clinical condition of the patient improved and he was discharged several days later.

Resistance to the newer quinolones in *H. influenzae* is rare. In a large Canadian study in 1992 and 1993, all of 1688 strains were susceptible to ciprofloxacin.² Jones found 1% of 7961 *H. influenzae* isolates to be moderately susceptible,³ and 1% resistant to lomefloxacin. Barriere & Hindler⁴ and Gould *et al.*⁵ described quinolone-resistant *H. influenzae* from patients with chronic respiratory tract infections. In an ongoing electronic surveillance in Dutch public health laboratories, only 0.19% of 2678 single isolates were resistant to ciprofloxacin (MIC > 1 mg/L). Therefore, strain 24482 is one of the first ciprofloxacin-resistant *H. influenzae* isolates in the Netherlands.

Since mutations in the DNA gyrase (*gyrA*) gene are the most common mechanism of fluoroquinolone resistance in other bacterial species,⁶ we sequenced the corresponding region of the *gyrA* gene of the *H. influenzae* strains Rd and 24482. DNA was extracted by heating a suspension of cells (10⁶ cfu/mL) for 15 min at 100°C and 10 μL of each sample was subjected to a ‘touchdown’ PCR with an annealing temperature ranging from 61°C to 50°C. Based on the *gyrA* sequence present in GenBank (accession number U32806) primers were developed corresponding to residues –43 to –25 (For; 5′-ATGCTATAATCGC-CACAA-3′) and 536–515 (Rev; 5′-ATCCCCACCGCA-ATACCAGAAG-3′) of the *gyrA* gene. DNA sequencing of both strands of the PCR products was performed in duplicate with an ABI 373A DNA sequencer according to the instructions provided by the manufacturer (Applied Biosystems, Foster City, CA, USA) using the same primers. The sequence of the Rd strain (positions 1–510) was identical to the sequence obtained from the database. The sequence of the quinolone-resistant isolate (positions 1–510, deposited in GenBank, accession number Z73213) differed in 11 bases. Eight of these replacements were silent and only three mutations resulted in amino acid substitutions, in codon 84 (TCC → TTA; Ser → Leu) and codon 88 (GAT → TAT; Asp → Tyr). Homologous double mutations have been found in codons 83 and 87 of several isolates of ciprofloxacin-resistant *Escherichia coli.*⁶

These identical substitutions in *E. coli* suggest that the resistance of our *H. influenzae* is due to the amino-acid substitutions at positions 84 and 88 of its *gyrA*-protein.

### Azithromycin uptake by tissue cultured epithelial cells

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Sir,

Azithromycin is an azalide antimicrobial agent that has been shown to concentrate and reach high intracellular concentrations in different types of human cells.¹⁻³ It has been postulated that polymorphonuclear leukocytes (PMNs) can transport the drug to the site of infection. It also appears that fibroblasts may act as a reservoir for the drug in tissues allowing activity against organisms and possibly transferring azithromycin to phagocytic cells.⁴

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**Correspondence**


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**Table.** MIC (mg/L) of ciprofloxacin and other antibiotics for *H. influenzae* 24482 and *H. influenzae* Rd

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg/L)</th>
<th>strain 24482</th>
<th>Rd strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>16/304</td>
<td>0.12/2.28</td>
<td></td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>0.25</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Co-amoxiclav</td>
<td>0.25/0.12</td>
<td>0.5/0.25</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>2</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>
Azithromycin has shown high intrinsic activity against *Chlamydia trachomatis*. In fact, a single oral dose of azithromycin is currently considered to be an alternative for the treatment of genital infections caused by this microorganism. Since *C. trachomatis* invades and multiplies within epithelial cells, the excellent response of *C. trachomatis* infections to azithromycin could be partially related to a potential capability of the drug to accumulate within these cells.

The purpose of this study was to evaluate the intracellular penetration of azithromycin into tissue-cultured epithelial cells relative to that in human phagocytic cells. The tissue-cultured epithelial cell lines McCoy and Hep-2 (Flow Laboratories, Irvine, UK) were grown in supplemented minimal essential medium (Flow Laboratories) containing 10% fetal calf serum (Flow Laboratories) without antibiotics. Cells were detached from tissue culture bottles with trypsin–EDTA (Flow Laboratories), washed and suspended in Hanks’ balanced salt solution (HBSS) at a concentration of 5 × 10^6 cells per mL. PMNs were recovered from venous blood of healthy donors by dextran sedimentation and a Ficoll–Hypaque (Pharmacia Biotech, Uppsala, Sweden) gradient. Peritoneal macrophages were isolated from peritoneal effluents of patients undergoing continuous ambulatory peritoneal dialysis (CAPD) as previously described. Cell preparations from CAPD donors always contained >75% peritoneal macrophages and <15% PMNs. Final cell suspensions were adjusted to 5 × 10^6 PMN, or peritoneal macrophages per mL in HBSS.

Uptake of [14C]azithromycin (42.15 μCi/mg; Pfizer, Greton, CT, USA) by phagocytic and non-phagocytic cells was determined by the technique described by Pascual et al. Cells were incubated in HBSS containing different extracellular concentrations of azithromycin (0.125–10 mg/L). After different incubation periods at 37°C, cells were separated from the extracellular solution by centrifugation through a silicone oil barrier in a microcentrifuge tube. A 10 μL aliquot of the extracellular medium and the entire cell pellet, obtained by cutting off the portion of the microcentrifuge tube containing the pellet, were placed in 3 mL of scintillation fluid (Ready Micro, Beckman Instruments, Inc., Beckman, Germany) and counted. After determination of the cell volume, the accumulation rate of azithromycin in cells (cellular to extracellular concentration ratio, C/E) was calculated as described previously.

The intracellular penetration of azithromycin into phagocytic and non-phagocytic cells is shown in the Figure. Azithromycin penetrated rapidly into both types of epithelial cells, reaching intracellular concentrations at least three times higher than the extracellular ones. At higher extracellular concentrations (10 mg/L) the intracellular concentrations of azithromycin in McCoy and Hep-2 cells were 35 ± 6.2 and 58 ± 10.8 mg/L respectively. This uptake was significantly lower than that observed in phagocytic cells. In fact, the C/E values of azithromycin in PMNs and peritoneal macrophages were greater than 23 and 60 respectively. At higher extracellular concentrations (10 mg/L), the intracellular concentrations of azithromycin in PMNs and peritoneal macrophages were 110 ± 19 and 606 ± 54 mg/L respectively.

Azithromycin reaches high tissue concentrations at the site of infection as a result of PMN transportation and high intracellular concentrations in fibroblasts. There is little information, however, about the ability of this agent to concentrate within epithelial cells, the host cell for *C. trachomatis*. The penetration of azithromycin into tissue cultured human endometrial cells exceeded that of erythromycin by as much as eight-fold. This penetration rate was not affected by the presence of intracellular *C. trachomatis*. This study only evaluated high extracellular concentrations of azithromycin (10 mg/L) and C/E ratio values were not calculated. We have observed that even at very low extracellular concentrations (0.125 mg/L) azithromycin concentrates several times within the cells used for susceptibility testing assays of *C. trachomatis*.

It is interesting to note that the uptake of azithromycin by human peritoneal macrophages is much higher than that observed in PMNs. This may be an advantage over other macrolides in the treatment of infections due to susceptible organisms in patients undergoing CAPD. Nevertheless further studies are needed to elucidate this point. Azithromycin concentrates several times in tissue-cultured epithelial cells. This phenomenon may partially explain the excellent response to this agent of genital infections caused by *C. trachomatis*.
References


Decrease in serum levels of valproic acid during treatment with a new carbapenem, panipenem/betamipron


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Sir,

Panipenem/betamipron, a new carbapenem, is a combined drug containing panipenem and betamipron, which has been released on the market in Japan. Panipenem is an antimicrobial agent and betamipron is an organic ion transport inhibitor for decreasing renal damage.\(^1\)

We found low serum concentrations of valproic acid during treatment with panipenem/betamipron in three epileptic patients. The serum concentrations of valproic acid and other antiepileptic drugs were measured 2 h after oral administration by fluorescence polarization immunoassay in all patients. The serum concentrations of panipenem and betamipron were determined by high-performance liquid chromatography. Informed consent for the administration of panipenem/betamipron and for taking blood and urinary samples for assays of drugs was obtained from patients.

The first case was a 10-year-old boy who had been diagnosed as having pleural empyema. He was taking valproic acid (8.3 mg/kg every 8 h), carbamazepine and clonazepam. The antibiotic treatment was changed to iv panipenem/betamipron (20 mg/kg every 6 h) and clindamycin on the third day. His convulsions increased on the sixteenth day after panipenem/betamipron was started. The serum concentration of valproic acid was 70.7 mg/L before admission and decreased to 4.6 mg/L with an appropriate serum carbamazepine level of 6.7 mg/L at this time. The low level of valproic acid continued although the daily dose was increased Panipenem/betamipron and clindamycin were continued because a clinical effect was observed and surgical treatment for pleural adhesions could not be done owing to the patient’s general condition. Panipenem/betamipron was administered for a total of 47 days. The serum levels of valproic acid were 25.8 mg/L and 45.8 mg/L on the third and eighth day respectively after stopping panipenem/betamipron.

The second case was an 8-year-old girl treated with a combination of valproic acid, carbamazepine, nitrazepam and phenytoin, who was admitted with recurrent pneumonia. The antibiotic treatment was changed to iv panipenem/betamipron (20 mg/kg every 8 h) on the tenth day, together with an iv antifungal agent, fluconazole. The serum level of valproic acid decreased after injection of panipenem/betamipron (Figure). The serum levels of panipenem and betamipron were 28.4 mg/L and 12.6 mg/L respectively at 1 h after injection, and 9.4 mg/L and 1.3 mg/L at 2 h, respectively. Panipenem/betamipron was discontinued on the 18th day because of clinical failure. The serum level of valproic acid returned to normal on the tenth day after stopping panipenem/betamipron.

A 10-year-old girl with pleural empyema was the third case. She had been treated with valproic acid (5 mg/kg every 12 h) and carbamazepine. The antibiotic treatment was changed to iv panipenem/betamipron (20 mg/kg every 6 h) on the third day because she was not improving. Her convulsions increased in frequency on the second day after the start of the drug. The serum level of valproic acid was 26.5 mg/L after injection of panipenem/betamipron. Valproic acid was substituted by phenobarbitone, which controlled her convulsions. Her clinical and laboratory findings were improved and panipenem/betamipron was given for 53 days in total. The fractional excretion of valproic acid (the level of urinary valproic acid × serum creatinine/ serum valproic acid × urinary creatinine × 100) was 0.06% at 0.5 h after the first injection of panipenem/betamipron and was increased to 0.21% at 18 h. The carbamazepine levels before and during panipenem/betamipron treatment were 9.0 mg/L and 9.2 mg/L, respectively.

The mechanisms of pharmacological interaction...