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Assessment of Cefazolin and Cefuroxime Tissue Penetration by Using a Continuous Intravenous Infusion

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A continuous intravenous infusion was used to assess the tissue penetration of cefazolin (14 subjects) and cefuroxime (15 subjects) in orthopedic surgery patients. Subjects were randomly assigned to receive a continuous intravenous infusion of cefazolin (mean, 178.6 mg/h) or cefuroxime (mean, 330.0 mg/h) at a rate estimated to achieve a target steady-state total concentration of 50 μg/ml in serum. The infusion was initiated 12 to 14 h before surgery, and blood and muscle tissue samples were collected intraoperatively at the times of incision and wound closure. Although there was a significant difference between the free concentrations of cefazolin (at incision, 9.3 μg/ml; at closure, 9.2 μg/ml) and cefuroxime in serum (at incision, 26.9 μg/ml; at closure, 31.8 μg/ml), there was no difference in the total concentrations in muscle at either surgical incision (cefazolin, 6.1 μg/g; cefuroxime, 5.6 μg/g) or wound closure (cefazolin, 7.7 μg/g; cefuroxime, 7.4 μg/g). There was a significant correlation between the pooled free serum and total muscle concentrations for cefazolin ($P = 0.001$); however, there was no correlation between these variables with the pooled cefuroxime data ($P = 0.403$). These findings indicate that the free drug concentration in serum alone is not consistently predictive of the total concentration of cephalosporin in muscle.

Although the relationships between the total and free drug concentrations in serum and their concurrent concentrations in tissues have been the object of much study, these relationships remain uncertain, and their clinical significance is controversial. Studies of antimicrobial tissue penetration have typically utilized a single dose of antimicrobial agent administered via a short intravenous (i.v.) infusion.

The goal of the present study was to assess under steady-state conditions the effect of serum protein binding on the tissue penetration of two cephalosporins, cefazolin and cefuroxime, administered by continuous i.v. infusion. The specific objectives of this study were to determine the concentrations of free and total drug in serum and total drug in muscle by using a continuous-infusion model in patients undergoing orthopedic surgery and to determine the relationships between these concentrations. Cefazolin and cefuroxime were selected as study drugs based on considerations for patient safety and differences in serum protein binding. Cefazolin is considered a drug of choice for surgical prophylaxis in total hip replacement and internal fixation of fractures, and cefuroxime has also been effective for these purposes (1, 6, 15). These drugs differ significantly in their degree of serum protein binding (cefazolin, 74 to 86%; cefuroxime, 33 to 50%), allowing for comparison of study parameters.

MATERIALS AND METHODS

Subjects. This study was approved by the institutional review board at the Medical College of Georgia and the Veterans Administration Medical Center, and all subjects provided written informed consent before being enrolled in the study. Subjects were hospitalized patients on the orthopedic surgery service at the two institutions who were undergoing primary total hip replacement or open repair of a hip or femur fracture. Subjects were required to be at least 18 years of age, have a creatinine clearance greater than 50 ml/min, and be within 15% of their ideal body weight. Exclusion criteria included history of a type I hypersensitivity reaction to penicillin or any reaction to a cephalosporin antibiotic, evidence of active cardiac or hepatic disease, history of severe hematologic disease or host defense impairment (absolute neutrophil count less than 2,000/mm^3), pregnancy or lactation, and evidence or suspicion of infection in the operative site at the time of surgery. In addition, subjects could not have received any antimicrobial agent within 72 h before surgery or received additional antimicrobial agents during the operation other than topical antimicrobial irrigation.

Study design, dosage calculations, and drug administration. Subjects were randomly assigned to receive a continuous i.v. infusion of cefazolin (Ancef; Smith Kline & French Laboratories, Philadelphia, Pa.) or cefuroxime (Zinacef; Glaxo Inc., Research Triangle Park, N.C.). The i.v. infusion rate was determined by using the model-independent relationship $k_0 = CL \times C_{ss}$, where k_0 is the i.v. infusion rate (milligrams per hour), CL is the total clearance of the study drug (liters per hour), and C_{ss} is the desired total concentration in serum at steady state (milligrams per liter). The target C_{ss} for all subjects was 50 μg/ml. The CL for each subject was estimated based on creatinine clearance (CL_{CR}) as predicted by the method of Cockcroft and Gault (4). Cefazolin CL, as shown below, was estimated from data collected in two previously published studies of 30 patients that received cefazolin preoperatively (7, 11). The range of creatinine clearance values in those patients was 50 to 120 ml/min. Cefazolin CL was calculated as $0.598(CL_{CR}) + 0.736$, where CLs are in liters per hour.

Cefuroxime CL, in liters per hour, was estimated as $V_{ss} \times$

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k_{el}. The estimated steady-state volume (liters) of distribution (V_{ss}) of cefuroxime was obtained from published information (0.19 liter/kg of body weight) (3). The elimination rate constant (k_{el}; h^{-1}) was estimated by using the subject's calculated CL_{CR} standardized to body surface area. A CL_{CR} of less than 65 ml/min per 1.73 m^2 was estimated to be associated with a k_{el} of 0.277 h^{-1}; likewise, creatinine clearances of 65 to 79, 80 to 104, 105 to 115, and greater than 115 ml/min per 1.73 m^2 were estimated to be associated with elimination rate constants of 0.347, 0.462, 0.554, and 0.6 h^{-1}, respectively (3).

The continuous i.v. infusion of cefazolin or cefuroxime was initiated 12 or 14 h, respectively, before the start of the operation to allow adequate time for the attainment of steady-state conditions before biologic sampling in the operating room. The study drug was infused via a syringe pump (Auto Syringe model AS2F; Travenol Laboratories, Hooksett, N.H.). Once the infusion was initiated, the rate was held constant until the infusion was discontinued in the postoperative recovery room.

Biologic sampling and drug assays. Venous blood samples of 10 ml were obtained at the time of the initial surgical incision and at wound closure. The serum was harvested and stored in glass vials at −80°C until assayed. A 1-g sample of viable muscle was obtained from the surgical site concurrent with the blood samples. Muscle samples were blotted dry with surgical gauze and stored at −80°C.

The concentrations of free and total drug in serum and total drug in homogenized muscle were determined by high-performance liquid chromatography (HPLC). The HPLC system included the following (all from Millipore Waters Associates, Milford, Mass.): pump (model 510), autosampler (WISP model 710B), and spectrophotometer set at a wavelength of 254 nm (Lambda-Max model 481). Peak heights were recorded on a thermal reporter-integrator (model 3390A; Hewlett Packard Co., Avondale, Pa.). The stationary phase for all assays consisted of a C_{18} precolumn attached to a C_{18} 25-cm reversed-phase column. The mobile phase for all cefazolin assays was 11% (vol/vol) acetonitrile in 0.1 M sodium acetate buffer (pH adjusted to 3.85) at a flow rate of 2.0 ml/min. The mobile phase for all cefuroxime assays was 10% (vol/vol) acetonitrile in 0.1 M sodium acetate buffer (pH adjusted to 3.85), also at a flow rate of 2.0 ml/min.

For determination of total cefazolin in serum, 500 μl of acetonitrile and 25 μl of cefoxitin internal standard (0.1 mg/ml) were added to 100 μl of serum test samples and vortexed. This mixture was centrifuged (model Centra 4; International Equipment Co., Needham Heights, Mass.) at 3,000 rpm for 10 min, and 10 μl of the clear eluent was injected onto the HPLC column. The concentration of free cefazolin in serum was obtained by ultrafiltration with an Amicon MPS filter system with a YMT membrane (Amicon Corp., Danvers, Mass.). Serum (1 ml) was centrifuged at 3,000 rpm for 6 min to yield 150 μl of ultrafiltrate. Then 25 μl of 8-chlorotheophylline internal standard (100 μg/ml) was added to 100 μl of protein-free filtrate and vortexed, and 10 μl was injected onto the HPLC column. To determine the total concentration of cefazolin in muscle, 2 ml of Sorensen phosphate buffer (pH 7.4) was added to 500 mg of frozen muscle. The frozen muscle sample was placed in an ice bath and homogenized for less than 1 min with a tissue homogenizer (Tissumizer TR5T; Tekmar Co., Cincinnati, Ohio). The homogenate was vortexed and then centrifuged for 10 min at 3,000 rpm. Solid-phase extraction of cefazolin from the homogenate was done on a C_{18} column (Prep-sep; Fisher Scientific Co., Pittsburgh, Pa.) by taking 500 μl of eluent from the centrifuged homogenate and washing it with 1 ml of purified water on the column, followed by extraction of the drug from the column with 1 ml of methanol. Then 20 μl of 8-chlorotheophylline internal standard (50 μg/ml) was added to 500 μl of the resulting eluent and vortexed, and 30 μl was injected onto the HPLC column.

The concentrations of total and free cefuroxime in serum and the concentration of total cefuroxime in muscle were determined as described above for cefazolin. 8-Chlorotheophylline was the internal standard for all cefuroxime assays.

The standard curve ranges for the cefazolin assays were 10 to 100 μg/ml, 2 to 30 μg/ml, and 2 to 15 μg/g for the total and free drug concentrations in serum and total drug concentration in muscle, respectively. The ranges for the standard curves of the cefuroxime assays were 10 to 100 μg/ml, 5 to 50 μg/ml, and 2 to 50 μg/g for the total and free drug concentrations in serum and total drug concentration in muscle, respectively. The standard curve for each of the study drugs in muscle was prepared with porcine muscle. Because of the limited amount of clinical material (0.5 to 1 g), drug stability tests were not undertaken. The sensitivities were 1 μg/ml and 1 μg/g for all serum and muscle assays, respectively. The r^2 for the standard curves ranged from 0.990 to 0.999. The intraday coefficients of variation were 5.9 and 8.9% for cefazolin and cefuroxime in muscle at 2 μg/g, respectively. For serum (total and free), they were usually less than 5%.

Data analysis. Mean concentration data obtained at surgical incision and wound closure were compared within subjects by using paired t tests. Comparisons between drugs were performed with independent t tests. The associations between concentrations were tested with the Pearson correlation coefficient. Two-tailed tests were used for all comparisons, and the a priori level of statistical significance was set at $P < 0.05$.

RESULTS

Of the 34 patients enrolled in the study, 5 were withdrawn for the following reasons: inability to start an i.v. to administer the study drug (2 patients), malfunction of the i.v. infusion pump (1 patient), inability to maintain the infusion (1 patient), and failure to begin the infusion at the proper time (1 patient). Of the 29 evaluable subjects, 14 received cefazolin and 15 received cefuroxime. Subjects in the two study groups did not differ significantly ($P > 0.05$) with regard to the following (means ± standard deviations): age (cefazolin, 48.1 ± 12.5 years; cefuroxime, 45.9 ± 17.6 years), calculated creatinine clearance (cefazolin, 76.6 ± 25.6 ml/min; cefuroxime, 88.5 ± 26.4 ml/min) and serum albumin (cefazolin, 4.8 ± 0.5 g/dl; cefuroxime, 4.5 ± 0.4 g/dl). The mean infusion rates of cefazolin and cefuroxime were 178.6 ± 46.9 and 330.0 ± 114.6 mg/h, respectively ($P < 0.001$). The mean duration of surgery for subjects receiving cefazolin and cefuroxime was 94.2 ± 21.5 and 105.4 ± 32.8 min, respectively ($P > 0.05$). Surgical procedures were begun between 8:00 a.m. and 2:00 p.m. and used a variety of anesthetic agents.

The mean concentration data obtained at surgical incision and wound closure for both antibiotics are summarized in Table 1. Comparisons within subjects of cefazolin concentrations obtained at incision and wound closure were not significant ($P > 0.05$). However, there were significant intrasubject differences in the cefuroxime total serum ($P < 0.05$), free serum ($P < 0.01$), and total muscle ($P = 0.001$) concentrations at incision and wound closure.

TABLE 1. Mean concentration data (mean ± standard deviation)

Antimicrobial agent	Time of sampling	Concn (μg/ml) in serum		Concn in muscle (μg/g)	Muscle/free serum ratio	Free serum/total serum ratio
		Free	Total			
Cefazolin (n = 14)	Incision	9.3 ± 3.3	52.8 ± 14.0[a]	6.1 ± 3.3	0.64 ± 0.24	0.17 ± 0.04[a]
	Closure	9.2 ± 2.8	51.8 ± 12.8	7.7 ± 4.9	0.82 ± 0.41	0.18 ± 0.05
Cefuroxime (n = 15)	Incision	26.9 ± 9.1[b]	36.2 ± 13.4[b]	5.6 ± 3.6	0.23 ± 0.20[b]	0.75 ± 0.04[b]
	Closure	31.8 ± 11.8	40.9 ± 15.5	7.4 ± 2.5[b]	0.24 ± 0.08[b]	0.79 ± 0.12

[a] n = 13.
[b] n = 14.

The concentrations of total cefazolin and cefuroxime in muscle were not statistically different (surgical incision, P = 0.74; wound closure, P = 0.86) despite significant differences between drugs in both total and free drug concentrations in serum. For cefazolin, there were significant correlations between total and free drug concentrations in serum at both surgical incision and wound closure and between free drug concentration in serum and total concentration in muscle at surgical incision. At wound closure, the correlation between free cefazolin concentration in serum and total concentration in muscle also approached statistical significance (P = 0.059). For cefuroxime, the only significant correlations were between total and free concentrations in serum at surgical incision and wound closure (Table 2). When the incision and closure concentration data for each drug were pooled, there were significant correlations between total and free concentrations in serum for both cefazolin and cefuroxime and between the concentration of free cefazolin in serum and total concentration in muscle (Table 3).

DISCUSSION

Generally it is believed that only free, unbound antimicrobial agents are able to interact with microorganisms to exert a pharmacologic effect (17). Most pathogenic bacteria reside in tissue sites outside of the vascular system; therefore, the tissue penetration of free drug is important for drug efficacy. In the present study we utilized a continuous i.v. infusion under steady-state conditions to control for the total concentration of drug in serum and drug clearance. At steady state, the concentrations of free drug in the vascular and extravascular compartments are expected to be similar. Despite significant differences in the concentrations of free cefazolin and cefuroxime in serum, we found no significant difference in the concentration of total drug in muscle. This was an unexpected finding and suggests that the concentration of free drug in serum alone is not a consistent predictor of the total concentration in tissue. The total concentration of drug in the tissue is dependent on the concentrations of both bound and free drug in the extravascular space. Since cefazolin protein binding in serum is significantly greater than that of cefuroxime, it would be anticipated that cefazolin protein binding in the extravascular space would also be greater. A possible explanation for the observation that cefazolin and cefuroxime had similar total concentrations in muscle despite having significant differences in their free concentrations in serum may relate to binding of cefazolin to proteins in the extravascular space. Possibly there is less free cefazolin than cefuroxime in the tissues, as would be predicted; however, the higher protein binding of cefazolin in tissue may have resulted in our observation that total concentrations of the two drugs in muscle were equivalent. Results of this study cannot be extrapolated to cefazolin and cefuroxime concentrations in serum outside the range of the study. Although in the present study we measured the concentration of total drug in tissue, it would have been desirable to also measure the concentration of free drug in muscle. However, homogenization of the tissue destroys the interrelationships of tissue components, resulting in a conglomerate containing muscle, interstitial fluid, cell cytoplasm, and possibly blood (2). Determination of the free drug concentration in this conglomerate would not be a reliable measure of free drug in the extracellular fluid. Therefore, determination of free drug in tissue homogenates is generally not performed.

TABLE 2. Correlations between concentration parameters

Concn parameters	Cefazolin (n = 14)		Cefuroxime (n = 15)	
	r[a]	P[b]	r	P
Incision				
Total serum and free serum	0.742	0.004[c]	0.992	<0.001[d]
Total serum and total muscle	0.339	0.257[c]	−0.112	0.702[d]
Free serum and total muscle	0.811	<0.001	−0.132	0.654[d]
Closure				
Total serum and free serum	0.547	0.043	0.955	<0.001
Total serum and total muscle	−0.197	0.501	0.297	0.303[d]
Free serum and total muscle	0.516	0.059	0.377	0.183[d]

[a] Pearson correlation coefficient.
[b] Two-tailed P value.
[c] n = 13.
[d] n = 14.

TABLE 3. Correlations between pooled (incision plus closure) concentration parameters

Concn parameters	Cefazolin (n = 28)		Cefuroxime (n = 30)	
	r[a]	P[b]	r	P
Total serum and free serum	0.643	<0.001[c]	0.966	<0.001[d]
Total serum and total muscle	0.002	0.993[c]	0.109	0.579[e]
Free serum and total muscle	0.607	0.001	0.165	0.403[e]

[a] Pearson correlation coefficient.
[b] Two-tailed P value.
[c] n = 27.
[d] n = 29.
[e] n = 28.

Although the mean total concentration of cefazolin in serum closely approximated the target concentration of 50 μg/ml, the mean total concentration of cefuroxime in serum fell considerably short of this goal. The reasons for not achieving the target concentration are not apparent but may relate to one of the components used to estimate cefuroxime clearance.

The bacteria most frequently associated with deep wound infections after orthopedic surgery are *Staphylococcus aureus* and *Staphylococcus epidermidis* (1). For cefuroxime, the MICs for 90% of *S. aureus* and *S. epidermidis* isolates are 1.4 and 0.98 μg/ml, respectively (10). Ninety-six percent of *S. aureus* isolates and 94% of *S. epidermidis* isolates are inhibited by cefazolin concentrations of 2.0 and 4.0 μg/ml, respectively (12). The concentration of cefuroxime in muscle exceeded the MIC for 90% of staphylococci in all subjects. Of 14 subjects receiving cefazolin, 1 had drug concentrations in muscle of less than 2 μg/g, and 3 had drug concentrations of less than 4 μg/g (range, 1.3 to 3.9 μg/g). No postoperative infections were noted in either study group. The optimal antimicrobial concentration in tissue for preventing postoperative infection and its relationship to the MIC for potentially infecting organisms are still unknown.

The significant differences in cefuroxime concentrations at the times of surgical incision and wound closure were an unexpected finding and indicate a change from the steady state. The increased cefuroxime concentrations at closure may have been due to intraoperative changes in drug clearance. Cefazolin and cefuroxime are both eliminated renally via glomerular filtration and tubular secretion. The renal clearances of cefazolin and cefuroxime are 50 to 62 and 150 ml/min per 1.73 m^2, respectively (8). General anesthetics have been shown to cause intraoperative reductions in renal blood flow and the glomerular filtration rate (5, 9, 14), and cefuroxime, having a significantly higher renal clearance, would be expected to be affected by these intraoperative changes to a greater extent than cefazolin.

We assessed antimicrobial tissue penetration by mechanically homogenizing wound muscle. As with any method of determining antimicrobial tissue penetration, tissue homogenization has certain limitations. First, blood may contaminate the tissue sample, resulting in a falsely elevated concentration of drug in tissue (2). Second, the process of homogenizing the tissue disturbs the anatomical relationships within the tissue, resulting in a dilutional effect on antibiotic in the extracellular space and underestimation of the antibiotic concentration at the site of most bacterial infections (13, 16). Previous experiences with the methods employed in this study have found blood contamination of muscle tissue to be negligible (7). Although tissue homogenization may result in underestimation of the antibiotic concentration in the extracellular space, both study drugs should be affected equally by this procedure.

Although the present study found a good correlation between free drug in serum and total drug in muscle for cefazolin, there was no such correlation for cefuroxime. Also, cefazolin and cefuroxime concentrations in muscle were similar, despite significantly different free drug concentrations in serum. These findings indicate that the free drug concentration in serum alone is not a consistent predictor of the total concentration in tissue. Further studies are needed to determine the clinical significance of this finding.

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LITERATURE CITED

1. **Abramowicz, M. (ed.).** 1989. Antimicrobial prophylaxis in surgery. Med. Lett. Drugs Ther. **31**:105–108.
2. **Bergan, T.** 1981. Pharmacokinetics of tissue penetration of antibiotics. Rev. Infect. Dis. **3**:45–66.
3. **Bundtzen, R. W., R. D. Toothaker, O. S. Nielson, P. O. Madsen, P. G. Welling, and W. A. Craig.** 1981. Pharmacokinetics of cefuroxime in normal and impaired renal function: comparison of high-pressure liquid chromatography and microbiological assays. Antimicrob. Agents Chemother. **19**:443–449.
4. **Cockcroft, D. W., and M. H. Gault.** 1976. Prediction of creatinine clearance from serum creatinine. Nephron **16**:31–41.
5. **Cousins, M. J., L. R. Greenstein, B. A. Hitt, and R. I. Mazze.** 1976. Metabolism and renal effects of enflurane in man. Anesthesiology **44**:44–53.
6. **Davies, A. J., R. M. Lockley, A. Jones, M. El-Safty, and J. C. Clothier.** 1986. Comparative pharmacokinetics of cefamandole, cefuroxime and cephradine during total hip replacement. J. Antimicrob. Chemother. **17**:637–640.
7. **DiPiro, J. T., J. J. Vallner, T. A. Bowden, Jr., B. A. Clark, and J. F. Sisley.** 1985. Intraoperative serum and tissue activity of cefazolin and cefoxitin. Arch. Surg. **120**:829–832.
8. **Gower, P. E., and C. H. Dash.** 1977. The pharmacokinetics of cefuroxime after intravenous injection. Eur. J. Clin. Pharmacol. **12**:221–227.
9. **Jarnberg, P., J. Santesson, and J. Eklund.** 1978. Renal function during neurolept anaesthesia. Acta Anaesthesiol. Scand. **22**: 167–172.
10. **Jones, R. N., P. C. Fuchs, T. L. Gavan, E. H. Gerlach, A. L. Barry, and C. Thornsberry.** 1977. Cefuroxime, a new parenteral cephalosporin: collaborative in vitro susceptibility comparison with cephalothin against 5,887 clinical bacterial isolates. Antimicrob. Agents Chemother. **12**:47–50.
11. **Jones, S. J., J. T. DiPiro, D. E. Nix, and N. A. Bhatti.** 1985. Prophylactic cephalosporins for open repairs of femoral fractures: comparative levels in serum, muscle, and hematoma. J. Bone Jt. Surg. Am. Vol. **67**:921–924.
12. **Knothe, H.** 1979. Microbiological activity of cefazedone as compared to cefazolin and cephalothin. Arzneim. Forsch. **29**: 378–381.
13. **LeBel, M., and M. Spino.** 1988. Pulse dosing versus continuous infusion of antibiotics: pharmacokinetic-pharmacodynamic considerations. Clin. Pharmacokinet. **14**:71–95.
14. **Mazze, R. I., M. J. Cousins, and G. A. Barr.** 1974. Renal effects and metabolism of isoflurane in man. Anesthesiology **40**:536–542.
15. **Patzakis, M. J., J. Wilkins, J. S. Bhatt, and A. Sarmiento.** 1987. Single-dose cefuroxime versus multidose cephapirin for prophylaxis in orthopaedic surgery. Contemp. Orthop. **15**(Sept.):33–38.
16. **Ryan, D. M., O. Cars, and B. Hoffstedt.** 1986. The use of antibiotic serum levels to predict concentrations in tissues. Scand. J. Infect. Dis. **18**:381–388.
17. **Wise, R.** 1986. The clinical relevance of protein binding and tissue concentrations in antimicrobial therapy. Clin. Pharmacokinet. **11**:470–482.