Journal of Chromatography, 223 (1981) 247—251
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 773

Note

Simultaneous determination of chloramphenicol and its succinate ester by high-performance liquid chromatography

MILAP C. NAHATA*

Colleges of Pharmacy and Medicine, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210 (U.S.A.)

and

DWIGHT A. POWELL

Children's Hospital Department of Pediatrics, College of Medicine, Children's Hospital, Columbus, OH 43205 (U.S.A.)

(First received July 24th, 1980; revised manuscript received November 7th, 1980)

Since its introduction in 1949, chloramphenicol (CAP) has been extensively used as an antibacterial and an antirickettsial agent [1]. Currently, it is the only drug that can be used effectively in patients with meningitis secondary to *Hemophilus influenzae* type b resistant to ampicillin [2]. However, CAP has been associated with a variety of adverse effects [3]. Two types of hematological toxicity have been reported: a rare idiosyncratic reaction [4] and a relatively common dose-related toxicity [5, 6]. Serum CAP concentrations of greater than 25 μg/ml and prolonged courses of therapy have been associated with dose-related bone marrow suppression [5, 7, 8]. Fatal toxicities such as "gray syndrome" in neonates [9—11] and "gray toddler syndrome" in infants [12] have been reported with CAP serum concentrations above 50 μg/ml. In humans, CAP is extensively metabolized. However, in premature infants, who lack an effective glucuronidation pathway, normal CAP doses can lead to accumulation causing serious side effects [11].

Although CAP pharmacokinetics in infants and children have been studied only recently, two reports [13, 14] have described an increase rather than decrease, in CAP concentrations with time following an intravenous dose of chloramphenicol sodium succinate (CAPS). This finding suggested the need for measuring both CAP and CAPS to account for incomplete or delayed hydrolysis of CAPS to CAP. CAP pharmacokinetic studies strongly suggest

0378-4347/81/0000—0000/$02.50

that CAP therapy should be individualized based on serum concentration measurements using a reliable, accurate and specific assay.

Several methods have been reported for the analysis of CAP in biological fluids [16—18], but high-performance liquid chromatography (HPLC) is the method of choice [19]. As yet, none of the published HPLC procedures provides an optimal method for the simultaneous measurement of CAP and CAPS in small sample volumes. Two methods [19, 20] did not employ an internal standard placing too much reliance on accurate volume transfers and on comparing the peak height of CAP to the standards for quantification. One of these methods [19] measured CAP and CAPS but required a relatively large sample (500 μl) of plasma or serum making it unsuitable for pediatric use. Other HPLC assays [21—26] used an internal standard but did not measure CAPS. Column temperature had to be maintained at nonambient conditions in two methods [20, 24]; no chromatogram was shown in one of the reports [22].

The purpose of this communication is to describe a simple and rapid HPLC procedure for simultaneous determination of CAP and CAPS in small volume samples using N-acetylchloramphenicol (NACAP) as an internal standard. To demonstrate its clinical utility serial blood samples were collected from a patient receiving CAPS for the treatment of *H. influenzae* meningitis. Simultaneous CAP and CAPS concentrations at steady state are described.

EXPERIMENTAL

Chemicals and reagents

Chloramphenicol, chloramphenicol monosuccinate ester and N-acetylchloramphenicol were obtained from Parke-Davis (Ann Arbor, MI, U.S.A.). Ampicillin and gentamicin sulfate were obtained from Sigma (St. Louis, MO, U.S.A.) and penicillin G from Squibb (Princeton, NJ, U.S.A.). Methanol and acetonitrile (glass-distilled) were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.).

Chromatographic equipment and conditions

The reversed-phase HPLC system consisted of Consta Metric pump II G (Laboratory Data Control, Riviera Beach, FL, U.S.A.), μBondapak C_{18} column, 30 cm × 3.9 mm, 10 μm (Waters Assoc., Milford, MA, U.S.A.), analytical fixed-wavelength UV detector, Model 153 (Beckman Instruments, Fullerton, CA, U.S.A.) and a recorder, Series 5000 (Fisher Recordall, Houston Instruments, Houston, TX, U.S.A.).

Methanol—water—acetic acid (37:62:1) was used as the mobile phase at a flow-rate of 1.5 ml/min. All chromatography was performed at room temperature. Chart speed of the recorder was set at 0.2 in./min.

Standards

CAP (10 mg) was dissolved in phosphate buffer saline, pH 7.2 (10 ml) while CAPS (10 mg) and NACAP (10 mg) were prepared in distilled water (10 ml) to give a concentration of 1 mg/ml. Appropriate amounts of these standard solutions were added to the serum samples to yield concentrations

of 0.5, 1, 3, 6, 10, 15, 20, 25, 30, 37, 45 and 60 μg/ml of CAP and CAPS. All samples were stored at −20°C as suggested by Nilsson-Ehle et al. [19].

Assay procedure

Known amounts of CAP and CAPS were added to 50 μl of serum placed in polypropylene 400-μl microcentrifuge tubes. Acetonitrile, 100 μl containing 10 μg of the internal standard was then added to the mixture. The resulting mixture was vortexed for 5 sec and then centrifuged at 9380 *g* for 5 min. The supernatant (50 μl) was injected into the HPLC instrument with the detector set at 0.01—0.02 a.u.f.s. (wavelength 254 nm).

Calculations

The concentrations of CAP and CAPS in the unknown plasma samples were calculated by comparing its CAP:NACAP and CAPS:NACAP peak height ratios with those obtained from CAP and CAPS standard curves.

Recovery and precision

CAP and CAPS were added to drug-free serum and then analyzed by the procedure described above but without any added internal standard. Fifty microliters of the supernatant were injected and peak heights corresponding to each compound measured. Absolute recovery was calculated by comparing these peak heights with peak heights obtained by direct injection of pure drug standards.

Precision of the method was evaluated by analysis of serum standards containing both CAP and CAPS at concentrations of 1, 10 and 40 μg/ml. These samples were analyzed nine times by one individual and eight times by another.

Clinical application

An 8-month-old infant with *H. influenzae* meningitis was receiving intravenous CAPS (Chloromycetin®, Parke-Davis) 100 mg/kg/day divided into four equal doses. A total of 185 mg CAPS was infused over 30 min during each 6-h interval. At steady state, serial blood samples were collected during a 6-h dosing interval. Serum samples were stored at −20°C and analyzed within four days.

RESULTS AND DISCUSSION

A typical chromatogram of NACAP, CAP and CAPS is shown in Fig. 1. The peaks are sharp and symmetrical allowing use of peak heights rather than peak areas to quantitate detector response. Detector response (peak height) was linear over 0.5—60 μg/ml range for both CAP and CAPS, with both curves passing through the origin. Peak height ratios of CAP:NACAP and CAPS: NACAP from extracted samples were also linear over 0.5—60 μg/ml concentration range.

The limits of detection were 0.5 and 0.2 μg/ml for CAP and CAPS respectively. Recovery of both compounds ranged from 96—103% while precision of CAP and CAPS varied from 2.5—6%. Daily variations in CAP and CAPS

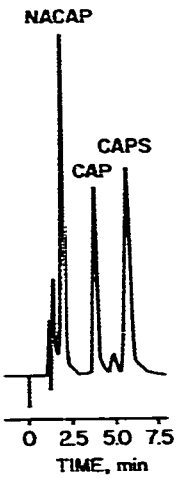

Fig. 1. Chromatogram obtained from serum sample containing NACAP (17.2 μg/ml); CAP (20 μg/ml) and CAPS (43 μg/ml), with retention times of 1.87, 3.9 and 5.75 min respectively (0.02 a.u.f.s.).

concentrations were < 5%. The retention times for NACAP, CAP and CAPS were 1.87, 3.90 and 5.75 min respectively. CAP and CAPS determination did not interfere with ampicillin and gentamicin which are commonly used with chloramphenicol clinically. Although stability of CAP in serum has been reported [19] no data were available for CAPS. In our experiments in serum stored at −20°C, CAP and CAPS were stable (> 95% activity) for at least

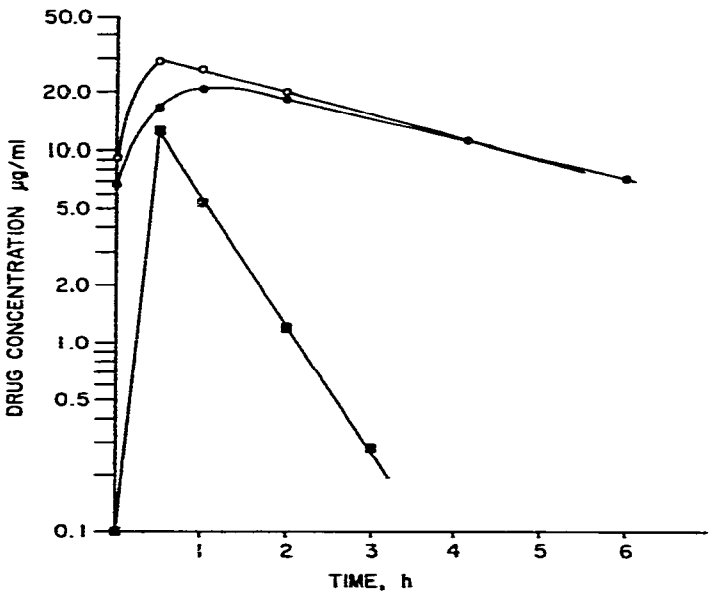

Fig. 2. Steady-state CAP (•), CPAS (▪) and CAP + CAPS (○) serum concentration—time curve obtained from a patient receiving 185 mg CAPS intravenously every 6 h.

two months and four days respectively. At −50° C, CAPS was stable for at least two weeks.

Steady-state CAP and CAPS serum concentration data from a patient receiving multiple doses of intravenous CAPS are shown in Fig. 2. CAP peak concentration of 20.66 μg/ml was achieved at 0.5 h after stopping the infusion. CAPS peak concentration of 12.58 μg/ml which accounted for about 26% of total drug (CAP + CAPS), was reached at the end of infusion. CAPS was detectable up to 3 h. The elimination of both CAP and CAPS appeared to be first-order demonstrating half-lives of 3.2 and 0.45 h respectively. Although the CAPS half-life has not been reported before, our CAP half-life value is consistent with the previously reported range of 0.87—17.8 h [13].

This assay system has proven simple, rapid, sensitive, specific and reproducible in simultaneously measuring CAP and CAPS. The small sample size required in this procedure makes it suitable for individualizing chloramphenicol therapy in pediatric population.

REFERENCES

1 H.C. Meissner and A.L. Smith, Pediatrics, 64 (1979) 348.
2 Committee on Infectious Diseases, Americam Academy of Pediatrics, Pediatrics, 54 (1974) 257.
3 F.A. Oski, J. Pediatr., 94 (1979) 515.
4 A.S. Widsberger, J. Amer. Med. Assoc., 209 (1969) 97.
5 J.L. Scott, S.M. Finegold, G.A. Belkin and J.S. Lawrence, N. Engl. J. Med., 272 (1965) 1137.
6 R.S. Daum, D.L. Cohen and A.L. Smith, J. Pediatr., 94 (1979) 403.
7 P.R. McCurdy, Blood, 21 (1963) 363.
8 S.B. Black, P. Levin and H.R. Shinefeld, J. Pediatr., 92 (1978) 235.
9 J.M. Sutherland, Amer. J. Dis. Child., 97 (1959) 761.
10 L.E. Burns, J.E. Hodgman and B. Cass, N. Engl. J. Med., 261 (1959) 1318.
11 C.F. Weiss, A.J. Glazko and J.K. Weston, N. Engl. J. Med., 262 (1960) 787.
12 A.W. Craft, J.T. Brocklebank, E.N. Hey and R.H. Jackson, Arch. Dis. Childhood, 49 (1979) 235.
13 C.A. Friedman, F.C. Lovejoy and A.L. Smith, J. Pediatr., 95 (1979) 1071.
14 J.P. Glazer, M.A. Danish, S.A. Plotkin and S.J. Yaffe, Pediatrics, 66 (1980) 573.
15 A. Windorfer and W. Pringsheim, Eur. J. Pediatr., 124 (1977) 129.
16 C.J. Least, N.J. Wiegand, G.F. Johnson and H.M. Solomon, Clin. Chem., 23 (1977) 220.
17 G.L. Resnick, D. Corbin and D.H. Sandberg, Anal. Chem., 38 (1966) 582.
18 J.M. Wal, J.C. Peleran and G. Bories, J. Chromatogr., 145 (1978) 502.
19 I. Nilsson-Ehle, G. Kahlmeter and P. Nilsson-Ehle, J. Antimicrob. Chemother., 4 (1978) 169.
20 G.W. Peng, M.A.F. Gadalla and W.L. Chiou, J. Pharm. Sci., 67 (1978) 1036.
21 R.L. Thies and L.J. Fischer, Clin. Chem., 24 (1978) 778.
22 R.H. Sample, M.R. Glick, M.B. Kleiman, J.W. Smith and T.O. Oei, Antimicrob. Ag. Chemother, 15 (1979) 491.
23 S.H. Petersdorf, V.A. Raisys and K.E. Opheim, Clin. Chem., 25 (1979) 1300.
24 J.R. Koup, B. Brodsky, A. Lau and T.R. Beam, Antimicrob. Ag. Chemother, 14 (1978) 439.
25 J. Crechiolo and R.E. Hill, J. Chromatogr., 162 (1979) 480.
26 J. Gal, P.D. Marcell and C.M. Tarascio, J. Chromatogr., 181 (1980) 123.