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Note

Determination of fentanyl and alfentanil in plasma by high-performance liquid chromatography with ultraviolet detection

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Fentanyl, 1-(2-phenethyl)-4-N-(N-propionylanilino)piperidine, is a synthetic narcotic analgesic widely used for the purpose of neuroleptic analgesia [1]. Alfentanil, N-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1H-tetrazol-1-yl)ethyl]-4-(methoxymethyl)-4-piperidinyl]-N-phenylpropanamide, is a narcotic analgesic, structurally similar to fentanyl and has about one third the (clinical) potency of fentanyl [2]. We have an interest in measuring plasma concentrations of these opioids associated with intravenous infusions for surgery. Gas chromatographic (GC) methods [3–5] are rather laborious and time-consuming, while radioimmunoassay [6,7] and radiochemical [8] methods suffer from a lack of selectivity. This lack of selectivity is thought to be at least partly responsible for the large variability in estimates of kinetic parameters for fentanyl [2].

We report a rapid and selective high-performance liquid chromatographic (HPLC) assay for fentanyl and alfentanil, of comparable sensitivity to currently used GC methods. The method should be useful in measuring plasma concentrations of these agents during intravenous infusion for surgery.

EXPERIMENTAL

Reagents

Fentanyl citrate and alfentanil·HCl were kindly supplied by Janssen Pharmaceutica (Sydney, Australia). All other chemicals were of HPLC or analytical

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reagent grade and obtained from Mallinckrodt (Melbourne, Australia) and Ajax Chemicals (Sydney, Australia). They were used as received.

Chromatographic systems

A Laboratory Data Control Constametric III pump (Riviera Beach, FL, U.S.A.), a UK6 Universal liquid chromatograph injector (Waters Assoc., Milford, MA, U.S.A.) and a Spherisorb nitrile, 5 μm, S5CN column (25 cm×4.6 mm, Phase Separations, Queensferry, U.K.) were used with a Lambda-max 481 LC spectrophotometer (Waters Assoc.) and Omniscribe (Houston Instrument, Austin, TX, U.S.A.) chart recorder. For the analysis of fentanyl the mobile phase was acetonitrile–0.01 *M* trisodium orthophosphate, adjusted to pH 5.0 with 1 *M* orthophosphoric acid (40:60, v/v). For the analysis of alfentanil the mobile phase was acetonitrile–0.01 *M* trisodium orthophosphate, adjusted to pH 2.6 with 1 *M* orthophosphoric acid (25:75, v/v). The mobile phase was filtered through a 0.22-μm filter under suction (Millipore, Bedford, MA, U.S.A.) and degassed. The flow-rate was 1.8 ml/min, the column was operated at ambient temperature (18–22°C) and the column effluent was monitored at 195 nm in both cases.

Plasma samples

Drug-free venous blood was obtained from healthy human subjects receiving no medication. Blood was also drawn via a radial artery cannula from two patients undergoing coronary artery bypass surgery and receiving either fentanyl 7 μg/kg (total dose 0.5 mg) or alfentanil 25 μg/kg (total dose 1.5 mg) as part of their anaesthetic regimen. Blood was collected into plastic tubes containing lithium heparin and centrifuged for 10 min at 1000 *g*. Plasma was separated and stored between −2 and −15°C in plastic tubes until assayed. A stability study revealed no degradation on storage after a two-month period.

Extraction of fentanyl and alfentanil

Fentanyl. Plasma (1 ml) was mixed in a stoppered glass tube with 0.2 ml of 0.5 *M* potassium hydroxide and extracted with 2 ml of heptane by vortexing for 2 min. The phases were separated by centrifugation (1000 *g*, 5 min) and the heptane phase was transferred to a 10-ml stoppered glass tube containing 0.2 ml of 0.01 *M* trisodium orthophosphate solution, adjusted to pH 3 with 1 *M* orthophosphoric acid, into which the fentanyl was extracted by vortexing for 2 min followed by centrifugation (1000 *g*, 5 min). A 100-μl aliquot of the aqueous phase was injected directly into the chromatograph.

Alfentanil. A similar extraction procedure was used for alfentanil, except 4 ml of a mixture of heptane–isoamyl alcohol (98.5:1.5) was used instead of 2 ml heptane.

RESULTS AND DISCUSSION

Under the chromatographic conditions described above fentanyl and alfentanil extracted from plasma had retention times of 7.2 and 6.2 min, respectively, and there was no interference due to any of the peaks present in plasma obtained

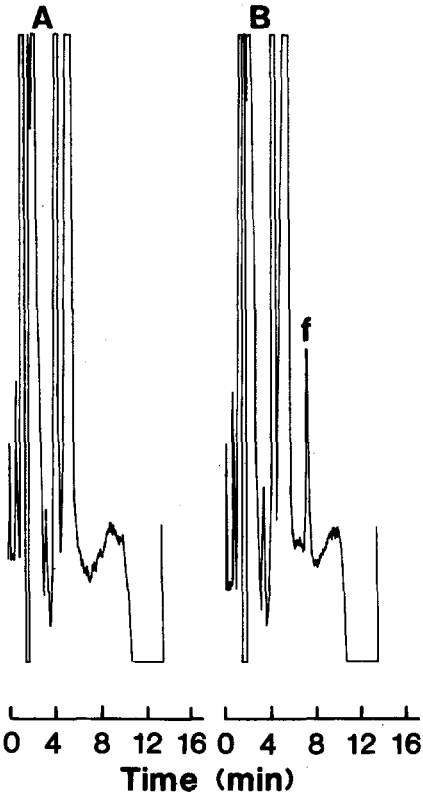

Fig. 1. Chromatograms obtained from plasma samples of a patient undergoing coronary artery bypass surgery and receiving fentanyl. (A) Blank and (B) sample containing fentanyl (f), 14 ng/ml.

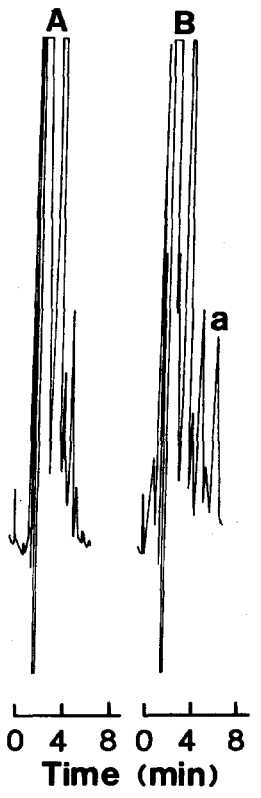

Fig. 2. Chromatograms obtained from plasma samples of a patient undergoing coronary artery bypass surgery and receiving alfentanil. (A) Blank and (B) sample containing alfentanil (a), 102 ng/ml.

from patients undergoing surgery (Figs. 1 and 2). Recovery of fentanyl and alfentanil, assessed by the injection of known amounts onto the chromatographic column, averaged 89.5 ± 1% ($n=5$) for fentanyl and 76.2 ± 3% ($n=5$) for alfentanil. The standard curve of peak height versus known concentration was linear over the range 0–1 μg/ml for alfentanil and over the range 0–100 ng/ml for fentanyl. The detection limit was 1.0 ng/ml (signal-to-noise ratio 2:1) in both cases. Accuracy and precision of the assay were assessed by replicate assays of known standards added to plasma obtained from unmedicated subjects. Replicate analysis of plasma at a fentanyl concentration of 20 ng/ml yielded 21.3 ± 0.5 ng/ml ($n=6$, within-day), 22.1 ± 2.1 ng/ml ($n=7$, day-to-day) and at a concentration of 2 ng/ml yielded 2.26 ± 0.1 ng/ml ($n=6$, within-day). Replicate analysis of plasma at an alfentanil concentration of 200 ng/ml yielded 202 ± 12.5 ng/ml ($n=7$, within-day), 205 ± 22.5 ng/ml ($n=5$, day-to-day) and at a concentration of 5 ng/ml yielded 4.96 ± 0.35 ng/ml ($n=7$, within-day).

These assays were used to measure plasma fentanyl and alfentanil concentra-

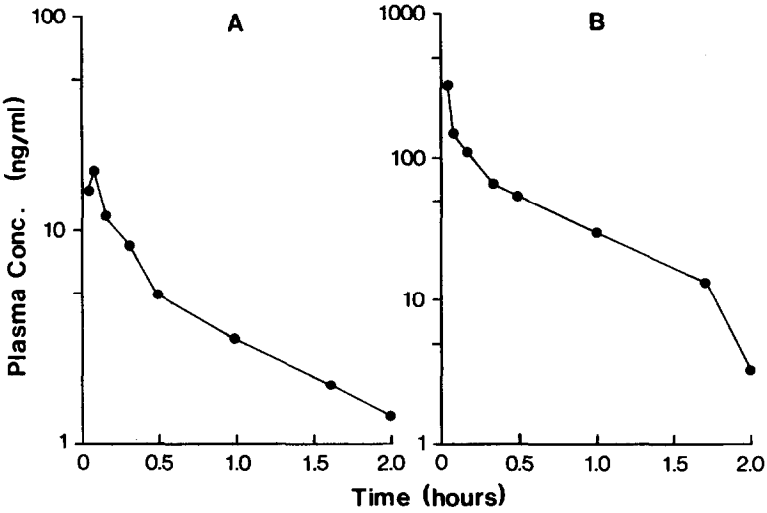

Fig. 3. Plasma concentrations in two patients undergoing coronary artery bypass surgery and receiving an intravenous bolus of (A) fentanyl, 7 μg/kg (total dose 0.5 mg) and (B) alfentanil, 25 μg/kg (total dose 1.5 mg).

tions in the two patients undergoing coronary bypass surgery (Fig. 3). Although these patients had also received a variety of other drugs (morphine, pethidine, promethazine, droperidol, atropine and codeine) no interference was observed in the chromatograms when these compounds were added to blank plasma. The data in Fig. 3 demonstrate that the method can accurately quantitate plasma fentanyl and alfentanil concentrations arising from intravenous dosage. While the method does not allow measurement of drug concentrations for more than a few hours after intravenous bolus dosage, this is also the case with GC and GC–mass spectrometric methods reported previously [3–5]. All of these methods have similar limits of sensitivity. However, the method will be very useful for measuring plasma fentanyl and alfentanil concentrations associated with infusions during surgery as concentrations required are usually greater than about 5 ng/ml for fentanyl [9] and 300 ng/ml for alfentanil [10].

In summary, the assay described has the advantage over existing assays of being selective and simple, in that it does not have the tedious derivatization and evaporation steps required for methods based on GC. The use of the more widely available UV detection and HPLC equipment should facilitate the acquisition of reliable pharmacokinetic data on fentanyl and alfentanil, especially during intravenous infusions within the therapeutic range.

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