Measurement of Cefaclor and Amoxicillin-Clavulanic Acid Levels in Middle-Ear Fluid in Patients with Acute Otitis Media

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Concentrations of cefaclor (CFC) or amoxicillin-clavulanic acid (AMX/CA) in middle-ear fluid collected preserving the stability and clearing the cell contents has been compared to those obtained using the traditional method. Sixty-seven children with effusive otitis media were treated orally with CFC (20 mg/kg body weight) or AMX/CA (20 mg/kg) (4:1 ratio). The concentrations in cell-free fluid (C−) appear higher than those in the total fluid (C+) (as assayed traditionally).

For a substantial percentage (8 to 15%) of children who receive antibiotic therapy for acute otitis media or effusive otitis media, infection is not clinically resolved (5). This clinical failure rate may be due to a variety of reasons, including resistance of the major causative bacteria (Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis), inadequate middle-ear fluid (MEF) penetration of the antibiotic, compounded viral infection, or physiological or immunological dysfunction (5, 6). The potential clinical efficacy of antibiotics in the treatment of acute otitis media is best predicted by direct testing of MEF for concentrations of antibiotics which can then be compared with MICs and localized patterns of resistance (3, 6). It has been suggested that clinical efficacy in acute otitis media depends on the ability of the antibiotics to achieve concentrations in MEF exceeding MICs at which 90% of the isolates tested are inhibited (MIC90s) against the major causative pathogens during an appropriate time (4, 6).

Homogenization of a fluid sample, centrifugation, and subsequent assay of the supernatant have been used to predict the activity of antibiotic concentrations. This method assumes that the fluid is homogeneous and that antibiotics as well as bacteria are evenly distributed through it. Fluids, however, are not homogeneous, and antibiotics as well as bacteria are not evenly distributed (10, 13). Within infected fluid, an antibiotic may concentrate into the cells and/or extracellular fluid, depending upon its physicochemical properties (10).

In the past, samples were obtained from subjects with serous otitis media, not from subjects with an acute otitis media (AOM). Since serous otitis is a chronic condition with less of an inflammatory component than AOM, levels measured may not reflect those achieved in AOM. Bioassays were used rather than high-performance liquid chromatography (HPLC). Handling of samples was uncertain, especially taking into consideration the instability of antibiotics such as cefaclor.

Differences in cell content of samples were not taken into account. Since different antibiotics are distributed unequally between the intracellular and extracellular compartments, and since inflammatory effusions have a high cellular content, not correcting for the cell content of the effusion may lead to calculations of inaccurate values of antibiotic concentration in the MEF (11, 12).

The primary objective of this study was to test the hypothesis that the concentrations of cefaclor or amoxicillin-clavulanic acid (co-amoxiclav) in MEF that was collected preserving the stability and clearing the cell contents and assayed using HPLC are greater than those with MEF that was collected and processed by a traditional method.

In this study, we measured the concentrations of cefaclor, amoxicillin, and clavulanic acid in the MEF of children undergoing therapeutic tympanocentesis for acute otitis media after administration of 20 mg of cefaclor suspension/kg of body weight and 20 mg (ratio, 4:1) of single-dose co-amoxiclav suspension/kg. A sparse sampling approach was used to establish these concentrations. The study was open label.

This study includes 67 subjects with a clinical diagnosis of acute otitis media. Informed consent was obtained pretherapy from parents after the nature of the study was explained.

Samples were obtained from 1 to 6 h after dosing (one sample for each subject).

For each subject, a 5-ml blood sample was collected for assay of plasma concentrations of cefaclor or co-amoxiclav within 2 min of middle-ear effusion collection.

Each blood-free sample of more than 300 µl was divided into two fractions for antibiotic assay (samples under 300 µl cannot suitably be divided in order to obtain a sufficient amount of fluid for two separate analyses. One total fraction (C+) was sonicated to lyse cells, homogenized by using an Ultra-Turrax blade homogenizer (three bursts, 6 s each) under constant cooling, and centrifuged, and the supernatant was assayed; supernatant included the content of the cells (most cells were inflammatory, i.e., granulocytes, macrophages, and lymphocytes, and to a lesser extent, cells from the surrounding tissue, i.e., epithelial cells, were present).

The second fraction (free-cell fluid [C−]) was cleared from the cells, and the fluid was assayed for antibiotics. The C− fraction was obtained via a velocity gradient technique (8, 9).

Briefly, the samples were centrifuged at 14,000 × g for 5 min in microtest tubes containing 200 µl of a mixture of silicone oils (556-550, 5:6 vol/vol; Dow Corning), separating the cells from extracellular fluid.

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Samples of less than 300 μl were cleared from cells as described before and assayed as extracellular fluid.

Serum as well as fluid was immediately stored at −80°C until assay. The time from collection to freezing was at maximum 20 min.

Samples were all assayed in parallel for each of the antibiotics. Cefaclor levels were assayed by HPLC as previously described (2). Briefly, duplicate samples of C− and C+ were combined with 20 μl of internal standard (acyclovir; 100 μg/ml) and 600 μl of acetonitrile (Bracco; Milan, Italy). The mixtures were then vortexed for 20 s and subsequently centrifuged for 2 min at 14,000 × g. The supernatant was removed, and 3 ml of dichloromethane (Bracco) was added. The mixtures were vortexed for 30 s and subsequently centrifuged at 2,500 rpm for 15 min (with a 95% estimated recovery). A 20-μl aliquot of each sample was analyzed by HPLC under isocratic conditions using as mobile phase 0.05 M ammonium acetate (Merck; Darmstadt, Germany) and acetonitrile (98:15 [vol/vol]; Merck) at a flow rate of 1 ml/min. Standard solutions were made up in drug-free human serum and phosphate buffer with concentrations ranging from 0.1 to 50 μg/ml. The HPLC system consisted of a Shimadzu liquid chromatograph equipped with a variable-wavelength UV detector, SPD-6A, LC-9A pumps, SIL-9A auto sampler, and a reverse-phase C18 column (Spherisorb). The method was sensitive (detection limits, 0.1 to 1 μg/ml) and reproducible (coefficient of variation, 2.9 to 4.5%) and gave high recovery rates (95% estimated recovery).

Amoxicillin and clavulanic acid were assayed simultaneously as previously described (1).

The column was m-Bondapak C18, and the mobile phase was methanol-phosphate-buffered saline buffer (pH 6; 0.2 M) (15:1 [vol/vol]) at a flow rate of 0.8 ml/min. The limits of detection for amoxicillin and clavulanic acid were found to be 0.05 and 0.08 mg liter−1, respectively. The method was reproducible (coefficients of variation, 2.9 to 3.2% and 3.2 to 4.5% for amoxicillin and clavulanic acid, respectively) and gave high recovery rates (>95% estimated recovery for both compounds).

The individual and mean concentrations of cefaclor and co-amoxiclav in the extracellular compartment of MEF and in plasma were tabulated and plotted by group. The standard descriptive statistics were calculated by group for the antibiotic concentration in the extracellular compartment of MEF and in plasma.

Of 67 patients enrolled, 9 could not be evaluated. All but four of the patients had experienced at least one episode of otitis media in the last 12 months before the study.

Cefaclor concentrations in MEF were obtained for 40 subjects (male/female ratio, 27/13; age range, 5 to 14 years; median 8). C− fraction was obtained for all subjects, while both C− and C+ fraction was obtained for 21 subjects only (Fig. 1).

The peak level was reached around 2 h after dosing in both groups. The concentrations in C− fluid appeared higher than those in the C+ fluid (as processed traditionally).

The area under the concentration-time curve (AUC) (Table 1) for cefaclor in C− fluid was higher than that in C+ and similar to that in serum.

Amoxicillin and clavulanic acid concentrations in MEF were obtained for 18 subjects (male/female ratio, 13:5; age range, 6 to 12 years, median 8). C− fraction was obtained for all sub-

<table>
<thead>
<tr>
<th>Sample</th>
<th>AUC (AUCmef/AUCs) (mg/liter·h)</th>
<th>Cefaclor</th>
<th>Amoxicillin</th>
<th>Clavulanic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>C− fluid</td>
<td>21.65 (1.07)</td>
<td>22.99 (1.03)</td>
<td>5.1 (1.05)</td>
<td></td>
</tr>
<tr>
<td>C+ fluid</td>
<td>14.23 (0.70)</td>
<td>16.80 (0.76)</td>
<td>3.61 (0.74)</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>20.18</td>
<td>22.21</td>
<td>4.87</td>
<td></td>
</tr>
</tbody>
</table>

* AUCmef/AUCs, ratio of AUC in MEF to AUC in serum.
TABLE 2. Percentage of C− MEF drug concentrations that exceed the MIC\textsubscript{50} and MIC\textsubscript{90} for S. pneumoniae, H. influenzae, and M. catarrhalis for at least 40% of the dosing interval\textsuperscript{*}

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Co-amoxiclav</th>
<th>Cefaclor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC\textsubscript{50} (µg/ml)</td>
<td>T &gt; MIC\textsubscript{50} (%)</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>0.25</td>
<td>100</td>
</tr>
</tbody>
</table>

\textsuperscript{*} MICs were obtained from reference 7. T > MIC\textsubscript{50} and T > MIC\textsubscript{90} time that levels in serum remain above the MIC\textsubscript{50} or MIC\textsubscript{90}, respectively, for the organism.

jects, while both C− and C+ fractions were obtained for 13 subjects only (Fig. 2).

The behavior was similar to that of cefaclor; nevertheless, the peak level was reached between 2 and 3 h postdosing. Clavulanic acid concentrations are often not measurable 6 h after dosing. The AUC (Table 1) of amoxicillin and clavulanic acid in C− fluid was also higher than that in C+ and similar to that in serum.

The present study shows that the tested beta-lactams rapidly and extensively penetrate into the MEF of pediatric patients with acute otitis media.

Beta-lactam antibiotics, such as cephalosporins and penicillins, are unevenly distributed in tissue, with a tissue/serum ratio of <1:1 for most sites. They are distributed mostly in the blood and extracellular fluid, which represent about 20% of the total body mass. Cefaclor and co-amoxiclav are distributed mainly in the extracellular fluids and appear to be underestimated inside the cellular fluids. The majority of common bacterial pathogens are confined to the blood and interstitial tissue fluid, which is precisely where beta-lactam antibiotics are found. Since the concentration of an antibiotic in interstitial tissue fluid often equates with its concentration in serum, estimation of the latter may be adequate to predict efficacy despite the fact that beta-lactams have low tissue/serum ratios. In fact, the AUC for cefaclor as well as that for co-amoxiclav in serum were similar to the AUCs in the C− fluid while higher than those in the C+ fluid. Apart from straightforward errors in processing and assaying total drug concentrations in tissue, the concentrations of beta-lactams in extracellular fluid (site of bacterial growth) are grossly underestimated.

On the basis of the pharmacodynamic characteristics of the beta-lactams, one would expect that an effective dosing regimen for otitis media would require drug concentrations in MEF exceeding the MICs of the causative pathogen, for at least 40% of the dosing interval (4). Comparison of the middle-ear C− concentrations of the antibiotics with published MIC\textsubscript{50}s can provide a guide to the potential for bacterial eradication and also clinical success with acute otitis media (Table 2).

In conclusion, the interpretation of the concentrations of antibiotics in the MEF must take account of the cell content into the fluid, which represents a kind of “dead space” for beta-lactams. Moreover, it could be unnecessary to directly measure MEF concentrations of drugs, such as beta-lactams, since these could be accurately predicted on the basis of the unbound plasma concentrations. This type of study and these data may be useful in clinical decision-making for the treatment of acute otitis media.

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REFERENCES