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Note

Quantitative determination of buprenorphine and its active metabolite, norbuprenorphine, in human plasma by gas chromatography–chemical ionization mass spectrometry

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Buprenorphine, 21-cyclopropyl-7α-[(*S*)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-*endo*-ethano-6,7,8,14-tetrahydrooripavine (BN), has both opioid agonistic and antagonistic properties [1]. It has been widely used to relieve cancer-related and postoperative pain. It appears that BN gave good results for the pain therapy when administered by sublingual and rectal routes, as well as parenteral routes, because of a considerable first-pass effect. However, BN is not available commercially except for injections as a dosage form in our country. So we have prepared BN sublingual tablets and suppositories from commercially available injections [2–4]. These preparations have been used for pain relief in patients, especially patients with cancer [5]. Studies of the disposition of BN in humans showed that its plasma concentration was very low after sublingual administration [6]. Further, an active metabolite, norbuprenorphine (NBN), has been isolated from human urine [7]. Therefore, it is necessary to have a microanalytical method to determine the concentration of BN and its active metabolite in plasma after administration of the BN preparation.

Several analytical methods have been reported for the determination of BN alone in plasma and serum. Radioimmunoassay [8] and radioreceptographic methods [9] have been reported for the determination of BN in human plasma. However, Bartlet et al. [8] reported that the antibody used did bind to the NBN

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and this could account for some of the observed BN levels at the later sampling times after intravenous administration of BN. High-performance liquid chromatographic methods using electrochemical detection have been reported for the determination of BN in human plasma [10,11]. These methods have a relatively high sensitivity. Gas chromatographic–electron-impact mass spectrometric (GC–EIMS) methods have been developed for the determination of BN in human plasma [12,13]. The method of Blom et al. [13] was based on the ring formation between the 6-methoxy group and the branched side-chain of BN by acid hydrolysis, followed by the preparation of the pentafluoropropionyl (PFP) derivatives. The lower limit of detection of BN was 150 pg/ml using 2 ml of plasma. Although they found the metabolite, NBN, in human plasma for a few hours after intravenous administration of BN, this metabolite was not quantified. Thus, simultaneous determination of BN and NBN in plasma has not yet been developed. On the other hand, several analytical methods have been reported for the determination of BN and/or NBN in urine [7,11,14,15].

In this paper, we describe a reliable GC–MS method in the chemical ionization (CI) mode for the determination of BN and its metabolite, NBN, in human plasma.

EXPERIMENTAL

Materials

BN·HCl, NBN and N-propylnorbuprenorphine (used as an internal standard, I.S.) were kindly supplied from Otsuka Pharmaceuticals (Japan). Pentafluoropropionyl anhydride (PFPA) was purchased from Pierce (U.S.A.). Sublingual tablets containing 0.1 mg of BN per tablet and suppositories containing 0.3 mg of BN per suppository were prepared according to the method reported previously [3,4]. All other solvents and reagents used were of analytical-reagent grade. Blood was collected in heparinized containers from several non-fasted human volunteers to generate a drug-free pool. Plasma was separated and frozen until use.

Gas chromatography–mass spectrometry

GC–MS analysis was performed using a mass spectrometer (JMA DX-300, JEOL, Japan) with a multiple-ion detector, a data processing system (JMA 2000, JEOL) and a gas chromatograph (GCG05, JEOL). SE-52 Chromosorb W, AW-DMCS (80–100 mesh, 1 m×2 mm I.D., Wako, Japan) was used for the separation. Helium was used as the carrier gas, and the flow-rate was 38 ml/min at a column temperature of 275°C. Injection port, separator and ion source temperatures were maintained at 280, 295 and 240°C, respectively. The mass spectrometer was operated in the CI mode with the electron current at 300 μA and the electron energy at 200 eV. Isobutane was used as reactant gas at a CI chamber pressure of ca. 1 Torr.

Extraction and derivatization

The extraction of BN and NBN from plasma was carried out according to the method of Cone et al. [14] with a minor modification. To 1 ml of plasma sample

in a 10-ml glass-stoppered centrifuge tube were added 1 ml of 0.05 *M* sulphuric acid, 4 ml of a mixture of ethyl acetate–heptane (4:1, v/v) and 100 μl of methanol containing 500 ng/ml I.S. The mixture was shaken for 5 min using a mechanical shaker, then centrifuged at 2270 *g* for 5 min. After the upper organic phase was aspirated off, 1 ml of 1 *M* sodium carbonate–hydrochloric acid buffer solution (pH 10.5) and 4 ml of the ethyl acetate–heptane mixture were added to the aqueous phase that remained. The mixture was shaken mechanically for 10 min and centrifuged at 2270 *g* for 5 min. The upper organic phase was transferred to a new tube and evaporated to dryness on a rotary vacuum evaporator, and the residue was dissolved in 0.5 ml of the same mixture of organic solvents as above. The mixture was transferred to a micro tube and evaporated. Then 30 μl of PFPA in 100 μl of toluene were added to the residue. After the mixture was allowed to stand for 1 h at room temperature, the excess reagent was evaporated to dryness under a stream of nitrogen at room temperature. The residue was dissolved in 10 μl of ethyl acetate, then an aliquot (1–4 μl) was injected into the GC–MS system.

Calibration curves

Solutions containing 0.2, 0.5, 1, 2, 5 and 10 ng/ml BN and solutions containing the same concentrations of NBN were prepared by dissolving BN·HCl and NBN in methanol, respectively. After 1 ml of each solution was placed into a centrifuge tube, the solvent was evaporated off and then 1 ml of drug-free human plasma was added to each residue. These samples were assayed according to the method described above. The peak-height ratios of *m/z* 596 for the PFP derivatives of BN and *m/z* 688 for that of the metabolite to *m/z* 584 (I.S.) were calculated and plotted against the known amounts of BN and the metabolite.

Human study

A healthy male volunteer (age 29 years and weight 60 kg), who gave informed consent to the study, received sublingually 0.2 mg of BN as two 0.1-mg tablets. Blood samples (ca. 5 ml) were collected via an indwelling venous cannula using a disposable syringe at 15, 30 and 45 min and 1, 1.5, 2, 3, 4, 5, 7 and 9 h after dosing. Two patients with cancer receiving BN preparations chronically who also gave informed consent were part of the study. One, a 56-year-old male, received three BN sublingual tablets per day in three divided doses for more than three months. The other, a 59-year-old male, received concomitantly eight BN sublingual tablets and four suppositories per day in four divided doses for more than eight months. Blood samples (ca. 5 ml) were drawn 2 h after the administration of the morning dose. The blood samples were immediately transferred into heparinized glass tubes. Plasma was immediately separated by centrifugation and stored at −80 °C until analysed.

RESULTS AND DISCUSSION

Chromatographic conditions

In the preliminary study, GC–MS analysis of BN and its metabolite, NBN, was examined in the EI mode. Fragment ions at *m/z* 524 $(M-89)^+$, 616 $(M-89)^+$

and 544 $(M-57)^+$ were monitored for the analysis of the PFP derivatives of BN, NBN and the I.S., respectively. However, this method was not satisfactory for analysis of the plasma concentration, since the background noise was too high on selected-ion monitoring (SIM) chromatograms of these compounds.

CI mass spectra of the PFP derivatives of BN, NBN and the I.S. using isobutane as a reactant gas are shown in Fig. 1. Pseudo-molecular ion peaks were not observed in their spectra. Ions at *m/z* 596 $(MH-18)^+$ for the PFP derivative of BN, *m/z* 688 $(MH-18)^+$ for that of NBN and *m/z* 584 $(MH-18)^+$ for that of the I.S. were observed as the base peaks. Thus, these fragment ions were chosen for the SIM analysis of the PFP derivatives of BN, NBN and the I.S. Among columns packed with 1% OV-17 Chromosorb W, 80–100 mesh (1 m×2 mm I.D.), with 1.5% OV-17/1.5% OV-210 Chromosorb W, 60–80 mesh (1 m×2 mm I.D.)

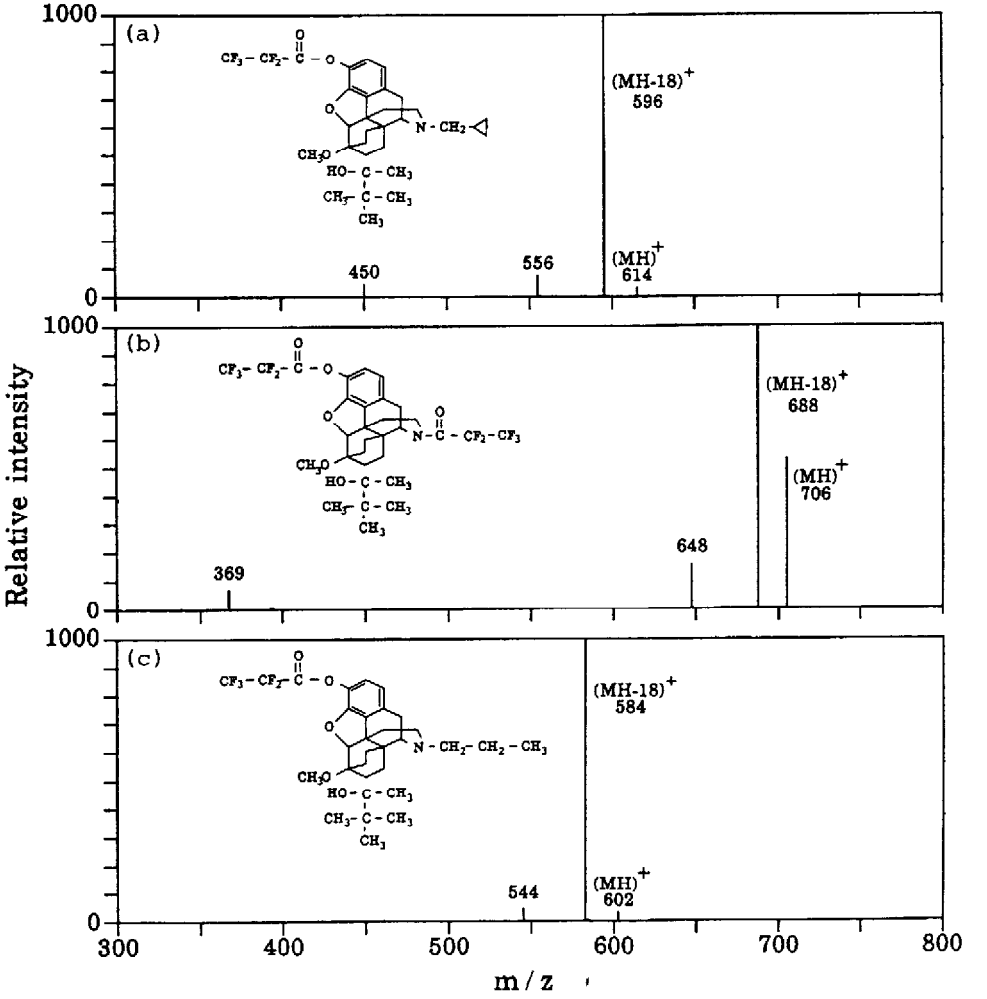

Fig. 1. Chemical ionization mass spectra of PFP derivatives of (a) buprenorphine, (b) norbuprenorphine and (c) N-propylnorbuprenorphine (internal standard).

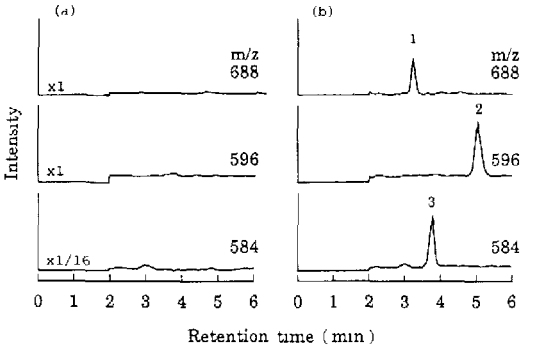

Fig 2. Selected-ion monitoring chromatograms of (a) blank plasma sample and (b) plasma sample containing 5 ng of buprenorpine, 5 ng of norbuprenorphine and 50 ng of N-propylnorbuprenorphine. Peaks: 1 = PFP derivative of norbuprenorphine; 2 = PFP derivative of buprenorphine; 3 = PFP derivative of N-propylnorbuprenorphine (internal standard).

and with 1% SE-52 Chromosorb W tested, the column packed with SE-52 Chromosorb W was the most suitable for the determination of BN and its metabolite in human plasma, since it produced the smallest background noise on the chromatogram of the PFP derivatives of BN under the selected GC–MS conditions. We also tried one-step extraction methods, but it was difficult to perform highly sensitive analyses because of the high background noise on the chromatogram. The use of both acidic and basic extractions seems to be the most suitable method to decrease the background noise. The reason for the choice of N-propylnorbuprenorphine as an internal standard is that its structure is similar to that of BN and it is converted into a similar derivative by the PFPA treatment.

Typical chromatograms obtained from drug-free human plasma and human plasma supplemented with BN, NBN and the I.S. are shown in Fig. 2. No interfering peaks were observed when blank human samples were analysed without adding the I.S. (Fig. 2). The retention times of the PFP derivatives of BN, NBN and the I.S. were 5.0, 3.2 and 3.7 min, respectively, and each peak was sharp.

Calibration curve and precision

The calibration curves were constructed by adding known amounts of BN and NBN to drug-free human plasma. The peak-height ratios of *m/z* 596 for the PFP derivative of BN and *m/z* 688 for that of the metabolite to *m/z* 584 for that of the I.S. were calculated by measuring the peak heights of the respective selected-ion current profiles. The resultant data exhibited good linearity over the range 0.2–10 ng for BN and the metabolite, respectively (regression equations: $y=0.12x+0.24$ for BN, $r=0.999$, and $y=0.067x+0.35$ for the metabolite, $r=0.998$). The lower limits of the quantitation were 0.2 ng for each of BN and NBN. Within- and between-assay variabilities in the analysis of BN and NBN were determined using plasma samples supplemented with known amounts of the compounds. The within- and between-assay coefficients of variation for peak-height ratios obtained by repeating the procedure four and six times in each sam-

TABLE I

WITHIN- AND BETWEEN-ASSAY VARIABILITY IN THE ANALYSIS OF BUPRENORPHINE AND NORBUPRENORPHINE

Concentration (ng/ml)	Coefficient of variation (%)			
	Within-assay ($n=4$)		Between-assay ($n=6$)	
	BN	NBN	BN	NBN
0.5	3.0	5.1	5.7	5.5
5	3.1	3.6	3.2	3 4

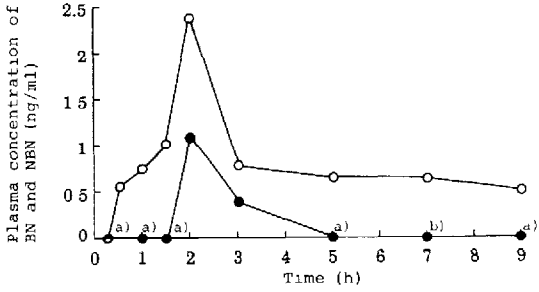

Fig. 3. Time courses of plasma concentrations of buprenorphine and its metabolite, norbuprenorphine, after administration of two sublingual tablets to a healthy volunteer. Data points: (○) buprenorphine; (●) norbuprenorphine; (a) not detected; (b) less than 0.2 ng/ml.

ple were less than 6% (Table I). The data for BN were better than those of the other assays [10–15].

Monitoring of plasma concentration

The method described here was applied to the determination of the plasma concentration of BN and NBN after sublingual administration of BN tablets to a healthy volunteer. Time courses of the concentrations of BN and NBN in plasma after a single administration of two sublingual tablets are shown in Fig. 3. BN was found in plasma 30 min after sublingual administration and the maximum plasma concentration of 2.4 ng/ml was observed 2 h after administration. Thereafter the concentration decreased and was 0.5 ng/ml after 9 h. NBN was found in plasma 2 and 3 h after administration, at concentrations of 1.1 and 0.4 ng/ml, respectively. Further, the present method was applied to determination of the concentration of BN and NBN in two patients with cancer receiving chronically BN sublingual tablets or suppositories. Plasma concentrations of BN and NBN in the patient who received sublingual tablets only were 1.8 and 2.0 ng/ml, respectively, and those in the patient who received both sublingual tablets and suppositories were 0.6 and 2.9 ng/ml, respectively.

These results demonstrate the usefulness of the present method of monitoring BN and NBN levels in clinical practice and for pharmacokinetic studies of BN

in humans. In our laboratory, this method is currently being used to study the disposition of BN and its metabolite in patients with cancer and postoperative patients after BN administration.

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