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Quantitative Determination of Buprenorphine in Human Plasma by High-Performance Liquid Chromatography

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ABSTRACT

A simple and rapid method for quantitative determination of buprenorphine in plasma by high-performance liquid chromatography was developed. The concentration of the drug in the plasma of the patients having been given buprenorphine was analyzed. The method developed seems to be reliable and simple enough for pharmacokinetic study of buprenorphine in human plasma.

Buprenorphine, a new synthetic analgesic, is about 30 times as potent as morphine, and is an agonist-antagonist to the opiate. The quantitation of the drug by radioimmunoassay[1-5,8] or radioreceptor assay[7] has been reported. These methods, however, have the possibility of cross reacting to other similar chemicals, and must be performed in a special laboratory.

A simple and rapid method for quantitative determination of buprenorphine by high-performance liquid chromatography was developed, and the concentration of the drug in human plasma was analyzed.

The method developed seems to be reliable and simple enough for pharmacokinetic study of buprenorphine.

MATERIALS AND METHODS

1) Chemicals

Buprenorphine hydrochloride (Otsuka Pharmaceutical Co), butorphanol tartate (given by Bristle Mayers Co) as an internal standard, methanol and acetonitrile (high-performance liquid chromatographic grade, Nakarai Chemicals), other chemicals (analytical grade) and water (bidistilled) were used. A phosphate buffer (pH 3.75) was made from 0.1 M potassium phosphate and 85% phosphate.

2) Samples

Non-heparinized arterial blood was collected from the radial artery of five patients who had received a non-deporalized muscle relaxant (pancronium bromide 6 mg) and inspired nitrous oxide 3 min following the administration of buprenorphine hydrochloride (6 μg/kg). The blood was collected from 2 to 180 min following the administration. The blood collected was put into a 10 ml glass tube and centrifuged at 3000 rpm for 15 min, and the plasma was kept frozen at −40°C until analysis.

3) Procedure

The extraction method described by Bartlett[2] was modified. One milliliter of plasma, 0.5 ml of 0.5 N hydroxyammonium and 100 μl of 10 μg/ml of butorphanol tartate (internal standard) solution were poured into a 12 ml screw-top tube. After mixing with a voltex mixer for 15 sec, 3.5 ml of diethyl ether was added, and mixed for 5 min. After being centrifuged at 3000 rpm for 5 min, the ether layer was collected. The ether extraction was performed twice. Both ether layers were combined and evaporated to dryness by using an evaporator in a water bath at 45°C. The residue was dissolved in 100 μl of methanol, and 20 μl of the methanol solution was analyzed by high-performance liquid

chromatography.

4) High-performance liquid chromatography

. The instrument used was a high-performance liquid chromatograph (Yanako L-4000W) equipped with an electrochemical detector (Yanako VMD-501) and a chromatogram processor (Yanagimoto 7000AS). The working electrode was made of glassy carbon. The electrode-applied voltage was set at +800 mV vs. the Ag/AgCl reference electrode. A 25 × 0.4 cm (i.d.) stainless tube packed with silica (Yanapak ODS-T) was used. The top of the column was fitted with a stainless precolumn and septum injector. The column temperature was maintained at 25°C. The eluent was a mixture of acetonitrile and a pH 3.75 phosphate buffer (35 : 65) with a flow rate of 1 ml/min. The sensitivity was set at ×1. The range was set at ×4 when the sample injected into the instrument contained less than 10 ng of buprenorphine, and at ×16 when more than 10 ng.

5) Calibration curve and quantitation

Several standard methanol solutions containing from 0.02 μg/ml to 7.5 μg/ml of buprenorphine hydrochloride and 10 μg/ml of the internal standard were prepared, and 20 μl of each standard methanol solution was analyzed by high-performance liquid chromatography.

The concentration of buprenorphine hydrochloride in the material was calculated by the calibration curve made by anlyzing 1, 10 and 100 ng of buprenorphine hydrochloride.

6) Recovery

Water samples and human plasma samples containing 0, 1, 10 or 100 ng per 1 ml and 1 μg/ml of the internal standard were prepared, and analyzed by the procedure described above.

RESULTS

1) Calibration curve

The peak area ratio of buprenorphine to the internal standard was plotted against the amount of buprenorphine hydrochloride (Fig. 1). The regression curves of buprenorphine hydrochloride ranging from 0.4 to 20 ng (a) and ranging from 10 to 150 ng (b) are shown as follows:

(a) $Y = (3.59 \cdot X + 1.395) \cdot 10^{-2}$, $r^2 = 0.9976$

(b) $Y = (5.917 \cdot X + 2.557) \cdot 10^{-3}$, $r^2 = 0.9989$

where,

Y: Peak area ratio

X: Amount of buprenorphine hydrochloride (ng)

r^2: Coefficient of correlation

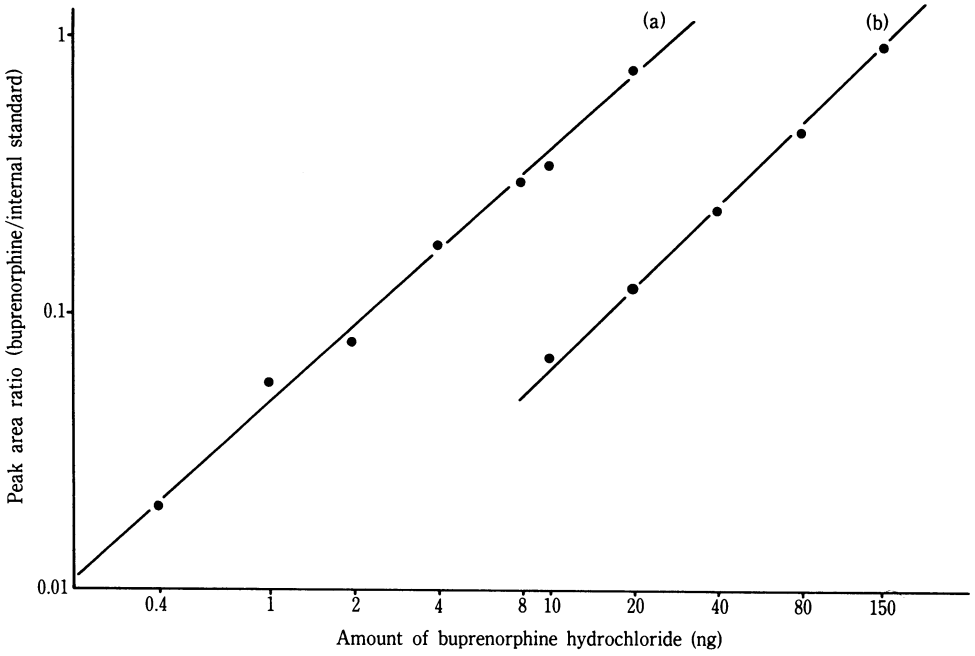

Fig. 1. Calibration curves. (a): Range ×4. (b): Range ×16.

Table 1. Extraction recovery and coefficient of variation

Amount added (ng)	Recovery (%)		Coefficient of variation (S/X, %)	
	Water	Plasma	Water	Plasma
1	97.8	87.9	5.12	10.4
10	100.2	118.8	2.38	12.8
100	96.7	104.4	2.51	2.16

X: Average. S: Standard deviation. n = 5.

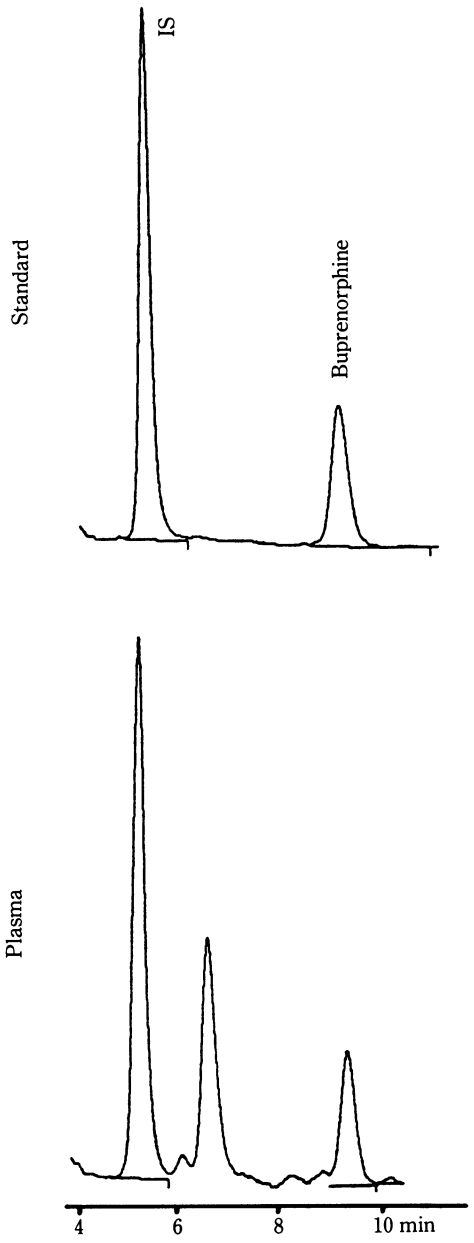

Fig. 2. High-performance liquid chromatograms. IS: Internal standard (butorphanol).

2) Recovery

The relative recoveries and coefficients of variation obtained are shown in Table 1. The relative recoveries of buprenorphine ranged from 87.9 to 118.8% in water and plasma. The coefficients of variation were from 2.38 to 5.12 % in water and from 2.16 to 12.8% in plasma.

3) Buprenorphine concentration in the plasma of five patients

The high-performance liquid chromatograms obtained by analyzing buprenorphine standard solution and plasma of one patient are shown in Fig. 2. Several unknown peaks were observed in the chromatogram of the plasma.

The buprenorphine concentrations in the plasma of five patients following administration of buprenorphine hydrochloride are shown in Table 2. The average concentrations plotted against the time following the administration are illustrated in Fig. 3.

The highest concentration was 68.9 ng/ml immediately following the administration, and 2 hr after the administration buprenorphine was detected in three patients out of five.

DISCUSSION

The retention time of buprenorphine depended on the pH value of the eluent. Buprenorphine and butorphanol (internal standard) were separated from other interfering substances in plasma by using a pH 3.75 phosphate buffer as an eluent, and suitable retention times of these chemicals were obtained. The addition of acetonitrile to the phosphate buffer reduced the retention time of these chemicals, yielded the symmetric peak waves, and increased the sensitivity.

Butorphanol was selected as an internal standard because its structure is similar to that of buprenorphine.

The method developed was as sensitive as both

Table 2. Concentrations of buprenorphine in the plasma of five patients given 6 μg/kg of buprenorphine by intravenous injection

Case	Time following administration (min)									
	2	5	10	20	30	60	90	120	150	180
No. 1	68.9	55.7	29.4	N.A.	N.A.	N.A.	N.D.	N.D.	N.D.	N.D.
No. 2	59.9	44.2	31.8	29.4	7.65	12.6	12.9	9.02	4.91	N.D.
No. 3	27.0	23.0	26.7	11.8	10.3	N.D.	N.D.	N.D.	N.D.	N.D.
No. 4	48.9	64.3	42.8	43.5	23.9	9.37	6.43	7.00	N.A.	N.A.
No. 5	50.4	38.9	40.6	25.6	27.1	17.5	10.1	11.3	7.80	7.28
Mean	51.0	45.2	34.3	27.6	17.2	9.87	5.89	5.46	3.18	1.82

N.D.: Not detected (< 0.2 ng/ml). N.A.: Not analyzed.

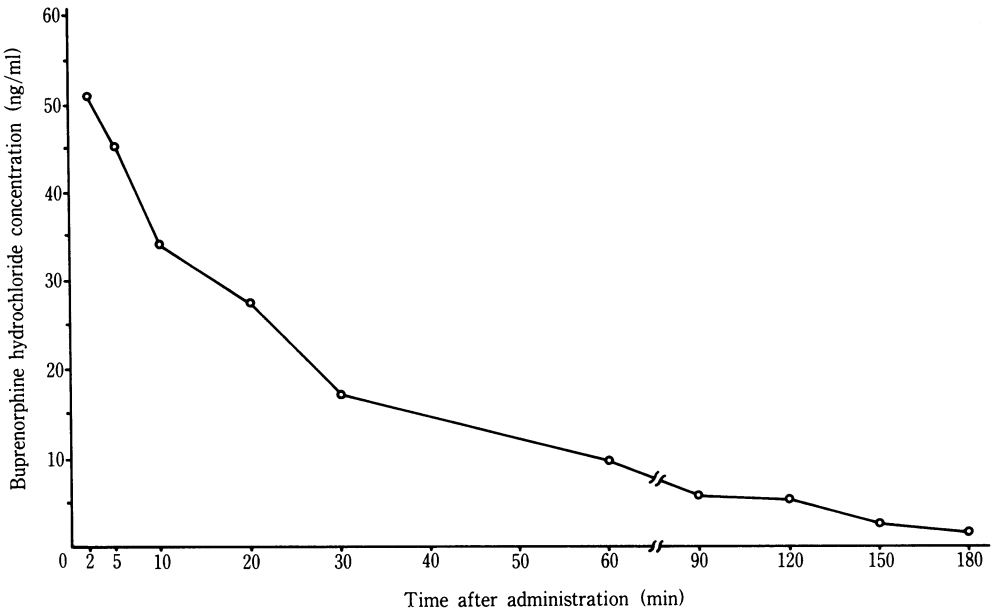

Fig. 3. The average concentrations of buprenorphine in the plasma of five patients given 6 μg/kg of buprenorphine by intravenous injection.

radioimmunoassay and radioreceptor assay, and the lowest detection limit was 0.2 ng/ml in plasma. The sensitivity, however, was lower than that of gas chromatography-mass spectrometry[6], and not sufficient to determine the concentration of buprenorphine in plasma following the administration by micro-drip infusion. Buprenorphine was detected in the plasma collected from one patient out of five who had been intravenously given 6 μg/kg of buprenorphine 180 min following the administration.

The method developed is simple and rapid in comparison to radioimmunoassay, radioreceptor assay and gas chromatography-mass spectrometry for the extraction method was simple and no cleanup procedure was necessary.

The buprenorphine concentrations in the plasma of five patients were higher than those having been determined by other methods in patients given the same amount of buprenorphine hydrochloride[1,2,4,8], and were the same as those in rats following intravenous administration of 200 μg/kg of buprenorphine[3]. One of the causes resulting in this significant difference seems to be the effect of general anesthesia. In this study, the buprenorphine plasma concentra-

tions in the patients under general anesthesia were determined. Cardiac output and hepatic blood flow were lowered by the general anesthesia, and the metabolic rate of buprenorphine seemed to slow down. Another cause was the possibility of interference by other substance in the analysis as the measurement of the buprenorphine at low concentration was occasionally disturbed by other substances.

There, however, were no significant differences in pharmacokinetic parameters. The plasma levels of buprenorphine declined very rapidly with the half-life period (t•1/2) being 8.2 min immediately after administration, which is as expected for buprenorphine is extremely lipophilic. This was followed by a slow terminal phase with t•1/2 ranging from 1 to 2 hr.

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