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THE ASSAY OF FENTANYL AND ITS METABOLITES IN PLASMA OF PATIENTS USING GAS CHROMATOGRAPHY WITH ALKALI FLAME IONISATION DETECTION AND GAS CHROMATOGRAPHY—MASS SPECTROMETRY

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SUMMARY

Fentanyl was determined using gas chromatography (GC) and alkali flame ionisation detection (AFID), in the plasma of patients who had received a high single dose (up to 60 μg/kg body weight). The relative standard deviation is 6% for 11 ng/ml while the calculated detection limit is 3.3 ng of fentanyl per 1 ml of plasma. The concentration of fentanyl in patients ranged from 40 to 3 ng/ml of plasma in the first hour after administration. In the plasma of patients treated with fentanyl two metabolites could be detected and identified using GC—AFID and GC—MS.

INTRODUCTION

The pharmacokinetics of fentanyl, 1-(2-phenethyl)-4-N-(N-propionyl-anilino)-piperidine (Fig. 1), have been investigated using either radioimmunoassay [1, 2] or an isotopic method with tritium-labelled fentanyl [3]. Re-

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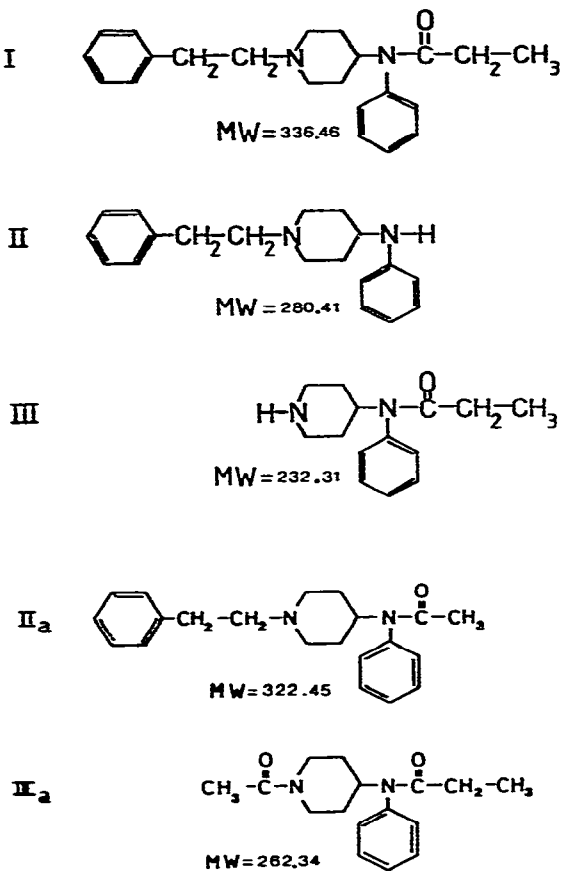

Fig. 1. Structural formulae of fentanyl (I), its metabolites II and III and their acetyl derivatives (IIa and IIIa).

sults indicated that a three-compartment model [1, 2] could be used to fit the data. Other than radioimmunoassay, no sensitive method for fentanyl determination has been described. Previously the metabolism of fentanyl in rats was studied [4, 5] and it was found that in plasma and urine of rats two metabolites can be identified; namely, 1-(2-phenethyl)-4-N-anilino-piperidine (structure II in Fig. 1) [4] and 4-N-(N-propionyl-anilino)-piperidine (structure III in Fig. 1) [5]. However, the presence of such metabolites in human patients has not been confirmed, which may be due to the lack of a sensitive and selective method for determining these metabolites in plasma. Although radioimmunoassay can meet the sensitivity requirements, appropriate assay materials for these potential metabolites are not currently available.

In the present study we report the results of an investigation into the application of gas chromatography (GC) and alkali flame ionisation detection (AFID) for the quantitative determination of fentanyl in plasma of patients who have received intravenous high single doses of fentanyl (up to 60 μg/kg). Moreover, the identification of two metabolites by means of gas chromatography—mass spectrometry (GC—MS) and GC—AFID) is reported.

EXPERIMENTAL

Gas—liquid chromatography

A gas chromatograph (Perkin-Elmer 3920), equipped with an alkali flame ionisation detector and a linear potentiometric recorder (Servogor, Type 541) was used. A glass column (120 cm × 2 mm I.D.) was filled with Chromosorb W HP (80—100 mesh) and loaded with 3% OV-17 (Chrompack, Middelburg, The Netherlands). The temperatures of the injection port, the oven and the interface were 290°C, 250°C and 300°C, respectively. For the assay of metabolite derivatives the oven temperature was set at 230°C. The carrier gas was helium (flow-rate 35 ml/min).

Gas chromatography—mass spectrometry

An LKB 2091—2130 gas chromatograph—mass spectrometer—computer system was used for the identification of the compounds eluted from the GC column. The gas chromatograph had a modified solid injector (Becker, Model 767) and a capillary SCOT column 10 m × 0.5 mm I.D. consisting of a Carbowax 20M stationary phase on a Tullanox support (Cabot Corp., Boston, MA, U.S.A.). Helium was used as the carrier gas (flow-rate 10 ml/min). The temperatures of the injector, column and separator were 260°C, 210°C and 210°C, respectively. The ion source temperature was 210°C. The electron energy was 70 eV, the trap current 50 μA and the accelerating voltage 3.5 kV.

Mass spectra were recorded and stored in a Digital PDP-11/05 computer.

Chemicals and reagents

In all experiments double-distilled water was used. All solvents and chemicals were of analytical grade, except for benzene, which was of pesticide grade (Merck, Darmstadt, G.F.R.). No pretreatment of reagents was carried out. All drugs and metabolites were kindly donated by Janssen Pharmaceutica (Beersse, Belgium).

Sample preparation

Blank pooled plasma and plasma samples containing fentanyl and its metabolites were stored at −20°C. After thawing all samples were ultrasonicated for 3 min. To extract fentanyl and its metabolites 0.1 ml of 4 *N* NaOH and 5.0 ml of benzene were added to 1 ml of plasma in a glass-stoppered centrifuge tube. The tube was shaken for 1 min and then centrifuged for 5 min at 900 *g*. Four millilitres of the upper layer were removed using a Hamilton syringe (Type 1005) and transferred to a glass-stoppered, tapered glass tube. This extraction procedure was repeated three times and the extracts were pooled. In order to derivatize the metabolites 0.5 ml of acetic anhydride and 10 μl of pyridine were added to the tube, which was subsequently heated in a water-bath at 75°C for 2 h. The organic reagents were evaporated to dryness in a water-bath at 75°C using a gentle stream of nitrogen. The residue was dissolved in 50 μl of a solution of the internal standard (papaverine 0.5 mg per 100 ml) in benzene. Ten microlitres of this final solution were injected into the gas chromatograph or evaporated on the tip of the solid injector of the GC—MS system. Peak heights of fentanyl and internal standard were measured and compared with

peak heights obtained after injecting standard amounts of fentanyl and internal standard.

Standard solutions

Standard solutions of fentanyl and its metabolites were prepared in methanol and also in water (2 mg per 100 ml) and were stored at 4°C. Standard solutions of the acetyl derivatives of the potential metabolites were freshly prepared.

RESULTS AND DISCUSSION

Chromatography

Until now the determination of fentanyl and its two potential metabolites (Fig. 1) in biological samples could be accomplished only by radioimmunoassay [6], by radioactivity measurement including an inverse isotope dilution method [5], or by separation using thin-layer chromatography [7].

One of the objectives of this present study was to search for the potential metabolites of fentanyl (II and III, Fig. 1) in man after intravenous administration of a single dose (up to 60 μg/kg) of fentanyl in coadministration with lorazepam and pancuronium bromide to patients undergoing heart surgery. It was decided to use GC in combination with AFID or MS because of the low concentrations expected to be present in the plasma samples. For reasons discussed under recovery and reproducibility of the extraction, it was found necessary to form acetyl derivatives of the metabolites prior to the evaporation of the organic extraction solvent. Therefore all GC measurements were performed with these acetyl derivatives (IIa and IIIa in Fig. 1).

Two stationary phases, OV-17 and Carbowax 20M, were examined for the analysis of fentanyl and its two metabolite derivatives. In order to elute fentanyl in a reasonable time the column temperature had to be 250°C, whereas a lower column temperature was necessary to resolve the metabolite derivatives from any coextracted endogenous substances. On both stationary phases symmetrical peak shapes were obtained. However, OV-17 gave the better selectivity and was used for subsequent experiments using AFID. GC—MS analysis was performed with Carbowax 20M as the stationary phase, because of serious bleeding of OV-17, which interferes with the MS measurements. The use of two stationary phases provides additional information for the identification of the metabolites.

Mass spectrometry

The mass spectra of fentanyl and the acetyl derivatives of its metabolites were recorded using electron impact ionisation at 70 eV and mass fragmentography and are shown in Fig. 2.

For mass fragmentography three *m/z* values were chosen: 245 for fentanyl, 231 for the derivative of metabolite II, and 231 and 274 for the derivative of metabolite III.

Quantitative aspects

The precision and linearity of the GC method were established by injecting

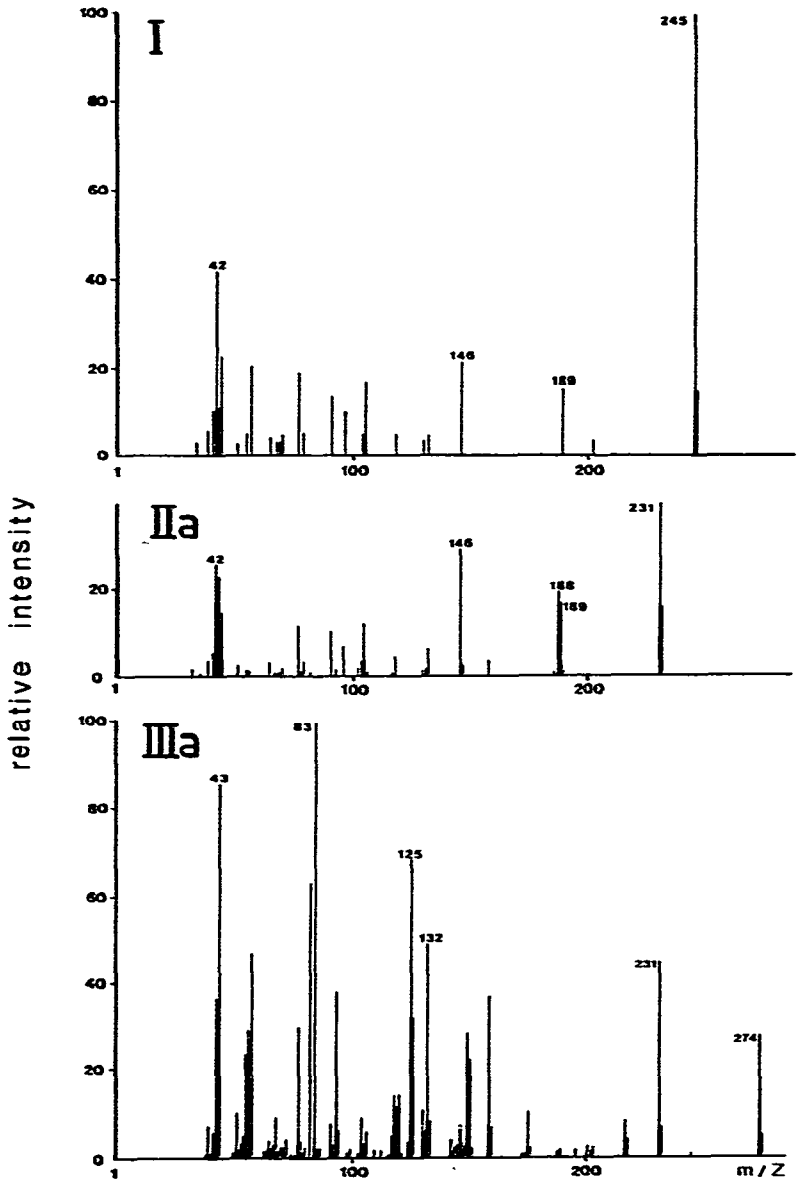

Fig. 2. Mass spectra of fentanyl (I) and the acetyl derivatives of the metabolites (IIa and IIIa).

10 µl of solutions of the solutes under investigation at different concentrations and subsequently measuring the peak heights. The regression analysis of peak heights vs. amount injected is found to be linear up to 8.5 µg/ml for fentanyl, up to 7.5 µg/ml for IIa and up to 8.5 µg/ml for IIIa, with correlation coefficients of 0.9996 and 0.9988, respectively, which indicates an acceptable degree of linearity.

The precision of the method was estimated from repeated injections (n=5) of standard solutions of the solutes in methanol and for 4.3 ng of I, 3.0 ng of IIa, and 6.0 ng of IIIa is 4.7, 6.6 and 5.0%, respectively.

The calculated limits of detection for a signal-to-noise ratio of 3 for the three solutes I, IIa and IIIa are 0.25 ng, 0.82 ng and 0.13 ng, respectively. The corresponding detection limits per ml plasma are 3.3 ng, 5.4 ng and 0.65 ng, respectively.

Recovery and reproducibility of extraction

The recovery and reproducibility of the extraction were determined by spiking blank pooled plasma samples with the solutes. For I (11 ng/ml of plasma) the recovery was 76.1% ± 6.0% (n=7) for II (14 ng/ml of plasma) the recovery was 74.1% ± 6.5% (n=7), and for III (22 ng/ml of plasma) the recovery was 96.2% ± 4.0% (n=7).

Initially, after the solutes were extracted, the organic solvent was evaporated using a gentle stream of nitrogen at 60°C. However, it was found that serious losses of compound III occurred with this method, because of its high volatility. Therefore a derivative-forming step was introduced by which a much less volatile derivative is obtained. Under these conditions no significant losses of the derivative of compound III were found during evaporation of the extraction solvent. It is therefore possible that Maruyama and Hosoya [4], who did not preacetylate the metabolites, could not detect metabolite III because it had disappeared during the evaporation of the extraction solvent (chloroform).

Determination of fentanyl in plasma samples of patients

The method developed for the analysis of fentanyl and its metabolites has been applied to plasma samples of patients receiving high doses of fentanyl. Fig. 3 shows a typical gas chromatogram of a blank pooled plasma extract and of a plasma extract of a patient. From all patients plasma sam-

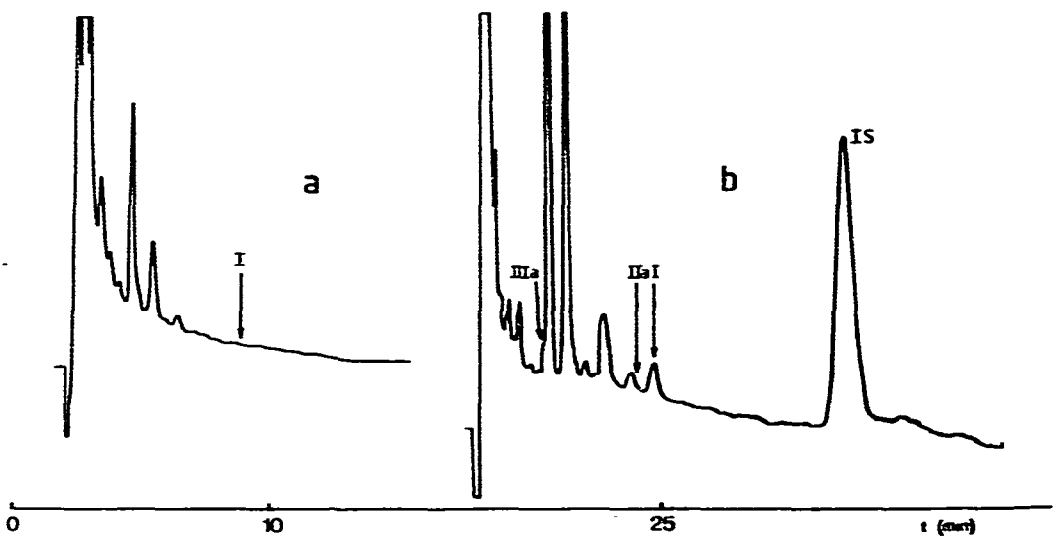

Fig. 3. Gas chromatogram of an extract of a blank pooled plasma sample (a) and of a plasma sample of a patient after the intravenous injection of fentanyl (b). Conditions: column, Chromosorb W HP coated with 3% OV-17; oven temperature, 250°C; AFID detection. IS = internal standard (papaverine).

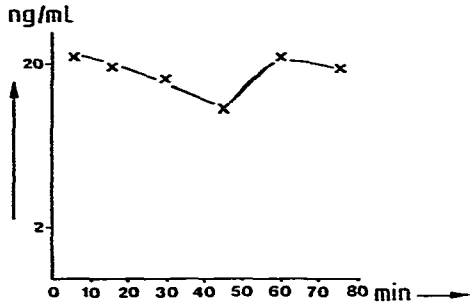

Fig. 4. Plasma concentration of fentanyl vs. time after intravenous administration of 3.5 mg of fentanyl (50 μg/kg) to patient C.

ples were taken at regular fixed time intervals up until 75 min post-administration. The blood levels of fentanyl ranged between 40 and 3 ng/ml of plasma. After this time the blood circulation was connected to a heart—lung machine which prevented more representative samples being taken.

Fig. 4 shows the time course of the plasma concentration of fentanyl in patient C, as determined by the GC method developed. In this patient a significant increase in the fentanyl concentration occurs at 60 min after administration of the drug. Such an increase has been found previously [8] and has been attributed to gastrointestinal recirculation of the drug.

From the time course of fentanyl in four patients, a mean half-life of 20 min is calculated; this is in accordance with earlier reports [1, 2].

The data also indicate that a rapid distribution of fentanyl occurs in the first minutes after administration.

Identification of metabolites

For the identification of the acetyl derivatives of the metabolites of fentanyl the column temperature was set at 230°C.

In the chromatograms of the plasma extracts of patients receiving fentanyl, peaks appear at the elution times of the acetyl derivatives of two potential metabolites. In order to obtain a more specific detection and positive identification, GC—MS analysis has been used. With this technique both metabolites could be identified in all the plasma samples taken from different patients 30 min after fentanyl administration. Typical GC—MS recordings of a test mixture and of plasma extracts of patients A and B are given in Fig. 5.

As can be seen from the ratio of IIIa and IIa in Fig. 5, it appears that significantly different metabolic routes or rates exist in patients. From the results of the GC—AFID measurements a rough estimate of the concentration of the metabolites can be obtained. For both metabolites the concentration ranges from 3 to 8 ng/ml of plasma.

CONCLUSION

GC with AFID has been found to be extremely useful for the determination of fentanyl in the plasma of patients. For the identification of the

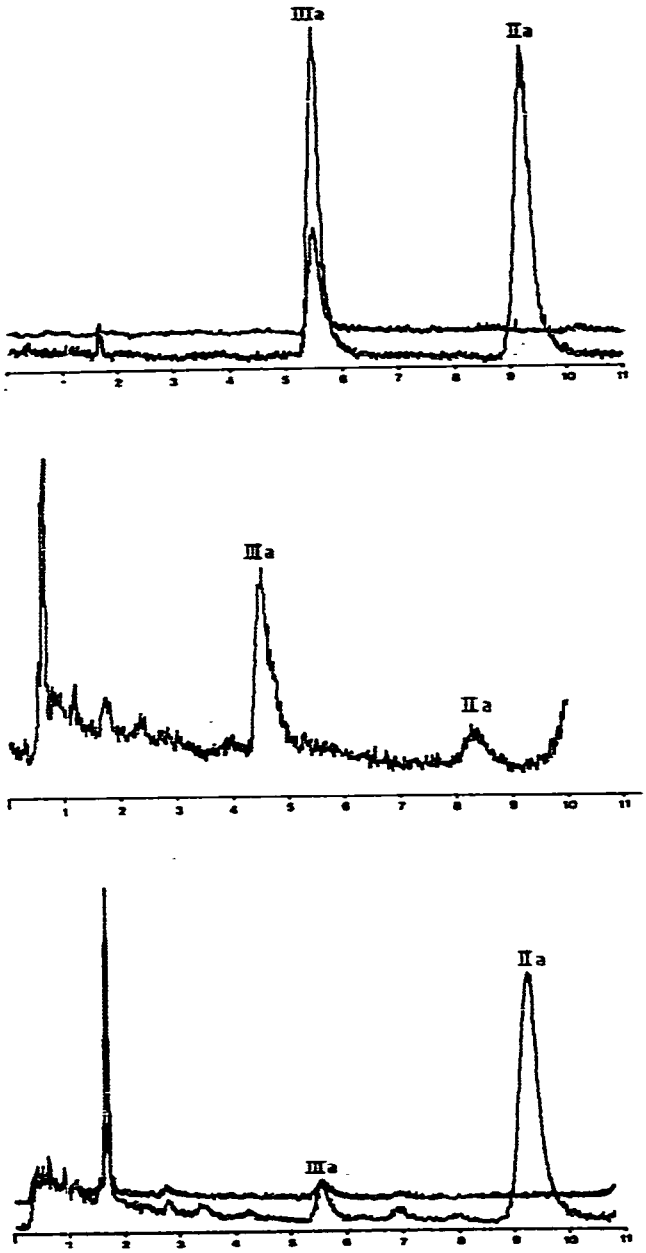

Fig. 5. GC—MS recordings of (top trace) a standard solution containing IIa and IIIa, (middle trace) an extract of a plasma sample of patient A, and (bottom trace) an extract of a plasma sample of patient B after intravenous injection of fentanyl. Conditions: column, capillary SCOT coated with 1% Carbowax 20M; oven temperature, 210°C; MS detection.

metabolites a GC—MS combination has been found necessary. With this combination two metabolites, which had previously been found in rats, could be positively identified in the plasma of patients.

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