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Pharmacodynamic Effects of Amoxicillin versus Cefotaxime against Penicillin-Susceptible and Penicillin-Resistant Pneumococcal Strains: a Phase I Study

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Serum bactericidal activity against a penicillin-susceptible strain and a penicillin-resistant strain of *Streptococcus pneumoniae* (amoxicillin and cefotaxime MICs, 0.001 and 1 μg/ml, respectively, and MBCs, 0.01 and 2 μg/ml, respectively) was measured in 12 healthy volunteers who each received an oral 875-mg dose of amoxicillin and an intramuscular 1-g dose of cefotaxime in a crossover fashion. The areas under the bactericidal activity-time curves for the two strains were found to be similar for both antibiotics despite the significantly higher ($P < 0.002$) AUC/MIC and peak level/MIC values for cefotaxime.

The incidence of pneumonia caused by penicillin-resistant pneumococci (PRP) is increasing worldwide (6), including Spain (11), at an alarming rate (2). Sensitivity determinations based on the breakpoints of the National Committee for Clinical Laboratory Standards (10) indicate that the penicillin-resistant strains are susceptible to cefotaxime. In any case, clinical data suggest that penicillins are effective in treating pneumococcal pneumonia attributable to resistant strains (11).

The serum bactericidal activity test may be useful in investigations of the clinical potential of antimicrobial agents (7, 13, 15), since the data reflect the relationship between in vitro activity and the concentrations achievable in vivo (5, 8). The area under the serum bactericidal activity-time curve (AUBC) is a more sensitive index of the pharmacodynamic effect than the area under the serum concentration-time curve (AUC) (9).

Since bacteremia occurs in 30% of the cases of pneumococcal pneumonia (3), we performed a phase I clinical trial to study the serum bactericidal activity and pharmacodynamic effects of amoxicillin and cefotaxime on both a penicillin-susceptible *Streptococcus pneumoniae* (PSP) strain and a PRP strain in order to explore the pharmacodynamic background that would contribute to explaining the similar clinical efficacies of penicillins and cephalosporins in the treatment of bacteremic pneumonia (11).

Twelve healthy male volunteers (mean age ± standard deviation, 22.64 ± 1.51 years; mean weight, 75.63 ± 9.02 kg; mean height, 179.50 ± 7.07 cm) were included in this open, randomized, controlled, crossover phase I clinical trial. The protocol was approved by the Research Ethics Committee of Hospital La Paz, Madrid, Spain. Written informed consent was obtained from all subjects before their inclusion in the study. Prior to the administration of 875 mg of amoxicillin orally or 1 g of cefotaxime intramuscularly (i.m.) (with a 7-day washout period between treatments), 15 ml of whole venous blood was collected from each subject. After drug administration, additional samples of 5 ml were collected at 15 min, 30 min, 1 h, 1.5 h, 2 h, 4 h, 6 h, and 8 h. Serum was separated for bioassay and determination of serum bactericidal titers (SBTs).

Amoxicillin and cefotaxime levels were determined by bioassay, using *Micrococcus luteus* ATCC 4698 as a reference organism, in 20-cm-diameter plates with 24 ml of Antibiotic Agar No. 1 (Difco) containing a final inoculum of 2.5×10^6 CFU/ml. Sixty-microliter aliquots of each serum sample were deposited into 10-mm-diameter wells in inoculated plates that were incubated at 37°C for 18 h. Standards containing from 0.078 to 25 μg/ml and from 0.937 to 30 μg/ml were prepared for amoxicillin and cefotaxime, respectively, in pooled serum obtained from the volunteers before drug administration. The Microstat program (Ecosoft, Inc., Indianapolis, Ind.) was used to determine the assay regression line (standard curve) and to extrapolate the antibiotic concentrations from the corresponding inhibition zone diameters. The reproducibility of the assay was determined from the means of the coefficients of variation, which were 1.92, 2.59, 0.48, and 0.75% for 25, 12.5, 6.25, and 1.56 μg of amoxicillin per ml, respectively, and 0.28, 0.53, 0.16 and 0.69% for 30, 15, 7.5, and 3.75 μg of cefotaxime per ml, respectively. The lower limits of detection were 0.19 and 0.20 μg/ml for amoxicillin and cefotaxime, respectively.

SBTs against *S. pneumoniae* strains selected on the basis of having the same penicillin, amoxicillin, and cefotaxime MICs and MBCs (for the PSP strain, 0.01 μg/ml for both concentrations; for the PRP strain, 1 and 2 μg/ml, respectively) were measured by the microdilution technique (16). Serum samples (50 μl) were diluted in noninactivated human serum (obtained from each volunteer before drug administration) containing 20% Todd-Hewitt broth (Difco). The final volume in each well was 100 μl, and the final inoculum concentration was approximately 7×10^5 CFU/ml. The inoculated plates were incubated at 37°C for 18 h and subcultured to plates of antibiotic-free blood agar medium which were incubated at 37°C for 18 h. The SBT was defined as the maximum dilution of serum that produced a 99.9% reduction of the initial inoculum.

Antibiotic concentration-time curves for each subject were analyzed through application of the noncompartmental approach by using the SIPHAR 4.0 program (Simed, Creteil, France). The AUBCs were calculated from plots of serum bactericidal titers versus time by using the trapezoidal rule.

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TABLE 1. Levels of drugs in serum and SBTs

Time (h)	Levels and SBTs for the drug							
	Amoxicillin				Cefotaxime			
	No. of serum samples[a]	Level in serum (μg/ml)[b,c]	SBT against[c,d]:		No. of serum samples[a]	Level in serum (μg/ml)[b,c]	SBT against[c,d]:	
			PSP (n = 12)	PRP (n = 12)			PSP (n = 12)	PRP (n = 12)
0.25	12	2.99* ± 1.45	32* (16–128)	2 (0–4)	12	17.12* ± 3.48	256* (256–256)	4 (2–4)
0.5	12	5.42* ± 1.20	128 (64–512)	4 (2–8)	12	22.93* ± 2.04	512 (256–512)	8 (2–8)
1	12	7.14 ± 1.16	384 (256–1,024)	4 (2–16)	12	26.81 ± 1.52	512 (512–512)	8 (4–8)
1.5	12	8.57* ± 1.49	512 (256–1,024)	8 (4–16)	12	25.89* ± 1.74	512 (256–512)	8 (4–8)
2	12	6.64 ± 1.51	256 (256–512)	6 (2–8)	12	15.29 ± 3.67	256 (128–512)	4 (2–8)
4	12	4.51 ± 0.93	128 (32–256)	2 (0–4)	12	8.24 ± 2.64	128 (32–128)	2 (2–4)
6	12	1.89 ± 0.84	24 (2–128)	2 (0–2)	10	2.15 ± 1.93	32 (2–64)	2 (0–2)
8	4	0.33 ± 0.56	1 (0–16)	0 (0–2)	0	ND[e]	0 (0–16)	0 (0–0)

[a] Number of serum samples tested with antibiotic concentrations over the detection limit (0.19 and 0.20 μg/ml for amoxicillin and cefotaxime, respectively).
[b] Values are means ± standard deviations.
[c] An asterisk indicates that the value is statistically significantly different from the corresponding value for the other antibiotic ($P < 0.002$).
[d] Values are medians (ranges).
[e] ND, not determined.

Due to the crossover design of the study, the statistical analysis was performed with a specific tests for paired data, analysis of variance, with drug treatment and study phase as covariables. Nonparametric tests were used in those cases in which the homoscedasticity assumptions were not fitted. Alpha error was adjusted by the Bonferroni method for multiple comparisons (1). A P value of <0.002 was considered statistically significant.

Serum drug levels and SBTs over time are shown in Table 1. Before drug administration, antibiotic levels and SBTs were below the limits of detection (SBT, <2). Cefotaxime was found to be present in serum at higher levels ($P < 0.002$) than amoxicillin up to 1.5 h after dosing. SBTs against the PSP were significantly higher for cefotaxime than for amoxicillin 15 min postdosing. No differences were found between amoxicillin and cefotaxime in SBTs against the PRP strain at all sample times and against the PSP from 15 min on.

Pharmacokinetic and pharmacodynamic parameters are presented in Table 2. Significant differences ($P < 0.002$) were found between the two antibiotics with respect to AUC, AUC/MIC, C_{max} (maximum concentration of a drug in serum), and therapeutic index (peak level/MIC) values. No significant differences were found between the AUBCs for the two drugs for either the PRP or the PSP strain.

In this study, we measured the serum bactericidal activities of amoxicillin and cefotaxime and their dependence on pharmacokinetic parameters, avoiding the standard in vitro susceptibility influence by using PRP and PSP strains with the same amoxicillin and cefotaxime MICs or MBCs. Serum bactericidal tests were performed with high concentrations of serum in order to simulate in vivo clinical conditions as closely as possible. When measuring the global serum bactericidal activity with time (AUBC), similar values were obtained with both antibiotics for both strains despite the significantly ($P < 0.002$) higher AUC/MIC and peak level/MIC ratios for cefotaxime than for amoxicillin (two and three times higher, respectively). This similarity of the ex vivo bactericidal activities of standard doses of amoxicillin and cefotaxime, despite the differences in the calculated pharmacodynamic parameters against pneumococcal strains (susceptible or resistant to penicillin) with equal in vitro susceptibilities to both drugs, may have clinical relevance if bloodstream bactericidal activity plays a role in the outcome (14).

The two strains and two antibiotics used in this study were also used in two previous experiments, a pharmacodynamic simulation (4) evaluating the ability of the antibiotics to reduce the initial inoculum after continuous exposure to simulated serum antibiotic concentrations and a neutropenic-mouse pneumonia model (12) measuring bacterial lung clearance and mortality rates. The results of this phase I study are in accordance with the results obtained in these previous studies, in which the significantly greater capability of amoxicillin to decrease the initial inoculum of PRP in the pharmacodynamic simulation was translated in the animal model to decreased PRP virulence versus untreated (control) and cefotaxime-treated animals, as measured by the lower mortality rates and the higher level of PRP lung clearance obtained with amoxicillin.

TABLE 2. Pharmacokinetic and pharmacodynamic parameters

Drug	Value for parameter[a]											
	C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{exp} (μg · h/ml)	Cl_{tot} (ml/min · kg)	V_d (liter/kg of body weight)	AUBC		AUC/MIC		TI	
							PSP	PRP	PSP	PRP	PSP	PRP
Amoxicillin	8.57* ± 1.49	1.50	2.06 ± 0.68	35.43* ± 6.92	25.68 ± 5.71	73.77 ± 20.59	1,307	24.5	3,543*	35.43*	857*	8.6*
Cefotaxime	27.11* ± 1.66	1.13 ± 0.23	1.41 ± 0.45	83.42* ± 15.31	12.34 ± 2.16	24.01 ± 5.22	1,344	23.75	8,342*	83.42*	2,711*	27.1*

[a] Values are means (± standard deviations where indicated). Values with asterisks are significantly different from the corresponding values ($P < 0.002$). Abbreviations: C_{max}, maximum concentration of drug in serum; T_{max}, time to maximum concentration of drug in serum; $t_{1/2}$, half-life; AUC_{exp}, experimentally determined AUC value; CL_{tot}, total clearance; V_d, volume of distribution; TI, therapeutic index (peak level/MIC).

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