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Note

High-performance liquid chromatographic determination of cephalexin in human plasma, urine and saliva

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Cephalexin, 7-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid (Fig. 1A) is one of the most commonly used oral cephalosporin antibiotics. Following oral administration, cephalexin is well absorbed and is largely eliminated by the kidney as unchanged drug [1—3]. Its elimination half-life of about an hour in normal subjects may be increased to 20—30 h in patients with severe renal dysfunction [4,5].

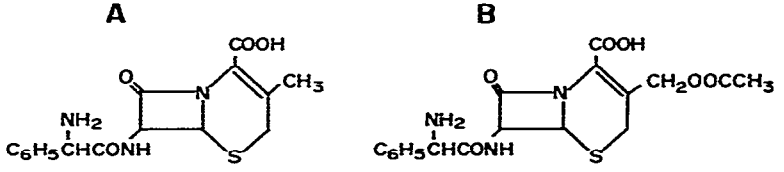

Fig. 1. Chemical structures of cephalexin (A) and cephaloglycin (B).

In severe systemic infections cephalexin dosage of 3—4 g daily for adults and 100 mg/kg/day for children have been recommended [1]. Recently, it was suggested that some pediatric patients with osteomyelitis may require as much as 200 mg/kg/day for a period of 6—16 months [6,7]. A good correlation has been observed between cephalexin serum concentration and concentration in synovial fluid of patients with suppurative arthritis [8]. Monitoring of cephalexin serum concentration is important to assure compliance and gastrointestinal absorption [9,10].

Several methods have been reported for the analysis of cephalexin using microbiological [3,11,12], spectrophotometric [13,14], fluorimetric [15—17] and polarographic [18] techniques but high-performance liquid chromatography (HPLC) is rapid, simple, sensitive and specific for cephalexin.

One HPLC method is available for determination of cephalexin in plasma [19] and two for urine [19,20]. However, no internal standard was employed placing too much reliance on accurate volume transfers and on comparing the peak of cephalexin to the standard for quantification. The procedure is unsuitable for pediatric use because a large sample (500 μl) of plasma was required. Furthermore, these methods are not preferred for routine monitoring of plasma or urine cephalexin concentration due to long retention time for plasma (16 min) and urine (8—16 min).

Since pK_a values of cephalexin are 5.2 and 7.3 [21], and plasma protein binding is about 15% [22], cephalexin may be present in saliva. Monitoring of salivary concentration has been shown to be of value for a number of drugs [23], especially in pediatric patients because saliva can be collected by non-invasive techniques. No methods have been reported for measurement of cephalexin in saliva.

This report describes a rapid, sensitive, simple, reproducible and specific HPLC technique for the determination of cephalexin in small volume samples of plasma, urine and saliva, using cephaloglycine (Fig. 1B) as an internal standard. To demonstrate its clinical utility plasma, urinary and salivary concentrations of cephalexin in a normal subject are presented.

MATERIALS AND METHODS

Chemicals and reagents

Cephalexin, cephaloglycin and tobramycin were obtained from Eli Lilly (Indianapolis, IN, U.S.A.), carbenicillin from Roerig (New York, NY, U.S.A.), gentamicin from Sigma (St. Louis, MO, U.S.A.), and chloramphenicol and its sodium succinate ester from Parke Davis (Ann Arbor, MI, U.S.A.). Methanol (glass distilled) HPLC grade, was purchased from Mallinckrodt (Paris, KY, 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, 3.9 mm × 30 cm, 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.).

Acetic acid (0.5%) was added to methanol—water (20:80) to prepare the mobile phase. It was pumped at 2 ml/min for analysis of plasma and saliva samples and at 1.7 ml/min for urine samples. Chart speed of the recorder was set at 0.2 in./min.

Standards

Cephalexin (10 mg) and cephaloglycin (10 mg) were each dissolved in distilled water (10 ml) to give a concentration of 1 mg/ml. Appropriate amounts of these standard solutions were added to the plasma, urine and saliva samples to yield concentrations of 0.2, 0.5, 1, 2, 5, 10, 20, 30, 40 and 50 μg/ml of cephalexin. Two standard curves were constructed for cephalexin in each body

fluid: one from 0.2—10 μg/ml using 0.5 μg of cephaloglycin and another from 10—50 μg/ml with 5 μg of cephaloglycin. All samples were stored at —20°C.

Assay procedure

Biological fluids (100 μl) containing known amounts of cephalexin were placed in polypropylene microcentrifuge tubes. Methanol (200 μl) containing internal standard was then added to the mixture. The resulting mixture was vortexed for 5 sec and then centrifuged at 9360 *g* for 5 min. The supernatant was transferred to another set of polypropylene test tubes and evaporated at 40°C under a gentle stream of nitrogen. The residue was dissolved in 75 μl of the mobile phase, vortex mixed for 30 sec, and 50 μl of this were injected onto the HPLC column. The detector was set at 0.005—0.08 a.u.f.s. (wavelength 254 nm).

Calculations

The concentrations of cephalexin in the unknown plasma, urine and saliva samples were calculated by comparing its cephalexin:cephaloglycin peak height ratios with those obtained from cephalexin standard curves for plasma, urine and saliva.

Recovery and precision

Cephalexin was added to drug-free plasma, urine and saliva 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 cephalexin measured. Absolute recoveries were calculated by comparing these peak heights with peak heights obtained by direct injection of pure standards.

Precision of the method was evaluated by analysis of plasma, urine and saliva standards containing cephalexin and cephaloglycin concentrations of 50, 25, 10 and 0.5 μg/ml. These samples were analyzed five times by two individuals.

Specificity

Commonly used antibiotics such as gentamicin (10 μg/ml), tobramycin (10 μg/ml), carbenicillin (15 μg/ml), chloramphenicol sodium succinate (20 μg/ml) and chloramphenicol (20 μg/ml) were tested using this procedure for potential interference with cephalexin and cephaloglycin.

Clinical application

A normal adult volunteer (age 29) received 1 g of cephalexin as an oral suspension (Keflex®, Eli Lilly). Blood and saliva (stimulated by paraffin wax) samples were obtained simultaneously at 0, 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 6.0 h after drug administration. Urine samples were collected hourly for the first 6 h and at normal voiding hours thereafter for a total of 24 h. The specimens were stored at —20°C and analyzed within a week.

RESULTS AND DISCUSSION

Typical chromatograms of cephalexin and cephaloglycin in plasma, urine and saliva are shown in Fig. 2. 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 (correlation coefficients > 0.99) over 0.2—50 μg/ml concentration range for cephalexin, with all curves passing through origin. Peak height ratios of cephalexin:cephaloglycin were also linear over the same concentration range.

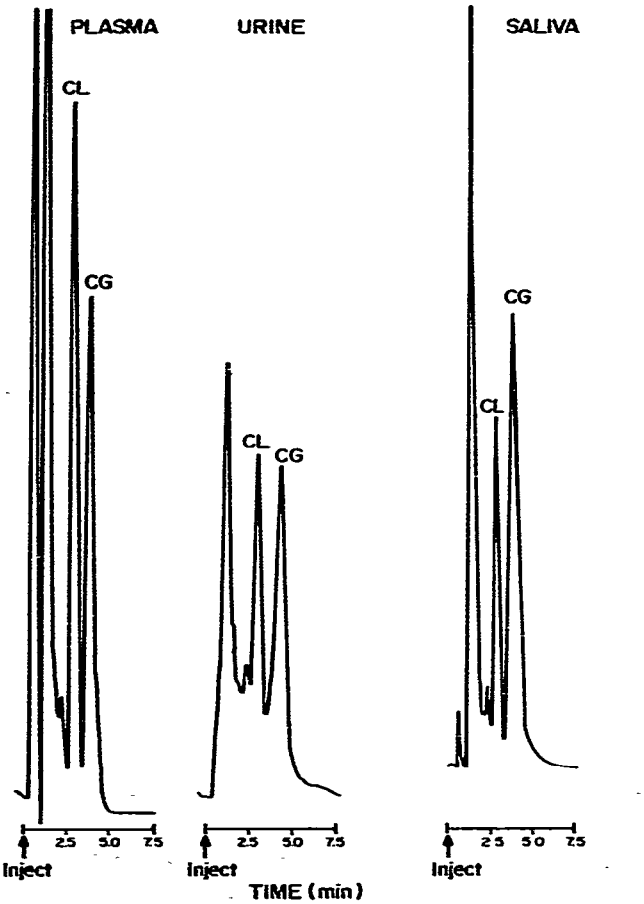

Fig. 2. Chromatograms of cephalexin (CL) and cephaloglycin (CG) in plasma (CL, 40 μg/ml; CG, 50 μg/ml), urine (CL, 30 μg/ml; CG, 27 μg/ml) and saliva (CL, 20 μg/ml; CG, 50 μg/ml). a.u.f.s.: 0.04.

The retention times for cephalexin and cephaloglycin were about 3.0 and 4.0 min respectively. This is a definite advantage over the currently available HPLC procedures [19,20] because this method uses an internal standard and allows rapid analysis of cephalexin in plasma, urine and saliva.

The limit of detection was 0.2 μg/ml for cephalexin in the three body fluids. Recoveries of cephalexin and cephaloglycin ranged from 96—102% (Table I)

TABLE I

RECOVERIES ON EXTRACTION OF CEPHALEXIN AND CEPHALOGLYCIN FROM PLASMA, URINE AND SALIVA

Each value is the mean of five determinations.

Compound	Concentration (μg/ml)	Recovery (% ± S.D.)		
		Plasma	Urine	Saliva
Cephalexin	50	101.9 ± 2.2	97.3 ± 3.3	98.1 ± 1.4
	25	97.6 ± 4.8	96.7 ± 2.7	96.5 ± 2.4
	10	96.8 ± 3.2	97.7 ± 3.8	102.0 ± 2.8
	0.5	97.1 ± 3.1	96.0 ± 4.9	97.7 ± 2.8
Cephaloglycin	50	98.1 ± 3.4	98.8 ± 2.3	98.7 ± 2.9
	25	102.0 ± 4.3	98.9 ± 3.2	100.3 ± 4.1
	10	97.8 ± 2.7	96.1 ± 4.8	97.7 ± 3.8
	0.5	96.5 ± 5.1	97.9 ± 3.7	98.1 ± 2.7

TABLE II

REPRODUCIBILITY OF CEPHALEXIN DETERMINATION IN PLASMA, URINE AND SALIVA

In all cases $n = 5$.

	Concentration (μg/ml)	Coefficient of variation (%)		
		Plasma	Urine	Saliva
Within-day	50	2.7	3.2	2.9
	25	4.2	4.5	3.4
	10	3.0	3.9	2.3
	0.5	2.4	6.0	4.8
Day-to-day	50	2.9	4.1	4.4
	25	3.2	3.4	3.7
	10	3.0	4.9	5.0
	0.5	5.0	3.8	4.2

while precision varied from 2.3—6% (Table II). Daily variations in cephalexin concentrations were < 5%. This assay was specific for cephalexin in that commonly used antibiotics (gentamicin, tobramycin, carbenicillin, chloramphenicol and its sodium succinate ester) did not interfere with its measurement. In these experiments cephalexin did not lose its potency during the storage for ⩽ 7 days prior to analysis.

Plasma concentrations of cephalexin are shown in Fig. 3. The peak plasma concentration of 32 μg/ml at 1 h after oral administration and an elimination half-life of about 1 h are consistent with reported findings [1, 24, 25]. Cephalexin concentration in saliva et 0.5 h and 1 h were 2.8 and 3.3 μg/ml, respectively. No cephalexin was detectable beyond 1 h after drug administration. Various factors including salivary flow-rate, pH fluctuation and low partition coefficient or lipid solubility of cephalexin may be responsible for relatively low concentrations of cephalexin in saliva [23,26].

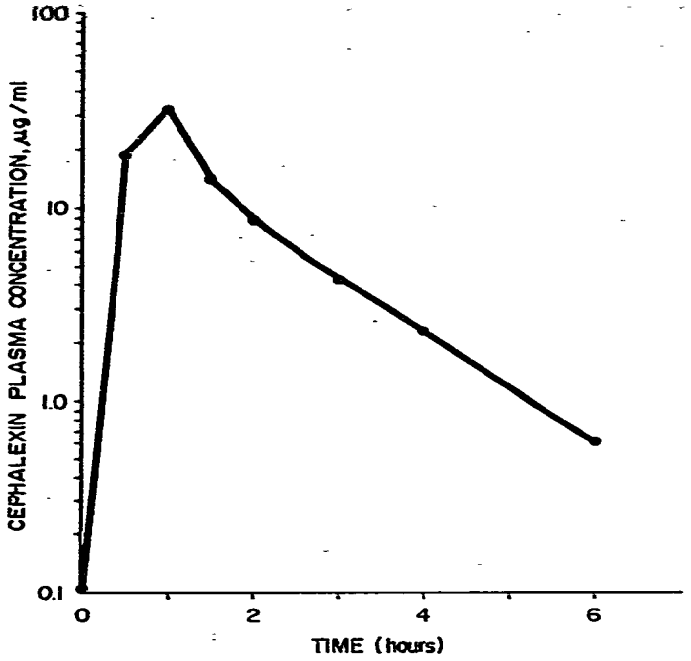

Fig. 3. Plasma concentrations of cephalexin following an 1-g oral dose.

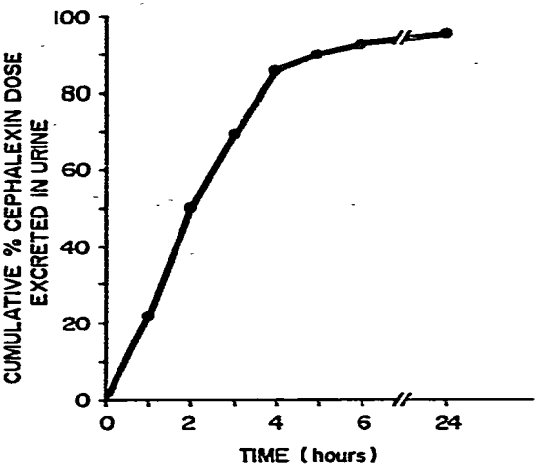

Fig. 4. Cumulative urinary excretion of cephalexin following oral administration of 1 g cephalexin.

Fig. 4 describes the cumulative urinary excretion of cephalexin during a 24-h period following drug administration. About 92% of the dose was excreted within 6 h while 95% was recovered within 24 h after cephalexin administration. These data are in agreement with other reports [1,19,20,24].

This assay system has proven simple, rapid, reproducible, sensitive and specific for determination of cephalexin in plasma, urine and saliva. The sample size required in this procedure makes it suitable for individualizing

cephalexin therapy in pediatric patients or for performing pharmacokinetic studies which requires multiple samples of biological fluids.

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