Postantibiotic Effects of ABT-773 and Amoxicillin-Clavulanate against *Streptococcus pneumoniae* and *Haemophilus influenzae*

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This study determined the postantibiotic effect (PAE) of ABT-773 versus that of amoxicillin-clavulanate against clinical isolates of *Streptococcus pneumoniae* and *Haemophilus influenzae*. The PAEs of ABT-773 and amoxicillin-clavulanate ranged from 2.3 to 6.0 h and 0 to 2.2 h against *S. pneumoniae* and from 2.7 to 9.1 h and 0 to 0.8 h against *H. influenzae*, respectively.

The ketolides represent a novel subclass of agents within the macrolide-lincosamide-streptogramin group. ABT-773, the newest member of the ketolide group, demonstrates good in vitro activity against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and other community-acquired respiratory tract pathogens (2, 6, 9). Due to enhanced accumulation into cells and tighter ribosomal binding, this new ketolide maintains in vitro activity against *msf-* and/or *ermB*-containing *S. pneumoniae* (3). The purpose of this study was to determine the postantibiotic effect (PAE) of ABT-773 versus that of amoxicillin-clavulanate against *S. pneumoniae* and *H. influenzae*.

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Clinical isolates of *S. pneumoniae* (*n* = 4) and *H. influenzae* (*n* = 4) were obtained from the microbiology laboratories at the University of Illinois at Chicago Medical Center (Chicago, Ill.) and Loyola University Medical Center (Maywood, Ill.). Two of the *S. pneumoniae* strains were resistant to penicillin and erythromycin (*msf* gene), while the other two strains were susceptible to both agents. Half of the *H. influenzae* strains were beta-lactamase positive, while the other half were beta-lactamase negative. The isolates were stored at −70°C in skim milk and underwent three subcultures prior to MIC and PAE studies. Control strains (*S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247) were used to validate MIC results.

ABT-773 (Abbott Laboratories, Abbott Park, Ill.) and amoxicillin-clavulanate (United States Pharmacopeia, Rockville, Md.) powders were prepared according to NCCLS guidelines or per the manufacturer’s recommendations (8). The media for MIC and PAE studies were cation-supplemented Mueller-Hinton broth with 5% lysed horse blood (Remel, Lenexa, Kans.) and *Haemophilus* test medium (Remel) for *S. pneumoniae* and *H. influenzae* isolates, respectively.

MIC studies were performed in duplicate per NCCLS guidelines using the microbroth dilution method (8). The organisms were freshly subcultured and incubated overnight in a 35°C incubator with 5% CO₂. The inoculum was prepared by direct suspension and adjusted with sterile saline until the turbidity matched a 0.5 McFarland standard using a spectrophotometer at 625 nm. Each suspension was further diluted in broth media to obtain a final inoculum of approximately 5 × 10⁵ CFU/ml. The exact inoculum size was determined via colony counts. The microtiter plate contents were incubated at 35°C (humidified air) and read at 20 h. The MIC was defined as the lowest concentration at which there was no visible growth.

The PAEs were determined in duplicate by the in vitro method using the repeated-washing technique (4). Concentrations of two and eight times the MIC were tested. The organisms were freshly subcultured and incubated overnight in a 35°C incubator with 5% CO₂. A 10⁴ CFU/ml suspension of each organism was exposed to each antibiotic for 1 h. A tube containing no antimicrobial agent was included as a growth control. Tubes containing 1/100 and 1/1,000 of the tested antimicrobial concentration were utilized to ensure that residual antibiotic remaining after washing would not interfere with bacterial growth. All tube contents were incubated on a shaking platform in a 35°C incubator. At the end of the exposure period, the antibiotics were removed by washing the tubes three times. The tubes were centrifuged, the supernatant was removed, and the bacterial pellet was resuspended with drug-free media. Viable counts were determined for all tubes at this time. The tubes were placed back on a shaking platform, with sampling performed every 1 to 2 h thereafter until the broth became cloudy. Each sample was serially diluted with sterile saline to produce 10-fold dilutions. The diluted samples were plated onto blood or chocolate agar (Remel) using a spiral plater (WASP Spiral Plater; Microbiology International, Frederick, Md.). Colonies were counted after 24 to 48 h of incubation at 35°C with 5% CO₂. The PAE was defined as *T − C*, where *T* was the time required for the count in the test culture to increase 1 log₁₀ above the count observed immediately after drug removal and where *C* was the time required for the count in the untreated control to increase 1 log₁₀ above the count observed immediately after washing (4). The lower limit of detection was 1.3 log₁₀ CFU/ml.

The results of the MIC and PAE studies are listed in Table...
1. ABT-773 achieved lower MICs for both penicillin-erythromycin-sensitive and -resistant *S. pneumoniae* than did amoxicillin-clavulanate. In contrast, amoxicillin-clavulanate achieved lower MICs than did ABT-773 for three of the four strains of *H. influenzae*. Differences in viable counts pre- and post-drug removal were modest (average, 0.28 CFU/ml; range, 0.02 to 0.59 CFU/ml). Viable counts from the tubes containing 1/100 and 1/1,000 of the antibiotic concentrations did not differ from the counts obtained with the controls (data not shown).

The PAEs of ABT-773 against both *S. pneumoniae* and *H. influenzae* were longer than those achieved following exposure to amoxicillin-clavulanate. Against *S. pneumoniae*, the PAEs ranged from 2.3 to 6.0 h for ABT-773, versus 0.0 to 2.2 h for amoxicillin-clavulanate. The PAEs ranged from 2.7 to 9.1 h for ABT-773 and 0.0 to 0.8 h for amoxicillin-clavulanate against *H. influenzae*. ABT-773 demonstrated a longer PAE against sensitive (5.1 to 6.0 h) than against resistant (2.3 to 2.7 h) strains of *S. pneumoniae*. No differences were noted in the PAEs of ABT-773 against beta-lactamase-positive versus beta-lactamase-negative strains of *H. influenzae*. A trend towards concentration-dependent activity was observed with ABT-773 against *H. influenzae* (2.7 to 4.5 h at two times the MIC versus 4.9 to 9.1 h at eight times MIC); however, this was not seen with *S. pneumoniae*. The significance of the long PAE of ABT-773 against *H. influenzae* is questionable, due to the higher MICs and achievable concentrations in serum of this new ketolide.

Limited data are available regarding the PAEs of ABT-773 against community-acquired pathogens (5, 6; N. Ramer, D. McDaniel, P. Johnson, D. Shortridge, and R. K. Flamm, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2137, 2000). Davies et al. determined the PAEs of ABT-773 and 10 other antibiotics against eight pneumococcal isolates (6). The organisms were exposed to 10 times the MIC of each agent for 1 h. Overall, ABT-773 demonstrated longer PAEs than did beta-lactams, macrolides, azithromycin, and clindamycin. The mean PAE of ABT-773 in that study was ≥5.6 h (range, 1.7 to ≥7.7 hours). We obtained similar results at a comparable (eight times the MIC) concentration, as well as at a lower (twice the MIC) concentration. Although their study did not provide individual PAE data on macrolide-susceptible strains, the mean PAE of ABT-773 ranged from 1.7 to 5.6 h against three strains of *S. pneumoniae* with high-level macrolide, azithromycin, and clindamycin resistance (MICs > 64 μg/ml). In comparison, our two macrolide-resistant strains containing the mefE gene demonstrated PAEs of 2.3 to 2.7 h. The mean PAE of amoxicillin in the study by Davies et al. was 1.7 h (range, 0.50 to 2.7 h), which was slightly higher than that of amoxicillin-clavulanate in our study.

Credito et al. determined the PAEs of ABT-773 and 11 other antibiotics against five strains of *H. influenzae* (5). Unlike the previously mentioned study, the antibiotic concentrations varied based upon achievable pharmacokinetic parameters. Agents tested at four times the MIC included ABT-773, erythromycin, clarithromycin, roxithromycin, and amoxicillin, while several other cephalosporins were tested at 10 times the MIC. Following 1 h of drug exposure, ABT-773 and azithromycin demonstrated longer PAEs than did the macrolides and beta-lactams. Both agents achieved a mean PAE of ≥6.1 h in the study by Credito et al. The PAE range was 4.9 to >8.0 h for ABT-773 and 4.4 to >7.4 h for azithromycin. In comparison, amoxicillin had a mean PAE of 0.5 h and a range of 0 to 2.6 h. In our study, the PAE ranges of ABT-773 were longer at eight times MIC (range, 4.9 to 9.1 h) and shorter at twice the MIC (range, 2.7 to 4.5 h) than were their results at four times the MIC.

Ramer et al. conducted a PAE study against a variety of respiratory pathogens, including six isolates of *S. pneumoniae* (two macrolide susceptible, two mef, and two erm) and two isolates of *H. influenzae* (Ramer et al., 40th ICAAC). The organisms were exposed to antibiotics at eight times the MIC for 2 h. Antimicrobials studied included ABT-773, erythromycin, ciprofloxacin, and cefuroxime. ABT-773 exhibited longer PAEs than did the other agents against both *S. pneumoniae* and *H. influenzae*. Despite a longer exposure period, their data were similar to our results. The data from their study also

### Table 1. MICs and PAEs of ABT-773 and A-C for *S. pneumoniae* and *H. influenzae*

<table>
<thead>
<tr>
<th>Organism and strain</th>
<th>MIC (μg/ml) of:</th>
<th>PAE (h) ± SD of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABT-773</td>
<td>A-C</td>
</tr>
<tr>
<td></td>
<td>8× MIC</td>
<td>2× MIC</td>
</tr>
<tr>
<td></td>
<td>8× MIC</td>
<td>2× MIC</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-S2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.004</td>
<td>0.03–0.015</td>
</tr>
<tr>
<td>SP-JH&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.004</td>
<td>0.008–0.004</td>
</tr>
<tr>
<td>SP-21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.03</td>
<td>8–4</td>
</tr>
<tr>
<td>SP-15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.06</td>
<td>2–1</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF-T&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2</td>
<td>0.5–0.25</td>
</tr>
<tr>
<td>HF-M&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2</td>
<td>0.5–0.25</td>
</tr>
<tr>
<td>HF-F&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2</td>
<td>1–0.5</td>
</tr>
<tr>
<td>HF-U&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2</td>
<td>2–1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Penicillin-erythromycin-sensitive isolates.<br>
<sup>b</sup> Penicillin-erythromycin-resistant isolates.<br>
<sup>c</sup> Beta-lactamase-negative isolates.<br>
<sup>d</sup> Beta-lactamase-positive isolates.<br>
<sup>e</sup> No standard deviation available.<br>
<sup>f</sup> A-C, amoxicillin-clavulanate.
indicated a trend towards slightly longer PAEs against sensitive than against resistant isolates of *S. pneumoniae*.

The PAE of ABT-773 is similar to that of another ketolide agent, telithromycin (HMR 3647) (1, 7, 10; M. R. Jacobs, S. Bajaksouzian, J. Chuang, M. P. Ronchetti, and P. C. Appelbaum, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-253, 1997). Munckhof et al. studied a wide range of telithromycin concentrations used against *S. pneumoniae* (7). A longer estimated maximum PAE for erythromycin-sensitive than for erythromycin-resistant strains of *S. pneumoniae* was noted (9.5 to 9.9 h versus 6.2 to 6.6 h). In addition, longer PAEs were achieved with higher concentrations (32 to 64 times the MIC). Boswell et al. also demonstrated concentration-dependent PAEs of telithromycin against *S. pneumoniae* and *H. influenzae* (1).

In summary, ABT-773 demonstrates good in vitro activity against community-acquired respiratory pathogens, including penicillin-erythromycin-resistant strains of *S. pneumoniae*. ABT-773 achieved prolonged PAEs against penicillin-erythromycin-sensitive *S. pneumoniae* and *H. influenzae*, compared to what was achieved by amoxicillin-clavulanate. Although the clinical significance of PAEs has not been established, additional in vitro and in vivo studies are needed to further assess this pharmacodynamic parameter.

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REFERENCES