Measurement of cefoxitin levels in tissue using high-pressure liquid chromatography

J. V. ROBBS, R. T. SALISBURY, K. I. ELSON, J. G. BROCK-UTNE

Summary
A high-pressure liquid chromatography (HPLC) method that successfully measured cefoxitin (a modification of the antibiotic cephamicyn C) levels in subcutaneous tissue, muscle, and aortic and peripheral arterial walls has been developed. Samples were obtained from 11 patients submitted to prosthetic aortic replacement. All patients received an intravenous bolus dose of cefoxitin 2 g just before induction of anaesthesia. Blood and tissue samples were taken at various intervals intra-operatively. The tissue samples were mechanically homogenised. Both plasma and the tissue homogenates were deproteinated with trichloracetic acid. The cefoxitin was separated by HPLC and measured by ultraviolet absorbance. The results show that the tissue concentration of the drug fell over a 4-hour period and that all levels exceeded the MIC90 (minimum inhibitory concentration that inhibits growth of bacteria at the 90% level) for most aerobic and anaerobic pathogens for at least 3 hours.

Materials
The analytical column was a μ-Bondapak C18, 300 mm x 3.9 mm x 10 μm (Waters Millipore), preceded by a guard column with ODS packing (Whatman's CSK1). The liquid chromatograph was a Hewlett Packard 1090, used in conjunction with an HP 1040 DV detector and an HP 3392 integrator. A high-pressure liquid chromatography (HPLC) method that successfully measured cefoxitin (a modification of the antibiotic cephamycin C) levels in subcutaneous tissue, muscle, and similar compounds in plasma and urine has been developed. In either case 25 μl of the resulting extract was injected into the column.

Collection
Blood samples (5 ml) were collected in heparinised tubes: they were centrifuged immediately and the plasma was removed. Tissue specimens were washed free of blood and dried with absorbent tissue. Both plasma and tissue samples were frozen and stored at -20°C.

Sample preparation
Tissue samples were thawed and sliced with a scalpel as finely and quickly as possible, on a glass plate. An amount of approximately 300 mg was weighed and homogenised in 1 ml distilled water at about 29,000 rpm. Both plasma and tissue homogenate were deproteinated with trichloracetic acid, using a previously described procedure. In either case 25 μl of the sample was examined, and the results often show large variations, even between identical samples. High-pressure liquid chromatography (HPLC) has already been used quite extensively to measure cefoxitin (a broad-spectrum semisynthetic cephamycin antibiotic with marked β-lactamase resistance) and similar compounds in plasma and urine.

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Chromatography
Cefoxitin was separated on the reverse-phase column described above. The mobile phase consisted of 50 mM phosphate buffer (pH 3.0) and acetonitrile, 79:21 by volume, flowing at 1.0 ml/min and at ambient temperature. Cefoxitin eluted at 7...
minutes and was detected and quantified by absorbance at 235 nm. Samples and external cefoxitin standards were injected alternately, and sample concentrations were determined from the peak area ratios. Assays were performed in duplicate whenever there was sufficient material, as in the case of the plasmas and the majority of tissue specimens. Recoveries were established by spiking multiple series of plasma or tissue with standard additions of cefoxitin over the range 5 - 50 µg/g.

**Results**

Detector response was found to be linear over a range of at least 1 - 200 µg/g. Recoveries were as follows (mean ± SE): plasma 57.5 ± 0.65%, arterial tissue 71.2 ± 3.11%, muscle 56.6 ± 2.03% and subcutaneous fatty tissue 118.2 ± 3.35%. The standard curves were reasonably linear, the correlation coefficients (r) being as follows: plasma 0.99, arterial wall 0.98, muscle 0.98 and subcutaneous tissue 0.99. The limit of detection was found to be about 1 µg/g at a signal-to-noise ratio of 4, and we have subsequently used related cephalosporin derivatives, such as cefoxitin, in the therapeutic range. The results of tests on 11 patients are illustrated graphically in Fig. 1. The drug concentrations in the aortic and peripheral artery walls as well as in muscle were maintained well above the MIC₉₀ (minimum inhibitory concentration that inhibits growth of bacteria at 90%) for most aerobic and anaerobic pathogens for at least 3 hours. The blood levels show the classic exponential decay following any intravenous drug administration. Unfortunately the subcutaneous tissue levels are too low to be of any significance pharmacokinetically.

**Discussion**

The risk of wound infection after abdominal surgery is still high. Pre-operative antibiotic prophylaxis, especially with cephalosporin derivatives, has been shown to be efficacious. The HPLC technique described here provides a rapid and reliable measurement of the antibiotic drug, even in tissues that we believe have not been reported before. Furthermore, the study shows that cefoxitin levels in plasma, muscle and aortic and peripheral arterial walls exceed the MIC₉₀ for most aerobic and anaerobic pathogens for at least 3 hours. The HPLC procedure is sensitive and reasonably precise and at least 30 assays, including standards, could be done during an average working day. We now feel that 1 - 2 ml blood samples would be adequate, and that this test could therefore possibly be used to measure blood cefoxitin levels in children.

We would have preferred to use an internal standard. The use of 3-isobutyl-1-methylxanthine has been reported, but in our hands this substance gave unacceptably variable recovery rates, even in plasma, in comparison with cefoxitin. However, we have subsequently used related cephalosporin derivatives successfully for this purpose.

Cefoxitin is well tolerated by patients and has proved itself a very potent antibiotic. Since it is rapidly cleared from the body it is important to assay drug and tissue levels to establish its pharmacokinetic profile. Thus the study shows that if adequate blood and tissue levels following intravenous administration of cefoxitin are to be maintained, a bolus injection every 3 hours is essential.

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**REFERENCES**