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Note

Determination of morphine in plasma by high-performance liquid chromatography with fluorescence detection

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Previously reported methods of measuring morphine concentration in plasma have used radioimmunoassay (RIA) [1], gas chromatography (GC) with electron-capture detection [2] or mass spectrometry [3] or high-performance liquid chromatography (HPLC) with electrochemical detection (ED) [4–8] or UV detection [9]. RIAs, while capable of detecting picogram amounts of morphine, lack specificity and often cannot distinguish between morphine, its metabolites and other structurally related compounds. Cross-reactivity of RIA antisera may be particularly important in patients receiving chronic doses of morphine since levels of morphine-3-glucuronide and morphine-6-glucuronide may be many times greater than that of the parent drug [10]. GC methods require extensive sample clean-up and derivatisation procedures prior to assay, making these procedures very time-consuming. HPLC–ED techniques are extremely sensitive although routine use of ED may require frequent electrode cleaning, and extremely stable conditions are required for good baseline stability.

In order to study the pharmacokinetics of morphine in cancer patients, we developed a simple method for determination of morphine by HPLC with fluorescence detection. This method requires only 0.5 ml of plasma and has a detection limit of 2.5 ng/ml.

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EXPERIMENTAL

Chromatographic system

The HPLC system consisted of a Model 6000A solvent delivery pump (Waters Assoc., Milford, MA, U.S.A.), a Model 7125 injector equipped with a 150-μl loop (Rheodyne, Cotati, CA, U.S.A.), a μBondapak C_{18} column (300 mm$\times$4 mm I.D., 10 μm), a Model FS970 fluorescence detector (Schoeffel Instruments, Westwood, NJ, U.S.A.) with an excitation wavelength of 210 nm and a 340-nm emission cut-off filter and a chart recorder (Rikadenki, Tokyo, Japan) with a chart speed of 5 mm/min. Chromatography was performed at ambient temperature using a mobile phase consisting of 8% methanol, 5% acetonitrile, 0.5 m*M* sodium edetate and 0.012 *M* potassium dihydrogen orthophosphate in distilled water. The flow-rate was 1.2 ml/min.

Materials

Methanol, acetonitrile, chloroform, butan-1-ol and hexane were HPLC grade and used as received. Potassium dihydrogen orthophosphate, boric acid, sodium borate and sodium edetate were AR grade. Standard solutions of 0.1 mg/ml nalorphine hydrobromide (Wellcome, Sydney, Australia) and morphine hydrochloride (Macfarlane Smith, Edinburgh, U.K.) were prepared and stored at 4°C.

Methods

All tubes used in the extraction procedure were soaked in cetrimide solution (ICI, Villawood, Australia) overnight, rinsed with distilled water and dried.

To 0.5 ml of plasma were added 100 μl of nalorphine hydrobromide solution (1 ng/μl), 500 μl of borate buffer (pH 8.9), 6 ml of chloroform and 300 μl of butan-1-ol. After mixing for 20 min on a rotating mixer (60 rpm) and centrifugation for 5 min (2000 *g*), the upper aqueous layer was aspirated and the organic layer transferred to a tube containing 2 ml of 5 m*M* ammonium sulphate buffer (pH 9.3). The mixture was mixed on a vortex mixer (Model 2601, SMI, Emeryville, CA, U.S.A.) for 1 min, centrifuged for 5 min (2000 *g*) and the upper aqueous layer was aspirated. The organic layer was then transferred to a tube containing 150 μl of 0.1 *M* hydrochloric acid. This mixture was mixed on a vortex mixer for 2 min, centrifuged for 5 min (2000 *g*), and the acid layer was transferred to a 15-ml centrifuge tube containing 15 μl of 1 *M* sodium hydroxide and 1 ml of hexane. This mixture was mixed on the vortex mixer for 20 s and centrifuged for 2 min (1500 *g*). The lower aqueous layer was transferred to a polyethylene microcentrifuge tube (Beckman, Palo Alto, CA, U.S.A.), and an aliquot (80–150 μl) was injected into the HPLC system.

Peak-height ratios (morphine/nalorphine) were used to calculate morphine concentrations, based on standard curves prepared from plasma samples spiked with morphine.

RESULTS AND DISCUSSION

Typical chromatograms from a plasma extract are shown in Fig. 1. Retention times of morphine and nalorphine were 6.4 and 14.4 min, respectively. The standard curve was linear over the range 2.5–200 ng/ml producing a correlation coefficient of 0.999. The inter-assay coefficient of variation at 200 ng/ml was 2.5% ($n=7$) and 6.3% at 2.5 ng/ml ($n=7$). The intra-assay coefficient of variation was 3.1% at 200 ng/ml ($n=7$) and 8.7% at 2.5 ng/ml ($n=6$). The extraction efficiency for morphine and nalorphine was 87.8 ± 3.9 and $88.0 \pm 3.2\%$, respectively.

This method has been used in our laboratory to measure plasma morphine concentrations in cancer patients. Since only 500 μl of plasma are required, the volume of blood needed to do pharmacokinetic studies in these patients is small. The addition of a small amount of sodium edetate to the mobile phase produced better chromatography, probably due to an ion-pairing effect. This method avoids the evaporation step frequently used as a final step in other morphine assays, resulting in faster sample preparation. The extraction procedure ensures that metabolites of morphine such as morphine-3-glucuronide and morphine-6-glucuronide do not interfere with this assay. Tubes used in

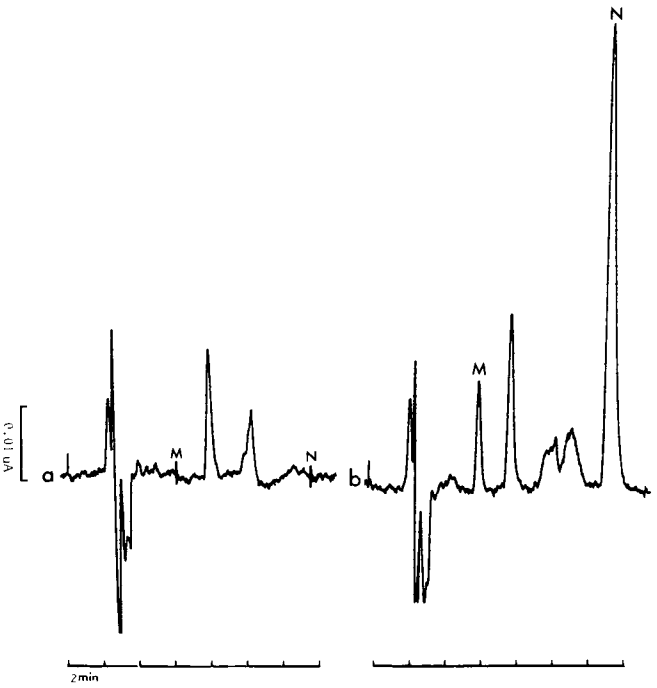

Fig. 1. Chromatograms of (a) blank pooled human plasma and (b) a plasma sample containing 20 ng/ml morphine obtained from a cancer patient receiving oral morphine. Peaks: M = morphine; N = nalorphine.

the extraction procedure were treated with cetrimide solution to prevent adsorption of morphine and nalorphine during extraction. Experiments using cetrimide and silanised tubes showed no difference in results obtained. However, a decrease in peak height of both morphine and nalorphine was observed when samples were left in cetrimide-treated tubes for several hours prior to injection. To overcome this, the final extract was transferred to and stored in polyethylene tubes.

Use of fluorescence detection for measurement of morphine in plasma has previously not been reported in the literature, although a method using HPLC with fluorescence detection for measurement of codeine in plasma has been described [11]. Fluorescence detection produces good selectivity, baseline stability and does not require any derivatisation of samples prior to assay. This method provides a simple, fast and accurate procedure which may be useful for routine monitoring of plasma morphine concentrations.

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