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Activities against *Streptococcus pneumoniae* of Amoxicillin and Cefotaxime at Physiological Concentrations: In Vitro Pharmacodynamic Simulation

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An in vitro model simulating amoxicillin and cefotaxime concentrations in human serum (after standard doses) was used to explore the activities of these drugs over time against penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae* strains. An initial inoculum reduction percentage of ≥90% was obtained with amoxicillin and maintained for 2 to 8 h, regardless of the strain tested. In contrast, experiments showed that cefotaxime had significantly ($P < 0.001$) less capability to reduce initial inocula of the penicillin-resistant pneumococci from 0.5 h on than amoxicillin, despite the same in vitro susceptibility to amoxicillin and cefotaxime in both strains.

The incidence of pneumonia caused by penicillin-resistant pneumococci is increasing worldwide (6), including Spain (13), at an alarming rate (3). Sensitivity based on the breakpoints of the National Committee for Clinical Laboratory Standards (11) indicates susceptibility to cefotaxime of the penicillin-resistant strains. In any case, clinical data suggest that penicillins are effective in treating pneumococcal pneumonia due to resistant strains (13), supporting the classical assumption of maximum efficacy when serum penicillin levels are 2 to 10 times above the MIC (4), a ratio currently achieved after treatment with penicillin or other equivalent β-lactam antibiotics, when the MICs for penicillin-resistant pneumococci are considered (6). The aim of this study was to evaluate the in vitro effect on *S. pneumoniae* viability of continuous exposure to variable concentrations of two antibiotics usually prescribed for pneumococcal respiratory tract infections: oral amoxicillin and intravenous cefotaxime (as the cephalosporin representative), since they have similar serum half-lives. A pharmacodynamic simulation method (9) was used, with concentrations similar to those obtained in serum after an oral dose of 875 mg of amoxicillin (5) or an intravenous dose of 1 g of cefotaxime (15).

Two clinical isolates, a serotype 3 penicillin-susceptible *S. pneumoniae* (PSP) strain and a serotype 9 penicillin-resistant (PRP) strain, were used throughout. Strains were selected on the basis of the same penicillin, amoxicillin, and cefotaxime MIC and MBC values (MIC, 0.01 μg/ml and MBC, 0.01 μg/ml for the PSP strain and MIC, 1 μg/ml and MBC, 2 μg/ml for the PRP strain). MICs and MBCs were confirmed by standard methodology five times for each strain (11).

Initial inocula were obtained by diluting Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) overnight cultures in new fresh broth; the cultures were incubated in a 36.5°C shaking bath until an A_{580} of 0.11 (Hitachi U-100 spectrophotometer) was achieved. Cultures were further diluted 1:10, yielding final inocula of 6×10^6 and 2.5×10^6 CFU/ml for the PSP and PRP strains, respectively, in 4 ml of broth containing the first antibiotic concentration (Table 1). Incubation was performed in Centriprep-10 (membrane pore size, 10,000 Da) concentrator tubes (Amicon Ltd., Beverly, Mass.) in a 36.5°C shaking bath for the first incubation period shown in Table 1. After incubation, an aliquot was taken for colony counting. Centrifugation-filtration at $2,000 \times g$ for 10 min (room temperature) was then performed, and the supernatant above the filter was carefully discarded. A bacterial suspension volume of 100 μl remained below the filter. New broth prewarmed at 36.5°C (4 ml) with the next drug concentration was added to the bacterial suspension. Tubes were incubated under the above-described conditions for the next incubation period. This methodology was performed throughout five incubation periods. Antibiotic experiments were performed three times for each strain. Each experiment was simultaneously controlled with tubes containing the initial inoculum in Todd-Hewitt broth without antibiotic addition and following the same centrifugation-filtration procedure. In addition, standard curves of bacterial growth in Todd-Hewitt broth without the centrifugation-filtration process were produced, with colony counting at the same times.

The antibiotic carryover effect, which occurred in the 100-μl bacterial suspensions remaining after the centrifugation-filtration steps, was considered. The amounts of antibiotics in the 100-μl bacterial suspensions after each incubation period were calculated by using $Y = 0.025X$, where X is the initial amount of antibiotic in 4 ml and Y is the resulting amount in the 100-μl suspension. Concentrations for each incubation period were calculated by adding the carried-over concentration of the 100-μl suspension to the antibiotic concentration in the new broth that was added. The final concentrations calculated are shown in Table 1.

The capability to decrease the initial inoculum over time on a CFU per milliliter basis was measured by calculating the initial inoculum reduction percentage (I.I.R.P.) at different incubation times ($t = x$ h) with respect to the $t = 0$ h inoculum (I) ($100 - [100 \times I_{t = x\ \mathrm{h}}/I_{t = 0\ \mathrm{h}}]$). Inter- and intragroup comparisons of colony counting (log CFU/ml) values and I.I.R.P. at the same sample times were performed by one-way analysis of variance. Statistical significance was defined as $P \leq 0.001$, because of the adjusted alpha error by Bonferroni's method for multiple comparisons.

Values of log CFU per milliliter versus time are shown in Table 1 for both strains, in Fig. 1 for the PSP strain, and in Fig.

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TABLE 1. Incubation periods, antibiotic concentrations, and colony counting values

Incubation periods (h)	Colony counting value (log CFU/ml) ± SD for control cultures with the indicated strain		Antibiotic concentration (μg/ml)		Colony counting value (log CFU/ml) ± SD for antibiotic cultures with the indicated strain			
					Amoxicillin		Cefotaxime	
	PSP	PRP	Amoxicillin	Cefotaxime	PSP	PRP	PSP	PRP
0–0.5	6.99 ± 0.04	6.71 ± 0.16	3.46	32.00	6.73 ± 0.04[a]	6.49 ± 0.04	6.10 ± 0.03[a,b]	6.68 ± 0.07[b]
0.5–2	7.24 ± 0.09	6.97 ± 0.24	11.98	12.48	5.54 ± 0.07	5.34 ± 0.03[a]	5.60 ± 0.04[b]	6.39 ± 0.06[a,b]
2–3	7.89 ± 0.06	7.32 ± 0.24	8.09	4.20	5.08 ± 0.04	5.28 ± 0.02[a]	5.39 ± 0.06[b]	5.74 ± 0.02[a,b]
3–6	8.20 ± 0.09	7.96 ± 0.30	2.14	0.49	3.88 ± 0.02[b]	5.20 ± 0.03[a,b]	3.91 ± 0.05[b]	5.84 ± 0.01[a,b]
6–8	8.55 ± 0.03	8.17 ± 0.14	0.54	0.01	3.55 ± 0.01[a,b]	5.32 ± 0.05[a,b]	3.13 ± 0.05[a,b]	5.96 ± 0.01[a,b]

[a] $P < 0.001$ for amoxicillin versus cefotaxime.
[b] $P < 0.001$ for the PSP strain versus the PRP strain in antibiotic groups.

2 for the PRP strain. No statistically significant differences were found between control experiments performed with the centrifugation-filtration procedure and those run in parallel with the standard methodology. In control Centriprep tubes, a continuous inoculum increase was observed, with a higher growth rate for the PSP strain. Statistically significant differences ($P < 0.001$) of viability were found between control and antibiotic experiments at every sample time. An I.I.R.P. of >90% was obtained from 2 to 8 h with amoxicillin against both strains and with cefotaxime against the PSP strain. In contrast, when the PRP strain was tested in the presence of cefotaxime, a maximum I.I.R.P. (81%) was obtained at 3 h, with bacterial regrowth leading to an I.I.R.P. of 68% at 8 h. Significantly lower ($P < 0.001$) log CFU per milliliter values and greater I.I.R.P. values for the PRP strain were found with amoxicillin than with cefotaxime from 0.5 h on. While significant differences were found in cefotaxime activity against both strains for all sampling intervals, similar amoxicillin activity was obtained against both strains during the first 3 h.

A 99.9% initial inoculum reduction has been defined as bactericidal activity (1) in an in vitro susceptibility test in which a predetermined number of bacteria is incubated for a predetermined time with a constant antibiotic concentration (7). But the optical density readings after 18 to 24 h can be influenced by pharmacodynamic factors operating in the initial parts of this period, giving identical results for different antibiotics (10). In this study, using two strains with identical responses to amoxicillin and cefotaxime after 18 to 24 h of in vitro susceptibility tests (MIC, MBC), we tried to explore the importance of these initial pharmacodynamic factors, taking into account antibiotic pharmacokinetics (simulating serum levels) and dosing intervals (8-h incubation) and using a higher inoculum than the one used in classical susceptibility tests to mimic tissue inoculum density ($\cong 10^6$ CFU/g) (2).

Although a therapeutic equivalent inoculum has never been established (2) and in consequence a therapeutic equivalent inoculum reduction for a time equal to the dosing interval is unknown, amoxicillin achieved an I.I.R.P. of 91.2% 2 h after exposure to the simulated serum concentrations, and this percentage was maintained at least until the time just before the

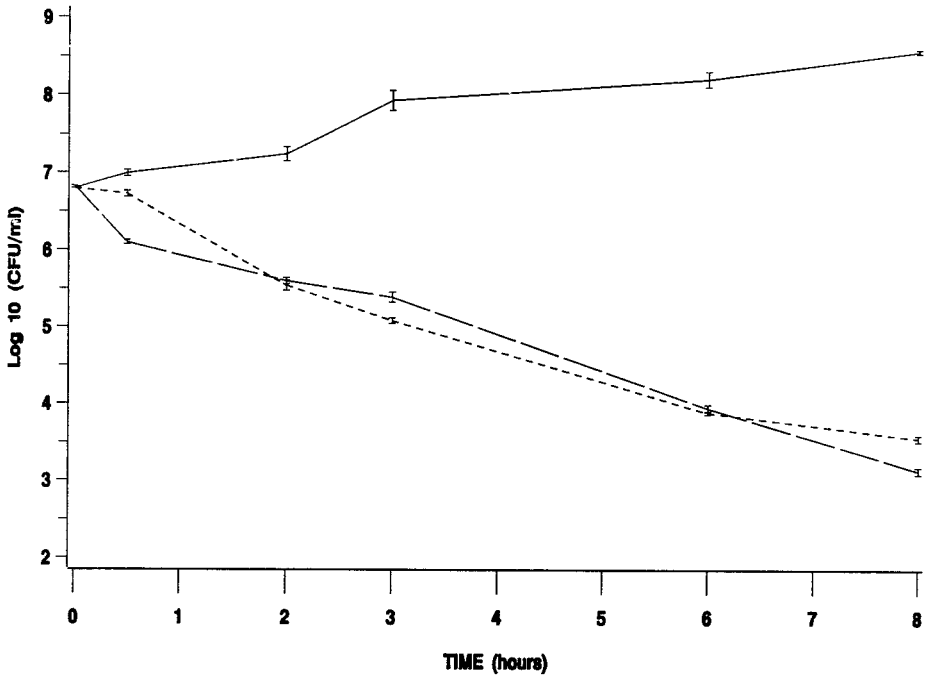

FIG. 1. Effect of amoxicillin (– – –) and cefotaxime (— —) physiological concentrations on PSP strain log CFU per milliliter values at the various sample times. ——, control (no antibiotic). Data represent the means ± standard deviations for three independent experiments.

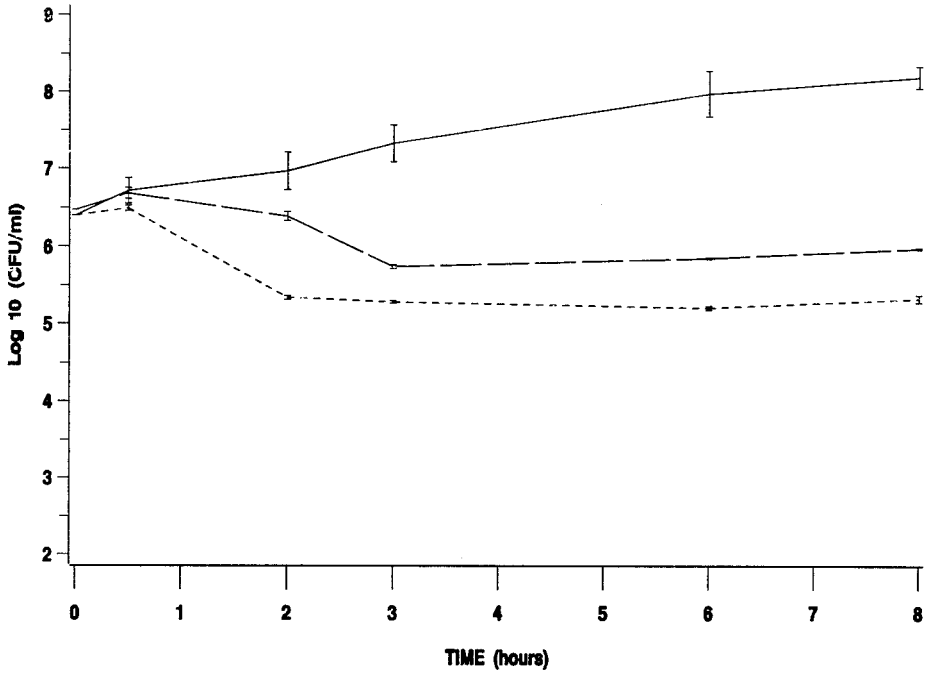

FIG. 2. Effect of amoxicillin (– – –) and cefotaxime (— —) physiological concentrations on PRP strain log CFU per milliliter values at the various sample times. ——, control (no antibiotic). Data represent the means ± standard deviations for three independent experiments.

next dose, in contrast to cefotaxime which had significantly lower reduction rates.

Although a therapeutic equivalent inoculum has not been defined, if virulence can be defined as a bacterial critical mass capable of producing injury to a given host, quantified as fatality rate and/or ability to invade tissues (8), the greater sustained capability for inoculum reduction against the PRP strain in simulated serum concentrations (determined as I.I.R.P.) of amoxicillin versus cefotaxime, despite their having the same standard in vitro susceptibility values for MIC and MBC, may explain the greater in vivo activity of amoxicillin (lower fatality rate and higher PRP lung clearance) versus cefotaxime obtained in a neutropenic murine pneumonia model with the same PRP strain (14). Breakpoints of $\leq$0.06 μg/ml for penicillin (applicable to other aminopenicillins) and $<$0.5 μg/ml for cefotaxime (12) have been historically used. Our results support the position that pneumococcal breakpoints should be reviewed if they can be extrapolated to other PRP strains. Further studies with a high number of PRP strains are warranted.

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