ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2005, p. 3095–3096 Vol. 49, No. 7
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.7.3095–3096.2005

Letters to the Editor

Specific Antibodies, Levofloxacin, and Modulation of Capsule-Associated Virulence in *Streptococcus pneumoniae*

Amoxicillin subinhibitory concentrations produced 100% survival in passively immunized mice infected with non-amoxicillin-susceptible, poorly or highly encapsulated *Streptococcus pneumoniae* strains (2, 6), with negligible values of time that serum levels exceeded the MIC.

We explored this phenomenon and its modulation by capsular production with levofloxacin against the same serotype 6B *Streptococcus pneumoniae* strain (6) (MIC of levofloxacin = 32 μg/ml) with two types of infecting inocula. (i) For the poorly encapsulated (PE) phenotype, the microorganism was grown in Todd-Hewitt broth supplemented with 0.5% yeast extract (Difco, Detroit, Mich.) until an absorbance of 0.3 at 580 nm (UV-visible spectrophotometer, Shimadzu UV-1203, Japan) was reached. (ii) For the highly encapsulated (HE) phenotype (4), after serial passages in mice, the microorganism was grown three times in Todd-Hewitt broth supplemented with 0.5% yeast extract (Difco, Detroit, Mich.) and enriched with 5% fetal bovine serum until an absorbance of 0.3 at 580 nm (UV-visible spectrophotometer, Shimadzu UV-1203, Japan) was reached.

Eight-to 12-week old female BALB/c mice weighing 19 to 22 g were used. The challenge dose with the PE and the HE inocula (2, 6) was 4×10^8 CFU/ml. Previously described methods (6) were followed for hyperimmune serum production and determination of protection with and without levofloxacin doses decreasing on a twofold basis from 25 mg/kg of body weight. Groups of 10 animals per dose were used. Experiments were carried out in duplicate. Treatment was initiated 1 h after the intraperitoneal challenge, and a second dose was administered 24 h later. Levofloxacin concentrations were determined by bioassay using *Escherichia coli* ATCC 25922 in pooled sera from five animals per sampling time (predosing, 15 min, 30 min, 1 h, 2 h, and 4 h).

Drug concentrations were analyzed by a noncompartmental approach using the WinNonlin Professional program (Pharsight, Mountain View, Calif.).

Survival curves were obtained by the Kaplan-Meier method. An ordinal log-rank test was used to compare different study groups. Due to multiple comparisons, a P value of ≤0.001 was considered significant.

Concentrations (μg/ml) of levofloxacin in serum obtained after a single 25-mg/kg dose were 144.54 at 15 min, 120.22 at 30 min, 4.67 at 1 h, 0.23 at 2 h, and undetectable at 4 h. Maximum concentration and area under the curve (AUC) were 144.54 μg/ml and 84.84 μg · h/ml.

Table 1 shows survival rates. No differences ($P = 0.85$) between the PE and the HE models were found with nonimmune serum or placebo controls. In the PE model, differences in survival rates between immunized and nonimmunized animals were nonsignificant ($P = 0.03$) with 6.25 mg/kg levofloxacin but significant ($P < 0.0001$) with the 12.5-mg/kg dose. Significant differences ($P < 0.0001$) were found, with higher survival rates in the PE than the HE model (0% from day 2 onwards), for each treatment regimen.

An AUC/MIC ratio of 25 to 30 has been classically related to favorable outcomes in humans infected with *S. pneumoniae* (1) despite data supporting lower values needed (5). Lower values are needed in rodents (3). In the present study, ratios of maximum concentration to MIC and AUC to MIC of 4.5 and 2.7, respectively, produced efficacy (80% survival) in the PE model. These values were not enough to produce efficacy when the strain was highly encapsulated (HE model), where an in-

TABLE 1. Survival rates produced by three levofloxacin doses over a 7-day follow-up period with both types of infecting inocula (PE and HE) in normal mice and previously immunized mice

Infecting inoculum	Dose and/or serum (dilution)[a]	% Survival on follow-up day							
		0	1	2	3	4	5	6	7
PE	Placebo	100	40	0	0	0	0	0	0
	Nonimmune serum	100	55	0	0	0	0	0	0
	HS (1/6)	100	60	25	10	0	0	0	0
	6.25 mg/kg	100	60	20	5	5	0	0	0
	6.25 mg/kg + HS (1/6)	100	70	50	20	20	20	20	20
	12.5 mg/kg	100	75	20	5	5	0	0	0
	12.5 mg/kg + HS (1/6)	100	90	55	40	40	40	35	35
	25 mg/kg	100	100	80	80	80	80	80	80
	25 mg/kg + HS (1/6)	100	100	100	100	100	100	100	100
HE	Placebo	100	0	0	0	0	0	0	0
	Nonimmune serum	100	0	0	0	0	0	0	0
	HS (1/6)	100	0	0	0	0	0	0	0
	12.5 mg/kg	100	40	0	0	0	0	0	0
	12.5 mg/kg + HS (1/6)	100	40	0	0	0	0	0	0
	25 mg/kg	100	40	0	0	0	0	0	0
	25 mg/kg + HS (1/6)	100	30	0	0	0	0	0	0

[a] HS, hyperimmune serum.

crease in capsule-associated virulence was noted. Human natural infections by *S. pneumoniae* occur with highly capsulated strains, suggesting that much higher AUC/MIC ratios are needed in natural infections.

We express our gratitude to L. Alou (IPM, Madrid, Spain) for the statistical analysis.

REFERENCES

1. **Andes, D.** 2001. Pharmacokinetic and pharmacodynamic properties of antimicrobials in the therapy of respiratory tract infections. Curr. Opin. Infect. Dis. **14:**165–172.
2. **Casal, J., L. Aguilar, I. Jado, J. Yuste, M. J. Giménez, J. Prieto, and A. Fenoll.** 2002. Effects of specific antibodies against *Streptococcus pneumoniae* on pharmacodynamic parameters of β-lactams in a mouse sepsis model. Antimicrob. Agents Chemother. **46:**1340–1344.
3. **Garcia-Olmos, M., A. Parra, G. Garcia-Calvo, C. Ponte, M. J. Gimenez, L. Aguilar, and F. Soriano.** 2003. Efficacy and pharmacodynamics of gemifloxacin versus levofloxacin in guinea pig pneumococcal pneumonia induced by strains with decreased ciprofloxacin susceptibility. Int. J. Antimicrob. Agents. **21:**568–573.
4. **Jansen, W. T. M., J. Gootges, M. Zelle, D. V. Madore, J. Verhoef, H. Snippe, and A. F. M. Verheul.** 1998. Use of highly encapsulated *Streptococcus pneumoniae* strains in a flow-cytometric assay for assessment of the phagocytic capacity of serotype-specific antibodies. Clin. Diagn. Lab. Immunol. **5:**703–710.
5. **Preston, S. L., G. L. Drusano, A. L. Berman, C. L. Fowler, A. T. Chow, B. Dornseif, V. Reichl, J. Natarajan, and M. Corrado.** 1998. Pharmacodynamics of levofloxacin: a new paradigm for early clinical trials. JAMA **279:**125–129.
6. **Tarragó, D., L. Aguilar, M. J. Giménez, A. Fenoll, and J. Casal.** 2004. Effects of amoxicillin subinhibitory concentrations on the cross-protection developed by pneumococcal antibodies in mouse sepsis caused by an amoxicillin-resistant serotype 6B *Streptococcus pneumoniae* strain. Antimicrob. Agents Chemother. **48:**4144–4147.

David Tarragó
Noelia Lara
Asunción Fenoll
Julio Casal*
Centro Nacional de Microbiología
Instituto de Salud Carlos III
Ctra. Majadahonda-Pozuelo Km. 2
28220 Majadahonda, Madrid, Spain

María-José Giménez
Lorenzo Aguilar
David Sevillano
Microbiology Department, School of Medicine
Universidad Complutense
Madrid, Spain

*Phone: 34-91-8223616
Fax: 34-91-5097966
E-mail: jcasal@isciii.es