1985

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ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1985, p. 487–490
0066-4804/85/040487-04$02.00/0

Vol. 27, No. 4

Intraoperative Ceforanide Pharmacokinetics and Protein Binding

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Received 10 September 1984/Accepted 2 January 1985

The pharmacokinetics and protein binding of ceforanide were studied in 15 patients undergoing cholecystectomies. Each patient received ceforanide (20 mg/kg) intravenously on arrival in the operating room, after which serial blood samples were collected during the elimination phase for determination of total and free ceforanide concentrations in the serum. A high-pressure liquid chromatography assay was used, with a centrifugal filtration system for free-drug determinations. Serum concentration data for each individual were subjected to linear regression to determine the elimination rate constants (total and free drug), volumes of distribution, and systemic clearances. The mean elimination rate constants were 0.41 and 0.50 h^{-1} for total and free ceforanide, respectively. The mean percentage of ceforanide bound to serum protein was 87.9%. The relationship of the free ceforanide concentration to the total concentration appeared to be linear. The data were fit to double-reciprocal and half-reciprocal relationships with good agreement, showing one binding site and an association constant range of 1.6×10^7 to 1.9×10^7 at these in vivo concentrations. The mean volume of distribution and mean systemic clearance of total drug were 100 ml/kg and 45.9 ml/min per 1.73 m^2, respectively. Ceforanide consistently produced higher intraoperative total drug concentrations compared with those of cefazolin and cefoxitin from similar studies.

The basic principle underlying the use of prophylactic antimicrobial agents is that the agent should be available during the time of potential bacterial contamination (2, 9). For surgical prophylaxis, this critical period begins with the initial incision and continues, in most cases, until the wound is closed. Optimal use of antimicrobial agents for surgical prophylaxis requires the agent to be present during this entire interval (2).

The availability of an antimicrobial agent at the site of potential contamination, the surgical wound, is related to the presence of antimicrobial agents in serum. Many factors affect tissue penetration by antimicrobial agents. When serum concentrations of beta-lactam antimicrobial agents are minimal, tissue concentrations are usually low. The transfer of an antibiotic into peripheral tissues is directly related to the area under the concentration versus the time curve for free drug (1). This area is partially determined by the free fraction of drug circulating in serum.

We previously investigated the intraoperative concentrations of cefazolin and cefoxitin (3). Considerable differences were observed in total drug concentrations throughout the operations. Cefazolin concentrations were two to three times higher than those of cefoxitin. Cefoxitin, the agent with a shorter elimination half-life, demonstrated that more frequent intraoperative readministration is needed.

Antimicrobial agents with relatively long elimination half-lives may be advantageous since they could provide adequate concentrations in serum throughout the surgical procedure. Ceforanide was chosen for this study because of its relatively long elimination half-life of 3.0 h (7, 10–12). The objective of this study was to determine the intraoperative pharmacokinetics and in vivo protein binding of ceforanide after preoperative, intravenous administration.

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MATERIALS AND METHODS

Subject selection. Hospitalized adult patients (18 to 70 years old) scheduled to undergo cholecystectomies were screened for study entry. The study protocol was approved by the Human Assurance Committee at the Medical College of Georgia, and written informed consent was obtained from each patient. All patients weighed between 60 and 90 kg, and none were more than 30% over ideal body weight. Evidence of renal or hepatic disease (creatinine clearance less than 60 ml/min and serum transaminase or alkaline phosphatase that was twice normal) was cause for patient exclusion. Neither antimicrobial agents nor probenicid was given for at least 1 week before the study. Serum creatinine, albumin, and total proteins were also determined, preoperatively, within 72 h. Albumin was determined by a colorimetric technique (SMA-II; Technicon Instruments Corp., Inc., Terrytown, N.Y.). The assay has demonstrated coefficients of variation from 2.9 to 3.4% at concentrations of 2 to 3.3 g/dl.

Drug administration. All patients received ceforanide (20 mg/kg) intravenously over 10 min on arrival in the operating room (drug supplied by Bristol Laboratories, lot 82L428). A second 1-g intravenous dose was given 12 h later.

Blood sampling. For each patient at least three intraoperative blood samples were collected (at initial incision, 1 h postincision, and wound closure). For operations lasting longer than 2 h, additional intraoperative blood samples were collected hourly. Serum was immediately separated from blood cells and stored at −25°C until analyzed.

Drug analysis. A high-pressure liquid chromatographic technique was developed with a Waters U6k injector and 440A detector set at 254 nm. A C_{18}, 25-cm column (Altech, Chicago, Ill.) was used with a flow rate of 2.0 ml/min of mobile phase (ratio of 0.1 M sodium acetate buffer [pH 4.0]/methanol, 90:10). Samples were prepared as follows. To 0.5 ml of patient plasma, 50 μl of sulfacetamide solution (1 mg/ml) was added as an internal standard; then 0.1 ml of 6%

TABLE 1. Patient and pharmacokinetic data

Patient	Age (years)	Wt (kg)	Ceforanide dose (g)	Total ceforanide				Free ceforanide	
				K_{el} (h^{-1})	$t_{1/2}$[a] (h)	V (ml/kg)	CL (ml/min per 173 m^2)	K_{el} (h^{-1})	$t_{1/2}$ (h)
1	62	60	1.2	0.31	2.26	101	33.5	0.62	1.12
2	35	65	1.3	0.79	0.88	67	60.8	0.54	1.29
3	31	60	1.2	0.43	1.63	104	48.0	0.41	1.68
4	43	78	1.6	0.35	1.96	101	41.1	0.38	1.84
5	22	85	1.7	0.42	1.66	112	59.9	0.62	1.12
6	60	66	1.3	0.20	3.48	108	26.3	0.17	4.11
7	32	79	1.6	0.38	1.83	111	55.9	0.49	1.41
8	27	64	1.3	0.49	1.42	113	59.4	0.44	1.57
9	24	77	1.5	0.50	1.38	94	57.4	0.70	0.99
10	44	90	1.8	0.27	2.56	103	33.0	0.40	1.72
11	34	65	1.3	0.35	1.97	100	39.5	0.54	1.29
12	63	61	1.2	0.20	3.47	107	24.0	0.23	2.96
13	26	70	1.4	0.50	1.39	83	46.4	0.71	0.97
14	22	86	1.7	0.34	2.04	115	49.6	0.32	2.14
15	19	57	1.2	0.61	1.13	84	53.5	0.87	0.80
Mean (±SD)	36.3 (±14.9)	70.9 (±10.7)	1.4 (±0.2)	0.41 (±0.16)	1.94 (±0.76)	100 (±13)	45.9 (±12.4)	0.50 (±0.19)	1.67 (±0.87)

[a] $t_{1/2}$, Half-life.

trichloroacetic acid was added to precipitate proteins, and the solution was vortexed for 1 min. Acetonitrile (1 ml) was then added, and the mixture was centrifuged for 10 min at 2,500 × *g*. The supernatant was transferred to a tube containing 5 ml of methylene chloride, vortexed for 1 min, and then shaken for 10 min. After centrifugation, 100 μl of the aqueous-phase solution was injected into the high-pressure liquid chromatography apparatus.

A standard curve for ceforanide-free acid was prepared by using final concentrations of 0, 2, 4, 8, 12, 16, 20, 30, 40, 60, 80, 100, and 120 μg of plasma per ml, with a resultant r of 0.999. The coefficients of variation were 1.7% at 2 μg/ml and 1.4% at 20 μg/ml.

Free ceforanide was separated from bound ceforanide by using a centrifugal filtration system (Amicon micropartition system with YMT membranes). Serum was placed in the filtration system and centrifuged at 3,000 rpm for 20 min. Then 0.2 ml of filtrate was collected and mixed with the internal standard (20 μl), and the sample was injected directly into the high-pressure liquid chromatography column under the conditions above. The YMT membrane was tested for any loss of ceforanide that may have been caused by absorption on the membrane during the filtration process. Six aqueous solutions of ceforanide with concentrations varying from 5 to 100 μg/ml were used in duplicate, and the mean change in concentration from nonfiltered solution was from +1.43% at 5 μg/ml to −0.71% at 100 μg/ml with a high value of 4.02% at 20 μg/ml.

Data analysis. For each subject, data for total and free ceforanide concentrations in serum versus time were subjected to linear regression analysis to determine individual intraoperative elimination rate constants (K_{el}) assuming a one-compartment model. Differences in K_{el} for total and free ceforanide were tested by the Wilcoxon Rank Sum test (4). From individual linear regression, concentrations were projected for standardized postinfusion times that corresponded to intraoperative events. Specifically, projected concentrations were determined for 30, 150, and 210 min postinfusion, corresponding to the appropriate time of initial incision and of closure for a 2- or 3-h operation, respectively.

The volume of distribution (V) was calculated for each individual from the back extrapolated total ceforanide concentration at time zero. Ceforanide systemic clearance (CL) was determined as the product of K_{el} and V.

Calculation of the binding parameters was performed in the following manner. First, the concentration of bound ceforanide in serum at every time point was calculated by subtracting the free concentration of ceforanide in serum from the total concentration. The number of moles of ceforanide bound per mole of albumin (r) was calculated by the following equation. r = [ceforanide concentration (μg/ml) bound in serum]/[MW (×10^6) of ceforanide]/[albumin concentration (g/100 ml) in serum]/[MW (×100) of albumin], (equation 1), where the molecular weight (MW) of ceforanide is 519 and the MW for albumin is 67,500. r can be related to the serum concentration of free moles of ceforanide (A) to yield the following familiar equation:

$$r = \frac{nK(A)}{1 + K(A)} \quad (2)$$

where n is the number of binding sites available on albumin and K is the association constant (15). Since all of the ceforanide concentrations were within a relatively narrow range, it was a preliminary assumption that there was only one principle class of binding sites available. Equation 2 can be transformed in at least three different ways to obtain a linear relationship between r and A. The double-reciprocal relationship (6) results as follows:

$$1/r = (1/nK)1/A + 1/n \quad (3)$$

The half-reciprocal relationship (8) is given as follows:

$$A/r = (1/n)A + 1/nK \quad (4)$$

The Scatchard (9) relationship can be expressed as follows:

$$r/A = Kn - Kr \quad (5)$$

Parameter estimates from equations 3, 4, and 5 were obtained by linear regression with a weighted least-squares method (1/y) (8).

RESULTS

The ages and weights of 15 patients who completed the investigation (3 male, 12 female) are given in Table 1. The

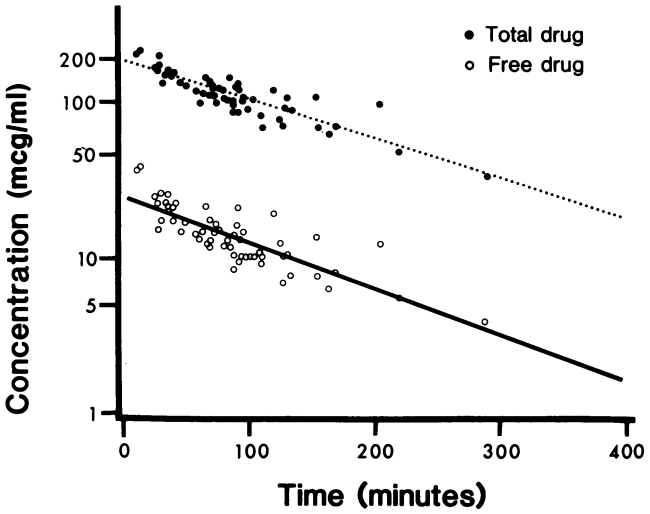

FIG. 1. Total and free ceforanide concentrations in serum versus time postinfusion.

mean dose of ceforanide administered was 1,420 ± 210 mg (range, 1,200 to 1,800 mg). The operations averaged 1.76 ± 0.8 h from incision to wound closure. Serum concentrations with the linear regression lines for total or free drug are shown in Fig. 1. These data imply that the K_{el}s for total and free ceforanide are approximately equivalent. However, from linear regression of the data for each individual, the mean K_{el}s for total and free ceforanide were 0.41 ± 0.16 and 0.50 ± 0.19 h^{-1}, respectively (Table 1). The K_{el} for free drug was significantly greater than that for total drug ($P < 0.05$ [Wilcoxon Rank Sum test]) (4) as indicated in Fig. 2. The mean percentage of ceforanide bound to serum proteins was 87.9%. The mean V and mean CL of total drug were 100 ml/kg and 45.9 ml/min per 1.73 m^2, respectively. The relationship of the free concentration of ceforanide in serum to total ceforanide was linear over the range of drug concentrations used in this study.

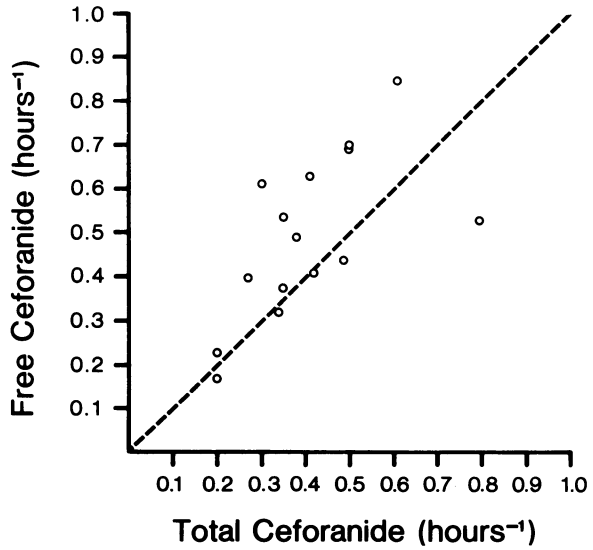

FIG. 2. Comparison of individual ceforanide elimination rate constants for free and total ceforanide. The broken line represents unity.

TABLE 2. Mean projected serum concentrations for total and free ceforanide

Time post-drug infusion (min)	Corresponding intraoperative event	Total drug (μg/ml ± SD)	Free drug (μg/ml ± SD)
30	Initial incision	165.0 ± 16.5	21.1 ± 3.9
150	Wound closure (2-h operation)	71.1 ± 28.2	8.3 ± 3.6
210	Wound closure (3-h operation)	52.4 ± 22.3	5.5 ± 3.6

Concentrations of total and free ceforanide were projected from the linear regression data of each individual for times corresponding to key intraoperative events, as described previously. These results are summarized in Table 2.

The number of binding sites and the association constant for the ceforanide-albumin in vivo binding interaction are given in Table 3. The results indicated that, at the therapeutic concentrations of ceforanide, a single binding site existed on albumin for this drug. The parameter values based on the half-reciprocal relationship are in close agreement with the parameter values obtained via the double-reciprocal method. The standard errors of the mean with a 95% confidence interval for the slope were 15 and 26% of the slope, respectively, for double-reciprocal and half-reciprocal relationships. The values obtained from the Scatchard relationship were different; however, the Scatchard relationship failed to provide a reasonable fit to the data. A plot of the binding data based on the double-reciprocal relationship is shown in Fig. 3.

DISCUSSION

Throughout the surgical procedures, ceforanide consistently produced high total drug concentrations in these patients relative to the values reported for cefazolin and cefoxitin by similar methods (3). It appears that a single preoperative dose of ceforanide would provide a sufficient intraoperative antimicrobial concentration even for long operations (over 4 h).

The mean elimination half-life determined in this study is somewhat shorter than previously reported for normal volunteers (1.9 h versus 2.6 to 3.0 h, respectively) although CL is similar (7, 10–12). The intraoperative serum protein binding for ceforanide is slightly greater than that reported for normal volunteers (87.9 versus 81%) (12). Differences in reported parameters may have resulted from the conditions during the surgical procedures. No other reports are currently available in the literature describing intraoperative ceforanide pharmacokinetics other than in open-heart surgery with a cardiopulmonary bypass (5, 10). The stress and trauma imposed by the surgical incision as well as the administration of a multitude of medications preoperatively

TABLE 3. In vivo protein binding data

Method	n of binding sites	K (M^{-1})	Slope	Intercept	F^a
Double reciprocal	1.03	1.65×10^7	5.8×10^{-8}	0.96	175.7[b]
Half reciprocal	0.91	1.97×10^7	1.09	5.5×10^{-8}	65.6[b]
Scatchard	3.51	3.4×10^6	-3.4×10^6	1.27×10^7	3.1

[a] F, Variance ratio.
[b] Significant ($P < 0.001$).

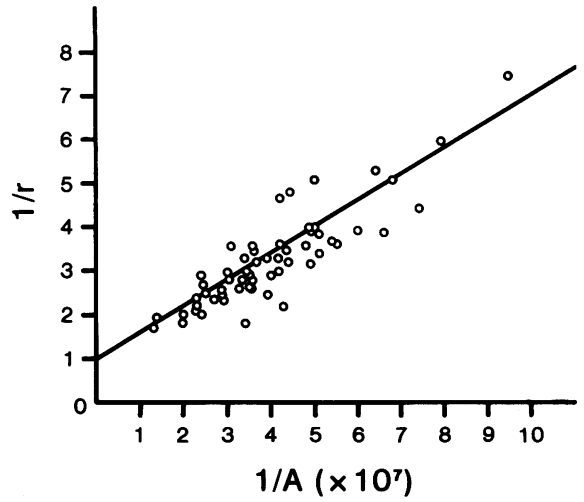

FIG. 3. Plot of the double-reciprocal relationship where r equals the number of moles of ceforanide bound per mole of albumin and A equals the moles of free ceforanide in serum.

and intraoperatively (e.g., anesthetic gases, anticholinergic agents, and opiate derivatives) may affect drug pharmacokinetics, but differences between the study group and reported data from normal volunteers are not dramatic.

In this investigation, attempts were made to determine ceforanide binding parameters. Low binding ratios were present and used to calculate the in vivo binding parameters. The value of 1.6×10^7 to 1.9×10^7 for the binding constant is high, and in relation to the in vitro binding of other antibacterial agents, such a large value has not been heretofore reported (14). Estimates of binding parameters in vivo have not been routinely examined until recently. The relevance of the binding parameters reported here should become more apparent as further in vivo protein binding studies are reported for other cephalosporins or under other conditions.

ACKNOWLEDGMENT

This work was supported in part by Bristol Laboratories.

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