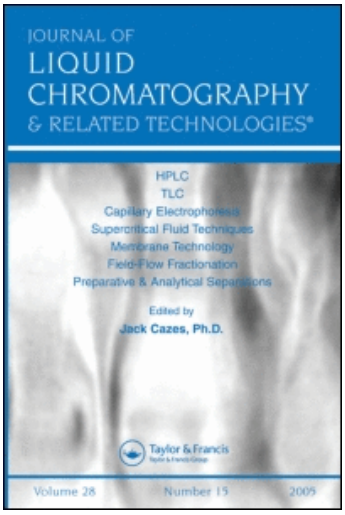

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HIGH PRESSURE LIQUID CHROMATOGRAPHIC ASSAY OF CEFAMANDOLE IN SERUM FOLLOWING INTRAVENOUS AND INTRAPERITONEAL ADMINISTRATION

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ABSTRACT

Following cesarean section 102 women were treated with cefamandole by either perioperative intravenous administration or intraperitoneal irrigation. High-pressure liquid chromatographic (HPLC) methods for the quantitation of the low serum levels of cefamandole following intraperitoneal lavage were developed. The antibiotic was assayed in the serum using a standard microbiological assay and two types of reverse phase column technology for HPLC. The two HPLC systems were almost identical in performance. Both HPLC methods were at least 10-fold more sensitive than the microbiological assay. The correlation between the three methods was 0.9739. The half-life of cefamandole was 37 min, which was not significantly different from the half-life of the drug in serum of non-pregnant women. The peak serum levels were 47.6 ± 36.8 μg/ml and 1.98 ± 1.5 μg/ml for the intravenous and intraperitoneal methods of administration, respectively.

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INTRODUCTION

Intrauterine intraperitoneal (IU/IP) irrigation and intravenous (IV) administration of antibiotics have been used effectively as prophylaxis in high-risk cesarean section patients (1,2). Rudd and associates used IU/IP lavage with cefamandole nafate to reduce post-cesarean section endometritis (2). Thirty patients received antibiotic via IU/IP irrigation and none developed endometritis. The reported serum levels of cefamandole using IU/IP lavage were frequently less than 0.5 μg/ml. Although the microbiological assay may be capable of measuring these low levels, the coefficient of variation of the assay has been very large (3). The purpose of this investigation was to develop a phase extraction procedure for cefamandole and to develop sensitive high-pressure liquid chromatographic (HPLC) assays for the determination of the wide range of levels of cefamandole anticipated in serum subsequent to IU/IP lavage and IV administration of the drug. Two types of column technology were compared, the conventional C_{18} μ-Bondapak steel column, and the new Radial Compression System (RCM-Z) containing a C_{18} μ-Bondapak compressible column. Serum levels were then determined following both IU/IP irrigation and the IV administration of the drug. In addition, the half-life of cefamandole was calculated from these data to determine if physiologic changes associated with pregnancy affected the clearance of the drug from these women (4).

MATERIALS AND METHODS

Patients

One hundred and two women at high-risk for infection following cesarean section were entered into this study. Informed consent was obtained. The patients received either 2 g of cefamandole IV over a 20 min infusion or 2 g of cefamandole in 800 ml of saline as an IU/IP lavage of the uterus and pelvis commencing at repair of the uterine incision. Using a bulb syringe, the irrigant was applied and simultaneously suctioned as follows: 200

ml to the uterine incision before closure, 200 ml to the bladder flap and closed uterine incision, 200 ml to the colic gutters with the patient in reverse Trendelenburg position, and 200 ml to the subfascial and subcutaneous space. Blood samples were obtained at 30, 60, and 120 min following both the IU/IP irrigation and IV administration of the drug. The serum was separated and frozen at -20°C until assayed.

Microbiological Methods

The serum concentration of cefamandole was measured by the agar diffusion technique (5). The test organism was Bacillus subtilis ATCC 6633. All specimens were assayed in duplicate.

HPLC Assay

The serum concentration of cefamandole was measured by modification of an existing HPLC assay for other penicillin and cephalosporin antibiotics (6,7). The HPLC analysis was performed on an extract of 0.5 ml of serum. The sera were extracted using a two phase extraction procedure. Acetonitrile and dichloromethane were used in the phase extraction which eliminates dilution of the specimens. A 50 µl aliquot was injected into the HPLC. The HPLC system was an ALC/GPC Model 204 liquid chromatograph attached to a Model 710 B Waters Intelligent Sample Processor (WISP), (Waters Associates, Milford, Mass.). All analyses were performed utilizing a 30 cm µ-Bondapak C_{18} steel column (Waters Associates, Milford, Mass.). The eluate was monitored at 254 µm with a Waters 440 detector. The detector sensitivity was 0.10 absorbance units (AU) full scale for the analysis of serum extracts from patients receiving intravenous cefamandole, and 0.01 AU for analysis of serum extracts from patients receiving IU/IP irrigation with cefamandole. Peaks were recorded on a 10 mv chart recorder (Houston Instruments, Houston, Texas) at a chart speed of 0.5 cm/min. The mobile phase consisted of 0.1 M sodium phosphate (85%) and acetonitrile (15%) at a pH of 6.0. The flow rate was

3.0 ml/min. Prior to use the mobile phase was degassed by filtration with a 0.45 μm FHUP filter (Millipore Corp., Bedford, Mass.).

In addition, assays were performed using the radial compression Z module (RCM-Z) (Waters Assoc., Milford, Mass) equipped with a column 10 cm long with an inside diameter of 8 mm containing a 10 μ C_{18} packing which should produce a more rapid and efficient separation than the 30 cm C_{18} steel column. The mobile phase for this system consisted of 0.1 M sodium phosphate (83%) and acetonitrile (17%) at a pH of 6.0. The flow rate was 4.0 ml/min. Prior to use the mobile phase was degassed by filtration as described previously.

Standard curves for both HPLC assays of cefamandole in serum were generated by extracting and assaying normal human serum spiked with cefamandole ranging from 0 to 100 μg/ml. In addition, three serum specimens containing an unknown quantity of cefamandole were assayed with the patients' specimens each time the assay procedure was performed (the quantities were unknown to the person performing the assay). The controls and the unknowns were prepared by the addition of a stock solution of cefamandole to serum with a microliter syringe (Hamilton Co., Reno, Nevada). Between-batch and within-batch recoveries were determined throughout the study using the spiked controls prepared as mentioned previously.

Statistics

Friedman's Chi-square nonparametric analysis was used to compare the three assay methods. Correlation coefficients for the three methods were done using multiple least squares linear regression. Comparison of the between-batch and within-batch data were done by linear least squares regression.

RESULTS

A chromatogram of the separation of cefamandole is depicted in Figure 1. Chloramphenicol, vancomycin, aminoglycosides, penicillins, cephalosporins or theophylline were without effect on

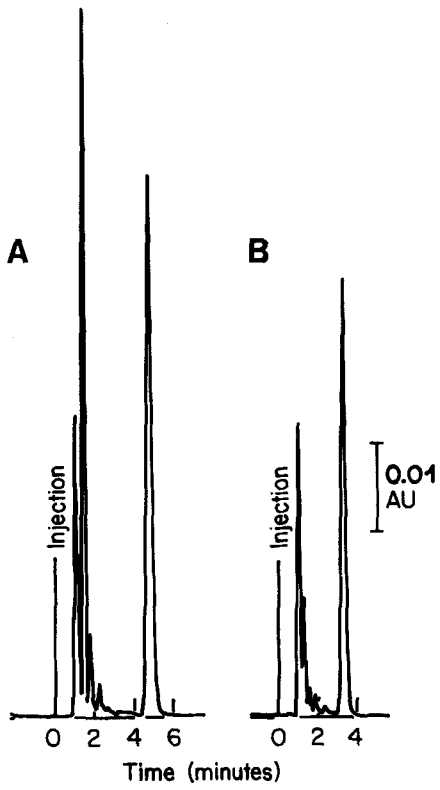

FIGURE 1. HPLC chromatograms of serum extracts of cefamandole (A) normal pooled human serum containing 47.5 µg/ml of cefamandole using a standard µ-Bondapak C_{18} steel column; (B) the same serum specimens using the Waters Radial Compression System (RCM-Z) equipped with a C_{18} column.

the separation when added to the specimens. Between-batch and within-batch reproducibility studies showed recoveries of at least 84% for the wide range of levels found using the two different methods of administering cefamandole. The within-batch and between-batch reproducibility data are depicted in Tables 1 and 2 and the correlation among the three methods of assay are shown in Table 3. When standard curves were prepared for quantitation of the low IU/IP irrigation levels, recoveries of greater than 90%

TABLE 1.

Results of Within-Batch Reproducibility With the C_{18} Column and the RCM-Z Module.

Cefamandole Concentration (μg/ml)	C_{18}				Average % Recovery	RCM-Z				Average % Recovery
	n	$\bar{x}$	SD	CV %		n	$\bar{x}$	SD	CV %	
1.0	9	0.84	0.05	5.95	84.0	8	0.90	0.04	4.44	90.0
5.0	7	4.93	0.18	3.65	98.6	6	4.76	0.17	3.45	95.2
24.4	9	23.80	0.74	3.11	97.9	10	24.5	1.00	4.08	100.0

$\bar{x}$, mean; SD, standard deviation; CV, coefficient of variation.

TABLE 2.

Results of Between-Batch Reproducibility With the C_{18} Column and the RCM-Z Module.

Cefamandole Concentration (μg/ml)	C_{18}				Average % Recovery	RCM-Z				Average % Recovery
	n	$\bar{x}$	SD	CV %		n	$\bar{x}$	SD	CV %	
1.0	5	0.87	0.13	14.83	87.0	4	0.83	0.10	12.05	83.0
5.0	5	4.57	0.49	10.72	91.4	4	4.77	0.38	7.97	95.4
24.4	3	24.28	0.88	3.62	99.5	4	23.74	1.45	6.11	97.3

$\bar{x}$, mean; SD, standard deviation; CV, coefficient of variation.

TABLE 3.

The Comparison of Concentration of Cefamandole by the Microbiological, the C_{18} Column and RCM-Z HPLC Methods.

Method of Administration	ASSAY		
	Microbiological	HPLC RCM-Z	HPLC C_{18}
IP	3.40	3.95	3.10
IP	2.60	2.23	1.70
IP	6.20	5.38	4.50
IP	4.50	2.72	2.30
IP	0.80	0.74	0.45
IP	2.95	2.80	2.10
IP	1.30	1.10	1.10
IP	0.60	0.76	0.45
IP	7.50	5.90	5.10
IP	3.60	5.10	4.20
IP	2.80	3.20	2.80
IP	1.30	1.00	0.86
IV	34.00	46.30	44.10
IV	15.20	21.00	18.60
IV	4.50	6.90	5.80
IV	26.50	21.90	17.10
IV	13.50	10.50	7.70
IV	4.30	3.50	2.80
IV	0.74	0.81	0.86
IP*	ND	0.31	0.67
IP*	ND	0.14	0.51
IV	46.00	34.90	33.80
IV	21.00	18.10	17.20
IV	4.20	5.10	3.90
IV	34.00	31.40	26.10
IV	14.00	13.60	11.00
IV	2.90	4.30	3.70
IV	53.00	55.50	45.40
IV	31.80	25.90	23.50
IV	102.00	100.50	106.90
IV	15.80	20.20	19.30
IV	47.50	37.00	36.00
IV	18.50	26.00	25.00

* Indicates that microbiological assay could not detect the presence of cefamandole in these specimens.

The correlation matrix for the above assays are as follows.

Microbiological to RCM-Z = 0.9792
Microbiological to C_{18} = 0.9739
RCM-Z to C_{18} = 0.9934

IP = Intraperitoneal.
IV = Intravenous.

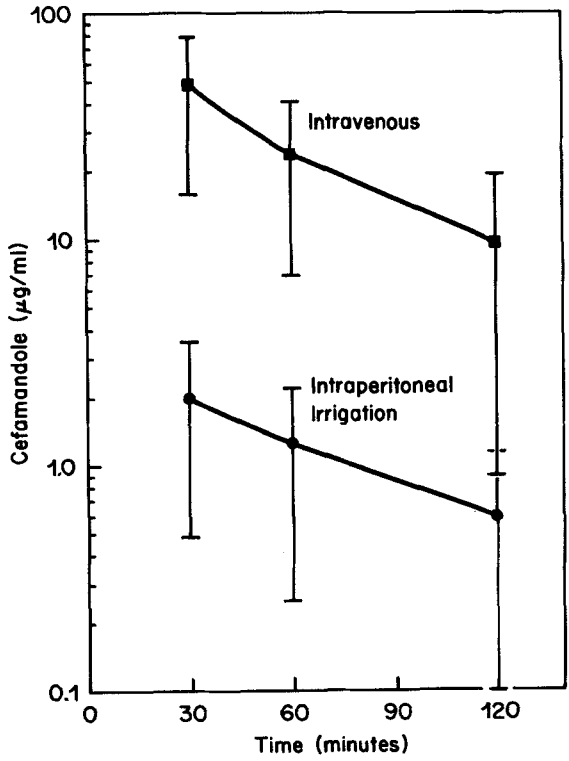

Figure 2. Semi-logarithmic plot of serum levels following the intravenous or intrauterine administration of 2 grams of cefamandole. Each time represents the mean and standard deviation. Forty-five or more values were used to achieve each point.

were obtained (data not shown). All methods were accurate and specific for cefamandole. The limit of detection for cefamandole by HPLC was < 0.05 µg/ml. The limit of the microbiological assay was approximately 0.50 µg/ml. Therefore the concentration of cefamandole in some specimens could not be measured by the microbiological assay method. The regression coefficient ($Y = \alpha + \beta X$; where X and Y represent the dependent and independent variables, β is the regression coefficient in the population and α is the value of Y when X is 0) for the comparisons of the RCM-Z method to the

TABLE 4.

Serum Concentration of Cefamandole Following Intravenous Infusion and Intrauterine Irrigation at Cesarean Section.

Administration	No. of Patients	30 Min.	60 Min.	120 Min.
IV Mean	53	47.6 ± 31.80**	23.67 ± 16.80	9.54 ± 9.67
Range		(15.0 - 165.6)	(3.5 - 80.9)	(0* - 34.7)
IU/IP irrigation Mean	49	1.98 ± 1.50	1.24 ± 0.99	0.58 ± .5.7
Range		0.1 - 5.1	(0.05 - 2.6)	(0 - 2.3)

** µg/ml ± standard deviation.
* Less than 0.01 µg/ml of cefamandole.

microbiological assay method, the C_{18} steel column method to the microbiological assay method, and the RCM-Z to the C_{18} steel column assay method was 0.51 = 0.953X, -0.87 + 0.957X, and 1.56 + 0.984X, respectively.

Friedman's Chi-square test indicated a significant difference in the comparison of the microbiological data with the HPLC C_{18} steel column data ($p < .001$). However, since these data had a very high correlation coefficient for all three methods, we do not feel that this significance was of practical importance.

Table 4 depicts the serum levels at the times collected. The serum levels decreased from 47.6 µg/ml at 30 min to 9.54 µg/ml at 120 min for the IV dose. The serum levels from the patients receiving cefamandole by IU/IP irrigation decreased from 1.98 µg/ml at 30 min to 0.58 µg/ml at 120 min. The disposition of cefamandole through the two hours is shown on Figure 2. The half-life for both methods of administration was 37 min.

DISCUSSION

The RCM-Z system provided a more rapid analysis of cefamandole than the standard C_{18} steel column. The C_{18} was slightly more sensitive than the RCM-Z as depicted in Figure 1. The decreased sensitivity of the RCM-Z is attributed to its shorter length and larger diameter. The C_{18} steel column has a lower flow rate, thus requiring less mobile phase for completion of the assay. Both HPLC assay methods worked well for the quantitation of cefamandole at the very low serum levels measured in this study.

In this report, IU/IP irrigation with cefamandole resulted in serum levels that were 4.5% of an equivalent IV dose. Although the cefamandole administered by lavage was present in the peritoneal cavity only momentarily, average serum concentrations of 1.98 µg/ml were present 30 minutes following irrigation. The serum concentrations measured in patients receiving cefamandole by IU/IP irrigation were 1.5 to 3 times higher than levels reported in two previous studies (1,8). This likely reflects idiosyncrasies in

the method of irrigation used as well as differences in the duration that the drug was in contact with the peritoneum before it was removed by suction. A second less likely explanation is that the microbiological assays used in the previous reports were less sensitive than the assays used in the present report. The sensitivity and precision of the microbiological assay probably decreases substantially at the low levels of cefamandole in the IU/IP lavage. Since the previous studies reported much lower cefamandole levels, this lack of sensitivity may also explain the discrepancy in results. Following either IU/IP irrigation or IV administration of cefamandole, large variations in serum concentration were measured in these women. We attributed this wide deviation to large differences in volume distribution in these patients who varied greatly in weight but who were all treated with a standard dose of the drug.

The serum half-life for cefamandole in non-pregnant individuals has been shown to be 34 minutes following an intravenous dose and 60 minutes following an intramuscular dose (9). In the present study a serum half-life of cefamandole of 37 minutes was measured in patients following either IU/IP irrigation or IV administration of the drug. It is well established that the glomerular filtration rate (GFR) increases during pregnancy, returning to normal only gradually over several days. It has been suggested that this change in renal function may affect the half-life of several drugs (4,5). From the data presented in this report, it would appear that pregnancy associated changes in GFR do not affect the renal excretion of cefamandole in patients treated with the drug in the peripartum period. The low but significant levels of cefamandole measured in the serum following rapid IU/IP irrigation likely reflects the rapid uptake of the drug across a large, well-vascularized space. Several reports have documented the efficacy of peritoneal lavage with antimicrobials at the time of cesarean section (1,2,8). The decreased incidence of postoperative infection in patients treated with IU/IP irrigation of cefamandole may be the result of either the

local bactericidal action of the drug or the result of a low but significant systemic level of cefamandole obtained in these patients. The results of this investigation justify the evaluation of the mean inhibitory concentrations of cefamandole for the bacteria recovered in these high-risk patients undergoing cesarean section.

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