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Changes in Adherence of Respiratory Pathogens to HEp-2 Cells Induced by Subinhibitory Concentrations of Sparfloxacin, Ciprofloxacin, and Trimethoprim

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Preincubation with subinhibitory concentrations of sparfloxacin, ciprofloxacin, and trimethoprim decreased the adherence of the respiratory pathogens *Klebsiella pneumoniae*, *Haemophilus influenzae*, and *Moraxalla* (*Branhamella*) *catarrhalis* to human larynx carcinoma HEp-2 cells. Subinhibitory concentrations of sparfloxacin did not change the adherence of *Pseudomonas aeruginosa* or *Streptococcus pneumoniae* 15.62, but adhesion of *S. pneumoniae* 15.42 was significantly enhanced by subinhibitory antimicrobial concentrations.

Adherence of bacteria to respiratory epithelial cells is an essential first step in the pathogenesis of respiratory tract infections. Before invasion of the tissue, the bacteria must firmly attach to respiratory epithelial cells to avoid elimination by mucociliary action. This concept is supported by in vitro studies in which the ability of respiratory pathogens to attach to isolated respiratory cells has been compared with their ability to produce respiratory tract infections (1, 10, 16, 17, 22, 24).

Antimicrobial agents may have profound effects on the adhesion of bacteria to cells (19, 20). The effect of antibiotics on adherence is neither predictable nor readily explained (4). Both increased and decreased adhesion has been described with various microorganism-antimicrobial agent combinations (19, 20). Since the penetration of antimicrobial agents into sputum is often incomplete, studies of the effects of sublethal concentrations may have relevance for the treatment of respiratory tract infections. Because of the availability of newer quinolones such as sparfloxacin with increased activity against respiratory pathogens (13, 26), we studied the effects of subinhibitory concentrations of sparfloxacin in comparison with those of ciprofloxacin and trimethoprim on the adherence of typical respiratory pathogens to the human larynx carcinoma cell line HEp-2.

The following antimicrobial agents were provided by the indicated manufacturers: sparfloxacin (lot T 89006), Rhone-Poulenc, Antony, France; ciprofloxacin, Bayer, Mijdrecht, The Netherlands; and trimethoprim, Centrafarm, Etten-Leur, The Netherlands. Fresh dilutions of all compounds were made daily.

The microorganisms used in the present study were routine clinical isolates collected between 1983 and 1990; the strains were identified by standard methods. The *Haemophilus influenzae* strains were nontypeable with serum against serotype b. The MICs of sparfloxacin and ciprofloxacin were ≤4 μg/ml; trimethoprim MICs were ≤16 μg/ml except for the two *Pseudomonas aeruginosa* strains (MICs, 256 μg/ml), as determined by standard methods (14). *Streptococcus pneumoniae* S3 and its unencapsulated mutant strain DW 3.7 were kindly provided by S. Geelen, Wilhelmina Children's Hospital, Utrecht, The Netherlands. All strains were maintained frozen at −70°C in skim milk until they were tested. The adherence method of Svanborg Eden et al. (23) was performed, with slight modifications (11). Bacteria were inoculated into Mueller-Hinton broth (Difco, Detroit, Mich.) (with 5% lysed horse blood for *S. pneumoniae*) or *Haemophilus* test medium (*H. influenzae*) and were grown in the absence (control) or presence of subinhibitory concentrations of antimicrobial agents at 37°C for 18 h. Bacteria were subsequently harvested by centrifugation and were resuspended in Hanks' balanced salt solution to a concentration of 2×10^8 CFU/ml; for *S. pneumoniae*, the bacterial inoculum was 2×10^9 CFU/ml. To ensure that all initial inocula were similar, the quantity of viable, antibiotic-exposed bacteria following resuspension was compared by dilution and plating with the quantity of control bacteria not exposed to antimicrobial agents. The results were accepted only if the initial inoculum of antibiotic-exposed bacteria was within 0.5 log unit of the control inoculum. The cultured HEp-2 cells (Flow Laboratories, Irvine, United Kingdom) were dissociated with 3 mM EDTA (E. Merck AG, Darmstadt, Germany) and were resuspended, counted, and diluted in Hanks' balanced salt solution to 2×10^5 cells per ml. Equal volumes (2 ml) of cells and bacteria were mixed and placed on a rotating rack at 37°C for 1 h. During this incubation, bacteria were exposed to the same subinhibitory antimicrobial concentrations that were used during the overnight preincubation, to prevent changes of the preexposed bacteria during the assay. Following incubation, unattached bacteria were separated from cells with adherent bacteria by three cycles of differential centrifugation. The final cell pellet was fixed on glass slides with a cytospin apparatus and was then stained (Diff-Quik; Baxter, Unterschleissheim, Germany). The attached bacteria on 40 separate cells were quantitated in blinded samples by direct light microscopy, and adherence was expressed as the mean number of bacteria attached per cell ± standard error of the mean (SEM).

Without antimicrobial agents, *Moraxella* (*Branhamella*) *catarrhalis* and *H. influenzae* adhered to HEp-2 cells in

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TABLE 1. Effects of subinhibitory concentrations of sparfloxacin, ciprofloxacin, and trimethoprim on the adherence of respiratory pathogens to HEp-2 cells

Strain and antimicrobial agent (no. of cells)[a]	Mean ± SEM no. of bacteria/cell		
	No antibiotic	1/4 MIC	1/8 MIC
Moraxella catarrhalis 18.38			
Control (360)	40.8 ± 1.3		
Sparfloxacin (120)		16.9 ± 1.2[b]	24.1 ± 1.9[b]
Ciprofloxacin (120)		19.9 ± 1.4[b]	20.9 ± 1.5[b]
Trimethoprim (120)		18.8 ± 1.6[b]	19.1 ± 1.5[b]
Moraxella catarrhalis 18.25			
Control (360)	13.6 ± 0.5		
Sparfloxacin (120)		8.4 ± 0.8[b]	11.1 ± 0.9[c]
Ciprofloxacin (120)		8.9 ± 0.8[b]	10.6 ± 0.8[b]
Trimethoprim (120)		10.5 ± 0.9[b]	12.7 ± 1.0
Klebsiella pneumoniae 1.209			
Control (360)	8.1 ± 0.4		
Sparfloxacin (120)		3.8 ± 0.4[b]	6.0 ± 0.5[b]
Ciprofloxacin (120)		4.3 ± 0.4[b]	4.6 ± 0.4[b]
Trimethoprim (120)		—[d]	5.6 ± 0.6[b]
Klebsiella pneumoniae 1.77			
Control (360)	5.7 ± 0.2		
Sparfloxacin (120)		—	2.1 ± 0.2[b]
Ciprofloxacin (120)		—	3.0 ± 0.3[b]
Trimethoprim (120)		—	—
Haemophilus influenzae 17.38			
Control (360)	28.9 ± 1.2		
Sparfloxacin (120)		6.6 ± 0.7[b]	12.5 ± 0.8[b]
Ciprofloxacin (120)		13.7 ± 0.9[b]	21.5 ± 1.4[b]
Trimethoprim (120)		—	—
Haemophilus influenzae 17.5			
Control (360)	11.1 ± 0.4		
Sparfloxacin (120)		5.6 ± 0.5[b]	7.5 ± 0.6[b]
Ciprofloxacin (120)		6.8 ± 0.6[b]	5.5 ± 0.5[b]
Trimethoprim (120)		6.1 ± 0.6[b]	9.1 ± 0.8[c]
Streptococcus pneumoniae 15.62			
Control (360)	1.52 ± 0.11		
Sparfloxacin (120)		1.33 ± 0.18	1.28 ± 0.20
Ciprofloxacin (120)		1.33 ± 0.20	1.83 ± 0.25
Trimethoprim (120)		1.58 ± 0.24	1.23 ± 0.17
Streptococcus pneumoniae 15.42			
Control (360)	2.51 ± 0.14		
Sparfloxacin (120)		3.50 ± 0.28[b]	2.83 ± 0.29
Ciprofloxacin (120)		3.81 ± 0.37[b]	3.58 ± 0.31[b]
Trimethoprim (120)		3.88 ± 0.40[b]	3.82 ± 0.48[b]
Pseudomonas aeruginosa 6.19			
Control (360)	4.5 ± 0.2		
Sparfloxacin (120)		4.3 ± 0.4	4.1 ± 0.3
Ciprofloxacin (120)		1.8 ± 0.2[b]	3.1 ± 0.3[b]
Trimethoprim (120)		3.3 ± 0.3[b]	3.8 ± 0.3
Pseudomonas aeruginosa 6.11			
Control (360)	11.7 ± 0.4		
Sparfloxacin (120)		—	11.0 ± 0.7
Ciprofloxacin (120)		—	8.7 ± 0.6[b]
Trimethoprim (120)		10.3 ± 0.8	12.1 ± 0.7

[a] Total number of cells counted in three independent experiments.
[b] $P < 0.01$ compared with control without antimicrobial agent (by unpaired Student's t test).
[c] $P < 0.05$ compared with control without antimicrobial agent (by unpaired Student's t test).
[d] —, no data; greater than 0.5-log-unit decrease in viability following an 18-h exposure to subinhibitory concentrations of antimicrobial agents.

greater numbers than the other respiratory pathogens did (Table 1). Pneumococci adhered poorly to the cells; a 10-fold higher inoculum than was used for the other bacteria was needed for the observation of significant bacteria-cell attachment. The adherence values obtained with HEp-2 cells in the present study were within the range of values obtained previously with normal respiratory epithelial cells (1, 5, 9, 15, 18, 21, 24).

Preincubation of the bacteria with sparfloxacin significantly decreased the number of adhering bacteria in a dose-dependent manner for *Klebsiella pneumoniae*, *H. influenzae*, and *M. catarrhalis*. Subinhibitory concentrations of sparfloxacin did not change the adherence of *P. aeruginosa* to HEp-2 cells. Adherence of *S. pneumoniae* 15.62 (capsular serotype 14) was not changed by subinhibitory concentrations of sparfloxacin, but adhesion of strain 15.42 (capsular serotype 11) was significantly enhanced by subinhibitory concentrations of sparfloxacin and the other antimicrobial agents. It has been shown that the pneumococcal capsule may interfere with adherence (21), that capsule loss may result in enhanced in vitro adherence and invasion (8), and that free pneumococcal oligosaccharide blocks adhesion (2). To test the hypothesis that the increase in adherence might be due to an antimicrobial agent-induced decrease in capsule production, the adherences of *S. pneumoniae* S3 and its unencapsulated mutant DW3.7 were measured. While the native S3 strain adhered poorly to HEp-2 cells (0.80 ± 0.26 bacteria per cell [±SEM]), a greater number of the uncapsulated mutant DW3.7 bacteria adhered to the cells (5.0 ± 0.70 bacteria/cell [±SEM]).

Preincubation of the bacteria with subinhibitory concentrations of ciprofloxacin and trimethoprim resulted in effects that were analogous to those of sparfloxacin; the only difference was that ciprofloxacin significantly decreased the adherence of the two *P. aeruginosa* strains, whereas sparfloxacin had no significant effect on adherence. Other investigators have described enhanced (12) or inhibited (25) epithelial cell adhesion of *P. aeruginosa*, depending on the antimicrobial agent used.

It is unlikely that the diminished viability of the bacteria after incubation with sublethal antimicrobial concentrations is responsible for the interference with adherence, because viability studies were performed with each incubation, and the results were accepted only if the initial inocula of antibiotic-exposed bacteria were within 0.5 log unit of the control inocula.

Interference with adherence of bacteria to cells after incubation with low concentrations of quinolones was described by Vosbeck et al. (27). Nalidixic acid at one-fourth the MIC caused a consistent increase in the adhesion of 10 *Escherichia coli* strains to Intestine 407 tissue culture cells. The mechanism for this increased adherence remains unclear; those investigators (27) observed increased hemagglutinating activity of *E. coli* strains after exposure to subinhibitory concentrations of nalidixic acid. Studies with the newer fluoroquinolone pefloxacin showed decreased adherence of six *S. aureus* strains to human buccal cells (6) and of *E. coli* T 1019 to urothelial cells (7). The present study shows decreased adherence of the respiratory pathogens *K. pneumoniae*, *H. influenzae*, and *M. catarrhalis*. The mechanism for this antimicrobial agent-induced decreased adherence is unclear. Adherence of *Klebsiella* and *Moraxella* species is mediated by fimbriae (4), while the adherence of *H. influenzae* is mediated by fimbriae (3) and nonfimbrial adhesins (18). Further studies should elucidate the mechanism of quinolone-induced interference with the bacteria-host cell interaction. Because the activities of newer quinolones include many airway pathogens, respiratory pathogens should be included in such studies.

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