Journal of Chromatography, 233 (1982) 386—391
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1408

Note

Sensitive high-performance liquid chromatographic analysis of moxalactam in biological fluids

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(First received April 13th, 1982; revised manuscript received June 30th, 1982)

Moxalactam (LY 127935; 6059 S) (Fig. 1) is a new parenteral semisynthetic β-lactam antibiotic in which an oxygen replaces the sulfur atom in the cephem

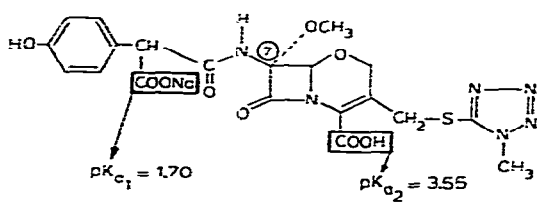

Fig. 1. Chemical structure of moxalactam.

ring. This antibiotic possesses two acidic functions: one on the cephem nucleus (as in cephalosporins), and the other on the side-chain. Moxalactam is a polar molecule, active in vitro against a variety of gram-positive and gram-negative bacteria including staphylococci, Enterobacteriaceae and *Pseudomonas* [1]. Plasma levels [2—4] and pharmacokinetics of moxalactam in normal volunteers [5—8] or in patients [9] have been determined by microbiological assay.

0378-4347/82/0000—0000/$02.75

Recent papers have described the use of high-performance liquid chromatographic (HPLC) methods for the quantitation of moxalactam in human urine [10] and to compare the pharmacokinetics of cefazolin and moxalactam after deproteination of the plasma [11]. Assay techniques used in pharmacokinetic studies of moxalactam must be capable of measuring plasma levels of at least 100 ng/ml. This report describes a sensitive (50 ng/ml) and selective ion-pair HPLC method for the quantitation of moxalactam present in biological fluids, and the application of this method in pharmacokinetic studies.

These results were presented in part at the 2nd Mediterranean Congress of Chemotherapy [12].

EXPERIMENTAL

Apparatus

A Waters Assoc. (Paris, France) HPLC system was used. This system includes a dual 6000A delivery system, a WISP 710 sample processor, and an M440 absorbance UV detector (280 nm fixed wavelength). An Omniscribe B 5000 recorder (Houston Instruments) (10 mV) was used. Data were analyzed by means of a computer program [13]. The column was a reversed-phase μBondapak C_{18} (10 μm; 30 cm × 3.9 mm I.D.) from Waters Assoc.

Drug standard, plasma and urine samples

Moxalactam (LY 127935) was supplied by E. Lilly Laboratories (Saint-Cloud, France). Five healthy adults (three women, two men) received a 1-g dose of moxalactam intravenously. The antibiotic was dissolved in sterile physiological saline (30 ml).

Blood samples (5 ml) were collected in glass tubes (Vacutainers, 10 ml) containing heparin, immediately before and at selected intervals following administration of the drug. The samples were immediately centrifuged at 1000 *g* for 10 min. The plasma fraction was carefully separated using a sera-clear and was then frozen at —70° C until taken for assay.

Urine samples were taken at selected intervals and they were also stored at —70° C until taken for assay, at which time they were directly injected into the chromatographic system after dilution with distilled water.

All standards were prepared by dilution of 1 g of moxalactam per 100 ml (primary stock solution) of distilled water to concentrations ranging from 2 μg/ml to 4 mg/ml. These standard solutions were used as the basis for repeatability tests and for the preparation of standard curves. Calibration curves were plotted from data obtained from standard solutions diluted with blank plasma (50 μl plus 950 μl blank plasma) to concentrations ranging from 0.1 μg/ml to 200 μg/ml.

Chemicals

Reagent grade ethyl acetate, acetonitrile (Uvasol grade), chloroform, hydrochloric acid, calcium chloride, potassium dihydrogen phosphate and disodium hydrogen phosphate (E. Merck, Darmstadt, G.F.R.) were used without further purification.

Procedure

In a 10-ml glass-stoppered centrifuge tube, 1.0 ml of plasma and 0.5 ml of 0.4 *M* hydrochloric acid were mixed with 7 ml of ethyl acetate and extracted by shaking for 5 min. After centrifugation, (5 min at 1000 *g*), a 6-ml portion of the organic phase was placed in a new 10-ml glass-stoppered centrifuge tube with 0.350 ml of phosphate buffer (pH 7). After the tube was shaken and centrifuged, the upper organic phase was discarded by aspiration, and the aqueous phase was washed with 5 ml of chloroform. Again the tube was shaken and centrifuged, and a 10—100 μl portion of the upper aqueous phase was chromatographed.

The mobile phase used was a solution of acetonitrile and phosphate buffer (pH 7) (170:822) containing per litre 25 mg of calcium chloride and 8 ml of tetra-*n*-butylammonium hydroxide (Pic A reagent, Waters). Prior to use, the eluate was passed through a Millipore filter (0.45 μm) and deaerated under vacuum.

The column flow-rate was 2 ml/min. The eluate was monitored at the UV wavelength of 280 nm and quantitation was based on the peak height.

RESULTS

A representative chromatogram from the plasma prepared with 5 μg/ml moxalactam and plasma samples obtained 8, 10, and 12 h after a 1-g intravenous injection are shown in Fig. 2. Neither control plasma nor urine samples gave any interfering peaks on the control chromatograms. Under the described chromatographic conditions, the retention time is 4.6 min. The relationship between plasma concentration of the drug and the peak height is linear: $Y = 1.02X + 0.68$, $r = 0.9995$, and $Y = 19.8X + 0.08$, $r = 0.9999$, in the calibration range 0.4—12.5 μg/ml and 12.5—200 μg/ml, respectively.

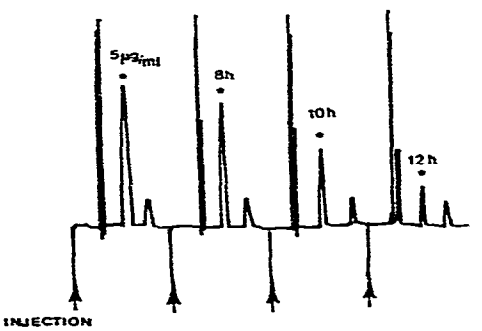

Fig. 2. Chromatograms of extracts of plasma spiked with moxalactam (5 μg/ml) and of plasma samples at various times after the intravenous administration of 1 g of moxalactam (a.u. = 0.05, injection volume = 25 μl).

The precision and accuracy of the plasma assays are summarized in Table I.

The mean plasma level after a 1-g intravenous injection of moxalactam (Fig. 3) was observed to decline in three phases. The average drug concentration at 12 h was 2.2 μg/ml and the biological half-life of the γ phase ($t_{½\gamma}$) was 2.74

TABLE I

WITHIN-DAY REPEATABILITY FOR PLASMA SAMPLES AT THREE CONCENTRATIONS

Concentration (μg/ml)	*n*	Injection volume (μl)	Sensitivity	Mean peak height (mm)	Standard deviation	Relative standard deviation (%)
40	10	10	0.05	172.6	3.9	2.3
5	6	40	0.05	75.3	2.7	3.6
0.5	9	40	0.01	39.5	5.1	12.9

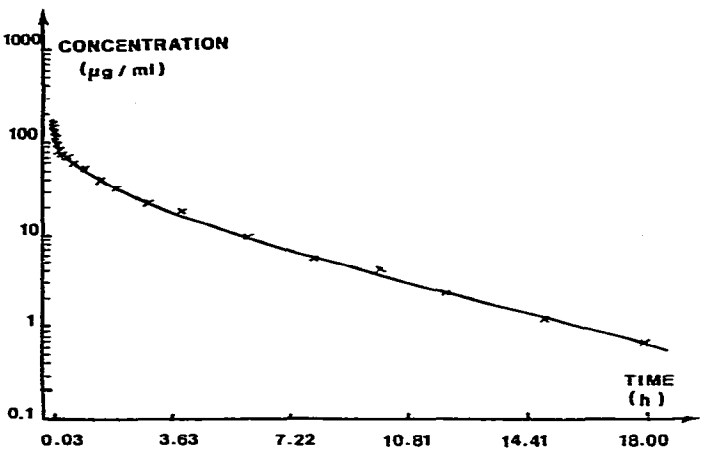

Fig. 3. Plasma concentration of moxalactam in a healthy volunteer following administration of 1 g intravenously. The concentration—time curve is fitted in a three compartment body model.

(± 0.28) h. For two subjects, plasma concentrations at 18 h were still at 0.53 μg/ml. The mean apparent volume of distribution was 0.26 l/kg. Approximately 57% of the administered dose was recovered in the urine within the first 12 h.

DISCUSSION

The extraction procedure is complex, due to the presence of the two acidic functions on the moxalactam molecule. The pK_a of the carboxylic acid groups was determined potentiometrically to be 3.55 for the group on the nucleus and 1.70 for the group on the chain (Fig. 1). To extract moxalactam by means of an organic phase, the pH has to be less than 1.7, as the pK_a for the acidic group on the side-chain is 1.7. Since the moxalactam molecule is polar, a polar organic solvent is required: ethyl acetate in this instance.

A reversed-phase system is used with a mobile phase at pH 7 to which counter-ion (tetra-*n*-butylammonium hydroxide) and calcium chloride were

added, since at pH 7 the two acidic functions of the moxalactam molecule are in ionised form.

The limit of sensitivity is at the 50 ng/ml level for plasma or urine samples (Fig. 4). This sensitivity level enables more accurate measurement of the plasma concentrations throughout pharmacokinetic studies. The small relative standard deviations amongst the averages obtained from repeatability tests affirm that this technique is accurate.

Fig. 4. Chromatogram of extract of plasma spiked with 0.625 μg/ml moxalactam (*) (a.u. = 0.02, injection volume = 50 μl).

Levels of moxalactam found to be present were lower than those of cefazolin, but generally higher than those of cephalosporins which have been studied in this laboratory (i.e. cefamandole, cefotaxime, cefoperazone, ceftazidime) [14, 15].

In summary, a method has been developed for the extraction and quantitation of moxalactam in plasma. This method enables determination of levels below 100 ng/ml of plasma. In terms of rapidity and selectivity, this method offers significant advantages over previously published microbiological procedures and has permitted the study of the pharmacokinetics of moxalactam.

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