0.16	8.4	−6.8
10.00	9.79 ± 0.77	7.9	−2.1
50.00	49.50 ± 4.44	9.0	−1
100.0	99.8 ± 6.0	6.0	−0.2
200.0	200.0 ± 14.7	7.3	0
500.0	500.2 ± 30.7	6.1	0.5
1000	1000 ± 49	4.9	0
1500	1498 ± 103	6.9	−0.1
Between-day (n = 24)			
2.00	1.81 ± 0.18	9.8	−9.4
10.00	9.15 ± 0.85	9.3	−8.5
50.00	48.66 ± 4.44	9.1	−2.7
100.0	98.0 ± 7.2	7.4	−2.1
200.0	201.7 ± 18.5	9.2	0.9
500.0	512.7 ± 47.7	9.3	2.6
1000	998 ± 61	6.1	−0.2
1500	1503 ± 120	8.0	0.2

New Zealand white rabbits (2 months old) weighing between 2.3 and 2.7 kg were used. In order to evaluate the pharmacokinetics of buprenorphine propionate, two studies were carried out. In study 1, the pharmacokinetic study of buprenorphine following IV administration of 6 μmole/kg (=3 mg/kg) was carried out. In study 2, the pharmacokinetic study of buprenorphine propionate following IV administration of 6 μmole/kg (=3.1 mg/kg) was carried out. All six rabbits involved in both of the studies received one of the treatments, randomly, at week 1 and the treatment at week 2. A 5 ml volume of blood was obtained from the artery of rabbits' ear at time zero and 1 ml at 1, 3, 5, 9, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min after IV administration of tested compounds. The blood was then spiked immediately into a chilled extraction solvent (ethyl acetate) with internal standard and shaken 10 s to quench the enzymatic hydrolysis reaction of ester compounds. The following extraction procedures and HPLC analysis were carried out as described above.

The concentration–time profiles of tested compounds were fitted by using the computer program PCNONLIN (version 3.0, Statistical consultants) [15,16]. Akaike information criteria, weighted residual sum of squares, and residual plots were used to judge the goodness-of-fit of the model to data [16]. A C-strip computer program was used to obtain the initial parameter estimations which were required for nonlinear regression analysis by the computer program PCNONLIN [15]. Pharmacokinetic parameters such as half-lives, clearance and area under the blood concentration-time graph (AUC) were calculated by standard formulae [15,16]. We compared the AUC of buprenorphine which was converted from buprenorphine propionate with those obtained by direct IV administration of buprenorphine. Two one-sided t-tests were used [17]. A P value less than 0.05 was considered significant.

3. Results and discussion

3.1. Chromatography

The chromatography of extracts from humans, rats, rabbits, dogs, and pigs were similar; here, we presented only the typical chromatograms of extracts from humans (Fig. 2).

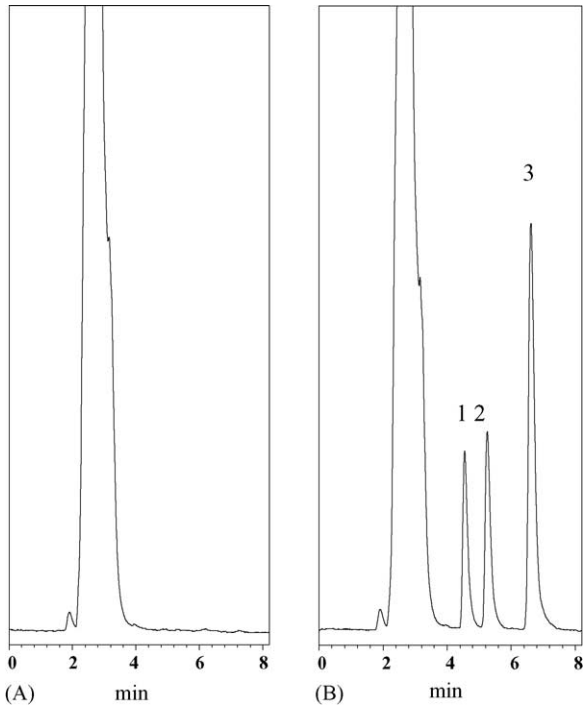

Fig. 2. Chromatograms of extracts from: (A) blank human blood, (B) human blood spiked with internal standard (1 μg/ml), buprenorphine propionate (1 μg/ml) and buprenorphine (100 ng/ml). Peak: 1, buprenorphine decanoate (internal standard); 2, buprenorphine propionate; 3, buprenorphine.

Table 2
Precision and accuracy of buprenorphine propionate determined by the HPLC method

Known concentration (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Accuracy (% mean deviation)
Within-day ($n = 10$)			
20.00	20.35 ± 1.09	5.3	1.7
30.00	31.14 ± 0.99	3.2	3.8
50.00	49.55 ± 1.65	3.3	−0.9
100.0	102.6 ± 6.5	6.4	2.6
200.0	200.6 ± 13.6	6.8	0.3
500.0	499.6 ± 40.7	8.1	−0.1
1000	999 ± 58	5.8	−0.1
1500	1501 ± 70	4.7	0.1
Between-day ($n = 24$)			
20.00	20.34 ± 1.08	5.3	1.7
30.00	32.06 ± 2.22	6.9	6.9
50.00	52.64 ± 5.04	9.6	5.3
100.0	100.0 ± 8.7	8.7	0
200.0	203.8 ± 19.9	9.8	1.9
500.0	503.7 ± 49.7	9.9	0.7
1000	1001 ± 96	9.6	0.1
1500	1486 ± 85	5.8	−0.9

These were extracts of drug-free blood, spiked samples with buprenorphine, buprenorphine propionate and internal standard. No interfering peaks were found in the blank blood nor in samples from both the humans and the animals.

3.2. Column retention time and low quantitation limit

Under the elution condition, the column retention time of buprenorphine, buprenorphine propionate and internal standard in human and animal blood samples were kept nearly constant (Fig. 2). The low quantitation limit of buprenorphine and buprenorphine propionate between human and animal bloods were similar and were 2.0 and 20 ng/ml, respectively (Tables 2 and 3).

3.3. Repeatability, precision and accuracy

The calibration curves for within-day ($n = 10$) and between-day ($n = 24$) analyses were obtained by plotting the peak area ratio versus concentration (Tables 2 and 3). Over the concentration range examined, the calibration curves for both within- and between-day analyses were linear, and the mean correlation coefficients were all more than 0.998 for both drugs. Precision and accuracy were determined by analyzing spiked blood samples at eight concentrations for buprenorphine (2–1500 ng/ml) and buprenorphine propionate (20–1500 ng/ml) with respect to the expected concentrations in a calibration graph, and the results are shown in Tables 2 and 3. For within-day analysis, the coefficients of variation were all within 9.0% for both analytes, and the

Table 3
Absolute recovery of buprenorphine, buprenorphine propionate and buprenorphine decanoate (internal standard) from spiked blood samples ($n = 6$)

Drug	Concentration (ng/ml)	Recovery (mean ± S.D.) (%)	Coefficient of variation (%)
Buprenorphine	2.00	92.8 ± 10.2	11
	10.00	97.0 ± 2.9	3.0
	50.00	93.7 ± 4.8	5.1
	100.0	92.2 ± 10.0	10.8
	200.0	94.2 ± 8.8	9.4
	500.0	92.1 ± 5.9	6.4
	1000	91.2 ± 4.8	5.3
	1500	92.3 ± 8.6	9.3
Buprenorphine propionate	20.00	94.3 ± 0.5	0.5
	30.00	89.7 ± 0.7	0.8
	50.00	84.6 ± 1.0	1.2
	100.0	90.9 ± 4.5	4.9
	200.0	92.5 ± 3.6	3.9
	500.0	88.2 ± 8.5	9.6
	1000	89.1 ± 4.8	5.4
	1500	84.2 ± 4.2	5.0
Buprenorphine decanoate	1000	95 ± 2	2.0

deviation from the expected concentration, as a measurement of accuracy, ranged from −6.8 to 0.5% for buprenorphine and −0.9 to 3.8% for buprenorphine propionate. For between-day analysis, all of the coefficients of variation for both drugs were within 10% and the deviation from the expected concentration ranged from −9.4 to 2.6% for buprenorphine and from −0.9 to 6.9% for buprenorphine propionate. These results indicate that the method is precise and accurate.

3.4. Recovery

The recoveries were determined by comparing peak areas of extracted standards with those of unextracted standards, across the range of standard curve. The mean recoveries of the analytes were 91–97% for buprenorphine and 84–94% for buprenorphine propionate over the constructed calibration concentration ranges.

3.5. Pharmacokinetic studies

This simple, precise and accurate HPLC method yielded satisfactory results for the simultaneous determination of buprenorphine and buprenorphine propionate in blood samples and has been used in pharmacokinetic studies of buprenorphine and buprenorphine propionate in rabbits following IV administration. The blood concentration–time profiles of both buprenorphine and buprenorphine propionate in rabbits are shown in Fig. 3; the pharmacokinetic parameters, listed in Table 4.

Following IV administration of buprenorphine, the concentrations of buprenorphine were successfully fitted to a three-compartment model with two distributions and one elimination phases (Table 4). Following IV administration of buprenorphine propionate, the concentrations of buprenorphine propionate declined rapidly. The concentrations of buprenorphine propionate followed a linear one-compartment model with an elimination half-life of 1.67 min (Table 4). On the contrary, the formation of buprenorphine occurred quite rapidly. The concentrations of buprenorphine which converted from buprenorphine propionate followed a three-compartment model (Table 4). When the AUC data of buprenorphine obtained by direct IV administration were compared with those converted from buprenorphine propionate, no significant differences were found (Table 4; $P > 0.05$ for each comparison). This meant that following IV administration, buprenorphine propionate was rapidly and totally

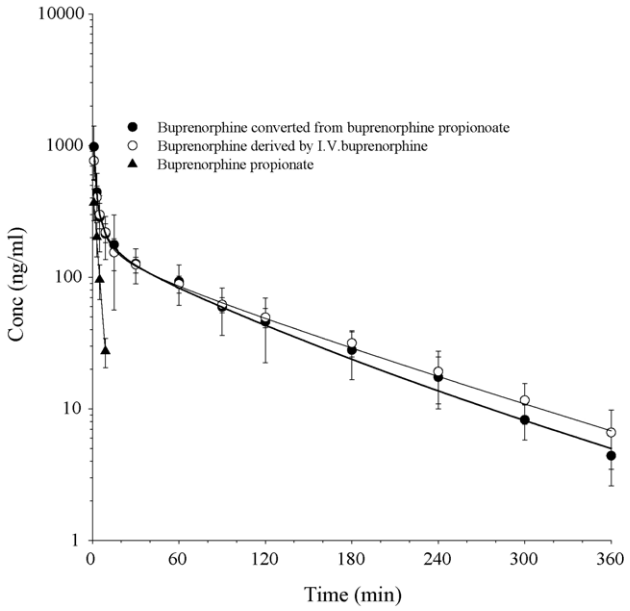

Fig. 3. Blood concentration–time profiles of buprenorphine propionate and buprenorphine in six rabbits following intravenous buprenorphine propionate (6 μmole/kg = 3.1 mg/kg) or buprenorphine (6 μmole/kg = 3.0 mg/ml).

Table 4
Pharmacokinetic parameters of buprenorphine propionate and buprenorphine in six rabbits after intravenous buprenorphine propionate or buprenorphine

Parameter	Unit	Buprenorphine propionate (mean ± S.D.)	Buprenorphine- converted (mean ± S.D.)	Buprenorphine-direct (mean ± S.D.)
A	ng/ml	1563 ± 973	1842 ± 819	3251 ± 524
B	ng/ml	–	272 ± 66	214 ± 106
C	ng/ml	–	101 ± 44	126 ± 30
α	l/min	0.543 ± 0.313	0.869 ± 0.364	1.092 ± 0.335
β	l/min	–	0.106 ± 0.091	0.085 ± 0.049
γ	l/min	–	0.008 ± 0.001	0.009 ± 0.002
$T_{1/2}(\alpha)$	min	1.672 ± 0.931	1.047 ± 0.249	1.268 ± 0.079
$T_{1/2}(\beta)$	min	–	22.25 ± 13.76	25.57 ± 15.58
$T_{1/2}(\gamma)$	min	–	79.81 ± 10.88	79.93 ± 17.05
$AUC_{0-\infty}$	ng min/ml	2440 ± 523	19984 ± 6164	19997 ± 2082
Cl_t	ml/min	1517 ± 521	438 ± 177	381 ± 39
Body weight	kg	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2

Equation for buprenorphine propionate: blood concentration (C) = $A\,e^{-\alpha t}$; equation for buprenorphine: blood concentration (C) = $A\,e^{-\alpha t} + B\,e^{-\beta t} + C\,e^{-\gamma t}$; A, B, C = intercepts; α, β, γ are the fist-order rate constants for the central, tissue, and deep tissue compartments; $T_{1/2}$ = half-life of the first-order rate constant; $AUC_{0-\infty}$ = area under the time-concentration graph to time infinity; Cl_t = total blood clearance. Buprenorphine-converted: buprenorphine which was converted from buprenorphine propionate. Buprenorphine-direct: buprenorphine which was obtained by direct intravenous administration of buprenorphine. The given doses of buprenorphine propionate and buprenorphine were 6 μmole/kg. There was no significant difference in AUC data between buprenorphine-converted and buprenorphine-direct by using two one-sided t-tests.

converted to its parent drug, buprenorphine. Buprenorphine propionate is a prodrug of buprenorphine.

The previous study found that buprenorphine propionate when prepared as a depot had a long-lasting effect [6]. The present study also found that buprenorphine propionate when added into blood was rapidly converted to buprenorphine. These results suggest that, as several other depots of ester-type prodrugs [8–10], the long-lasting effect of IM depot of buprenorphine propionate is due to a slow release of buprenorphine propionate from its oil vehicle. Once buprenorphine propionate is released from its vehicle and absorbed into the blood stream, buprenorphine propionate will convert to buprenorphine rapidly and exerts its pharmacologic effect [8–10].

In conclusion, a simple, sensitive, and accurate HPLC method has been developed for the simultaneous determination of buprenorphine and its prodrug, buprenorphine propionate, in blood. The method has been successfully applied to pharmacokinetic studies of buprenorphine and buprenorphine propionate in rabbits. The results have demonstrated that buprenorphine propionate is a prodrug of buprenorphine.

Acknowledgments

This work was supported in part by the National Science Council of the Republic of China, Grant NSC 92-2314-B-384-008.

References

[1] S.E. Robinson, CNS Drug Rev. 8 (2000) 377.
[2] P.J. Hoskin, G.W. Hanks, Drugs 30 (1991) 326.
[3] T.M. Tzschentke, Psychopharmacology 161 (2002) 1.
[4] A.L. Stinchcomb, R. Dua, A. Paliwal, R.W. Woodard, G.L. Flynn, Pharmaceut. Res. 12 (1995) 1526.
[5] P. Buchwald, N. Bodor, J. Med. Chem. 42 (1999) 5160.
[6] J.J. Wang, Patent of the Republic of China, No. I226830 (2005).
[7] K.S. Chu, J.J. Wang, O.Y.P. Hu, S.T. Ho, Y.W. Chen, Anesth. Anal. 97 (2003) 806.
[8] H.C. Ansel Jr., L.V. Allen, N.G. Popovich, Pharmaceutical Dosage Forms and Drug Delivery Systems, sixth ed., Lippincott Willams & Wilkins, Philadelphia, 1999, p. 101.
[9] H.C. Ansel Jr., L.V. Allen, N.G. Popovich, Pharmaceutical Dosage Forms and Drug Delivery Systems, seventh ed., Lippincott Willams & Wilkins, Philadelphia, 1999, p. 229.
[10] H.C. Ansel Jr., L.V. Allen, N.G. Popovich, Pharmaceutical Dosage Forms and Drug Delivery Systems, seventh ed., Lippincott Willams & Wilkins, Philadelphia, 1999, p. 397.
[11] L.H. Pao, C.H. Hsiong, O.Y.P. Hu, S.T. Ho, J. Chromatogr. B 746 (2000) 241.
[12] S. Pirnay, S. Bouchonnet, F. Hervé, D. Libong, N. Milan, P. d'Athis, F. Baud, I. Ricordel, J. Chromatogr. B 807 (2004) 335.
[13] A. Ceccato, R. Klinkenberg, P. Hubert, B. Streel, J. Pharmaceut. Biomed. Anal. 32 (2003) 619.
[14] W.J. Liaw, S.T. Ho, J.J. Wang, O.Y.P. Hu, J.H. Li, J. Chromatogr. B 714 (1998) 237.
[15] C.M. Metzler, D.L. Weiner, PCNONLIN User's Guide, Statistical Consultants, Lexington, KT, 1989.
[16] A.J. Sedman, J.G. Wagner, J. Pharm. Sci. 65 (1976) 1006.
[17] L. Shargel, A.B.C. Yu, Applied Biopharmaceutics and Pharmacokinetics, third ed., Prentice-Hall International, 1997 (Chapter 10).