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Note

High-performance liquid chromatographic assay for measurement of cefoxitin in serum

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Cefoxitin is a new semi-synthetic cephamycin that is highly resistant to hydrolysis by β-lactamase [1]. This property makes cefoxitin highly active against many bacterial species which are resistant, in vitro and in vivo, to the available cephalosporin antibiotics. Such bacterial pathogens include indole-positive *Proteus*, *Serratia marcescens*, *Bacteroides fragilis* and *Providencia* spp. [2]. Recent clinical trials have shown that cefoxitin is effective in treating a variety of serious infections [3].

Present methods for the analysis of cefoxitin in plasma are bioassay techniques using either a cup-plate technique [1, 4] or disk-agar diffusion [5]. Bioassay procedures lack chemical specificity and require incubation for 12 to 15 h. Buhs et al. [6] have reported on the use of anion-exchange resins with high-performance liquid chromatography (HPLC) to quantitate cefoxitin in the urine. This method is not ideally suited to rapid clinical analysis because of the long retention time of cefoxitin (19 min).

The present study was undertaken to develop a rapid, simple and sensitive assay for cefoxitin in serum using reversed-phase HPLC. The HPLC assay was used to monitor drug levels in adult patients with serious infections and the results compared with a microbiological assay.

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MATERIALS AND METHODS

Antibiotic

Sodium cefoxitin was supplied by Merck, Sharp and Dohme Labs. (West Point, PA, U.S.A.).

Patients

Serum cefoxitin concentrations were determined for patients in a clinical study evaluating the efficacy of intravenous cefoxitin in aerobic and anaerobic infections. Fourteen patients with bacterial infections including endocarditis, pneumonia, pyogenic cellulitis, abscess and pyogenic arthritis were treated with cefoxitin. Cefoxitin was administered as 1 or 2-g doses, diluted in 100 ml of either normal saline or 5% dextrose in water, given over 30 min. Doses were given at 4- or 6-h intervals.

Sample preparation

Clotted blood was centrifuged and serum pipetted into a sterile tube. The serum samples were stored at −20°C if they were not analyzed immediately.

After thawing, 0.5 ml of serum was mixed with 0.5 ml of 5% trichloracetic acid (TCA) dissolved in methanol. The sample was then mixed by vortexing and kept on ice for 30 min. The TCA solution was made fresh every 3 to 5 h. As the TCA solution ages a new peak appears on the chromatogram with a retention time of 10 min. The peak is first observed approximately 3 h after the methanolic TCA is prepared. As the TCA solution ages the peak will increase in size. This peak does not interfere with the cefoxitin analysis but prolongs the assay time since the peak must come off the column before injection of the next sample. The sample was centrifuged for 5 min at 2000 *g* in a Sorvall SP centrifuge to remove the precipitated serum proteins. The supernatant was collected and filtered through a 0.22-μm filter (GSWP; Millipore, Bedford, MA, U.S.A.), and 25 μl were then injected into the chromatograph.

Standard curves were prepared by adding known amounts of an aqueous solution of sodium cefoxitin to water or blank serum. Concentrations of cefoxitin in serum were calculated by the peak-height ratio method. Cephalothin (25 μg/ml final concentration in the serum) can be used as an internal standard.

HPLC procedure

Chromatography was carried out on an ALC/GPC 204 liquid chromatographic system (Waters Assoc., Milford, MA, U.S.A.). A Pye Unicam LC3 variable-wavelength detector (Cole Scientific, Calabasis, CA, U.S.A.) set at 235 nm monitored the effluent from the column. A reversed-phase column of μBondapak C_{18}, 10-μm particle size (30 cm × 3.9 mm; Waters Assoc.) was used to separate cefoxitin from other UV absorbing compounds in the serum. The solvent system was a mixture of acetonitrile—acetic acid—0.005 *M* potassium dihydrogen phosphate (25:0.5:74.5, v/v/v). The flow-rate was 2 ml/min.

The microbiological assay for measuring cefoxitin in sera was performed at Merck, Sharp and Dohme using the cup-plate technique of Sonneville [7] with *Bacillus subtilis* MB 36.

RESULTS

The ultraviolet absorption of cefoxitin in 0.005 *M* potassium dihydrogen phosphate was characterized by maxima at 235 nm and 263 nm. The extinction coefficients at each wavelength were 16,400 and 9500 respectively. Therefore, 235 nm was chosen as the most sensitive wavelength to use for the cefoxitin assay.

The retention time of cefoxitin was 3.2 min. No interfering peaks were observed in serum containing no cefoxitin. Fig. 1 shows a chromatogram of a cefoxitin standard and a typical chromatogram of serum from a patient on cefoxitin therapy.

The method was linear for concentrations of cefoxitin from 2 μg/ml to 150 μg/ml. Standard curves in both distilled water and blank serum gave similar results. The coefficients of variation for replicate samples obtained on the same day were 2.3% (n = 3); 2.4 (n = 3); 2.6 (n = 6); 3 (n = 5) and 10 (n = 3) for 3, 6, 10, 30 and 50 μg/ml respectively. Blank serum samples with 10 or 30 μg/ml cefoxitin were frozen and thawed on four different days to determine day-to-day variation of the method. The coefficients of variation were 4 and 5% (n = 4) respectively.

The practical limit for the detection of cefoxitin is 1 μg/ml. Levels below 1 μg/ml of cefoxitin can be measured if desired by increasing the injection

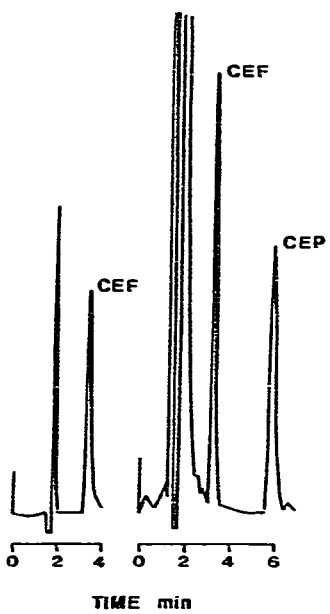

Fig. 1. On the left is a representative chromatogram of cefoxitin (CEF) in blank serum (30 μg/ml). Attenuation was set at 0.08. The peak at 2.1 min is present when blank serum with no cefoxitin is injected. On the right is a typical chromatogram from a patient receiving cefoxitin therapy with cephalothin added as an internal standard (25 μg/ml final concentration in the serum). Attenuation was set at 0.04. The retention time of cefoxitin (CEF) is 3.2 min and 5.8 min for cephalothin (CEP).

volume and/or lowering the attenuation. These manipulations can cause the solvent front to mask the cefoxitin peak.

Sera from patients receiving cefoxitin intravenously were assayed first by the HPLC method. Table I shows the concentrations (using HPLC) in the sera of each patient at the end of the cefoxitin infusion (time 0) and at 30, 120, and 240 min post-infusion. The mean values after a 2-g infusion were 106, 38, 13 and 7 μg/ml, respectively.

A duplicate serum sample was frozen and sent to Merck, Sharp and Dohme Labs. to determine the cefoxitin present using the microbiological assay. The data in Table II show the concentrations of cefoxitin in the sera of patients when measured microbiologically.

The correlation between serum cefoxitin concentrations obtained by HPLC and the microbiological assay was determined by linear regression analysis. The equation for the line was $y = 0.77\,x + 3.9$ (y = microbiological assay, x = HPLC data). The correlation coefficient was 0.93. These results indicate the HPLC assay tends to give higher values than the microbiological assay but that there is still an acceptable correlation between the two methods.

TABLE I

SERUM CONCENTRATIONS OF CEFOXITIN DETERMINED BY HPLC AT VARIOUS TIMES AFTER INTRAVENOUS ADMINISTRATION*

Patient number	Minutes post-infusion			
	0**	30	120	240
2 g every 4 h				
55	122	38.4	15.2	43.5
56	91.4	18.8	6.1	3
57	130	93	37.1	15
58	n.d.§§	21	5.3	3.2
60***	n.d.	21.3	8.9	3.7
62	n.d.	15.1	3.3	18.9
63	138	54.3	28.2	13.8
64	114	41	10.7	101
66	83	32	6.1	1.4
67	60.4	40	12.7	6.7
$X \pm$ S.D.	106±24	38±22	13±10	7±5§
1.5 g every 4 h				
61	n.d.	31.4	5.5	4.8
1.0 g				
59 every 6 h	n.d.	10.5	–	–
65 every 4 h	38.2	18.9	2.4	2.2

*Cefoxitin concentrations are reported as μg/ml serum.

**Infusion time for cefoxitin was 30 min. Time 0 is at the end of the 30 min-infusion.

***Patient 60 received 2 g every 6 h.

§This mean excludes patients 55, 62 and 64. In this group of patients the blood sample was accidently taken just after the 30-min infusion of cefoxitin instead of just before.

§§n.d. = Not determined.

TABLE II

SERUM CONCENTRATIONS OF CEFOXITIN DETERMINED BY THE MICROBIOLOGICAL ASSAY AT VARIOUS TIMES AFTER INTRAVENOUS ADMINISTRATION*

Patient number	Minutes post-infusion			
	0**	30	120	240
2 g every 4 h				
55	119.7	40.6	16.3	77.7
56	34	17.0	7.9	4.0
57	117.2	52.4	24.2	13.7
58	n.d.§§	27.3	7.7	4.3
60***	n.d.	23.8	11.0	3.7
62	n.d.	10.4	1.8	13.0
63	82.4	36.9	17.5	16.3
64	114.2	34.7	10.8	83.2
66	78.2	33.1	7.1	1.6
67	53.9	31.1	15.3	14.3
$\bar{X}$ ± S.D.	86±31	31±11	12±6	8±6§
1.5 g every 4 h				
61	n.d.	49.5	8.0	3.6
1.0 g				
59 every 6 h	n.d.	12.7	5.0	2.1
65 every 4 h	32.9	13.4	6.0	2.3

*Cefoxitin concentrations are reported in μg/ml serum.

**Infusion time for cefoxitin was 30 min. Time 0 is at the end of the 30 min-infusion.

***Patient 60 received 2 g every 6 h.

§ The mean excludes patients 55, 62 and 64. In this group of patients the blood sample was accidently taken just after the 30-min infusion of cefoxitin instead of just before.

§§ n.d. = Not determined.

DISCUSSION

A rapid, reliable and sensitive procedure was developed for the analysis of cefoxitin in serum. The mean cefoxitin concentrations as determined by either HPLC or the microbiological assay are comparable to those determined by Heseltine et al. [3] using a microbiological assay technique. Their means were: 108, 72, 12 and 10 μg/ml for the end of infusion (time 0) and 30 min, 2 h and 4 h post-infusion respectively. In the current study using HPLC the means were 106, 38, 13 and 7 respectively.

The possible use of cefoxitin in selected dental infections prompted us to measure cefoxitin in the saliva of three patients. Saliva was diluted in half with the HPLC solvent and passed through a 0.22-μm filter before injection. Saliva samples were obtained at the end of the 30-min infusion of 2 g of cefoxitin. In one patient additional samples were collected at 30 and 60 min post-infusion. No cefoxitin (<1 μg/ml) was found in any of the samples.

Monitoring the serum level is important in adjusting the dosage of cefoxitin in renal insufficiency since greater than 90% of the drug is eliminated into the urine [1]. Cefoxitin concentrations can be determined by HPLC in approximately 40 min compared to 12—15 h using a conventional microbiological assay.

HPLC is an easy and versatile method of determining cefoxitin in serum and saliva.

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