Journal of Chromatography, 530 (1990) 186–191
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5349

Note

Simultaneous high-performance liquid chromatographic determination of ciprofloxacin, fenbufen and felbinac in rat plasma

KOHJI NAORA, YOSHIHIRO KATAGIRI, NOBUHIRO ICHIKAWA, MASAKAZU HAYASHIBARA and KIKUO IWAMOTO*

Department of Pharmacy, Shimane Medical University Hospital, 89-1 Enya-cho, Izumo, Shimane 693 (Japan)

(First received February 19th, 1990; revised manuscript received April 3rd, 1990)

Ciprofloxacin (CPFX) is a new quinolone agent with potent, broad antibacterial activity. It is well absorbed and widely distributed into various body tissues and fluids when given orally [1,2]. Recently, it was reported that severe convulsion was induced in some patients who were given new quinolone antibacterial agents and non-steroidal anti-inflammatory drugs, including fenbufen (FNB) [3,4], which was later reported to potentiate the convulsant activity of various quinolones in mice [5]. FNB is readily biotransformed into 4-biphenylacetic acid (felbinac, FLB), which possesses anti-inflammatory activity [6–8]. Therefore, in order to investigate possible pharmacokinetic interaction between these drugs, it is necessary to develop a simple, sensitive, selective and simultaneous assay method for the quinolone, FNB and FLB in biological fluids.

We have already developed high-performance liquid chromatographic (HPLC) methods for simultaneous determination of quinolone involving ofloxacin, enoxacin or norfloxacin with FNB and FLB [9–11], and examined the effects of FNB and FLB on the pharmacokinetics of these three quinolones [12–14]. However, no investigation has been carried out on the pharmacokinetic interaction of the newer quinolone, CPFX, with FNB and FLB.

This paper describes a novel analytical method for the simultaneous determination of CPFX, FNB and FLB in rat plasma.

EXPERIMENTAL

Chemicals

Ciprofloxacin hydrochloride was kindly supplied by Bayer (Leverkusen, F.R.G.). A standard solution of CPFX was prepared in distilled water and stored in amber vials to protect it from light. Other details were as described previously [9–11].

0378-4347/90/$03.50 © 1990 Elsevier Science Publishers B.V.

Animals and drug treatments

Male Wistar rats (Clea Japan, Tokyo, Japan), 10 weeks old, were cannulated in the right jugular vein. Drug administration, blood sampling and plasma separation were performed as reported previously [9–11].

Analytical procedure

Pretreatment of the sample, including the extraction procedure and the HPLC analysis, was carried out in accordance with our previous methods [9–11], except that the pH of the mobile phase was adjusted to 2.35 and the detection wavelength was set at 275 nm. This pH was selected following the detailed examinations described below.

Effect of the pH of mobile phase on the separation

The rats were administered 10 mg/kg FNB intravenously. Plasma samples were collected, spiked with CPFX, and chromatographed in the mobile phases with pH values ranging from 2.0 to 2.5. The peak resolution was examined.

Calibration graphs

Three drugs were dissolved in drug-free plasma to give the following concentration ranges: CPFX, 0.2–20 μg/ml; FNB, 0.2–80 μg/ml; FLB, 0.4–40 μg/ml. Calibration graphs were constructed by plotting the peak-height ratio of each compound to that of the internal standard against the concentration.

Accuracy

Six samples of plasma obtained after the coadministration of CPFX and FNB were spiked with 0.5 or 3.0 μg/ml CPFX and 1.0 or 10 μg/ml FNB and FLB. The accuracy of the present method was evaluated by comparing the found amount of the drug with the theoretical added amount.

RESULTS

The effect of the pH of the mobile phase on the resolution of the CPFX peak from other peaks was examined in detail. The partial chromatograms (0–8 min) obtained at three different pH values are shown in Fig. 1. At pH 2.5, the CPFX peak overlapped that of a substance that seemed to be one of the metabolites of FNB. The retention time of CPFX was found to decrease as the pH of the mobile phase was lowered. At pH 2.0, these two peaks were well resolved but the CPFX peak was too close to that of the internal standard, nalidixic acid. The most satisfactory separation was obtained at pH 2.35. The change in the pH did not affect the separation of other peaks.

The chromatograms in Fig. 2 are representative for the analyses of plasma blank and plasma sample obtained from a rat treated with CPFX and FNB intravenously. No peak for endogenous materials was detected in the chroma-

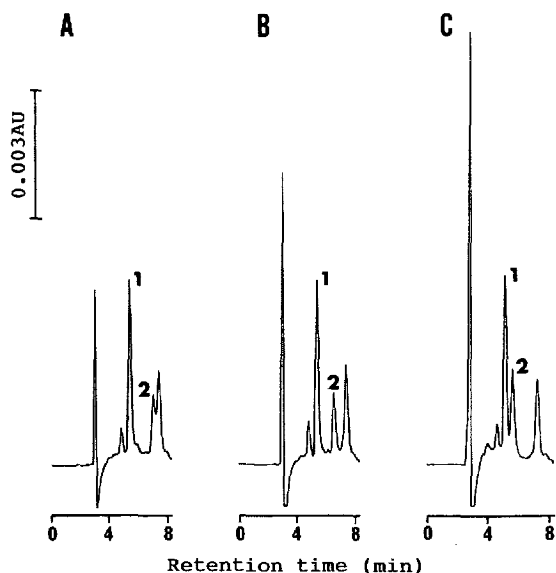

Fig. 1. Partial chromatograms obtained at different mobile phase pH: (A) pH 2.5; (B) pH 2.35; (C) pH 2.0. Peaks: 1 = nalidixic acid; 2 = CPFX.

togram of the plasma blank. CPFX, FNB and FLB were eluted at retention times of *ca.* 6.2, 9.0 and 10.0 min, respectively, as symmetrical and well resolved peaks without any serious interference. The retention times for nalidixic acid and N-phenylanthranilic acid as the internal standards were *ca.* 5.0 and 16.1 min, respec-

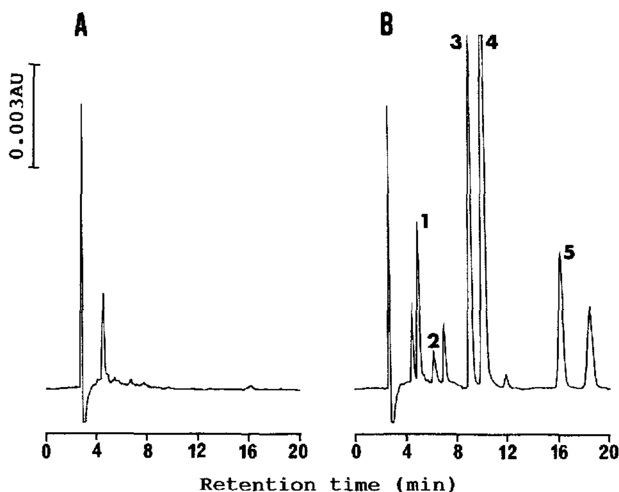

Fig. 2. Chromatograms of (A) a plasma blank and (B) a plasma sample 1 h after bolus intravenous administration of 5 mg/kg CPFX and 10 mg/kg FNB to a rat. Peaks: 1 = nalidixic acid (internal standard); 2 = CPFX; 3 = FNB; 4 = FLB; 5 = N-phenylanthranilic acid (internal standard).

TABLE I

ACCURACY OF THE ASSAY FOR CPFX, FNB AND FLB ADDED TO RAT PLASMA

Drug	Added (μg/ml)	Recovery (mean ± S.D., $n=6$) (%)	Coefficient of variation (%)
CPFX	0.50	102.0 ± 3.3	3.3
	3.0	106.3 ± 2.7	2.6
FNB	1.0	107.7 ± 2.3	2.2
	10.0	99.9 ± 0.8	0.8
FLB	1.0	99.3 ± 2.4	2.4
	10.0	98.8 ± 0.6	0.6

tively. Although some peaks that seemed to be derived from minor metabolites of FNB appeared in the plasma obtained from drug-treated rat, any interference could be observed with CPFX, FNB, FLB and the internal standards.

Calibration curves were generated by least-squares linear regression analysis. Satisfactory linearity was observed in the ranges 0.2–20 μg/ml for CPFX, 0.2–80 μg/ml for FNB and 0.4–40 μg/ml for FLB. The regression equations of the calibration curves were $y = 0.462x - 0.076$ ($r = 0.9998$) for CPFX, $y = 0.469x + 0.066$ ($r = 1.0000$) for FNB and $y = 0.212x - 0.007$ ($r = 0.9999$) for FLB, where y is the peak-height ratio of the drug to the internal standard, x is the concentration (μg/ml) of the drug in plasma and r is the coefficient of correlation. The lower limits of determination were 0.2 μg/ml for CPFX or FNB and 0.4 μg/ml for FLB. These calibration and sensitivity data for each drug were not affected by the presence of other drugs in the plasma.

Table I shows the results of the accuracy test for the determination of CPFX,

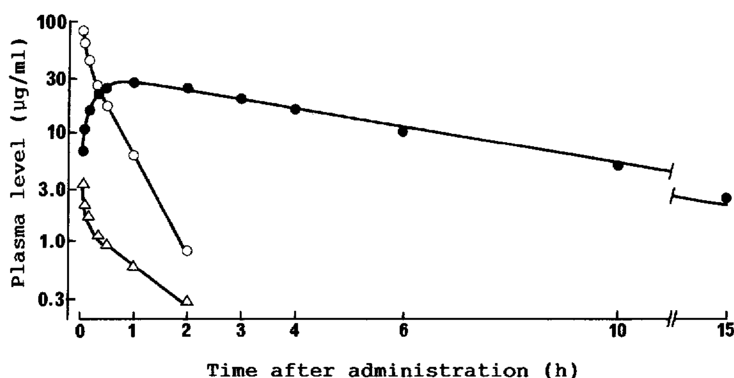

Fig. 3. Plasma concentrations of (△) CPFX, (○) FNB and (●) FLB after bolus intravenous administration of 5 mg/kg CPFX and 10 mg/kg FNB to a rat.

FNB and FLB in rat plasma. The recoveries for CPFX were 102.0 and 106.3% at concentrations of 0.5 and 3.0 μg/ml, respectively. Similarly, FNB and FLB showed recoveries of *ca.* 100% at both low and high drug concentrations. The coefficients of variation were less than 3.3% for each drug.

Fig. 3 shows the typical plasma concentration *vs.* time profiles for CPFX, FNB and FLB in a rat that had received an intravenous dose of 5 mg/kg CPFX and 10 mg/kg FNB concomitantly. Plasma concentrations of both CPFX and FNB were found to decline quite rapidly in a bi-exponential fashion. It was observed that FLB, active metabolite of FNB, was formed immediately after the dosage and then disappeared relatively slowly in a mono-exponential fashion.

DISCUSSION

In previous papers [9–11] we have described HPLC procedures by which each of three quinolones, ofloxacin, enoxacin and norfloxacin, was simultaneously determined in the rat plasma with FNB and FLB. For these three quinolones, identical HPLC conditions and extraction procedure were applicable, except for the detection wavelength. In the present study, we first attempted to adapt the previous method for the simultaneous determination of norfloxacin, FNB and FLB [11] to CPFX with the alternation of detection wavelength alone.

The retention time of CPFX was found to be longer than that of norfloxacin under the original conditions (Fig. 1A), probably due to an increase in lipophilicity caused by substitution of the cyclopropyl group for the ethyl group at position 1 of the quinolone nucleus in norfloxacin. As the result of this peak shift, the peak of CPFX was interfered by a peak that appeared to be derived from a metabolite of FNB other than FLB. The separation of these peaks was then attempted by changing the pH of the mobile phase. The reduction in the pH of the mobile phase from 2.5 to 2.0 shortened the retention time of CPFX from 6.6 to 5.7 min but did not affect the retention time of the interfering peak (Fig. 1C). The best resolution was achieved at pH 2.35 (Fig. 1B).

Recently, various HPLC methods to determine CPFX in serum or plasma have been described [15–24]. Most of these required relatively large amounts of the sample (300 μl to 1.0 ml). However, it is desirable that sample volume is as small as possible during a time-course study, in which blood is repeatedly collected from one animal. The present assay method required only 50 μl of plasma, and the lower limits of determination of CPFX, FNB and FLB were 0.2, 0.2 and 0.4 μg/ml, respectively. All of these levels are far below the therapeutic ranges. Thus, this determination method is suitable even for the pharmacokinetic study of the interaction among CPFX, FNB and FLB in small animals such as rat.

As is clear from the results shown in Table I, both reproducibility and recovery in the determination of each drug were satisfactory over a wide concentration range. The present analytical method was found to be suitable for detailed pharmacokinetic studies of these drugs, as exemplified in Fig. 3.

REFERENCES

1 M.Neuman, *Clin. Pharmacokin.*, 14 (1988) 96.
2 D. E. Nix and J. M. DeVito, *Clin. Pharm.*, 6 (1987) 105.
3 *Information on Drug Adverse Reactions*, No. 81, Ministry of Health and Welfare, Tokyo, 1986 p. 1.
4 *Information on Drug Adverse Reactions*, No. 98, Ministry of Health and Welfare, Tokyo, 1989 p. 2.
5 S. Hirai, K. Tanaka, S. Makino and H. Narita, *Yakugaku Zasshi*, 109 (1989) 119.
6 F. S. Chiccarelli, H. J. Eisner and G. E. Van Lear, *Arzneim.- Forsch.*, 30 (1980) 707.
7 F. S. Chiccarelli, H. J. Eisner and G. E. Van Lear, *Arzneim.-Forsch.*, 30 (1980) 728.
8 A. E. Sloboda, E. L. Tolman, A. C. Osterberg and J. Panagides, *Arzneim.-Forsch.*, 30 (1980) 716.
9 Y. Katagiri, K. Naora, N. Ichikawa, M. Hayashibara and K. Iwamoto, *J. Chromatogr.*, 431 (1988) 135.
10 Y. Katagiri, K. Naora, N. Ichikawa, M. Hayashibara and K. Iwamoto, *Jpn. J. Hosp. Pharm.*, 15 (1989) 292.
11 Y. Katagiri, K. Naora, N. Ichikawa, M. Hayashibara and K. Iwamoto, *Chem. Pharm. Bull.*, 37 (1989) 2858.
12 Y. Katagiri, K. Naora, N. Ichikawa, M. Hayashibara and K. Iwamoto, *J. Pharm. Pharmacol.*, 41 (1989) 717.
13 K. Naora, Y. Katagiri, N. Ichikawa, M. Hayashibara and K. Iwamoto, *J. Pharmacobio-Dyn.*, 13 (1990) 90.
14 Y. Katagiri, N. Ichikawa, K. Naora, M. Hayashibara and K. Iwamoto, *Jpn. J. Hosp. Pharm.*, 15 (1989) 427.
15 F. Jehl, C. Gallion, J. Debs, J. M. Brogard, H. Monteil and R. Minck, *J. Chromatogr.*, 339 (1985) 347.
16 B. Joos, B. Ledergerber, M. Flepp, J.-D. Bettex, R. Lüthy and W. Siegenthaler, *Antimicrob. Agents Chemother.*, 27 (1985) 353.
17 A. Weber, D. Chaffin, A. Smith and K. E. Opheim, *Antimicrob. Agents Chemother.*, 27 (1985) 531.
18 D. E. Nix, J. M. DeVito and J. J. Schentag, *Clin. Chem.*, 31 (1985) 684.
19 F. Vallée, M. LeBel and M. G. Bergeron, *Ther. Drug Monit.*, 8 (1986) 340.
20 K. Borner, H. Lode, G. Höffken, C. Prinzing, P. Glatzel and R. Wiley, *J. Clin. Chem. Clin. Biochem.*, 24 (1986) 325.
21 I. Nilsson-Ehle, *J. Chromatogr.*, 416 (1987) 207.
22 W. M. Awni, J. Clarkson and D. R. P. Guay, *J. Chromatogr.*, 419 (1987) 414.
23 C. M. Myers and J. L. Blumer, *J. Chromatogr.*, 422 (1987) 153.
24 K. Tyczkowska, K. M. Hedeen, D. P. Aucoin and A. L. Aronson, *J. Chromatogr.*, 493 (1989) 337.