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Journal of Chromatography B, 675 (1996) 336–341

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

Rapid determination of chloroprocaine and its major metabolite, 2-chloroaminobenzoic acid, in plasma by high-performance liquid chromatography

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Received 6 April 1995; revised 17 August 1995; accepted 5 September 1995

Abstract

A sensitive and specific high-performance liquid chromatographic method for determination of the 2-chloroprocaine, local anesthetic of ester type, and its major metabolite 2-chloroaminobenzoic acid, has been developed and validated. A single-step extraction procedure is employed followed by high-performance liquid chromatographic separation using reversed-phase column and analysis using variable length UV detection. Lidocaine was used as internal standard for 2-chloroprocaine measurement and *p*-aminobenzoic acid was used as internal standard for 2-chloroaminobenzoic acid analysis. The analysis of spiked plasma demonstrated good accuracy and precision of the method with limit of detection 0.1 μg/ml for 2-chloroprocaine and 0.5 μg/ml for 2-chloroaminobenzoic acid. The method has been used for pharmacokinetic studies in laboratory animals.

Keywords: Chloroprocaine; 2-Chloroaminobenzoic acid

1. Introduction

2-Chloroprocaine (CHP) is the local anesthetic of ester type commonly used in obstetric anesthesia. CHP is rapidly hydrolyzed by plasma pseudocholinesterase (PCHE) to the pharmacologically inactive metabolites 2-chloroaminobenzoic acid (CABA) and 2-diethylaminoethanol [1]. The rapid and theoretically complete hydrolysis by PCHE with the lack of measurable maternal and fetal plasma concentrations, as well as the lack of the placental transfer of the drug, were previously well documented in the pharmacokinetic studies in human and might contribute to the low level of systemic toxicity observed after CHP administration [2–4].

Until recently, little attention has been paid to the kinetic analysis of CHP because of the lack of simple techniques to monitor plasma concentrations. In addition, PCHE present in blood rapidly decomposes CHP in vitro, making drug analysis difficult and meaningless. Detection of CHP and CABA in pharmacological and clinical investigations has been attempted almost exclusively by utilizing labour intensive procedure of derivatization to ester-linked compounds, followed by multistep extraction and analysis by gas chromatography [2,5].

In order to study in more detail the pharmacokinetics of CHP and CABA we developed a

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0378-4347/96/$15.00
SSDI 0378-4347(95)00376-2

simple and rapid high-performance liquid chromatographic (HPLC) method which is sufficient to study their concentrations in plasma. CHP was extracted and analysed by the modified HPLC method used previously for measurement of plasma concentrations of bupivacaine [6]. CABA was extracted and analysed by the modification of the HPLC method used previously for measurement of concentrations of *p*-aminobenzoic acid in plasma [7,8].

2. Experimental

2.1. Chemicals and reagents

Solvents of HPLC grade and all other chemicals were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Chloroprocaine standard (Nescaine) was obtained from Penwalt (Rochester, NY, USA) and CABA standard was obtained from Sigma (St. Louis, MO, USA). Blood tubes (Vacutainer, volume 5 ml) containing 10 mg of potassium oxalate and 12.5 mg sodium fluoride (NaF) were obtained from Beckton-Dickinson (Rutherford, NJ, USA).

2.2. Drug standard

Drug plasma standards were prepared by spiking blank control plasma containing 2.5% NaF with an appropriate μl volume of working drug solutions to obtain plasma standards with the following concentrations of CHP: 0.1–10 μg/ml and CABA: 0.5–100 μg/ml.

2.3. Sample preparation

2.3.1. CHP

Internal standard lidocaine (50 μl of 50 μg/ml of aqueous stock solution) was added to experimental and standard plasma samples (1 ml) which were subsequently made basic by addition 0.2 ml of 2 *M* NaOH and extracted with diethyl ether. They were vaporized to dryness under N_2, dissolved in 150 μl of mobile phase and 50 μl injected into the column.

2.3.2. CABA

Internal standard *p*-aminobenzoic acid (50 μl of 3 mg/ml of aqueous stock solution) was added to experimental and standard plasma samples (0.5 ml) which were subsequently made acidic with 0.5 ml of 30% perchloric acid. Samples were vortex-mixed and centrifuged for 10 min at 1000 *g*. Supernatant was combined with 10 ml of diethyl ether, shaken for 20 min and diethyl ether layer collected and evaporated to dryness under N_2. The dry residue was dissolved in 150 μl of mobile phase and 100 μl injected into the column.

2.4. Instrumentation

The HPLC Waters 810 system (Millipore, Milford, MA, USA) consisted of a Waters 501 pump, an automated injection system Waters WISP 712, a Waters 486 variable-wavelength UV detector set at 210 nm for CHP analysis and 285 nm for CABA UV absorbance monitoring. The HPLC column was μBondapak C_{18}, 150×3.9 mm (Millipore). For CHP estimations the column was equilibrated at 1 ml/min with the mobile phase which consisted of acetonitrile–0.05 *M* Na_2HPO_4, pH 5.8 (30:70, v/v). For CABA estimations the column was equilibrated at 1 ml/min with the separate mobile phase consisting of acetonitrile–0.2 *M* phosphate buffer, pH 4.0 (10:70, v/v). The chromatographic system was operated at room temperature.

2.5. Calibration curve

CHP and CABA concentrations were ascertained separately from peak-height ratios with the appropriate internal standards (lidocaine and *p*-aminobenzoic acid, respectively) and subsequent comparisons to a calibration curves run concurrently with known CHP and CABA concentrations. All determinations were done in triplicates.

2.6. Extraction recovery

The extraction recovery of CHP and CABA was determined from two sets of three extracted samples and three unextracted reference standards injected directly on column. All determinations were done in triplicates.

2.7. Stability of CHP and CABA in the plasma samples

The stability of CHP and CABA was tested in plasma samples stored at −20°C for 1 month prior to HPLC analysis and extraction. Two sets of plasma samples were spiked with 5 μg/ml of CHP and CABA (in triplicates), and then subjected to various cycles of freezing and thawing. The experimental design of the stability test is summarized in the Table 2.

2.8. Intra- and inter-day reproducibility

Six sets of dog plasma samples at two concentrations of CHP and CABA (1 and 10 μg/ml) were analyzed on the same day to obtain intra-day reproducibility data. Additional sets of two concentration levels in dog plasma were analyzed on three different days to obtain inter-day reproducibility data.

2.9. Animal study

With the approval of our Institutional Animal Care Committee, 6 mixed-breed dogs were brought under deep anesthesia with methohexital (4 mg/kg, i.v.), intubated and mechanically ventilated. Anesthesia was continued with 1.5% isoflurane in oxygen. After a hemodynamically stable period of 15 min, CHP (2 mg/kg) was given as an intravenous bolus. Blood samples of 5 ml were drawn from the arterial line at 0, 0.5, 1, 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 120, 150 and 180 min into vacutainer tubes containing 2.5% sodium fluoride following CHP administration. The blood samples were kept on ice until separation of plasma was accomplished by centrifugation. Plasma samples were stored at −20°C until analysis. All blood samples were measured in duplicates.

3. Results and discussion

Fig. 1 shows three chromatograms of CHP in plasma of a dog after intravenous injection of 2 mg/kg CHP. A drug-free sample, a low concentration of 0.24 μg/ml, 60 min after injection, and a high concentration of 2.91 μg/ml, 1 min after injection are shown. Calibration curves were linear

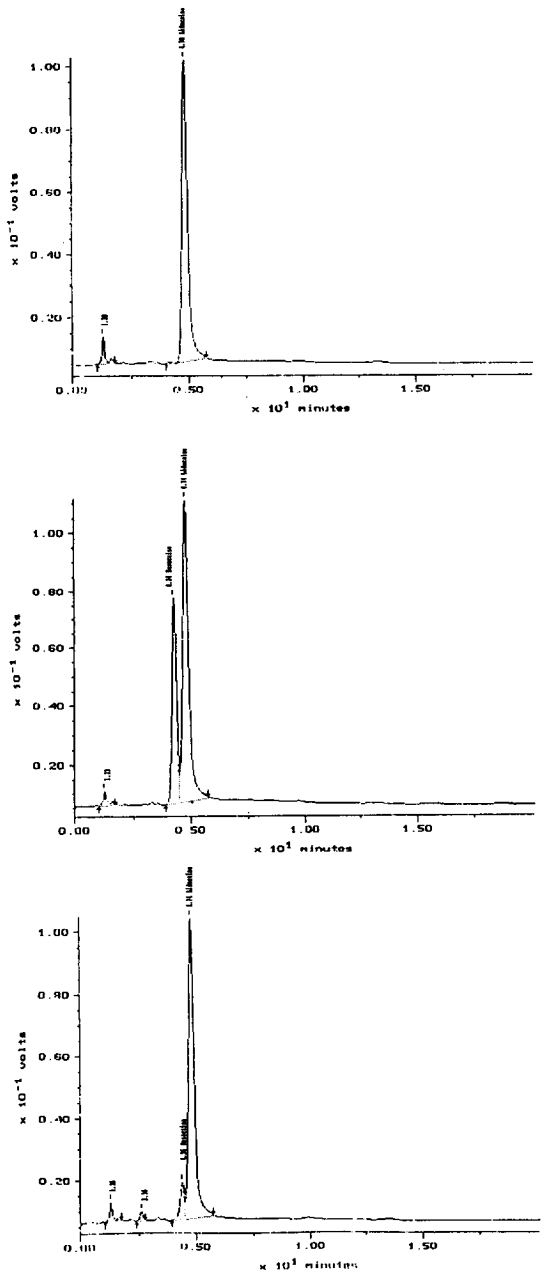

Fig. 1. Three chromatograms of CHP in plasma of a dog after intravenous injection of 2 mg/kg CHP. A drug-free sample (upper panel), high concentration of 2.91 μg/ml, 1 min after injection (middle panel) and a low concentration of 0.24 μg/ml, 60 min after injection (lower panel) are shown. Lidocaine was used as internal standard.

in the range from 0.1 to 10 μg/ml (r=0.998). The limit of detection (defined as peak-to-background noise ratio>3) was 0.1 μg/ml.

Fig. 2 shows three chromatograms of CABA in plasma of a dog after intravenous injection of 2 mg/kg CHP. A metabolite-free sample, high CABA concentration of 14.6 μg/ml, 1 min after CHP injection and low CABA concentration of 1.2 μg/ml, 8 min after CHP injection, are shown. Calibration curves for CABA were linear in the range of 0.5 μg/ml to 100 μg/ml of plasma (r=0.989). The limit of detection (defined as peak-to-background noise ratio>3) was 0.5 μg/ml.

The extraction recovery of CHP and CABA was determined from three extracted samples and three unextracted reference standards (1 and 10 μg/ml concentrations for each compound). The results are shown in Table 1. The recovery was in the range of 75 to 85% in both CHP and CABA samples.

The stability of CHP and CABA was tested in different biological matrices stored at room temperature and −20°C prior to HPLC with and without presence of NaF prior to the HPLC analysis. Two sets of blood, and plasma samples (for CHP and CABA) were spiked with known amounts of CHP and CABA and then subjected to various cycles of freezing and thawing. The experimental design of the stability test is summarized in Table 2. These investigations have shown that no major difference was observed in the mean calculated concentrations of the spiked blood and plasma samples compared to each theoretical values (Table 2). However, in order not to damage the biological specimens, they should be collected into the tubes containing the appropriate amount of NaF.

The summary of inter-day and intra-day precision expressed as the coefficient of variation (C.V.) for both CHP and CABA is presented in Table 3. The inter-day precision for CHP is 6.1 at a concentration of 1 μg/ml and 3.6 at a concentration of 10 μg/ml (n=6). The intra-day precision for CHP expressed as the C.V. is 5.9 at a concentration of 1 μg/ml and 2.1 at a concentration of 10 μg/ml.

The inter-day precision for CABA is 8.9% at a concentration of 1 μg/ml and 1.89% at a concentration of 1 μg/ml (n=6). The intra-day precision for CABA expressed as the C.V. is 9.2% at a

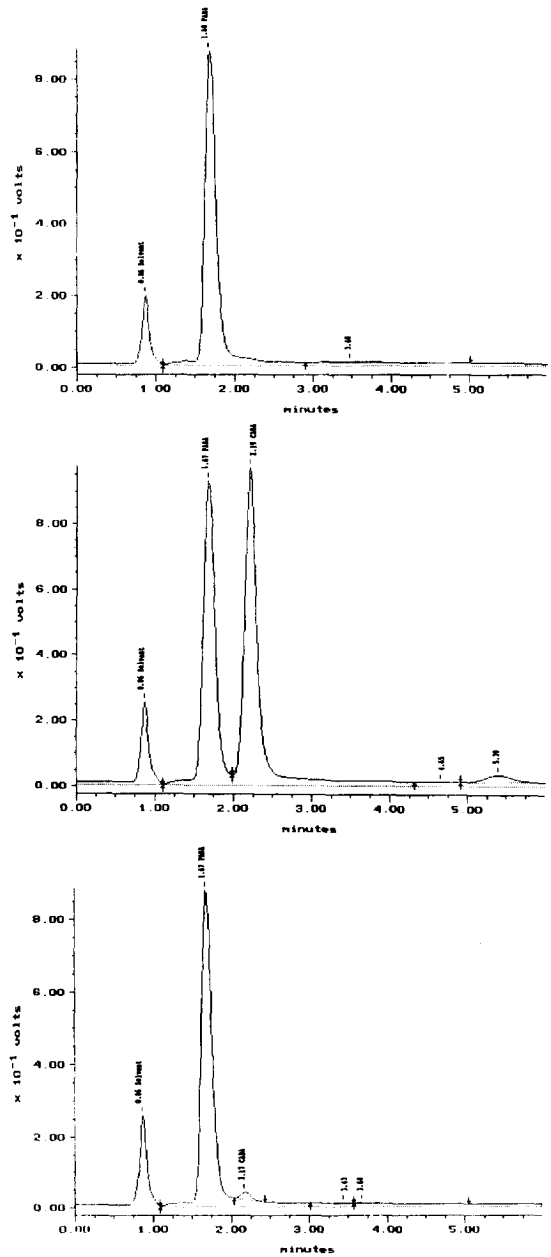

Fig. 2. Three chromatograms of CABA in plasma of a dog after intravenous injection of 2 mg/kg CHP. A metabolite-free sample (upper panel), high CABA concentration of 14.6 μg/ml 1 min after CHP injection (middle panel) and low CABA concentration of 1.2 μg/ml 8 min after CHP injection (lower panel) are shown. p-Aminobenzoic acid (PABA) was used as internal standard.

Table 1
Recovery and coefficient of variation.

Amount added (μg/ml)	Recovery (%)	C.V. (%)
CHP		
1	76.4	12.4
10	81.2	10.4
CABA		
1	79.9	11.4
10	84.3	9.8

concentration of 1 μg/ml and 1.55% at a concentration of 10 μg/ml ($n=10$).

The applicability of this technique was exemplified by the results of the CHP and CABA plasma concentrations measured after intravenous administration of a single 2 mg/kg dose of CHP to six dogs. Fig. 3 shows the plasma concentration–time curves of CHP and CABA in experimental animals. The elimination of CHP is biphasic with apparent elimination half-life of 23 min.

Table 2
Results of stability test for CHP and CABA in the blood and plasma samples

	B1	B2	S1	S2	P1	P2	P3
CHP							
Theoretical concentration (μg/ml)	5	5	5	5	5	5	5
Mean measured concentration (μg/ml)	1.87	4.96	4.98	5.02	5.12	4.94	4.97
C.V. in (%)	13.5	3.7	2.3	2.1	4.2	3.9	5.7
CABA							
Theoretical concentration (μg/ml)	5	5	5	5	5	5	5
Mean measured concentration (μg/ml)	7.92	5.12	5.05	4.89	5.17	4.92	4.98
C.V. (in %)	10.9	5.2	4.8	6.2	4.9	3.9	6.1

B1: Heparinized blood samples spiked with 5 μg/ml of CHP and CABA, kept on ice for 30 min before separation of plasma and extraction; B2: Blood samples containing 2.5% NaF, spiked with 5 μg/ml of CHP and CABA, kept on ice for 30 min before separation of plasma and extraction.S1: Physiological saline spiked with 5 μg/ml of CHP and CABA, kept on ice for 30 min before extraction in plastic tubes; S2: Physiological saline spiked with 5 μg/ml of CHP and CABA, kept on ice for 30 min before extraction in glass tubes. P1: Plasma samples (with 2.5% NaF) spiked with 5 μg/ml of CHP and CABA, followed by immediated extraction procedure and HPLC analysis; P2: Plasma samples (with 2.5% NaF) spiked with 5 μg/ml of CHP and CABA, kept frozen at −20 °C for 1 month, thawed and extracted; P3: Plasma samples (with 2.5% NaF) spiked with 5 μg/ml of CHP and CABA, extracted, dried and kept frozen for −20°C for 1 month, before reconstitution in the mobile phase and HPLC analysis.

Table 3
Precision data for the determination of chloroprocaine (CHP) and its metabolite 2-chloroaminobenzoic acid (CABA) in plasma

Concentration added (μg/ml)	n	Concentration measured (mean ± S.D.) (μg/ml)	C.V. (%)
CHP			
Inter-day			
1	6	0.989±0.06	6.07
10	6	9.987±0.36	3.6
Intra-day			
1	6	1.014±0.06	5.9
10	6	10.05±0.21	2.1
CABA			
Inter-day			
1	6	1.12±0.2	8.9
10	6	9.96±0.18	1.8
Intra-day			
1	10	1.09±0.1	9.2
10	10	10.28±0.16	1.5

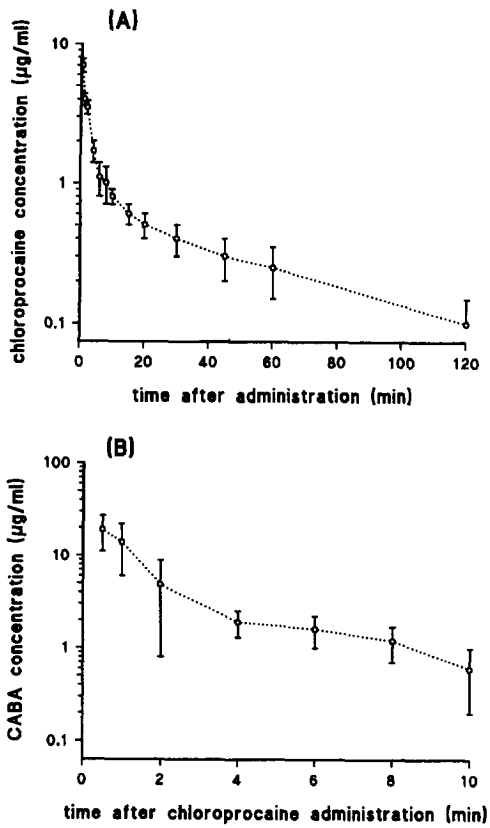

Fig. 3. Plasma concentration–time curve of chloroprocaine (CHP) (upper panel) and its metabolite, 2-chloroaminobenzoic acid (CABA) (lower panel) in dogs (mean±S.E.M., $n=6$) after intravenous administration of 2 mg/kg chloroprocaine.

In conclusion, this HPLC method is simple and rapid, and makes possible the pharmacokinetic analysis of CHP and its major metabolite CABA.

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