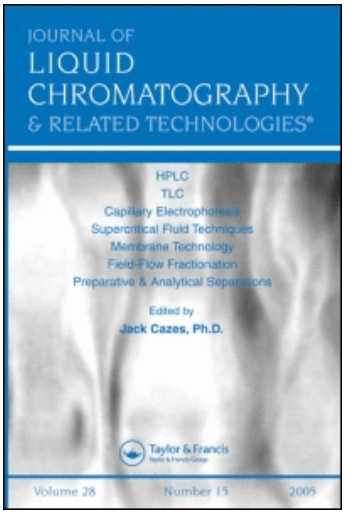

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SERUM AND PELVIC TISSUE CONCENTRATIONS OF CEFTRIAXONE AND CEFAZOLIN AT HYSTERECTOMY

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ABSTRACT

Ceftriaxone and cefazolin concentrations were assayed by high-pressure liquid chromatography in serum and pelvic tissue. Specimens were obtained at uterine removal subsequent to a 1-g intramuscular preoperative dose given to 117 women scheduled for elective vaginal or abdominal hysterectomy. The mean serum concentration of cefazolin was 43.2 ± 13.1 and 39.8 ± 15.4 μg/ml after vaginal and abdominal hysterectomy, respectively. For ceftriaxone they were 59.2 ± 16.8 and 56.1 ± 18.3 μg/ml for vaginal and abdominal hysterectomy, respectively. Mean tissue concentrations of ceftriaxone were 22.5 ± 10.4, 17.4 ± 6.9, 27.9 ± 10.7, and 16.4 ± 6.3 μg/g for vagina, myometrium, fallopian tube, and ovary, respectively, and respective mean tissue concentrations for cefazolin were 15.8 ± 7.6, 14.4 ± 8.5, 15.6 ± 8.0, and 12.4 ± 5.8 μg/g. Pelvic tissue concentrations of cefazolin were similar, but concentrations of ceftriaxone in fallopian tube and vagina were higher than those in ovary and myometrium. Tissue to serum ratios of ceftriaxone remained constant throughout the time intervals studied, whereas cefazolin ratios increased with time.

INTRODUCTION

Ceftriaxone is a new third-generation cephalosporin antibiotic with a 6-hour half-life (1). Cefazolin, a first-generation

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cephalosporin with a 1.5-hour half-life, is effective in reducing infection after hysterectomy (2). We determined serum and pelvic tissue concentrations in specimens from elective hysterectomy patients. The serum and tissue levels were determined by high-pressure liquid chromatography (HPLC) and were compared to a microbiological assay.

MATERIALS AND METHODS

Patients and Specimens

One hundred and seventeen women scheduled for elective vaginal or abdominal hysterectomy gave informed consent, and were assigned one of the two drugs by a computer-generated randomization list. On call in the operating room, each woman received 1 g of either ceftriaxone or cefazolin by deep intramuscular injection and the time of injection was recorded. At uterine excision, the time was noted, a blood sample was drawn, and the serum was separated and frozen at -20°C until assayed for antibiotic concentration. In addition, vagina was collected after vaginal hysterectomy and myometrium, a segment of fallopian tube, and ovarian tissue were obtained from the abdominal hysterectomy patients. All tissue specimens were maintained at -20°C until processed and assayed for antibiotic concentrations.

Sample Preparation

Both serum and whole tissue specimens were processed as described previously (3, 4). There was no attempt to determine the concentration of antibiotic in extracellular tissue fluid. There were no modifications in the acetonitrile dichloromethane phase extraction procedure. All specimens were assayed in duplicate. Tissue concentrations of both antibiotics were corrected for blood contamination by the cyanomethemoglobin colorimetric method (5).

HPLC Assay

Serum and tissue specimens were assayed by a modification of the reverse-phase HPLC assay method for cefoperazone as previously

described (3). Modifications in the procedures were in the mobile phases for both ceftriaxone and cefazolin. The mobile phase for ceftriaxone consisted of 10% methanol and 90% 0.10 M sodium phosphate buffer, pH 8.0. The flow rate was 2.3 ml/min and the UV detector was operated at 254 nm and 0.1 absorbance units (AU) for serum and for tissue concentration the detector was operated at 0.01 AU.

The mobile phase for cefazolin was 12% acetonitrile and 88% 0.10 M sodium phosphate buffer, pH 6.1. The flow rate was 2.0 ml/min and the UV detector was operated at 254 nm and 0.1 AU for serum and 0.01 AU for tissue specimens.

Between-group and within-group reproducibility studies were made by injecting either ceftriaxone or cefazolin at concentrations of 50 µg/g and 10 µg/g into a pre-weighted portion of normal gynecologic tissue with a micro syringe. These tissue were then extracted with 0.1 M PO_4 buffer pH 6.1 as described previously (3). All determinations in these recovery studies were done a minimum of five times.

The components of the HPLC system consisted of a 30-cm C_{18} µ Bondapak column (Water's Associates, Milford, Mass.), a model 6000-A pump, a model 710 B Water's Intelligent Sample Processor, and a 440 UV detector (Water's Associates, Milford, Mass.). Peaks were recorded on a 10 mv chart recorder (Linear Instruments, Inc., Reno, Nevada) at a chart speed of 0.50 cm/min.

Microbiological Assay

Twenty serum and tissue specimens were assayed for ceftriaxone and cefazolin concentrations by the microbiological assay. The bioassay for ceftriaxone was done using *E. coli* ATCC 10536. For cefazolin the bioassay was done using a *Bacillus subtilis* spore suspension (Difco Laboratories, Detroit, Michigan). Each petri plate contained 10 ml of antibiotic assay agar number one (Difco Laboratories, Detroit, Michigan). Fifteen microliters of serum or tissue extracts of the specimens and spiked serum or tissue extracts were added to 6 mm sterile filter paper discs.

The plates were incubated at 35°C for 12-18 hrs. Zone diameters were measured using a dial calipers.

Statistical Analysis

Least square linear regression was used to correlate the HPLC and microbiological bioassay data. An analysis of variance followed by the Students' Newman-Keuls multiple comparison procedure was used for the statistical analysis of these data.

RESULTS

Chromatograms of cefazolin and ceftriaxone are shown in Figures 1 and 2, respectively. The HPLC assay was linear when both serum and tissue were spiked with ceftriaxone and cefazolin in concentration from zero to 200 μg/ml (g). The correlation between concentration on peak height was > .99 for both antibiotics. The correlation between the microbiological bioassay and the HPLC assay for both antibiotics was > .90. Between-group and within-group recovery in pelvic tissue was > 95% for both ceftriaxone and cefazolin (data not shown).

The women in both antibiotic groups had clinical and surgical variables that were similar; 51 women underwent abdominal hysterectomy, and 66 had vaginal hysterectomy. There was a significant difference in the interval from dose to sample time in the two surgical procedures ($P < .05$). The mean collection times were 142.9 ± 37.5 and 164.1 ± 45.4 min following preoperative dose for vaginal and abdominal hysterectomy, respectively. Although the times to specimen collection were different, serum concentrations of both antibiotics were not significantly different for the two procedures. The mean serum concentrations of ceftriaxone were 59.2 ± 16.8 and 56.1 ± 18.3 μg/ml for the vaginal and abdominal hysterectomy. Similarly, the mean serum cefazolin levels were 43.2 ± 13.1 and 39.8 ± 15.4 μg/ml. Serum concentrations of cefazolin were 71.9% of the ceftriaxone levels. The mean tissue concentrations of ceftriaxone were 22.5 ± 10.4, 17.4 ± 6.9, 27.9 ± 10.7, and 16.4 ± 6.3 μg/g for vagina, myometrium, fallopian tube,

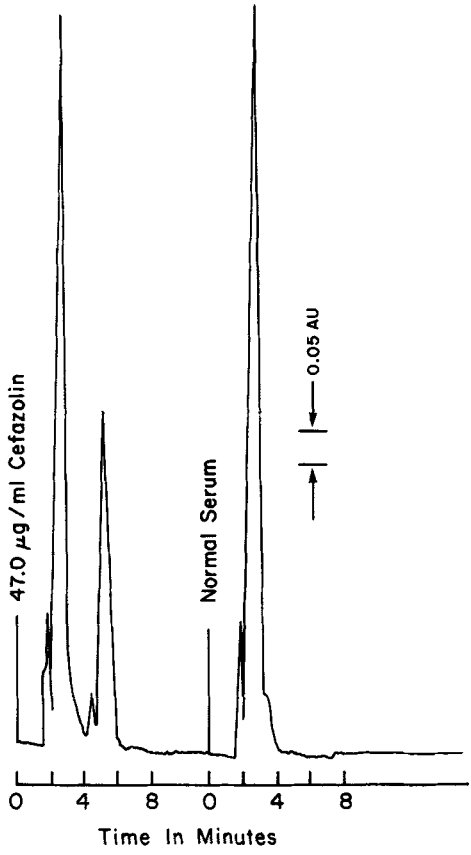

FIGURE 1

The HPLC serum assay of cefazolin. The chromatogram of cefazolin on a C_{18} µ Bondapak column at an absorption wave length of 254 nm. The mobile phase was 12% acetonitrile and 88% 0.1 M PO_4 buffer pH 6.0. The flow rate was 2.0 ml/min. The chromatograms were prepared with the sensitivity of the chromatograph at 0.10 absorbance units (AU).

and ovary, respectively. The mean tissue levels for cefazolin were 15.8 ± 7.6, 14.4 ± 8.5, 15.6 ± 8.0, and 12.4 ± 5.8 µg/g for vagina, myometrium, fallopian tube and ovary, respectively. The tissue to serum ratio of ceftriaxone remained constant throughout the time intervals studied with the initial ratio being 0.342 ± 0.12, and after 6 hours it was 0.383 ± .05. Cefazolin tissue to serum ratios appeared to increase with time. Initially the mean

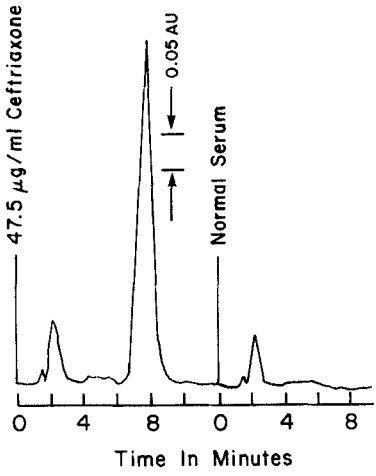

FIGURE 2

The HPLC serum assay of ceftriaxone. The chromatogram of ceftriaxone on a C_{18} µ Bondapak column at an absorption wave length of 254 nm. The mobile phase was 10% methanol in 90% 0.1 M PO_4 buffer pH 6.0. The flow rate was 2.3 ml/min. The chromatograms were prepared with the sensitivity of the chromatograph at 0.10 absorbance units (AU).

tissue ratio was 0.293 ± .06, and at > 200 min the ratio was 0.544 ± .17. Tables 1 and 2 represent a summary of serum and pelvic tissue and the ratio of tissue concentrations to simultaneous serum concentrations.

DISCUSSION

There were significant differences in the tissue concentrations of ceftriaxone, whereas they were similar for cefazolin. The fallopian tube and vagina tissue concentrations of ceftriaxone were significantly higher than myometrium and ovarian tissue concentrations ($P < .05$).

In a previous study, the concentrations of ceftriaxone in serum and gynecological tissue were studied in 31 patients following a 2-g injection (6). These investigators showed that fallopian tube concentrations were significantly higher than those found

TABLE 1

Mean (± SD) Serum and Pelvic Tissue* Concentrations of Ceftriaxone Following Hysterectomy.

Time Minutes	Specimens Per Unit Time	Concentrations				RATIO		
		Serum µg/ml	Myometrium µg/g	Fallopian Tube µg/g	Ovary µg/g	M/S	T/S	O/S
Abdominal Hysterectomy								
90 - 120	4	57.7 ± 22.6	17.3 ± 4.3	28.3 ± 12.4	11.5 ± 1.8	.300	.490	.199
120 - 160	8	58.0 ± 15.1	18.6 ± 8.4	30.1 ± 11.6	18.5 ± 6.6	.321	.519	.319
160 - 200	8	54.3 ± 22.5	15.5 ± 5.9	28.5 ± 9.9	13.8 ± 5.1	.285	.525	.254
200 - 255	3	53.7 ± 17.8	19.3 ± 10.1	20.2 ± 10.1	24.3 ± 8.2	.359	.376	.453

Time Minutes	Specimens Per Unit Time	Concentration		RATIO
		Serum µg/ml	Vaginal Tissue µg/g	V/S
Vaginal Hysterectomy				
85 - 120	10	64.2 ± 22.4	24.4 ± 12.1	.380
120 - 160	15	57.7 ± 12.2	22.1 ± 10.7	.383
160 - 240	6	52.8 ± 9.6	18.2 ± 2.8	.344

* All tissue concentrations were corrected for blood contamination
M/S - Myometrium to serum
T/S - Fallopian tube to serum
O/S - Ovary to serum
V/S - Vagina to serum

TABLE 2

Mean (± SD) Serum and Pelvic Tissue Concentrations of Cefazolin Following Hysterectomy.

Time Minutes	Specimens Per Unit Time	Concentrations				RATIO		
		Serum µg/ml	Myometrium µg/g	Fallopian Tube µg/g	Ovary µg/g	M/S	T/S	O/S
Abdominal Hysterectomy								
105 - 140	9	45.8 ± 17.9	14.1 ± 7.9	17.5 ± 10.9	11.3 ± 6.5	.305	.382	.247
140 - 180	13	41.7 ± 12.4	13.1 ± 5.1	14.7 ± 6.1	13.7 ± 5.7	.314	.353	.329
180 - 200	4	32.5 ± 5.7	24.2 ± 14.9	18.6 ± 5.0	12.3 - 6.2	.744	.527	.378
200 - 390	2	17.8 ± 10.5	11.0 ± 5.1	13.4 ± 1.9	6.8 ± 8.4	.618	.753	.382

Time Minutes	Specimens Per Unit Time	Concentration		RATIO
		Serum µg/ml	Vaginal Tissue µg/g	V/S
Vaginal Hysterectomy				
90 - 120	7	47.4 ± 14.4	11.3 ± 6.5	.238
120 - 160	14	44.3 ± 16.3	16.3 ± 7.8	.368
160 - 220	14	40.0 ± 8.4	16.9 ± 8.2	.422

* All tissue concentrations were corrected for blood contamination
M/S - Myometrium to serum
T/S - Fallopian tube to serum
O/S - Ovary to serum
V/S - Vagina to serum

in the myometrium and endometrium. They further reported that after 5 h, tissue concentrations were > 20 μg/g of tissue.

In another study, protein binding at doses > 1500 mg was thought to give higher protein-free drug levels in blister and tissue levels. They found that the blister and tissue levels were linear with the change in dose (7).

Data from the 2-g study (6) and those in our study indicate that the serum to tissue ratios for ceftriaxone do not change appreciably, and appear to be linear. Accordingly, the high proten binding (95%) as compared to cefazolin which is 80% protein bound, the unusually long elimination half-life in tissue, and the high levels make the drug interesting for study, and predictably useful clinically since even with a 1-g injection tissue concentration of > 20 μg/g were observed over 3 hours post-dose.

Ceftriaxone is the only antibiotic we have assayed in pelvic tissue that appears to have an affinity for the fallopian tube and vaginal tissue. In a previous study, we found no interregimen or intertissue differences in concentrations of cefoperazone or cefoxitin, as was true for cefazolin in the current study (3). The significance of these differences could be attributed to the longer half-life, or the higher protein binding capacity of ceftriaxone. These tissue/serum ratios for ceftriaxone are different from cefoperazone and cefoxitin as we previously reported (3).

In summary, the concentration of ceftriaxone and cefazolin were determined through about 4 hours subsequent to a 1-g intramuscular preoperative dose given to women undergoing hysterectomy. Mean cefazolin serum levels were significantly lower than serum levels of ceftriaxone ($P < .01$). Similarly, mean tissue concentrations of cefazolin were 56 to 82% of ceftriaxone tissue levels. The longer half-life of ceftriaxone is probably responsible for the higher serum and tissue concentrations of ceftriaxone.

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