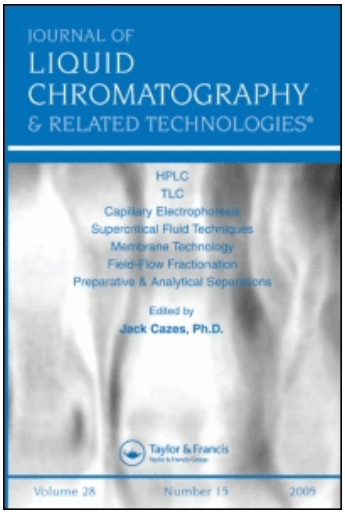

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Roger E. Bawdon[a]; William J. Novick[b]; David L. Hemsell[a]; William D. Welch[c]
[a] Department of Obstetrics and Gynecology, The University of Texas Health Science Center at Dallas, Dallas, Texas [b] Hoechst-Roussel Pharamaceuticals, Inc., North Somerville, New Jersey [c] Department of Pathology, The University of Texas Health Science Center at Dallas, Dallas, Texas

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HIGH-PRESSURE LIQUID CHROMATOGRAPHIC ASSAY OF CEFOTAXIME AND DESACETYLCEFOTAXIME IN HUMAN MYOMETRIUM

[1]Roger E. Bawdon, Ph.D., [3]William J. Novick, Ph.D.,
[1]David L. Hemsell, M.D., and [2]William D. Welch, Ph.D.
[1]Department of Obstetrics and Gynecology
[2]Department of Pathology
The University of Texas Health Science Center at Dallas
5323 Harry Hines Boulevard
Dallas, Texas 75235
[3]Hoechst-Roussel Pharamaceuticals, Inc.
Route 202-206 North
Somerville, New Jersey 08876

ABSTRACT

An analytic high-pressure liquid chromatographic (HPLC) procedure for the assay of desacetylcefotaxime and cefotaxime in gynecologic tissue was developed. Normal individuals undergoing elective hysterectomy were subjects in this study. Blood and myometrium were removed up to four hours after a 1-g intramuscular dose of cefotaxime. Since cefotaxime is unstable in homogenized tissue at room temperature, the specimens must be maintained at 4°C during homogenization and extraction. Mean serum desacetylcefotaxime and cefotaxime levels were 3.2 ± 2.0 µg/ml and 6.8 ± 4.4 µg/ml, respectively. The mean myometrium concentrations of desacetylcefotaxime and cefotaxime were 8.4 ± 10.0 µg/g and 6.3 ± 8.9 µg/g, respectively. The cefotaxime to desacetylcefotaxime ratios in serum and tissue were 2.12 and 0.75, respectively. Our results suggest that in antimicrobial synergistic studies evaluating serum and tissue levels, the optimal ratio of one part cefotaxime to at least one part desacetylcefotaxime.

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INTRODUCTION

The pharmacokinetics of cefotaxime and desacetylcefotaxime in serum have been well documented (1, 2). The serum concentration of both antimicrobials is easily measured in serum using high-pressure liquid chromatography (HPLC) (3, 4). Direct tissue and bile concentrations of desacetylcefotaxime have not been determined because of body metabolites which interfere with the assay (5). The rapid elution of desacetylcefotaxime from reverse-phase HPLC columns also contributed to the problem. Desacetylcefotaxime has been quantified in bile by transformation into a lactone, and then determined by HPLC (1). The HPLC assays of desacetylcefotaxime in bile was 1.43 times higher than cefotaxime after 90 minutes. At 240 minutes the desacetylcefotaxime was 2.2 times higher than the cefotaxime levels (6).

There are other problems associated with the assay of cefotaxime and desacetylcefotaxime, related to hemolysis of red cells and tissue destruction. Both processes result in the release of enzymes that promote the rapid deacetylation of cefotaxime to the desacetylcefotaxime metabolite (4). This study examines the metabolism of cefotaxime and desacetylcefotaxime subsequent to tissue homogenization, and the development of a direct tissue (myometrium) assay for desacetylcefotaxime.

MATERIALS AND METHODS

To determine the fate of cefotaxime in tissue, normal myometrium was collected, maintained at 4°C, and spiked with 50 μg/g of cefotaxime (cefotaxime and desacetylcefotaxime were obtained from Hoechst-Roussel Pharmaceuticals, Inc., Somerville, New Jersey). Additional specimens were spiked with cefotaxime and 10 mM sodium p-hydroxymecuribenzoate (PHMB) (Sigma Chemical Co., St. Louis, Missouri), an enzyme inhibitor. These specimens were maintained at 4°C and homogenized with a Polytron (Brinkman Instruments, Inc., Westbury, New York). They were then centrifuged at 3,000 X g for five minutes at 4°C and the supernatant divided into

2.0 ml aliquots and incubated at 4°C, 25°C, and 37°C for two hours. At the end of the incubation period, the reactions were stopped by processing through the Sep-pak C_{18} column (Water's Associates, Milford, Massachusetts) extraction procedure.

The Sep-pak cartridges were attached to the barrel of a 10 cc disposable syringe which serves as a solvent reservoir. The plunger of the syringe is used to force the specimen through the Sep-pak cartridges. To prepare the cartridges for the specimens, the cartridges were rinsed with 3.0 ml of HPLC grade methanol followed by 5.0 ml of 0.1 M sodium phosphate buffer pH 6.1. The flow rate through the cartridge was about 5 ml/minute.

To each of the cartridges was added 1.0 ml of the standards or tissue extract. The specimens were pushed through the Sep-pak C_{18} column with the syringe plunger. One ml of 0.1 M sodium phosphate buffer was added to rinse all the specimen into the column and pushed through as before. An additional 5.0 ml of the 0.1 M sodium phosphate buffer was added and pushed through the column. The cefotaxime and desacetylcefotaxime were then eluted through the column with 1.0 ml of 50% methanol in water (v/v).

The most rapid HPLC assay of cefotaxime in serum and tissue may be obtained by using the previously described acetonitrile dichloromethane extraction methodology with modification of only the mobile phase (7). The HPLC mobile phase consisted of 92% 0.10 M sodium phosphate pH 6.1 and 8% acetonitrile. The flow rate was 2.5 ml/minute. The mobile phase for the assay of desacetylcefotaxime in tissue was 95% 0.1 M sodium phosphate pH 6.1 and 5% acetonitrile. For cefotaxime the 8% acetonitrile mobile phase was used subsequent to the Sep-pak extraction. There was no interference between the assay of cefotaxime and desacetylcefotaxime.

Samples from 15 women undergoing elective hysterectomy were assayed. A 1-g IM dose of cefotaxime was given on call to the operating room, and the injection time was recorded. At the time of uterine removal, a blood sample was obtained, normal appearing myometrium was removed and the time recorded. The serum and uterine tissue specimens were maintained at -20°C until assayed.

The sera from these women were assayed for cefotaxime and desacetylcefotaxime as described above. The standard curve for serum assays consisted of normal serum which was spiked with 0-100 µg/ml of both cefotaxime and desacetylcefotaxime. Similarly, tissue or tissue extracts were spiked with 0-25 µg/g for tissue assay controls. The tissue was extracted by the addition of five parts 0.1 M sodium phosphate buffer pH 6.1 to one part tissue. The tissues were then disrupted with the Polytron and processed through the Sep-pak C_{18} column as described earlier. Between- and within-batch recoveries were made on both the Sep-pak and the acetonitrile dichloromethane phase extraction procedures. Linear least squares regression analysis was used to analyze the between- and within-batch data.

RESULTS AND DISCUSSION

The chromatograms of cefotaxime and desacetylcefotaxime in serum using the phase extraction system are shown in Figure 1. This method is extremely sensitive for both antibiotics in serum, and there was no interference from body metabolites. Between- and within-batch recoveries of the HPLC extraction procedures for both were > .95 (data not shown).

The Sep-pak C_{18} tissue extraction eliminated interference from body metabolites, and the standard curve from 0 to 100 µg/g was linear and had a > .98 correlation with peak height. Chromatograms of desacetylcefotaxime from spiked myometrium are shown in Figures 2 and 3. Cefotaxime resolves late in the 5% acetonitrile mobile phase, and should be assayed using the 8% acetonitrile mobile phase to increase sensitivity and decrease column retention time. Desacetylcefotaxime may occasionally be resolved by the 8% acetonitrile mobile phase, but was completely resolved with the 5% acetonitrile mobile phase.

The results of incubating spiked tissue extract at various temperatures and with PHMB are shown in Figure 4. There is a linear relationship between temperature and the amount of

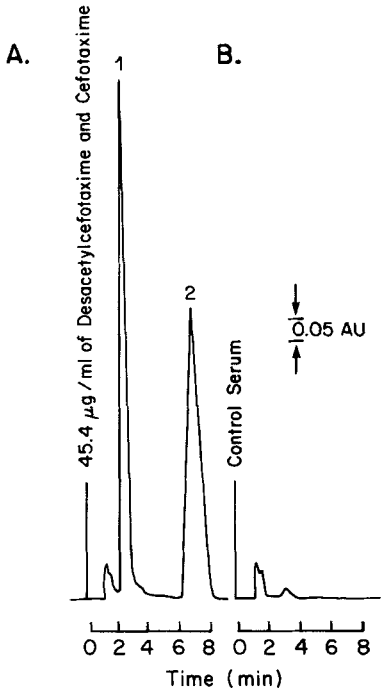

Figure 1. Chromatograms of desacetylcefotaxime and cefotaxime in serum subsequent to a 1-g IM dose of cefotaxime. The serum was extracted with acetonitrile and dichloromethane. The retention times were 3.0 minutes and 7.0 minutes for desacetylcefotaxime and cefotaxime, respectively. AU: Absorbance units. (1) desacetylcefotaxime and (2) cefotaxime. (A) Control serum spiked with desacetylcefotaxime and cefotaxime.

desacetylcefotaxime formed. Approximately 8.0 µg/g of desacetylcefotaxime was formed after the incubation of 50 µg/g of cefotaxime at 4°C for two hours with a 50% reduction after the addition of PHMB. At 25°C PHMB reduced the formation of desacetylcefotaxime by 84.4%, from 24 µg/g to 4.2 µg/g. At 37°C, 34.5 µg/ml of desacetylcefotaxime was formed after two hours incubation. It is therefore essential that all tissue specimens be maintained at 4°C after collection and during extraction for the accurate determination of the desacetylcefotaxime concentrations

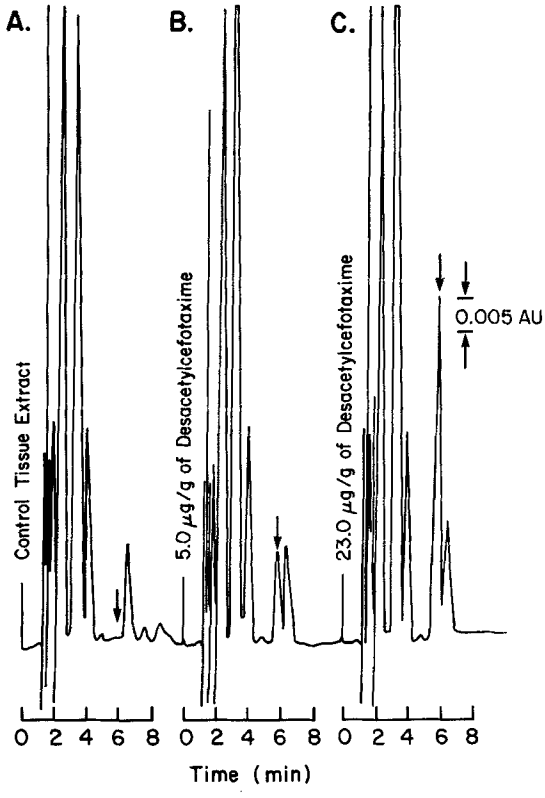

Figure 2. Chromatograms of desacetylcefotaxime as extracted from myometrium using the Sep-pak C_{18} extraction column. The HPLC mobile phase was 5% acetonitrile and 95% 0.10 M sodium phosphate buffer pH 6.1. Retention time was 5.8 minutes. AU: Absorbance units (A) myometrium spiked with no desacetylcefotaxime. (B) tissue extract with 5.0 µg/g desacetylcefotaxime added, and (C) tissue extract with 23 µg/g of desacetylcefotaxime added. Arrows indicate desacetylcefotaxime peak.

in tissue. As long as this temperature is maintained, little desacetylcefotaxime is formed and the addition of PHMB is not necessary. Enzymes capable of deacetylation such as those found here in myometrium tissue may also be present in lysed blood (4). The serum and tissue concentrations of cefotaxime and desacetylcefotaxime levels are shown in Table 1. The mean serum concen-

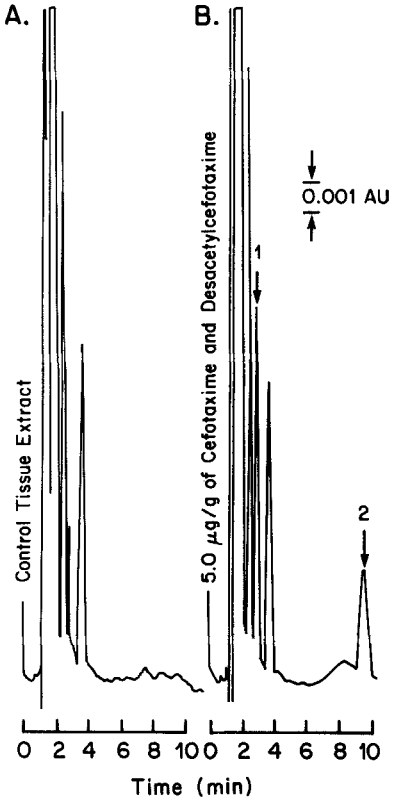

Figure 3. Chromatograms of desacetylcefotaxime and cefotaxime as extracted from myometrium using Sep-pak C_{18} extraction column. The HPLC mobile phase was 8% acetonitrile and 92% 0.10 M sodium phosphate buffer pH 6.1. Retention time was 3.0 minutes for desacetylcefotaxime and 9.6 minutes for cefotaxime. AU: Absorbance units (A) Control tissue extract and (B) 5 µg/g of desacetylcefotaxime and cefotaxime added to myometrium. (1) desacetylcefotaxime and (2) cefotaxime.

trations of cefotaxime and desacetylcefotaxime were 6.8 ± 4.4 and 3.2 ± 2.0, respectively. The mean tissue concentrations were 6.3 ± 8.9 µg/g and 8.4 ± 10.0 µg/g for cefotaxime and desacetylcefotaxime, respectively. The ratio of serum cefotaxime to serum desacetylcefotaxime was 2.12, and for myometrium the ratio was .75. The ratio of serum cefotaxime and desacetylcefotaxime is

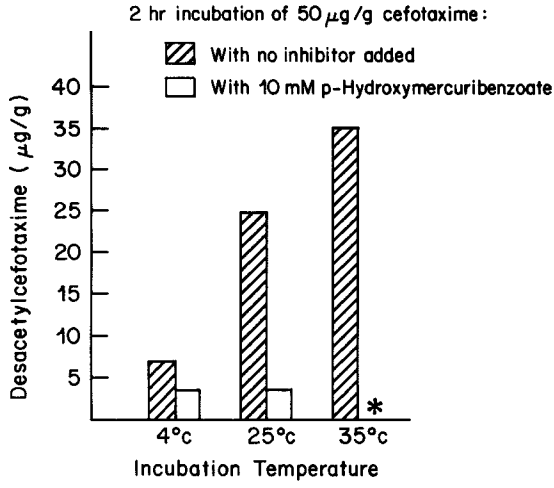

Figure 4. Formation of desacetylcefotaxime from 50 μg/g of cefotaxime added to tissue by the enzymes of myometrium. *PHMB was not added to specimens at 35°C.

TABLE 1

Concentration of cefotaxime and desacetylcefotaxime in serum and myometrium from 15 women.

	Serum μg/ml		Tissue μg/g	
†Time Collected	Cefotaxime	Desacetyl-cefotaxime	Cefotaxime	Desacetyl-cefotaxime
2:15	7.4	3.0	0.8	2.6
2:05	7.2	4.4	5.4	0.7
3:05	2.3	1.5	2.8	2.2
3:00	12.7	2.8	3.9	5.5
1:25	10.8	1.2	31.4	3.3
1:25	12.0	1.5	4.6	4.3
6:10	2.2	2.0	0.7	4.6
2:10	2.4	7.4	1.1	5.1
2:30	2.5	2.4	4.1	3.0
1:57	9.8	7.8	23.1	4.3
2:40	1.8	1.8	1.5	9.4
1:50	9.5	3.5	2.6	18.0
1:25	14.1	2.1	9.2	38.0
2:10	3.9	3.5	2.6	21.4
5:00	3.0	2.7	1.3	3.1
	6.8 ± 4.4	3.2 ± 2.0	6.3 ± 8.9	8.4 ± 10.0

* Standard deviation

† Time in hours and minutes subsequent to a 1-g IM dose

essentially the same as reported previously (8). The ratio of cefotaxime to desacetylcefotaxime in myometrium is also very similar to the ratio reported in human bile (6). Since there is reported antibacterial synergy of cefotaxime and desacetylcefotaxime, and the tissue ratio of these antibiotics is about one part cefotaxime to at least one part desacetylcefotaxime, we suggest that in vitro antimicrobial synergy between cefotaxime and desacetylcefotaxime studies be done at a minimum of a 1:1 ratio. It will be interesting to determine if these ratios are similar in other tissues.

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