Journal of Chromatography, 613 (1993) 359–364
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6700

Short Communication

Determination of nalmefene in plasma by high-performance liquid chromatography with electrochemical detection and its application in pharmacokinetic studies

James Z. Chou*, Henrik Albeck and Mary Jeanne Kreek

Laboratory of Biology of Addictive Diseases, Rockefeller University, 1230 York Avenue, Box H19, New York, NY 10021 (USA)

(First received September 21st, 1992; revised manuscript received November 30th, 1992)

ABSTRACT

A method for measuring human plasma levels of nalmefene after oral and intravenous administration is presented. The method consists of a solid-phase extraction procedure followed by HPLC analysis. A cyanopropyl column is used for the solid-phase extraction and 60% (v/v) acetonitrile in dilute sodium pentanesulfonic acid solution for elution. The concentrated and filtered eluate is injected into the HPLC system, which is equipped with an electrochemical dual-electrode detector. A phenyl column is used in this HPLC system with a mobile phase containing 30% (v/v) acetonitrile in dilute sodium pentanesulfonic acid solution. A signal-to-noise ratio of 4.5 is obtained when a 1 ng/ml spiked plasma sample is analyzed. To determine the applicability of this method for human pharmacokinetic studies, nalmefene levels in plasma were measured at time points up to 24 h following oral and intravenous administration of 30 mg of nalmefene hydrochloride to two subjects. These studies demonstrated that the proposed method is sufficiently sensitive to study the pharmacokinetic profile of nalmefene in man.

INTRODUCTION

Nalmefene (6-desoxy-6-methylenenaltrexone) [1], a potent specific opiate receptor antagonist, is a potentially useful agent in clinical treatment. Similarly to naloxone, another widely used specific opioid antagonist, nalmefene, is metabolized in humans primarily by glucuronidation [2] whereas a third opioid antagonist, naltrexone, is metabolized in man by an enzyme-dependent oxidation–reduction pathway [3,4]. However, nalmefene has a longer half-life and greater oral bioavailability than naloxone and naltrexone [2–7]. The ability to measure nalmefene levels in human plasma is important for pharmacokinetic studies and for diverse clinical studies in which nalmefene may be used as an opioid antagonist for various indications.

This study was conducted to develop a method for the determination of nalmefene in plasma after oral and intravenous administration to humans. HPLC and radioimmunoassay (RIA) have been employed in the separation and determination of nalmefene [8,9]. However, the method reported here has improved sensitivity and selectivity, partly because a dual-electrode detection

* Corresponding author.

0378-4347/93/$06.00

system is employed, similar to the procedure for the determination of naloxone in plasma previously developed in our laboratory [10].

EXPERIMENTAL

Reagents and materials

The mobile phase consisted of 30% (v/v) acetonitrile (UV grade, Burdick & Jackson, Muskegon, MI, USA), 70% (v/v) distilled, deionized water (obtained with a Milli-Q water purification system; Millipore, Bedford, MA, USA), 5 m*M* sodium pentanesulfonic acid monohydrate (HPLC grade, Fisher Scientific, Fair Lawn, NJ, USA) and 0.0045% (v/v) orthophosphoric acid (85%, HPLC grade, Fisher). The pH of the mobile phase was 3.1. For the solid-phase extraction, cyanopropyl columns, 100 mg/ml Bond-Elut (Varian/Analytichem, Harbor City, CA, USA) were used. Nalmefene hydrochloride was provided by Baker Cummins Pharmaceuticals (Miami, FL, USA) and nalbuphine by DuPont (Wilmington, DE, USA).

HPLC system

The HPLC system used in this study was similar to that employed in the pharmacokinitic determination of naloxone in plasma [10]. Thus, only the modifications and key features are described. A phenyl column (Nova-Pak Radial-Pak, 100 mm × 8 mm I.D., 4 μm particle size; Waters Assoc., Milford, MA, USA) was used and a 0.2-μm graphite filter (ESA, Bedford, MA, USA) was inserted to protect the column and another one to protect the detector. The flow-rate of the mobile phase was 1.2 ml/min. Three electrochemical cells were involved in screening and detection: a guard cell (−0.3 V); detector 1 (+0.23 V); and detector 2 (+0.35 V). The output of detector 2 was digitized by an HP 35900 interface (Hewlett-Packard, Melville, NY, USA) and the data were transferred to a computer. The chromatograms were integrated and analyzed with an HPChem-Station, version 3.0 (Hewlett-Packard).

Solid-phase extraction

The plasma samples were centrifuged (15 min, 4000 *g*) and the supernatants were pipetted into glass culture tubes. For standards, 700 μl of blank plasma (from blood bank) were mixed with 200 μl of nalmefene standards of 15.6 ng/ml and 100 μl of nalbuphine (in the mobile phase). For samples, 700 μl of sample plasma were mixed with 200 μl of mobile phase and 100 μl of nalbuphine. The 1-ml Bond Elut CN extraction columns were activated with 1 ml of acetonitrile followed by 1 ml of water. The sample was applied to the mini-columns and a partial vacuum was applied to initiate the flow. The columns were then washed with deionized water five times and dried by drawing air through for 1 min. The elution agent consisted of 1 ml of 60% acetonitrile in 1 m*M* sodium pentanesulfonic acid aqueous solution. The eluate was placed in a speed vacuum concentrator (Savant Model SVC200H and VP190 two-stage pump; Savant Instruments, Farmingdale, NY, USA) for 1 h to reduce its volume. The final volume of the eluate was *ca.* 200 μl. The sample was filtered through a 0.2-μm nylon filter (Vangard International Lida Manufacturing, Kenosha, WI, USA), centrifuged for 15 min at 3000 *g* to remove any particles and 50 μl of the filtrate were analyzed by HPLC. Standards and unknown samples were analyzed in duplicate.

RESULTS AND DISCUSSION

Method

To obtain good selectivity, the potentials of the two electrodes are set such that the extraneous materials are oxidized by the first electrode and the compound of interest is oxidized by the second electrode, which makes the measurements. This was achieved by plotting the measured detector 2 response (to a known concentration of nalmefene standard while holding detector 1 at 0 V) against the voltage setting of detector 2. A voltammogram was constructed (Fig. 1) and the potentials of the two electrodes were set at 0.23 and 0.35 V.

Various amounts of acetonitrile in aqueous so-

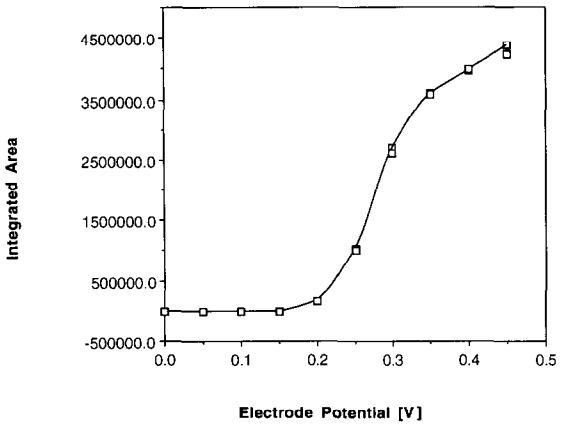

Fig. 1. Hydrodynamic voltammogram for nalmefene. The detector response was recorded for the same 20-μl injection of a 5 ng/ml nalmefene standard. Detector 1 was set at 0.0 V while the potential of the detector 2 was varied from 0.0 to 0.45 V in increments of 0.05 V. The integrated area under the peak is plotted on the vertical axis. The solid line is a cubic spline interpolation through these data points.

lution were tested as mobile phases so that the separation of different components could be achieved within a reasonable length of time. The mobile phase that satisfied these criteria for this study consisted of an acidic aqueous solution of 30% (v/v) acetonitrile and sodium pentanesulfonic acid (ion-pairing agent). Naloxone, naltrexone and nalbuphine were all possible internal standards. However, the peaks produced by naloxone and naltrexone were too close to the solvent peaks and the peaks from the residue of the plasma proteins. Nalbuphine was found to be a satisfactory internal standard as its peak was clearly separated from the extraneous earlier peaks and the nalmefene peak. With a flow-rate of 1.2 ml/min, the retention times (t_R) of the nalbuphine and nalmefene peaks were *ca.* 7 and 12 min, respectively, as shown in Fig. 2c. The chromatograms of blank plasma (taken before the intravenous injection of nalmefene) with (Fig. 2b) and without (Fig. 2a) the internal standard nalbuphine are also shown, demonstrating a stable baseline throughout the region where a nalmefene peak was expected. Many extraneous early peaks ($t_R \approx$ 2–5 min), a few small "bumps" ($t_R \approx$ 10 min) and a late peak ($t_R \approx$ 14.5 min) were

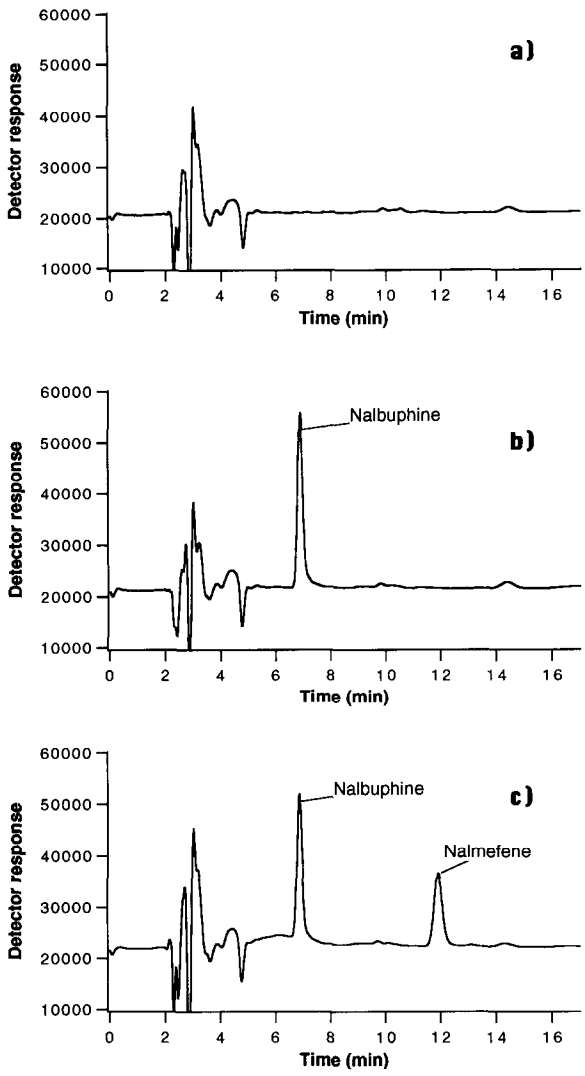

Fig. 2. Typical chromatograms from injections of sample extract. (a) The plasma sample was obtained 15 min before nalmefene was given; (b) nalbuphine (internal standard) was added to the above plasma sample; (c) the plasma sample was obtained 5 min after the subject had received 30 mg of nalmefene · HCl intravenously. Nalbuphine was also added as an internal standard.

always observed, probably due to the residual proteins in plasma that the CN-bonded mini-column did not remove completely.

Calibration graphs were obtained with nalmefene standards and nalmefene-spiked plasma samples in the range 1–500 ng/ml. Even in this wide range of concentrations the assay showed a high degree of linearity for a line either forced

TABLE I

CHARACTERISTIC PARAMETERS OF NALMEFENE ASSAY

Concentration (ng/ml)	Parameter[a]	Value
15.6	Intra-assay C.V. (%)	3.0
	Inter-assay C.V. (%)	3.5
	Accuracy (%)	3.2
62.5	Intra-assay C.V. (%)	1.8
	Inter-assay C.V. (%)	2.0
	Accuracy (%)	2.7
125	Intra-assay C.V. (%)	3.0
	Inter-assay C.V. (%)	2.9
	Accuracy (%)	2.8

[a] Intra-assay C.V.: on each of three different days four plasma spiked samples containing different amount of nalmefene · HCl were analyzed; the average C.V. was calculated and is reported here. Inter-assay C.V.: the C.V. calculated from all these samples at a given concentration. Accuracy: the deviation of the average value for samples (calculated from standards) compared with the expected spiking values of 15.6, 62.5 and 125 ng/ml.

through the origin or a least-squares linear regression through these points, with high correlation coefficients of 99.9 and 99.8% for nalmefene standards and nalmefene-spiked plasma curves, respectively. The intra- and inter-assay coefficients of variation (C.V.) were calculated at several nalmefene spiking concentrations and the results at three concentrations are shown in Table I, demonstrating the characteristic parameters of the assay. A signal-to-noise ratio of 4.5 was obtained for a 1 ng/ml spiked plasma sample.

The sensitivity of the method was sufficient to detect nalmefene levels in human subjects up to 24 h after 30 mg of the drug had been administered either intravenously or orally. We did not study samples with lower concentrations. However, it is expected that increased sensitivity could be achieved by injecting a larger volume of the final eluate, instead of the injection volume of 50 μl used in this study.

Applications

The procedure was applied to a pharmacokinetic study of nalmefene in two subjects. Two volunteer subjects were given a dose of 30 mg of

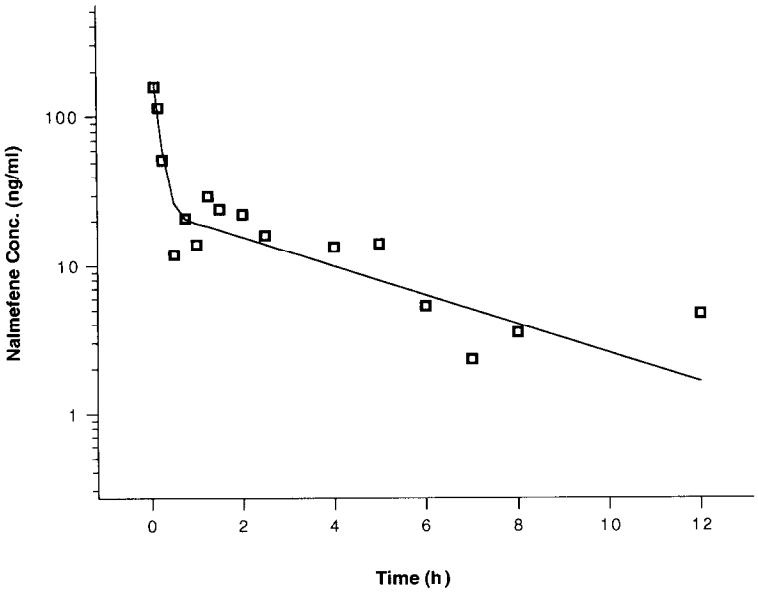

Fig. 3. Time profile of the nalmefene level in plasma after a subject had received a bolus injection of 30 mg of nalmefene · HCl intravenously. The open squares represent data points and the solid line is a (non-linear least-squares) double exponential fit to the data. Blood specimens were taken for 12 h after administration of nalmefene.

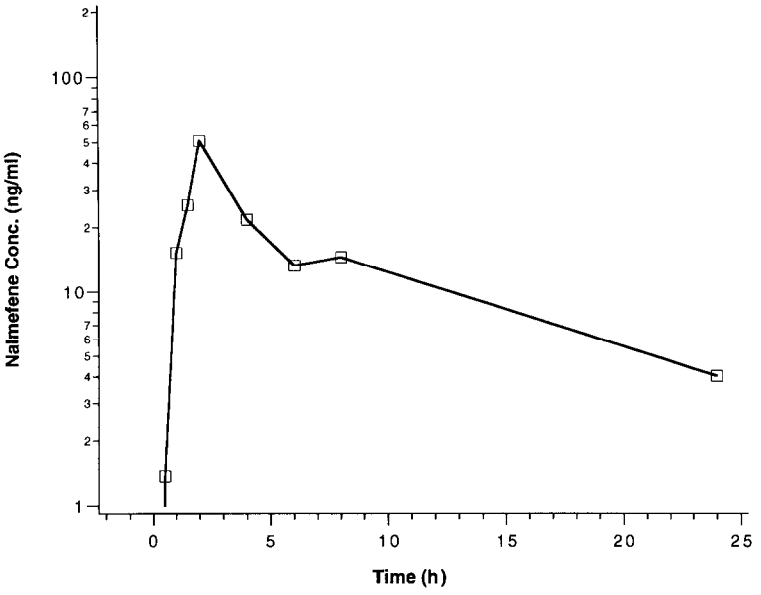

Fig. 4. Time profile of the nalmefene level in plasma after an oral administration of 30 mg of nalmefene · HCl to a subject. Blood specimens were obtained for 24 h after administration of nalmefene.

nalmefene hydrochloride intravenously (subject I) or orally (subject II) and blood was drawn at regular intervals. Both subjects were females, aged 45 (subject I) and 70 years (subject II). The plasma samples were analyzed according to the procedures described earlier. The areas of the nalmefene and nalbuphine peaks were calculated by the HP computer program. The ratio of nalmefene to nalbuphine peak areas, when normalized by the known standards, gives an accurate measurement of the concentration of nalmefene in each sample. The time profile of free nalmefene levels in plasma from 0 to 12 h is shown after subject I had received 30 mg of nalmefene · HCl intravenously (Fig. 3) and from 0 to 24 h after subject II had received 30 mg of nalmefene · HCl orally (Fig. 4). In subject I, who received nalmefene intravenously, the plasma level peaked at an early time and decreased as time elapsed. With oral administration, the drug absorption period delayed the peak plasma concentration of nalmefene, which occurred within *ca.* 2 h after dose administration.

Following intravenous administration, the decrease in nalmefene plasma levels with time appeared to deviate from a "smooth" decline. This is not likely to be a method-related effect. In both clinical studies, blood samples were drawn from an indwelling cannula, without flushing with saline. At each time point, the cannula line was cleared by discarding the first 1–2 ml initial volume of blood; then the blood samples collected in heparinized tubes were centrifuged in a refrigerated centrifuge immediately. The plasma portion was separated and the plasma samples were frozen immediately at −40°C until the time of this analysis. All tubes were thawed and analyzed at the same time. Although it is possible that different CN-bonded mini extraction columns may not yield identical results in extracting nalmefene from individual plasma samples, tests using nalmefene-spiked plasma demonstrated that the variance was minimal (Table I). Measurements of precision and accuracy presented in Table I also showed that the method is consistent and reliable over a wide range of nalmefene concentrations, including the range in which most of the uneven decline of nalmefene levels occurred.

Other opioid antagonists and agonists such as naloxone [10] and methadone [11,12], and other

basic amine drugs, have been reported to exhibit similar plateaux and "bumps" in their plasma disappearance curves. One hypothesis which has been proposed to explain this previously documented phenomenon is that when a subject is allowed to eat during the study, the normal surge of bile acids undergoing enterohepatic cycling through the liver may release tissue-bound nalmefene back into the circulation by a detergent effect [10–12]. This pharmacokinetic phenomenon was observed in both subjects. In the subject receiving nalmefene intravenously, a small plateau was seen at 1, 4 and 8 h (Fig. 3). In the subject receiving nalmefene orally, a plateau was observed at around 8 h (Fig. 4), although the details of the plasma disappearance curve were not available owing to the paucity of data points for subject II. Further studies are in progress to explore this pharmacokinetic phenomenon.

Figs. 3 and 4 demonstrate that this method is sufficiently sensitive to follow the nalmefene concentration changes after intravenous and oral administration for up to 24 h and thus the pharmacokinetic profile of nalmefene can be obtained. A modest increase in sensitivity over the existing HPLC method with electrochemcial detection [5,8] provides the potential for this method to monitor even lower nalmefene levels in plasma.

ACKNOWLEDGEMENTS

We thank Drs. Elliot Hahn and Gregory Smith of Baker Cummins Pharmaceuticals for supplying the nalmefene standards, Dr. Isabelle Maisonneuve for many helpful suggestions and discussions, Janel Maki for editorial assistance and proof reading and Shirley Pinto and Kevin Reid for technical assistance. This work was supported in part by NIH–NIDA Research Center Grant I-P50-DA05130, Aaron Diamond Foundation (M.J.K.,H.A. and J.Z.C.), an Aaron Diamond Postdoctoral Fellowship (J.Z.C.) and an NIH-NIDA Research Scientist Award, K05-DA00049 (M.J.K.).

REFERENCES

1 E. F. Hahn, J. Fishman and R. D. Heilman, *J. Med. Chem.*, 18 (1975) 259.
2 R. Dixon, J. Gentile, H. B. Hsu, J. Hsiao, J. Howes, D. Garg and D. Weidler, *J. Clin. Pharmacol.*, 27 (1987) 233.
3 J. P. Gonzalez and R. N. Brogden, *Drugs*, 35 (1988) 192.
4 M. E. Wall, D. R. Brine and M. Perez-Reyes, *Drug Metab. Dispos.*, 9 (1981) 369.
5 R. Dixon, J. Howes, J. Gentile, H. B. Hsu, J. Hsiao, D. Garg, D. Weidler, M. Meyer and R. Tuttle, *Clin. Pharmacol. Ther.*, 39 (1986) 49.
6 J. Fishman, H. Roffwarg and L. Hellman, *J. Pharmacol., Exp. Ther.*, 187 (1973) 575.
7 A. R. Aikenhead, D. R. Derbyshire, C. A. Pinnock, K. Achola and G. Smith, *Anesthesiology*, 61 (1984) A381.
8 J. Hsiao and R. Dixon, *Res. Commun. Chem. Pathol. Pharmacol.*, 42 (1983) 449.
9 R. Dixon, J. Hsiao, W. Taaffe, E. Hahn and R. Tuttle, *J. Pharm. Sci.*, 73 (1984) 1645.
10 H. Albeck, S. Woodfield and M. J. Kreek, *J. Chromatogr.*, 488 (1989) 435.
11 K. Nakamura, D. L. Hachey, M. J. Kreek, C. S. Irving and P. D. Klein, *J. Pharm. Sci.*, 71 (1982) 39.
12 M. J. Kreek, M. Oratz and M. A. Rothschild, *Gastroenterology*, 75 (1978) 88.