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Note

High-performance liquid chromatography and preliminary pharmacokinetics of nicomorphine and its metabolites 3-nicotinoyl- and 6-nicotinoylmorphine and morphine

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Nicomorphine (Vilan®) is the 3,6-dinicotinoyl ester of morphine and may be described as a morphinomimetic prodrug with an approximate equipotency with morphine. Since 1960 nicomorphine has frequently been used clinically as a central analgesic during anaesthesia, and more recently also to relieve post-operative pain by epidural administration [1].

From studies with radioactively labelled nicomorphine in rats, it is known that this substance rapidly disappears from the blood and enters the brain, with the concomitant appearance of its metabolites 6-nicotinoylmorphine (6NM) and morphine in the blood and the brain [2]. In humans no pharmacokinetic and/or metabolic studies have been described. To investigate the pharmacokinetics of nicomorphine in humans, a high-performance liquid chromatographic analysis (HPLC) in combination with UV and electrochemical detection has been developed to analyse nicomorphine and its metabolites in serum samples obtained

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from three groups of patients receiving 30, 20 and 10 mg of nicomorphine, respectively (i.v.). This note describes the HPLC analysis and the preliminary kinetic profile of nicomorphine and its metabolites.

EXPERIMENTAL

HPLC of nicomorphine

For nicomorphine measurements, a Spectra-Physics 3500 B high-performance liquid chromatograph (Spectra-Physics, Eindhoven, The Netherlands) was used, with a Spectroflow variable-wavelength detector (Model 773, Rotterdam, The Netherlands) operated at 265 nm. The detector was connected to a recorder (BD 7, Kipp & Zonen, Delft, The Netherlands). A stainless-steel column (150×4.6 mm I.D.) packed with Hypersil 5 ODS, particle size 5 μm (Chrompack, Middelburg, The Netherlands) was used. A guard column (75×2.1 mm I.D.) tap-filled with pellicular reversed-phase material (Chrompack) was placed between the sampling valve and the analytical column. The injection volume was 50 μl.

The solvent contained water, tetramethylammonium hydroxide (TMAOH) and acetonitrile: 7 ml of TMAOH (10% solution in water, Merck, Darmstadt, F.R.G.) was adjusted to pH 4.0 with phosphoric acid (total volume 300 ml with water), after which 150 ml of acetonitrile were added. The flow-rate was 1.6 ml/min at a pressure of 9.1 MPa. Nicomorphine had a retention time of 2.7 min ($k' = 1.6$).

HPLC of nicomorphine metabolites: morphine, 3-mononicotinoylmorphine and 6-mononicotinoylmorphine

A Spectroflow 400 high-performance liquid chromatograph (Kratos, Rotterdam, The Netherlands) equipped with an electrochemical detector (Model 5100 A, ESA, Kratos) was used. The analytical cell (Model 5010, ESA) consisted of two units, which could be regulated separately. The potentials of detector cell 1 and detector cell 2 were +0.13 V and +0.80 V, respectively. Both cells were connected to a two-channel 10-mV recorder (BD 41, Kipp & Zonen).

A stainless-steel column (150×4.6 mm I.D.) packed with Hypersil 5 ODS, particle size 5 μm, was used in combination with a guard column. The injection volume was 20 μl. The solvent contained 0.5 g of sodium chloride dissolved in 350 ml of 0.001 *M* disodium hydrogen phosphate and 50 ml of 0.001 *M* potassium dihydrogen phosphate, and an organic part consisting of 300 ml of methanol and 100 ml of acetonitrile (pH 7.4). The water and the organic part were separately filtered (0.5 μm), then mixed and degassed with a gentle stream of helium. The flow-rate was 1 ml/min at a pressure of ca. 11.1 MPa. In this case, HPLC-grade water (Fisons) was used to minimize the background current.

All the other reagents were of analytical-reagent grade and were obtained from Merck. The chromatographic procedures were carried out at room temperature (21°C). Under these conditions morphine, 6NM and 3-nicotinoylmorphine (3NM) had retention times of 3.1, 6.6 and 7.0 min, respectively, and k' values of 1.2, 3.7 and 4.0, respectively.

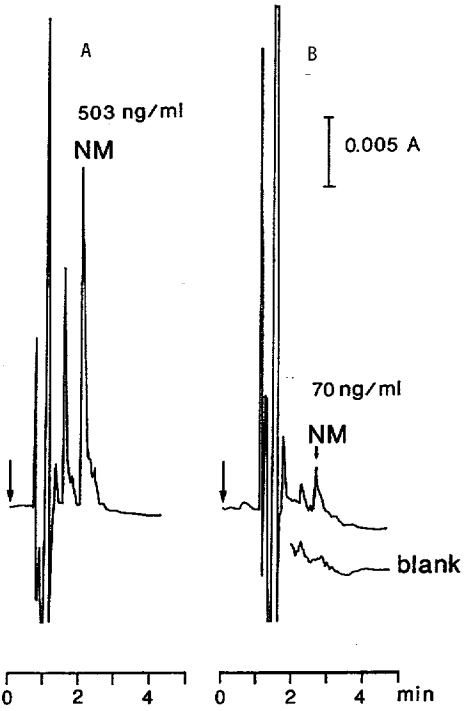

Fig. 1. HPLC profiles of (A) nicomorphine (NM) at a relatively high serum concentration of 503 ng/ml and (B) a blank serum sample and nicomorphine in a relatively low serum concentration of 70 ng/ml.

Drugs

Nicomorphine, 6NM, 3NM and morphine were obtained from Nourypharma (Oss, The Netherlands).

Patients

Samples were taken from patients in the Department of Anaesthesiology of the Sint Radboud Hospital. Blood samples were taken just before and at regular time intervals during and after anaesthesia: 1, 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, 30, 45, 60, 90, 120, 240, 360 and 480 min after the administration of nicomorphine.

Sample preparation

Serum aliquots (0.5 ml) were extracted with the Baker-10 extraction system (Baker Chemicals, Cat. No. 70180, Deventer, The Netherlands) fitted with 1-ml disposable extraction columns packed with cyanopropylsilane bonded to silica gel (Cat. No. 7021-1). The extraction column was conditioned with two column volumes of methanol, two column volumes of water and 1 ml of 500 m*M* diammonium sulphate (pH 9.3). The serum was brought to the top of the column, and the column was washed with 1 ml of 5 m*M* diammonium sulphate (pH 9.3). The sample was eluted with five 0.5-ml aliquots of chloroform–propanol (90:10). This organic phase was evaporated to dryness under a stream of nitrogen at 37°C. The residue was dissolved in 200 μl of water.

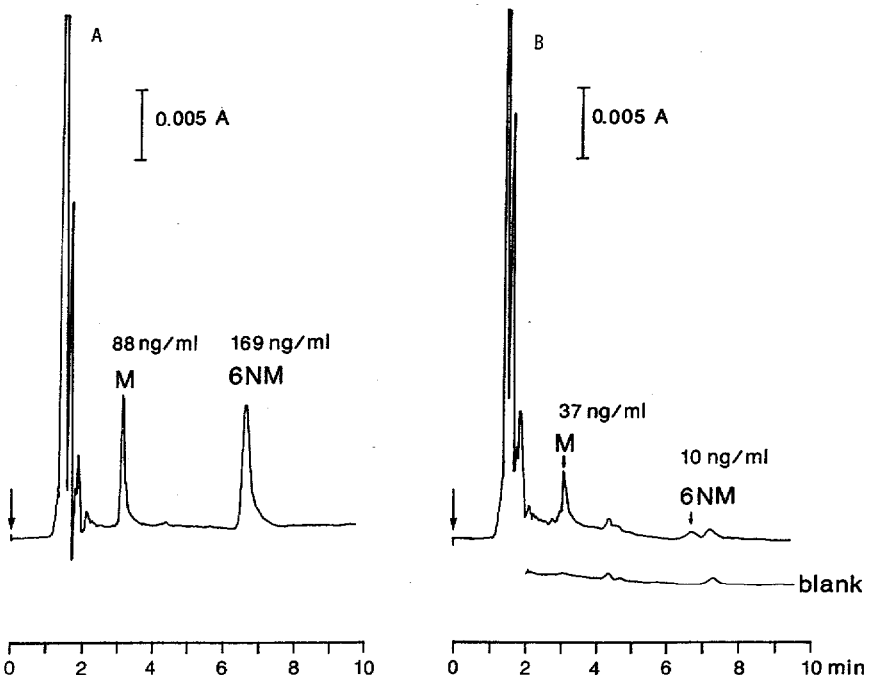

Fig. 2. HPLC profiles of (A) nicomorphine metabolites 6-nicotinoylmorphine (6NM) and morphine (M) at a relatively high serum concentration of 169 and 88 ng/ml, respectively, and (B) a blank serum sample and the metabolites at a relatively low serum concentration.

Recovery and reproducibility

Calibration curves were prepared by adding various amounts of stock solution to blank serum. The calibration samples for electrochemical detection (morphine and 6NM) and UV detection (nicomorphine) were prepared separately. The calibration graphs were linear for morphine in the concentration range 15–300 ng/ml ($r=0.9975$), for 6NM and 3NM in the concentration range 15–300 ng/ml ($r=0.9915$ and 0.9810, respectively) and for nicomorphine in the concentration range 70–800 ng/ml ($r=0.9965$).

After the extraction of patient samples, the sample has a volume of 200 μl. This enables the simultaneous injection of the same sample on both HPLC systems: a 20-μl injection volume for electrochemical detection and 50 μl for UV detection. In this way only 0.5 ml of serum were needed to determine all known metabolite concentrations. The detection limit for morphine and 6 NM is 10 ng/ml, for 3NM 30 ng/ml and for nicomorphine 40 ng/ml.

RESULTS

Fig. 1A and B shows chromatograms of nicomorphine in a relatively high concentration of 503 ng/ml (A) and in an low concentration of 70 ng/ml (B) in the serum of a patient. The compound is well separated from the low concentrations of endogenous compounds present in blank serum.

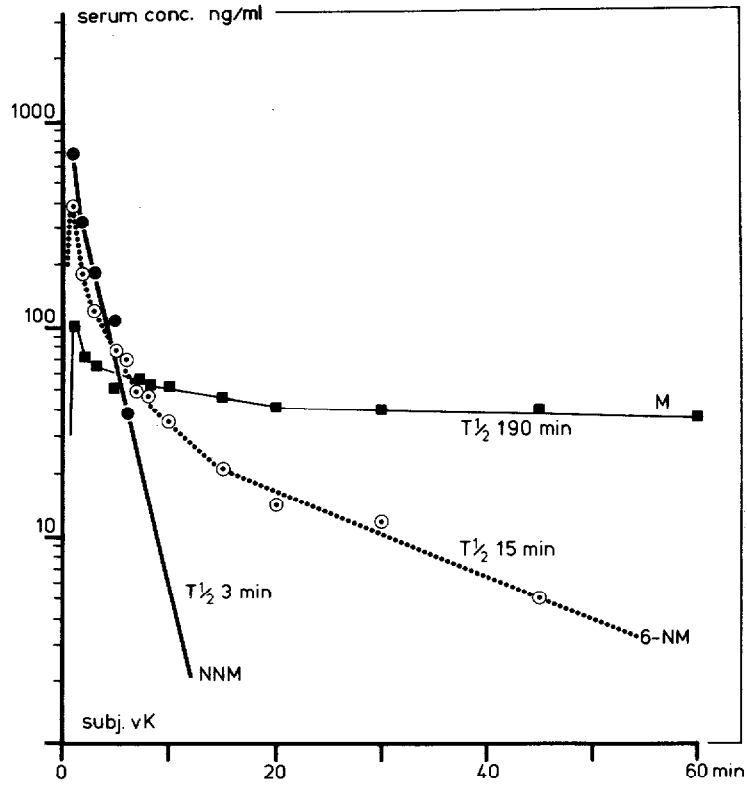

Fig. 3. Serum concentration-time curves of nicomorphine (NNM) and its metabolites 6-nicotinoylmorphine (6-NM) and morphine (M) in a patient after an i.v. dose of 20 mg of nicomorphine.

Fig. 2A and B shows chromatograms of the metabolites 6NM and morphine in relatively high (A) and low (B) serum concentrations. The compounds are well separated from endogenous compounds present in blank serum.

The recoveries of the extraction procedure for morphine, 6NM, 3NM and nicomorphine were 87, 96, 91 and 69%, respectively. The reproducibility was ca. 5% for the concentration ranges of all compouds mentioned above.

Fig. 3 shows the serum concentration–time curve of nicomorphine, 6NM and morphine in a patient after an i.v. dose of 20 mg of nicomorphine. Nicomorphine is detectable in serum for only 10 min, and it is rapidly converted into 6NM and morphine. The metabolite 3NM is not detectable in the serum of patients.

DISCUSSION

The analysis of morpine by means of HPLC with electrochemical detection is well documented [3–6]. Nicomorphine is much less electrochemically active than its metabolites, and a combination of different analytical methods is often used to measure nicomorphine and its metabolites, such as HPLC with radioimmunoassay [7,8] and HPLC of [^{14}C]nicomorphine with off-line radioactivity measurement [8,9]. The method described here requires a relatively simple extraction

and the analysis of the extract by two different HPLC systems. Figs. 1–3 show that the result is a measurable pharmacokinetic profile of nicomorphine and its metabolites. The metabolite 3NM is not present in the serum of patients, which can only be demonstrated when the method enables its measurement. A full kinetic investigation of nicomorphine at different dose levels will be published elsewhere.

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