CHROMBIO. 2548

Note

Quantitation of levorphanol in plasma using high-performance liquid chromatography with electrochemical detection

RUDOLPH LUCEK* and ROSS DIXON*

*Department of Pharmacokinetics, Biopharmaceutics and Drug Metabolism,
Hoffmann-La Roche Inc., Nutley, NJ 07110 (U.S.A.)*

Levorphanol, (—)-3-hydroxy-N-methylmorphinan (Fig. 1), is one of the most widely used oral and parenteral narcotic analgesics in the management of patients with moderate to severe pain associated with cancer and terminal illness. As a congener of morphine, it has similar properties and actions, 2 mg of i.m. levorphanol having equal analgesic potency as 10 mg of i.m. morphine [1]. Levorphanol also has good oral efficacy with an i.m./p.o. ratio of about one half [1].
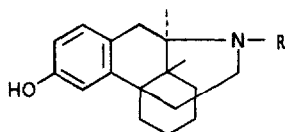
R = CH₃ LEVORPHANOL

= CH₂CH = CH₂ LEVALLORPHAN

Fig. 1. Structures of levorphanol, (—)-3-hydroxy-N-methylmorphinan, and levallorphan, internal standard.

At the present time there are only two reported procedures with the necessary sensitivity for the quantitation of plasma concentrations of the drug encountered in the clinical situation. Dixon et al. [2] reported a radioimmunoassay (RIA) while more recently Min and Garland [3] developed a gas chroma-

*Present address: Key Pharmaceuticals Inc., Miami, FL 33769, U.S.A.

tography—mass spectrometry (GC—MS) procedure and confirmed the selectivity of the original RIA. However, neither of these methods is readily accessible to most investigators; the RIA requires the production of specific antiserum while GC—MS involves costly instrumentation.

To overcome these limitations, the present study describes the development of a simple high-performance liquid chromatographic (HPLC) procedure utilizing electrochemical detection (ED) to determine levorphanol concentrations as low as 1 ng/ml in human plasma.

EXPERIMENTAL

Reagents and materials

All reagents were of analytical reagent grade. Solvents were supplied by Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Levorphanol was supplied by Hoffmann-La Roche (Nutley, NJ, U.S.A.).

High-performance liquid chromatography

The HPLC—ED system was composed of a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A solvent delivery pump, Model U6K universal liquid chromatograph injector equipped with a 2-ml sample loop and a Waters reversed-phase column, 300 mm × 4 mm I.D., containing μBondapak C_{18} (10 μm particle size). A 2-μm precolumn filter (Waters 84560) was placed before the reversed-phase column. Compound detection was achieved with an amperometric detector system (LC-4B, Bioanalytical Systems, Lafayette, IN, U.S.A.). The amperometric detector with a glassy carbon electrode and Ag/AgCl reference electrode was set in the oxidation mode at an applied potential of +1.00 V.

Chromatography was performed at ambient temperature using a mobile phase composed of acetonitrile—0.01 M sodium chloride (30:70) containing 0.1 mM EDTA. The aqueous phase was adjusted to pH 4.8 with 1 M hydrochloric acid before mixing with the acetonitrile. The flow-rate was 1.0 ml/min.

Plasma extraction

To a 1-ml aliquot of plasma containing either known or unknown concentrations of levorphanol, 20 ng of levallorphan (Fig. 1) was added as an internal standard. After mixing, 1 ml of 1 M borate buffer (pH 9) was added and the sample extracted by shaking for 10 min with 10 ml of hexane—ethyl acetate (9:1). Following centrifugation (2500 rpm for 10 min), 9 ml of the organic extract was taken and evaporated to dryness at 50°C under nitrogen in a conical tube. The residue was dissolved in 200 μl of the mobile phase of which a 100-μl aliquot was injected for HPLC—ED analysis.

Calculations

Calibration curves were derived by plotting peak height ratios of levorphanol to internal standard response versus concentration and fitting by least-squares regression analysis using a power function. Correlation coefficients were typically 0.999. Calibration curves were run daily. The standards were taken through the extraction procedure and handled in an identical manner to the unknown, thereby avoiding any corrections for recovery in the unknowns.

Based on the response of unextracted standards, the extraction procedure gave a levorphanol recovery of 104%.

RESULTS AND DISCUSSION

Assay characteristics

Under the chromatographic conditions specified, levorphanol and levallorphan, internal standard, eluted at 6.5 and 9.5 min, respectively (Fig. 2). No interfering peaks were noted in the chromatograms in the retention region of levorphanol or the internal standard when plasma extracts were chromato-graphed. Calibration curves derived from plasma fortified with levorphanol and extracted by the procedure outlined above were constructed over the
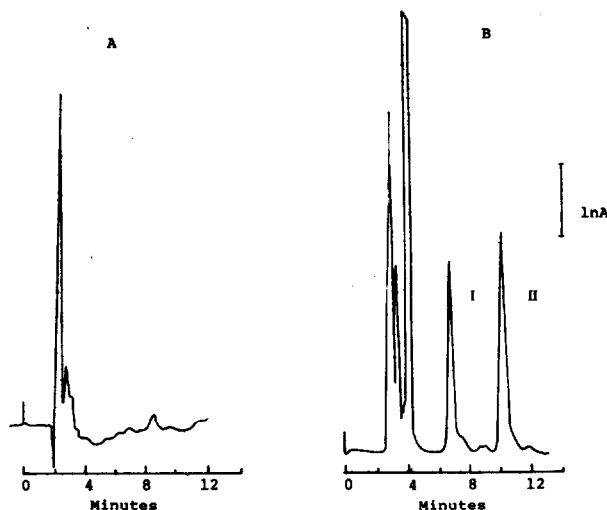

Fig. 2. Chromatograms of plasma extracts: (A) control plasma; (B) 10 min following a 5-mg i.v. dose of levorphanol in man. Column: μBondapak C_{18}, 300 mm $\times$ 4 mm, mobile phase: acetonitrile–0.01 M sodium chloride, pH 4.8, (30:70); flow-rate 1.0 ml/min; applied potential, +1.00 V. Peaks: I = levorphanol, 15.8 ng/ml; II = levallorphan, internal standard, 20 ng/ml.

TABLE I

RETENTION TIME AND DETECTOR RESPONSE FOR COMMON NARCOTIC ANALGESICS

No detector response was observed for codeine, morphine, hydrocodone, oxycodone, hydromorphine, meperidine, propoxyphene, 1-α-acetylmethodol, acetaminophen or caffeine at concentrations of 125 ng/ml.

	Retention time (min)	Response (mm/ng/ml)
Levorphanol	6.4	1.50
6-Acetylmorphine	4.2	1.08
Oxymorphine	3.2	0.20
Pentazocine	14.4	1.07
Heroin	4	0.22

concentration range 1.25—50 ng/ml. Over the concentration range 1.25—50 ng/ml the intra- and inter-assay coefficients of variation ($n = 3$) did not exceed 11.8% and 9.5%, respectively.

Selectivity of assay

To determine the possibility of interference from other commonly used drugs and/or their metabolites which may be present in patient's plasma, the assay selectivity was evaluated by assaying plasma samples containing 125 ng/ml of the following compounds: heroin, 6-acetylmorphine, codeine, morphine, hydrocodone, oxycodone, hydromorphine, meperidine, propoxyphene, oxymorphine, 1-α-acetylmethodol, acetaminophen and caffeine. No chromatographic interference was found in the determination of levorphanol or the internal standard levallorphan from any of the compounds tested. No detector response was found for any of the compounds tested except heroin, 6-acetylmorphine, oxymorphine and pentazocine. All of which, were well separated from levorphanol (Table I).

Analysis of clinical plasma samples

In order to assess the clinical usefulness of the HPLC—ED procedure, plasma samples from patients who had received levorphanol i.v., i.m. and p.o. were assayed for drug content. Concentrations of levorphanol determined in two patients (A and B) who had received 2-mg and 5-mg i.v. doses of the drug, respectively, are given in Fig. 3. Patient A had been receiving daily 2-mg doses of levorphanol i.m. prior to this present i.v. administration which may account
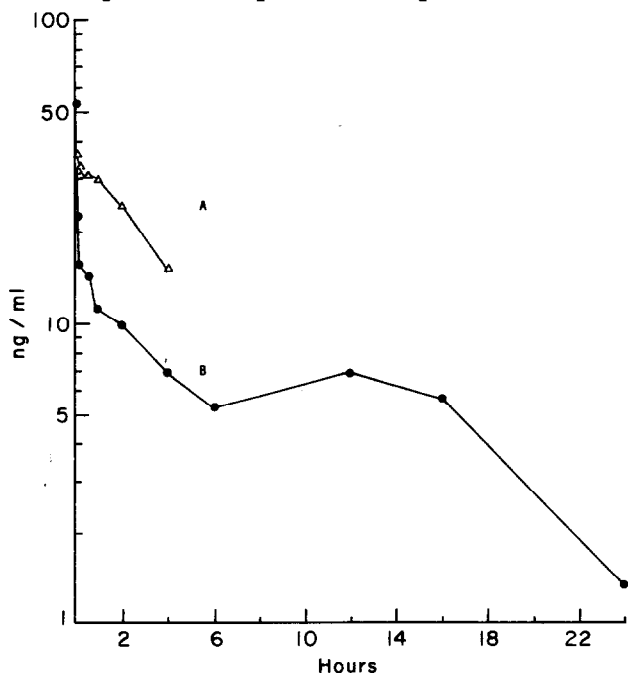

Fig. 3. Plasma concentration—time curves of levorphanol in two patients after receiving levorphanol: (A) 2 mg levorphanol i.v. (preceded by 2 mg levorphanol i.m. daily); (B) single 5-mg i.v. dose of levorphanol.

for the high concentrations of levorphanol found relative to the concentrations observed in patient B who received only a single 5-mg i.v. dose. Due to limited data obtained from these two patients, no pharmacokinetic interpretation was attempted.

Steady-state plasma concentrations of levorphanol were quantitated in a patient who had been receiving 16 mg of drug orally twice daily. The concentrations ranged from 65 to 87 ng/ml up to 3 h after drug administration. When the same patient was switched to 8 mg of levorphanol i.m. with the same dosing schedule, similar plasma concentrations were observed and ranged from 58 to 76 ng/ml. It should be noted that the i.m. and p.o. doses which result in equivalent plasma concentrations of levorphanol have an i.m./p.o. ratio of one half which is equivalent to the i.m./p.o. efficacy ratio reported previously [1].

In conclusion, the HPLC—ED method provides a simple approach for the quantitation of levorphanol at clinical doses.

REFERENCES

1   R.W. Houde, S.L. Wallenstein and W.T. Beaver, in C. De Stevens (Editor), Analgesics, Academic Press, New York, 1975, p. 75.
2   R. Dixon, T. Crews, E. Mohacsi, E. Inturrisi and K. Foley, Res. Comm. Chem. Pathol. Pharmacol., 32 (1981) 545.
3   B.H. Min, W.A. Garland and J. Pao, J. Chromatogr., 231 (1982) 194.