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Note

Measurement of 2-chloroprocaine in plasma by selected ion monitoring

BETTY R. KUHNERT*, PAUL M. KUHNERT and A.L.P. REESE

Department of Obstetrics and Gynecology, Perinatal Clinical Research Center of Case Western Reserve University, Cleveland Metropolitan General Hospital, 3395 Scranton Road, Cleveland, OH 44109 (U.S.A.)

and

D.R. KNAPP

Department of Pharmacology, Medical University of South Carolina, Charleston, SC 29403 (U.S.A.)

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2-Chloroprocaine (2CP) (Nesacaine®) has become a popular and widely used short acting local anesthetic agent. One reason for its popularity is its rapid hydrolysis to inactive metabolites by plasma cholinesterases; it is therefore unlikely to reach toxic levels in plasma. Due to this rapid breakdown, it is also difficult to measure in plasma.

However, 2CP is not completely broken down immediately and low levels are measurable in plasma. We have recently reported that plasma levels of 2CP can be measured following epidural anesthesia in obstetric patients [1]. In addition, we have reported a very high plasma level in a patient who accidently received an apparent intravenous injection of 2CP through an epidural catheter [2]. One might also expect that other patients with decreased cholinesterase activity for genetic or toxicological reasons could also accumulate high plasma levels of 2CP. Consequently, there is a need for a rapid and specific analytical method for measuring 2CP.

We have reported that with elaborate collection and extraction procedures, gas chromatography with nitrogen—phosphorus detection can be used to measure 2CP in plasma [1]. We would now like to report an easier, more rapid and sensitive technique using selected ion monitoring (SIM) gas chromatography—mass spectrometry.

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MATERIALS AND METHODS

Reference compounds

Reference 2-chloroprocaine hydrochloride was provided by the Pennwalt Corp. (Rochester, NY, U.S.A.). The internal standard, procaine hydrochloride, was purchased from Applied Science Labs. (State College, PA, U.S.A.). A stock solution of standard 2CP was made up to the equivalent of 1 mg/ml free base in 0.01 *N* hydrochloric acid. The internal standard stock solution was also made up to the equivalent of 1 mg/ml free base in 0.01 *N* hydrochloric acid.

Apparatus

A Hewlett-Packard 5995A quadrupole table-top mass spectrometer equipped with a direct probe inlet was used to obtain 70-eV electron impact mass spectra of the reference compounds. For selected ion monitoring the gas chromatograph was interfaced to the mass spectrometer with a glass jet separator. The chromatograph was fitted with a 1.0 m × 2 mm I.D. AW DMCS treated glass coil packed with 3% OV-1—OV-17 (6:1) coated on an 80—100 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The instrument conditions were: carrier gas flow-rate, 20 ml/min; injection port temperature, 250°C; oven temperature, programmed from 220°C (0 min) at 16°C/min to 240°C; and the entire run time was 2 min. The optics of the mass spectrometer were optimized by autotuning at *m/z* 100. The ion intensity at *m/z* 86 was monitored for both 2-chloroprocaine and procaine with a window width of 0.1 a.m.u.

Procedures

Blood was obtained from pregnant patients undergoing epidural anesthesia for Cesarean section. Samples were collected in heparinized Vacutainers to which 0.3 ml of a 0.2 g/ml solution of cholinesterase inhibitor, echothiophate iodide (Ayerst Labs., New York, NY, U.S.A.) had been added. Standard curves were prepared using echothiophate-inhibited blood bank plasma after careful checking for interfering contaminants. Patient blood samples were separated by centrifugation and the plasma was stored frozen until analyzed.

Patient plasma samples (0.3—1 ml) and spiked plasma were extracted using a procedure similar to that described by Lesko et al. [3]. Following addition of 25 ng of procaine, the internal standard, the samples were made basic with 0.5 ml of 2 *M* sodium carbonate solution saturated with sodium chloride. Following extraction with 5 ml of diethyl ether, the samples were centrifuged and the organic layer transferred to a clean tube. The ether was carefully evaporated under nitrogen at room temperature and the extract reconstituted with 30 μl of benzene or toluene. A 2-μl aliquot of the final solution was injected into the gas chromatograph—mass spectrometer system.

Standard curves were prepared and the samples were quantitated using the Hewlett-Packard software for automatic quantitation of selected ion monitoring data by area normalization on the *m/z* 86 peak for procaine [4]. Standard curves ranged from 1.5—200 ng/ml.

RESULTS AND DISCUSSION

The mass spectra of the 2CP and procaine standards are shown in Fig. 1. The *m/z* 86 peak was chosen for selected ion monitoring because it was the base peak in both compounds and permitted increased sensitivity by monitoring only one ion.

The selected ion chromatogram of a plasma extract containing 6.25 ng/ml 2CP and 25 ng/ml internal standard is shown in Fig. 2. The retention times of procaine and 2CP are approximately 1.0 and 1.6 min, respectively. Frequently a small non-interfering unknown contaminant peak can also be seen between the two peaks of interest.

Calibration curves were linear over the ranges studied. The least squares linear regression line which describes a typical curve is $y = 0.25x + 1.63$. Using this curve, samples containing less than 2 ng/ml 2CP could be quantitated.

The precision of the method was determined by repeated analysis of spiked samples containing low (6.28 ng/ml) and high (100 ng/ml) concentrations of 2CP. The relative standard deviation (coefficient of variation) was 8 and 4% for five low and high samples, respectively.

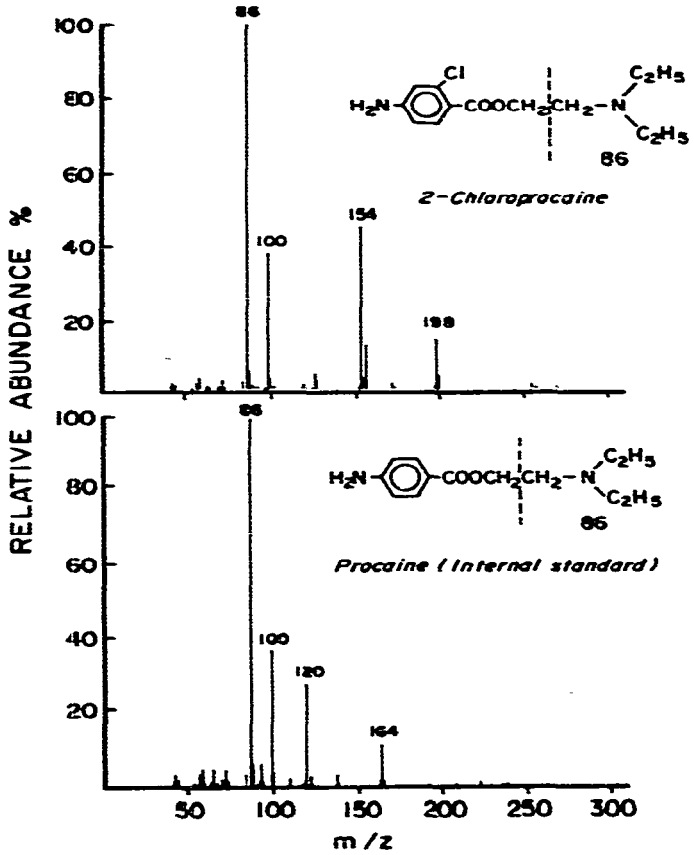

Fig. 1. Mass spectra of 2-chloroprocaine and procaine.

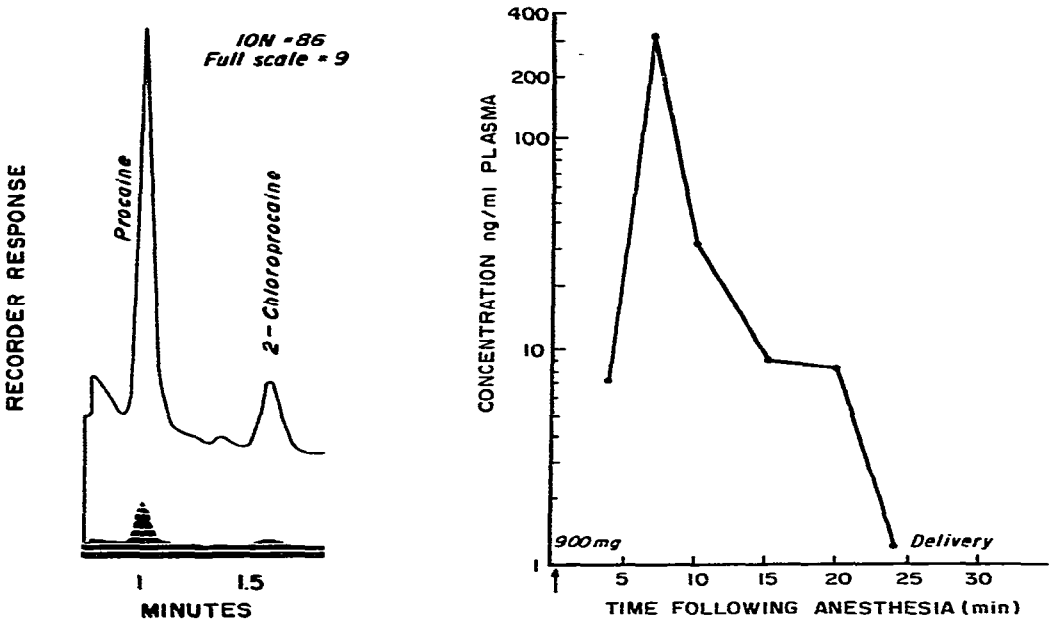

Fig. 2. Selected ion chromatogram of a plasma extract containing 6.25 ng/ml 2CP and 25 ng/ml procaine (internal standard).

Fig. 3. 2-Chloroprocaine in maternal plasma following epidural anesthesia for Cesarean section.

Using the method described, 2CP elimination curves in plasma were obtained during labor or Cesarean section (Fig. 3). Also, 2CP levels could be determined in umbilical cord vein and artery at delivery.

This method is very sensitive, quantitative, easy to perform, and it utilizes mass spectrometry equipment increasingly common in hospital chemistry and other laboratories. In addition, the automatic quantitation feature of the HP 5995A system greatly decreases analysis time; one technician can extract, analyze and quantitate 35 samples and standards in one day.

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