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Detection of Picogram Levels of Sufentanil by Capillary Gas Chromatography

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Sufentanil and its two primary metabolites, N-(4-[methoxymethyl]-4-piperidinyl)-N-phenyl propanamide (MPPP), and N-(4[hydroxymethyl]-1-[2-thienylethyl]-4-piperidinyl)-N-phenyl propanamide (desmethyl sufentanil), were detected by capillary gas chromatography with a nitrogen–phosphorous detector. The detection limit for sufentanil and its metabolites is 30–50 pg/ml with minimal interfering substances in the chromatograms. This method allowed for the detection of serum sufentanil in the terminal elimination phase of sufentanil in a patient receiving 1.5 μg/kg and will allow for studies to determine the pharmacokinetics and metabolism of sufentanil in a wide variety of patient groups now receiving this agent. (Key words: Anesthetics, intravenous: sufentanil. Metabolism: metabolites. Pharmacokinetics: sufentanil.)

SUFENTANIL, N - (4 - [methoxymethyl] - 1 - (2 - [2 - thienyl]ethyl)-4-piperidinyl)-N-phenylpropanamide, a new thienyl analog of fentanyl, is a highly potent synthetic narcotic analgesic for use in surgical procedures.[1,2] Previously, sufentanil pharmacokinetics in dogs were determined by gas chromatography¶·** with the use of a method initially developed for fentanyl analyses.[3] This gas chromatographic assay, however, did not have sufficient resolution or sensitivity necessary for determination of terminal elimination phase kinetics for sufentanil. To increase the sensitivity for sufentanil, an assay using capillary gas chromatography was developed. This system also detects the two primary metabolites of sufentanil (fig. 1), one of which retains some pharmacologic activity.††·‡‡

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†† Meuldermans W, Hurkmans R, Hendricks J, Woestenborghs R, Thijssen J, Lenaerts F, Heykants J: Plasma levels, excretion and metabolism of tritium-labeled sufentanil after intravenous administration in dogs. Janssen Preclinical Research Report R33, November 1980, pp 800–808.

‡‡ Heykants J, Meuldermans W, Michiels M: Pharmacokinetic features of intravenous sufentanil in rats, dogs and man. Janssen Preclinical Research Report, R33, May 1981, pp 800–811.

Materials and Methods

Reagents and Materials

Sufentanil citrate, fentanyl citrate, N-(4-[methoxymethyl]-4-piperidinyl)-N-phenyl propanamide (MPPP), and N-(4-[hydroxymethyl]-1-(2-[2-thienyl]ethyl)-4- piperidinyl)-N-phenylpropanamide (desmethyl sufentanil) were supplied by Janssen Pharmaceutica (Beerse, Belgium). Hexane and methanol (UV Grade) were obtained from Burdick and Jackson Laboratories (Muskegon, Michigan) and 100% ethanol from U. S. Industrial Chemicals (Houston, Texas). Outdated human serum was obtained from the Arizona Health Science Center Blood Bank (Tucson, Arizona).

Standards

Serum sufentanil standards were prepared by adding 250 μl of a 1 ng/μl sufentanil base stock solution, 250 μl of water, mixing, then adding 9.5 ml of serum for a final concentration of 25 ng/ml. Intermediate concentrations were prepared by serial dilution with additional serum. Desmethyl sufentanil and MPPP standards were similarly prepared. Fentanyl internal standard (1 ng/μl) was prepared in ethanol from a 1 μg/μl stock ethanol solution.

Extraction

A modification of the extraction procedure of Gillespie *et al.*[3] was used. Glassware was rinsed with ethanol before use to reduce chromatographic inferences. Aliquots (2 ml) of standards or samples were placed in screw-cap culture tubes and 20 μl of fentanyl internal standard added. Each tube then was alkalinized with 0.5 ml of 2 N NaOH and 8 ml of hexane–methanol (19:1) extraction solvent added. The tubes were capped, shaken for 20 min, then centrifuged. The upper organic phase was transferred to a clean tube containing 2 ml of 0.1 N HCl, capped, shaken, and centrifuged. The organic phase was removed and discarded. The aqueous phase then was alkalinized and reextracted with fresh extraction solvent. The organic phase was transferred to silanized conical tubes and evaporated under nitrogen in a 37° C water bath. Ethanol (200 μl) then was added to each tube, vortexed for 15 s, then reevaporated to dryness. The samples then were reconstituted in 15 μl of toluene just before injection of 5–8 μl injected onto the column.

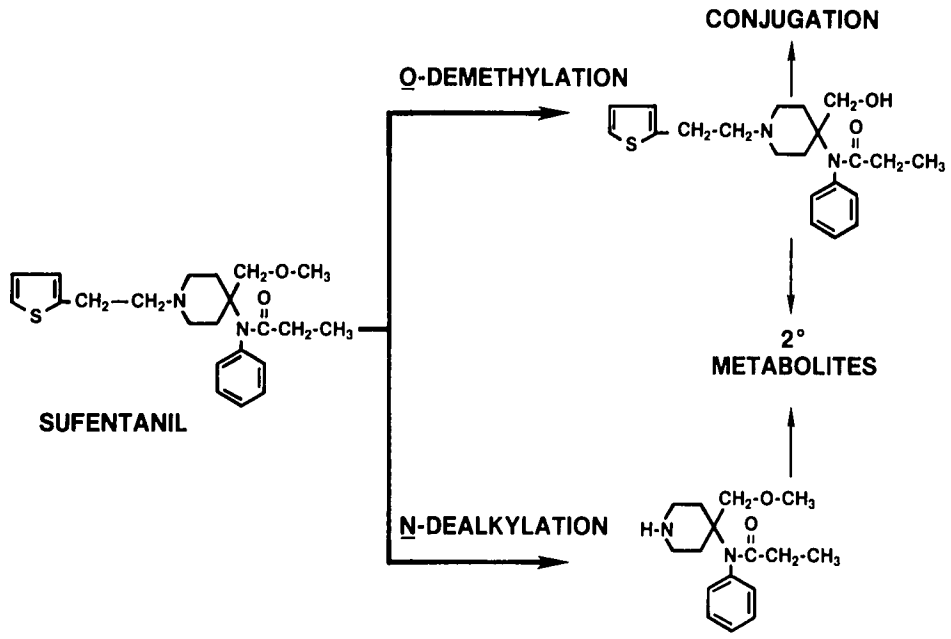

FIG. 1. Biotransformation of sufentanil.

EQUIPMENT

A Hewlett-Packard® 5880 gas chromatograph (Avondale, Pennsylvania), equipped with a split–splitless capillary injection system and a nitrogen—phosphorous detector, was used for the analyses. To maximize sensitivity, analyses were made in the splitless mode. Separation was accomplished using a 12.5 m × 0.25 mm (i.d.) bonded methyl/silicone phase fused silica capillary column (Hewlett-Packard). Linear velocity of the helium carrier gas was maintained at 34 cm/s. An initial oven temperature of 100° C for 1.5 min was temperature programmed at 20° C/min to 200° C; the rate then was decreased to 10° C/min until a final temperature of 265° C was reached. The final temperature was held for 6 min. The detector gases were set at the following flow rates: hydrogen 4 ml/min, air 50 ml/min, and nitrogen 30 ml/min. The nitrogen–phosphorous detector collector bead current was adjusted for optimal performance. Peak areas obtained from Hewlett-Packard® Level 4 printer plotter were used for quantification of sufentanil. Detector and injector temperatures were 300° C and 265° C, respectively.

HUMAN SUFENTANIL SERUM CONCENTRATIONS

A consenting adult male patient received 1.5 μg/kg sufentanil intravenously over a 1-min duration. Blood samples (5 ml) were collected over a 24-h period by an indwelling arterial line, and they were placed in polypropylene tubes. The serum was separated from whole blood by centrifugation (1,000 × *g*), transferred to polypropylene tubes, and frozen at −20° C until analyzed.

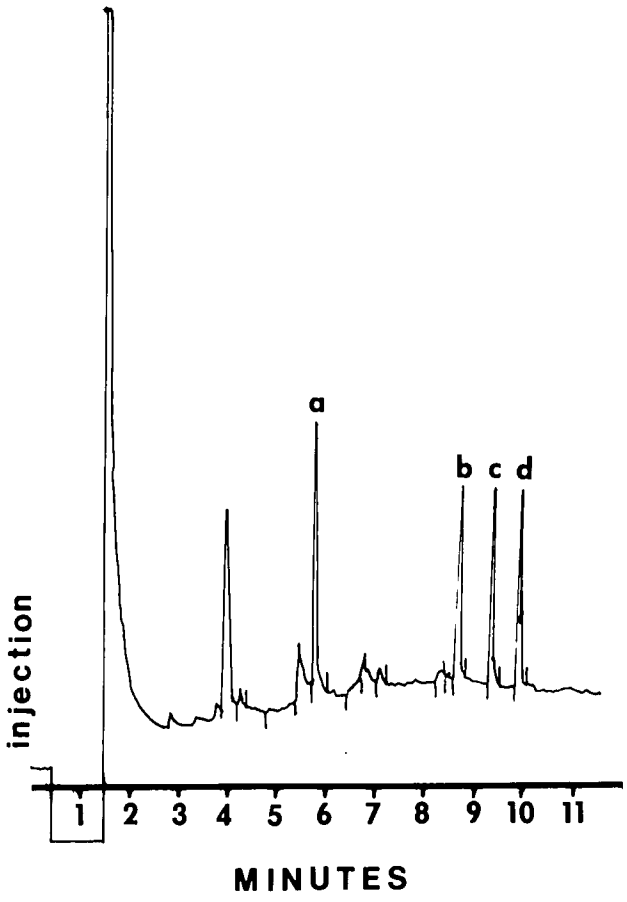

FIG. 2. Typical gas chromatographic profile for the detection of sufentanil (c), desmethylsufentanil (d), MPPP (a), and fentanyl internal standard (b).

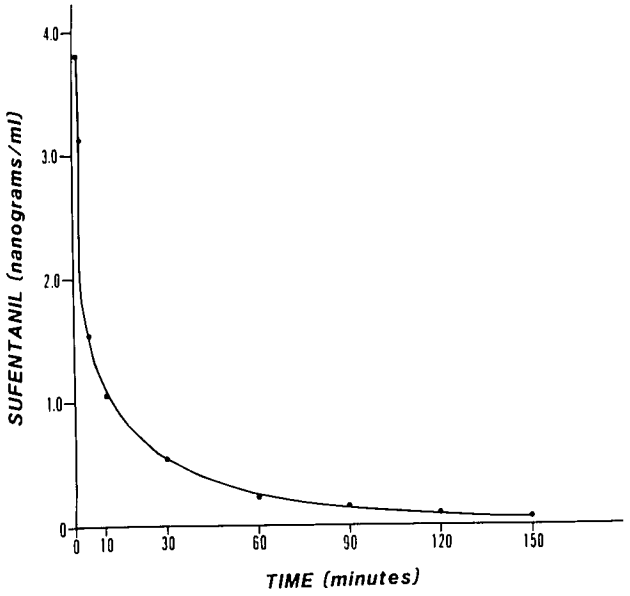

FIG. 3. Concentrations of sufentanil in the serum of a patient receiving a 1.5 μg/kg iv dose.

Results

Sufentanil, desmethyl sufentanil, MPPP, and fentanyl (internal standard) are readily separated from each other on the capillary gas chromatographic column (fig. 2). The use of temperature programming facilitates the separation and allows for the rapid elution of considerable amounts of possible interfering substances. With most samples, sufentanil and its metabolites are completely resolved. However, there still may be occasional interferences by substances of unknown origin or structure.

The assay is linear from 0.03 to 5 ng/ml, which is in the range of serum values found during the terminal elimination of sufentanil after a 1.5–5 μg/kg dose.[4] Sensitivity limits of 30–50 pg/ml serum were obtained by using 2 ml of serum. Greater sensitivity may be obtained if larger volumes of serum are extracted and analyzed. The retention times for sufentanil, desmethylsulfentanil, MPPP, and fentanyl (internal standard) with the use of this method are 11.6, 12.7, 4.9, 10.4 min, respectively. The recovery of known quantities of sufentanil from human plasma samples are 82 ± 7% for sufentanil concentrations of 1.5 ng/ml.

Plasma concentrations of sufentanil in an adult patient undergoing elective surgery were quantifiable 150 min after a single 1.5 μg/kg dose of sufentanil (fig. 3). The estimated terminal elimination half-life from these data is 123 min. Small concentrations (50–150 pg) of both of the metabolites desmethyl sufentanil and MPPP were detected in the samples before 30 min, whereas at the later time points neither of the metabolites were detected.

Discussion

In previous pharmacokinetic studies, plasma sufentanil concentrations were determined with the use of gas chromatographic[3] or radioimmunoassay techniques.[4] However, in our laboratory the gas chromatographic techniques have been plagued with lack of sensitivity and the appearance of interfering substances in the chromatographic profiles. We have also found the radioimmunoassays have not been readily available and the patients' serum has a variable "blank" response during anesthesia that often invalidates the analyses. The absence of appropriate assays to monitor the clearance of sufentanil in patients is evident by the lack of publications describing the disposition of this narcotic analgesic.

A recent complication with a patient with renal failure while using sufentanil[5] prompted us to reexamine gas chromatographic techniques to detect sufentanil at low levels (<100 pg/ml) in patients' serum. By using capillary gas chromatography, sufentanil could be separated from the previous interfering substances and detected at these lower levels. In addition, the two primary metabolites of sufentanil,§§ desmethyl sufentanil and MPPP, were separated and could be quantified. This is the first report of a technique to detect the metabolites of sufentanil. The detection limit for sufentanil and its metabolites is approximately 20–30 pg/ml.

Serum fentanil concentration following a 1.5 μg/ml dose of sefentanil by this gas chromatographic assay are similar to those recently reported in 10 patients with the use of a custom radioimmunoassay technique.[4] Attempts in our laboratory to use a sufentanil radioimmunoassay system supplied to us by Janssen Pharmaceutica were unsuccessful. A crucial step in radioimmunoassays is to determine the level of nonspecific plasma binding of the radioligand. In our hands, the level of nonspecific plasma binding varied considerably between patients and within a single patient's samples. This invalidates the radioimmunoassay, since accurate blank values cannot be obtained once the patient has received sufentanil.

Even though this assay can detect 20–30 pg/ml with sufentanil in the serum, studying the pharmacokinetics of sufentanil with this assay will be difficult. At doses of 1.5 μg/kg of sufentanil the expected serum concentrations during the terminal elimination phase will be at or less than the limits of detection. Larger blood samples (up

§§ Meuldermans W, Hurkmans R, Hendricks J, Woestenborghs R, Thijssen J, Lenaaerts F, Heykants J: Plasma levels, excretion and metabolism of tritium-labeled sufentanil after intravenous administration in dogs. Janssen Preclinical Research Report R33, November 1980, pp 800–808.

to 20 ml) will be required at these times to have enough sufentanil for detection.

This gas chromatographic assay offers a method to study the biotransformation of sufentanil. This is of particular importance, since one of sufentanil's metabolites (desmethyl sufentanil) retains pharmacologic activity that is one-tenth that of sufentanil or equivalent to the activity of fentanyl.¶¶ In addition, sufentanil is metabolized identically to alfentanil. Alfentanil has been reported to undergo polymorphic metabolism.[6] Thus, if in certain patients sufentanil is predominantly metabolized to the desmethyl metabolite, a prolongation of the pharmacologic effects of sufentanil may be observed. This obviously merits further investigation before this agent is accepted into wide use in anesthesiology.

¶¶ Heykants J, Meuldermans W, Michiels M: Pharmacokinetic features of intravenous sufentanil in rats, dogs and man. Janssen Preclinical Research Report, R33, May 1981, pp 800–811.

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