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An improved HPLC assay for ciprofloxacin in biological samples

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ABSTRACT A simple and sensitive high performance liquid chromatographic method was developed for determination of ciprofloxacin (Cip) in 0.1 ml of rabbit and human serum and CSF. Separation of Cip and pipemidic acid (internal standard) was achieved on Nucleosil C_{18} (4.6 mm × 250 mm) using fluorescence detection with λ_{exc} 274 nm and λ_{emi} 418 nm. The mobile phase was composed of acetonitrile and phosphate buffer 10 mmol · L^{-1} (pH 2.7) containing tetrabutylammonium hydrogen sulfate 5 mmol · L^{-1} (18 : 82, vol : vol) and the flow rate was set at 1.0 ml · min^{-1}. The retention times were 5.9 min for Cip and 4.0 min for pipemidic acid. The inter-day coefficient of variation was <6.97% (n = 5) and the intra-day coefficient of variation was < 3.33% (n = 5). The limits of detection were 3 ng · ml^{-1} serum and 5 ng · ml^{-1} CSF, ($r \geqslant 0.9996$). Application of this method was demonstrated with simultaneous measurements of concentration-time profiles of Cip in rabbit serum and CSF during iv infusions at constant rates of 0.33, 1.0, and 2.5 mg · kg^{-1} · h^{-1}.

KEY WORDS ciprofloxacin; high pressure liquid chromatography; pharmacokinetics

Ciprofloxacin (Cip) is a new fluoroquinolone antibiotic, which exhibits excellent activity against a broad spectrum of bacteria and is highly effective in the treatment of a wide variety of infectious diseases. The minimum inhibitory concentrations (MIC) of Cip ranges from 0.008 to 2.0 μg · ml^{-1}[1,2]. For pharmacokinetic studies in tissue sites where sample volume is small and where drug penetration is poor, it is necessary that the method of analysis be quite sensitive, requiring small sample volumes and be extremely accurate. Unfortunately most of the existing assays do not meet this criteria. Among the high-performance liquid chromatographic (HPLC) methods of analysis for Cip in biological fluids[3—13], nearly half of these methods did not use internal standards. The detection limits of most of these methods were above 0.01 μg · ml^{-1}, which do not make them useful for measuring Cip concentration around the MIC. In addition, all of these methods need a sample size of 0.2 — 0.5 ml. In this article, we described an improved HPLC assay for Cip in biological fluids and its application in the determination of Cip pharmacokinetic curves in rabbit serum and CSF during iv infusion.

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MATERIALS AND METHODS

Chemicals and reagents Cip standard powder, pipemidic acid (internal standard) and tetrabutylammonium hydrogen sulfate were obtained from Sigma Chemical Co. HPLC-grade acetonitrile and dichloromethane were purchased from Mallinckrodt Specialty Chemical Co. Other chemicals and reagents were all of analytical grade. Deionized HPLC grade water was used throughout the experiments.

Chromatographic condition The chromatographic system consisted of a Waters 510 solvent delivery pump, a WISP 710A automatic injector, a Waters 730 data module, and a Kratos 980 programmable fluorescence detector. Separation was accomplished on a Nucleosil C_{18} column (4.6 mm × 250 mm, Alltech Associates, Inc). Detection was at an excitation wave-

length of 274 nm and an emission wavelength of 418 nm with a sensitivity of 0.005 AUFS. The mobile phase was composed of acetonitrile and phosphate buffer 10 mmol · L^{-1} (pH 2.7) containing tetrabutylammonium hydrogen sulfate 5 mmol · L^{-1} (18 : 82, vol : vol). The flow rate was set at 1 ml · min^{-1}.

Sample preparation 20 μl of pipemidic acid (0.3 μg), the internal standard, was added to 100 μl of human or rabbit serum or CSF in a 1.5 ml polypropylene micro-centrifuge tube. 120 μl of Na_2SO_4 solution (25%) and 20 μl of deionized water were then added to the tube. After mixing for a few seconds on a vortex mixer, 1.1 ml of CH_2Cl_2 was added to each sample. The biphase mixture was shaken for 10 min on a Eberbach shaker, and then centrifuged for 7 min at 1000×g. After separating the 2 layers, the organic phase was completely transferred to a second micro-centrifuge tube, and gently brought to dryness by flowing over the sample at 55 ℃. The sample was reconstituted with 75 μl of mobile phase on a vortex mixer for 30 s. 40 μl of the reconstituted sample was injected onto the column via the automatic injector.

Establishment of standard curves A standard stock solution of Cip was prepared in HPLC grade water at a concentration of 32 μg · ml^{-1}. This stock solution was further diluted with water, pooled rabbit serum or CSF to get concentrations of 6400, 3200, 1600, 800, 400, 100, 50, 25, 12.5, 6.25 ng · ml^{-1} in serum, or 800, 400, 100, 50, 25, 12.5, 6.25 ng · ml^{-1} in CSF. In these concentration ranges, 2 standard curves were constructed. One served for the concentration range from 6400 to 100 ng · ml^{-1} for serum samples or from 800 to 50 ng · ml^{-1} for CSF samples, and the other, from 100 to 6.25 ng · ml^{-1} or 50 to 6.25 ng · ml^{-1}, in serum and CSF respectively. The standard curves were freshly prepared each day. For quality control purposes, 2 "check" samples for each curve were prepared independently. The concentrations of "check" samples for the first standard curve were 12.5 and 50 ng · ml^{-1} for both serum and CSF samples; and those for the second were 200 and 1600 ng · ml^{-1} for serum samples, and 100 and 400 ng · ml^{-1} for CSF samples. Concentrations of Cip in rabbit serum and CSF were calculated from the standard curves constructed by plotting the ratio of the peak area for Cip to that for the internal standard against the labeled concentrations. When serum or CSF samples had Cip concentrations higher than the highest point of the standard curve, the samples were diluted with pooled rabbit serum or CSF.

Validation procedures To test the inter-day reproducibility, 5 aliquots of each check sample (for both serum and CSF) were assayed one by one on 5 consecutive days. For intra-day reproducibility, 5 aliquots of the serum check samples of 50 and 200 ng · ml^{-1} and the CSF check samples of 50 and 400 ng · ml^{-1} were analyzed on the same day. Accuracy and precision were evaluated by calculating the recovery values from the standard curve and the coefficients of variation (CV) among the aliquots, respectively.

Sampling Six New Zealand white rabbits with neutropenia (white blood cell count $\leqslant 2\times10^9$ L^{-1}) weighing 2.4±0.3 kg were anesthetized with iv urethane 1.5 g · kg^{-1}. A metal cannula (0.6 mm×35 mm) was introduced into the cisterna magna through the atlanto-occipital membrane with a 3-dimensional geared introducer and maintained in the position with dental acrylic (Lang dental MFG Co Inc, Wheeling IL). An iv catheter needle was inserted into a marginal ear artery for sampling. At 9 h after introduction of the cannula, the animals were infused with Cip (Miles Pharmaceuticals, West Haven CT, USA) through a marginal ear vein at a rate of 0.33, 1.0 and 2.5 mg · kg^{-1} · h^{-1}, respectively. The serum and CSF samples were collected periodically from the appropriate cannula and stored at −20 ℃ until assay.

RESULTS

Chromatography Typical chromatograms resulting from serum and CSF are shown in Fig 1. Both Cip and the internal standard appeared as sharp and well-resolved peaks with retention times of 5.9 and 4.0 min, respectively. During the analysis of samples, no interference due to endogenous substances was detected. The limits of detection of the assay were 3 ng · ml^{-1} for serum and 5 ng · ml^{-1} for CSF resulting in a signal-noise ratio of 4 : 1.

Accuracy and reproducibility The standard curves for serum and CSF within all the concentration ranges studied were linear ($r\geqslant$ 0.9995). The inter-day CV were <6.97%,

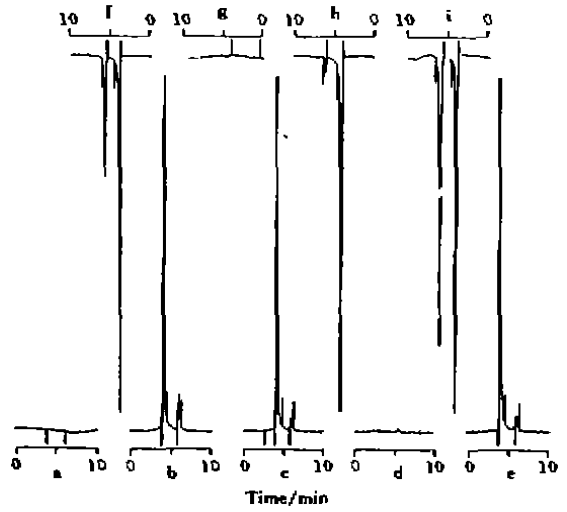

Fig 1. Chromatograms for rabbit CSF containing ciprofloxacin (Cip) 0 (a), 25 (b), and 8.1 ng · ml^{-1} (c). human serum containing Cip 0 (d), and 8.25 ng · ml^{-1}(e). water solution of Cip 100 ng · ml^{-1}(f). rabbit serum containing Cip 0 (g), 8.25 (h), and 171 ng · ml^{-1}(i).

the intra-day CV <3.33%, and the variation in the percentage recovery was <7.04% (Tab 1).

Tab 1. HPLC for ciprofloxacin in rabbits (n=5).

	Added/ng · ml^{-1}	Recovery/%	CV/%
	Inter-day		
Serum	1.25	93.4	4.94
	50	92.6	5.74
	200	105.6	6.20
	1 600	103.5	6.80
CSF	1.25	99.5	4.42
	50	98.2	5.83
	100	103.2	6.97
	400	98.5	4.52
	Intra-day		
Serum	50	99.4	2.61
	200	100.7	1.06
CSF	50	101.2	3.33
	400	98.0	2.08

Rabbit iv Cip pharmacokinetic curves

The concentration-time profiles of Cip in the serum and CSF of 3 rabbits during iv infusion at 3 different rates were shown in Fig 2. The measured Cip serum concentration ranges were 1254—4359, 605.3—1399, and 198.8—234.5 ng · ml^{-1}, and the CSF concentration ranges, 54.6—516.4, 7.7—87.2, and 1.9—10.1 ng · ml^{-1}, respectively. It was found that this HPLC method offers a ng · ml^{-1} level of quantitative measurement and could be used for direct determination of the Cip concentrations using infusion rates from 2.5 to 0.33 mg · kg^{-1} · h^{-1}.

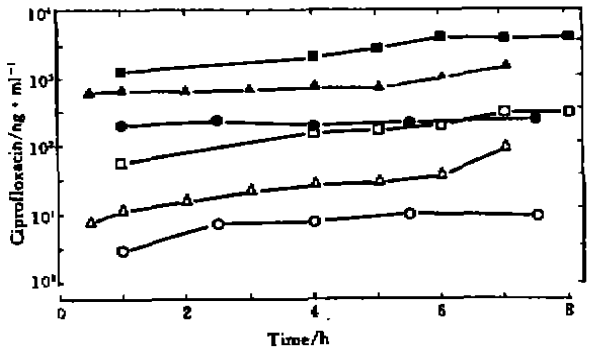

Fig 2. Cip concentration in rabbit serum (solid) and CSF (open) during iv infusion of 2.5 (□), 1.0 (△), or 0.33 (○) mg · kg^{-1} · h^{-1}. (n=3).

DISCUSSION

Presently, the factors influencing the therapeutic activity of antibiotics is still limited[14] and under study. These factors are related not only to the complex interaction among the hosts, microoganisms and antibiotics, but also to the difficulty in the accurate determination of drug concentrations at the site of infection[15]. The HPLC assay of Cip developed here offers a ng · ml^{-1} level sensitivity and requires only a microliter of sample volume. Thus it provides a simple, sensitive and accurate analytical method for the pharmacokinetic and pharmacodynamic study of Cip, especially in CSF samples. In addition,

since the sample volume is only 0.1 ml at most, it allows the experiment to be carried out in animals without depleting their normal physiological pool of CSF. This insures that the results are more reliable and accurate than those experiments where the CSF pool is abnormally low.

Cip is one of the drugs with strong fluorescence. Compared with uv detection, fluorescence detection provides not only a higher sensitivity but also higher selectivity. In developing this assay method, it was found that the peak of maximal absorbance of Cip in the mobile phase described in the procedure was 274 nm which is slightly shorter than 277 and 278 nm reported in the literature[3,5,9,10,11,13]. The maximal fluorescence was obtained at 418 nm without any interferences compared with the reported emission wavelength ($\geqslant$ 445 nm)[5,9,10,11,13]. Furthermore, it was observed that the glass surface absorbed Cip and facilitated the degradation of Cip under light. As a result, any contact of Cip with glass should be avoided throughout the assay procedures.

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改进的 HPLC 测定生物样品中环丙沙星浓度的微量分析法

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摘要 介绍一种测定生物样品中环丙沙星(Cip)的 HPLC 方法及其应用实例. 采用 Nucleosil C_{18}为固定相, 18 : 82 乙腈—磷酸缓冲液 10 mmol · L^{-1}(含硫酸四丁基铵 5 mmol · L^{-1}, pH 2.7) 为流动相, 荧光检测. 本法对血清和脑脊液中 Cip 的最低检测浓度分别为 3 ng · ml^{-1}和 5 ng · ml^{-1}, 具有简便、灵敏、准确且样品需量少 (0.1 ml) 等优点.

关键词 环丙沙星; 高压液相色谱法; 药物动力学

药代动力学

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Protective effect of dipfluzine on experimental brain edema in rats

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ABSTRACT The effects of dipfluzine (1-diphenylmethyl-4-(3-(4-fluorobenzoyl))-piperazine, Dip), a new calcium antagonist developed in China, on experimental brain edema in ♀ Wistar rats with bilateral carotid artery ligation were compared with those of cinnarizine (Cin). Dip 25—100 mg · kg^{-1} ip protected the rats against the characteristic signs of global cerebral ischemia that correlate well with the development of brain edema. Its effects were more potent than those of Cin; and the effects of both drugs were more potent by both pretreatment and posttreatment than those by posttreatment alone. Dip 50 mg · kg^{-1} ip attenuated the reduction in cerebral blood flow (CBF) and the infarct size after occlusion, but did not alter CBF before ischemia. These findings suggested that Dip may be potentially useful to treat ischemic brain edema in part by preserving CBF in the ischemic zone.

KEY WORDS dipfluzine; cinnarizine; cerebral infarction; brain edema

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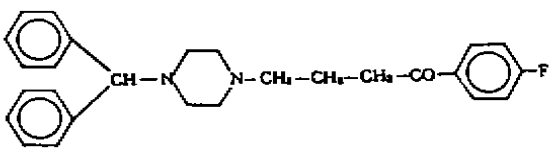
Brain edema is a serious complication in acute cerebrovascular disorders, yet there are few drugs available for its therapeutic intervention. Dipfluzine (1-diphenylmethyl-4-(3-(4-fluorobenzoyl))-piperazine, Dip), a novel diphenylpiperazine calcium channel blocker first developed by Department of Chemistry, Beijing University, has been demonstrated to possess selective and more potent dilatory effects on cerebral vessels than cinnarizine (Cin) did *in vitro* and *in vivo*[1,2]. Accordingly, Dip may be of prophylactic or therapeutic value for ischemic brain edema. The present study was to evaluate the therapeutic effects of Dip and Cin for experimental brain edema induced by bilateral common carotid artery ligation in ♀ Wistar rats.



Dipfluzine