In vivo activity of amoxicillin/clavulanic acid and erythromycin in experimental otitis media caused by Streptococcus pneumoniae plus Haemophilus influenzae

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Abstract

A gerbil model of acute otitis media induced by Streptococcus pneumoniae plus Haemophilus influenzae was used to assess the efficacy of amoxicillin/clavulanic acid (A/C) (1.5/0.3, 2.5/0.5 and 10/2 mg/kg) and erythromycin (2.5, 10, 20 and 50 mg/kg) with or without acetaminophen. The amoxicillin/clavulanic acid MIC was 1/0.5 mg/l for both organisms and the erythromycin MICs were 0.12 and 4 mg/l for S. pneumoniae and H. influenzae, respectively. The organisms were inoculated directly into the middle ear (ME) and antibiotic treatment started 2 h post-inoculation and continued at 8 h intervals for three doses. Acetaminophen was administered at 50 mg/kg. Samples for bacterial counting were obtained from the ME on day 2. Amoxicillin/clavulanic acid peri-MIC concentrations in ME were effective in eradicating both organisms. Despite the inflammation induced by S. pneumoniae, erythromycin did not eradicate H. influenzae at ME concentrations (2.4 mg/l for erythromycin 50 mg/kg) higher than those obtained in humans but lower than the MIC.

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Keywords: S. pneumoniae; H. influenzae; Acute otitis media; Animal model

1. Introduction

Otitis media is the inflammation of the middle ear (ME), associated to the presence of fluid and/or otorrhoea. Streptococcus pneumoniae and Haemophilus influenzae are the bacterial pathogens most commonly involved, and both organisms can be isolated in mixed culture from 10–24% of patients [1–5].

We have shown in previous experimental models that S. pneumoniae induces acute otitis media (AOM) while H. influenzae induces otitis media with effusion (OME) [6]. Beta-lactam antibiotic concentrations in ME fluid were lower in OME than in AOM, probably due to the lower inflammatory reaction associated with OME [6]. Doses of amoxicillin/clavulanic acid (A/C) that achieve ME concentrations in OME [7] that are similar to those obtained in humans after standard doses [8] are effective in eradicating H. influenzae (with an MIC of 1 mg/l, a value similar to the MIC50/MIC90 in Spain), but eradication did not occur with lower doses [7]. In contrast, these lower doses in a mixed (S. pneumoniae plus H. influenzae) AOM model were able to eradicate both organisms due to the higher antibiotic concentration in the ME fluid, probably due to a greater inflammatory reaction [6].

Amoxicillin/clavulanic acid and macrolides are commonly prescribed for the empirical treatment of otitis media often together with an anti-inflammatory or analgesic drug such as acetaminophen [9–12]. As erythromycin achieves higher concentrations in cerebrospinal fluid in the presence of meningeal inflammation [13], we speculated that, if this antibiotic is administered for the empirical treatment of AOM caused by S. pneumoniae plus H. influenzae, both organisms might be eradicated. The second objective was to know if acetaminophen, could prevent the development of OME and to study its possible interference with the ME antibiotic pharmacokinetics as well as therapeutic efficacy.

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2. Materials and methods

2.1. Bacteria
Two clinical isolates were used: S. pneumoniae serotype 23F (MIC benzylpenicillin, 2 mg/l) isolated from a patient with bacteremia and a biotype II β-lactamase producing, non-serotypable H. influenzae (MIC amoxicillin, 8 mg/l) isolated from a patient with otitis media.

2.2. Pharmacological compounds
Amoxicillin trihydrate and lithium clavulanate (SmithKline Beecham Pharmaceuticals, Worthing, UK) and erythromycin (Panther Laboratories, Agent-Lot et Garonne, France), amoxicillin/clavulanic acid 5:1 (Augmentin; SmithKline Beecham Pharmaceuticals, Worthing, UK), and erythromycin lactobionate (Panther Laboratories, Agent-Lot et Garonne, France) were used in the in vitro studies.

For in vivo (therapeutic) use, propacetamol chlorhydrate, a pro-drug of acetaminophen, was used (Pro-efferalgan; Zamboni, Madrid, Spain) and were managed as previously described [6]. Groups of six animals per sample were used in the in vitro studies.

2.3. In vitro studies
MICs and MBCs were determined by a microdilution broth method following previously described procedures [14,15]. Modal values of three separate determinations were recorded.

2.4. Animals
Eight to 9-week-old adult female Mongolian gerbils (Meriones unguiculatus) weighing 49 ± 5 g each were purchased from the Centre d’Élevage R. Janvier (Le Genest St. Isle, France) and were managed as previously described [6]. The study was performed in accordance with prevailing regulations regarding the care and use of laboratory animals in the European Community [16].

2.5. Experimental otitis
Overnight cultures of the organisms were kept in aliquots at −70 °C. The day before the experiment, a freshly thawed aliquot of H. influenzae was added to brain heart infusion broth (BHI) (Oxoid Ltd., Basingstoke, UK) enriched with 2% Fildes extract (Oxoid), incubated at 35 °C in 5% CO₂ for 4 h. On the day of the experiment, a freshly thawed aliquot of S. pneumoniae was incubated for 4 h at 37 °C in a 5% CO₂ atmosphere in BHI enriched with 5% horse serum (bio-Merieux, Marcy l’Etoile, France). The number of viable bacteria in the different inocula was determined by colony counting. Animals were inoculated bilaterally with a mixture of both broths diluted in BHI to obtain approximately 10⁵ cfu of H. influenzae and 10⁶ cfu of S. pneumoniae per 20 μl, introduced directly in the ME bulla. The tympanic membrane was left intact and swelled without rupture during the inoculation. A normal tympanic aspect and correct inoculation were verified with an operating microscope. AOM was defined as otorrhoea through a perforation in the tympanic membrane and/or inflammatory signs with changes in the membrane’s normal yellowish-pink appearance to a grey, dark brownish-yellow, or whitish opaque area, with a very rough surface texture. OME was defined as no inflammatory signs of tympanic membrane with air fluid levels and ME fluid with or without signs of negative ME pressure. Three animals (six ears) were inoculated with 20 μl of BHI/ear to investigate the possible role of the broth as inducer of otitis media.

Blood cultures were performed in four animals 20 h after bacterial inoculation to check for the presence of bacteremia in infected animals.

2.6. Treatment regimen and efficacy studies
Amoxicillin/clavulanic acid (1.5/0.3, 2.5/0.5 and 10/2 mg/kg) and erythromycin (2.5, 10, 20 and 50 mg/kg) were administered subcutaneously (sc) in 500 μl at 2, 10, and 18 h post-inoculation (pi). The experiments were carried out in parallel administering the antibiotics alone or combined with 50 mg/kg of propacetamol sc administered 30 min before each antibiotic dose. Animals in the control groups received pyrogen free sterile distilled water instead of antibiotic with and without propacetamol. Groups of 6–8 animals were used per treatment and control groups.

Treated and control animals were studied longitudinally for otorrhoea, weight, and behaviour. Otoscopic aspect and ME samples obtained by washing the ME fossa with 20 μl of saline solution injected and withdrawn via the eptympanic membrane with a 0.33 mm needle to determine bacterial counts in ME washing fluid, were obtained from both ears on day 2 pi. Aliquots of serial 10-fold dilutions in saline were plated on sheep blood agar and chocolate agar and incubated for 24 h at 35 °C in a 5% CO₂ atmosphere. Bacterial counts are expressed as log₁₀ cfu/20 μl; the lowest detectable bacterial count was 4 cfu/20 μl (0.60 log₁₀ cfu/20 μl). To evaluate the presence of polymorphonuclear (PMN) leukocytes, 3 μl of ME samples were spread over a 6 cm² slide surface to be Gram stained, and observed under a high-power (1000×) microscope. The number of cells in 10 fields was calculated and expressed as mean [S.D.] per field.

2.7. Pharmacokinetic studies
Serum levels of acetaminophen were determined in healthy animals 15 and 30 min after sc administration of 50 mg/kg of propacetamol, by fluorescence polarization immunoassay (FPIA) in an Asysym autoanalyzer (Abbott Cientifica, S.A., Spain). Groups of six animals per sample...
time were killed with CO₂ and were exsanguined by intracardiac puncture. The limit of detection was 1 µg/ml. Serum levels of amoxicillin, clavulanic acid and erythromycin were determined in healthy animals after a single sc injection of the same doses used for the treatment. Groups of six animals per antibiotic dose and collection time (15, 30, 60 and 120 min after drug administration) were killed as described for acetaminophen.

Antibiotic concentrations in ME fluid without washing were also determined in groups of 10 animals bilaterally inoculated under the same conditions as previously described in the experimental otitis model. Amoxicillin/clavulanic acid (1.5/0.3 mg/kg) and erythromycin (50 mg/kg) were administered alone or combined with propacetamol, 46 h after bacterial inoculation. ME samples were obtained 90 min later, via the tympanic membrane, with a 0.38 mm needle. Aliquots of ME samples having ≥2 µl of exudate were pooled and frozen at −70 °C for later determination of antibiotic levels. This procedure permits collection of ME samples at a time when effusion is consistently abundant with high bacterial density and without otorrhoea.

Antibiotic concentrations were determined by microbiological assay using Micrococcus luteus ATCC 9341 for amoxicillin and erythromycin and Klebsiella pneumoniae NCTC 11228 for clavulanic acid. Standard curves for determination of antibiotic concentrations were derived from standard solutions prepared in pooled gerbil sera for serum levels and in 0.1 M phosphate buffer pH 6.0, for ME fluid. Assay variability for individual samples was <10%.

Antibiotic concentration–time curves for each antimicrobial were analysed by a non-compartmental approach using the WinNonlin program (Pharsight, Mountainview, CA). Time over MIC was calculated graphically from the semilogarithmic representation of the concentration–time curve and the regression line representing the apparent elimination rate constant. The relationship between the maximum antibiotic serum concentration obtained (concentration at 15 min after antibiotic administration: C₁₅) and the MIC of the infecting microorganism (serum inhibitory quotient: C₁₅/MIC) and the relationship between the ME antibiotic concentration after 90 min of drug administration and the MIC of the infecting microorganism (ME inhibitory quotient: ME level/MIC) were calculated.

2.8. Statistical analysis

The number of ears with a positive count divided by the total number of ears was calculated to give the percentage of positive ears in each group of animals. To detect differences in eradication rates in each group, the Fisher’s exact test was used. Otorrhoea at day 1 was analysed using the chi-square test. Bacterial counts were expressed in untransformed data as arithmetic means in log10 cfu per 20 µl of ME sample, culture negative samples being included in the calculation of means assuming a value at the detection limit. Analysis of covariance (ANCOVA) was used to compare the reduction of log10 cfu and loss of body weight at day 2 in each group controlled for the log10 cfu or weight basal values and for the differences by groups. The data on number of PMN cells in the ME sample were analysed by the ANOVA. When the ANCOVA or ANOVA P-value was significant (P < 0.05), contrast between groups was made by use of the Tukey-Kramer test to adjust the type I experiment-wise error.

3. Results

3.1. In vitro studies

For H. influenzae, median MICS/MBCs were 1/1 and 4/4 mg/l, for amoxicillin/clavulanic acid and erythromycin, respectively. For S. pneumoniae, median MICS/MBCs were 1/1 and 0.12/0.12 mg/l for amoxicillin/clavulanic acid and erythromycin, respectively. For amoxicillin/clavulanic acid (2/1) data are referred as the amoxicillin concentration.

3.2. Experimental otitis and therapeutic efficacy

Since no significant differences could be found between the administration or not of acetaminophen in the different groups, results have been grouped according to the antibiotic regimen administered and in one untreated control group.

After inoculation of S. pneumoniae (6.24 ± 0.07 log10 cfu) and H. influenzae (4.78 ± 0.15 log10 cfu) bilateral AOM was observed in all ears from animals which did not receive antibiotics with 100% of culture positive ME samples. S. pneumoniae was isolated in 93.3% and H. influenzae in 96.7% of the samples with bacterial counts (log10 cfu) of 3.10 ± 1.10 and 5.40 ± 1.18, respectively. In these samples a mean polymorphonuclear cells of 8.3 ± 7.8 per field with intra- and extracellular organisms was found. Animals showed lethargy, with an important weight loss (11 ± 3.82%) and 76.7% of ears showing otorrhoea. One of the four animals from which blood culture was taken was positive for both organisms inoculated.

Table 1 presents the comparative therapeutic results. Any dose of amoxicillin/clavulanic acid showed a significant (P < 0.001) reduction in the number of culture-positive ME specimens and in the colony counts of both organisms compared with control animals. Erythromycin, at any dose, did not significantly reduce the number of culture-positive ME specimens due to its failure to eradicate H. influenzae. Erythromycin significantly (P < 0.001) reduced S. pneumoniae colony counts but H. influenzae counts remained at levels similar to the control group regardless of the dose administered (from 2.5 to 50 mg/kg).

Table 2 shows otoscopic results and presence of otorrhoea. All ears from the control group showed AOM with a high percentage of ears with otorrhoea while more than 90% of those treated with any dose of any antibiotic showed OME, the animals having a very low percentage
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No. of ears</th>
<th>No. of ME a culture-positive samples (%)</th>
<th>Bacterial counts log10 cfu b mean (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. pneumoniae</td>
<td>H. influenzae</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>28 (93.3)</td>
<td>29 (96.7)</td>
<td>30 (100)</td>
</tr>
<tr>
<td>A/C</td>
<td>1.5 c</td>
<td>24</td>
<td>2 (8.3)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>A/C</td>
<td>2.5 c</td>
<td>24</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>A/C</td>
<td>10 c</td>
<td>24</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2.5</td>
<td>24</td>
<td>0 (0.0)</td>
<td>22 (91.7)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10</td>
<td>24</td>
<td>0 (0.0)</td>
<td>21 (87.5)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>20</td>
<td>24</td>
<td>0 (0.0)</td>
<td>24 (100)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50</td>
<td>24</td>
<td>0 (0.0)</td>
<td>22 (91.7)</td>
</tr>
</tbody>
</table>

a Middle ear washing fluid.
b Values are the mean ± S.D. of the number of colonies recovered from 1 ml of washing fluid.
c Referred to amoxicillin.
d Differences statistically significant vs. controls.
e Differences statistically significant vs. treated groups with erythromycin.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No. of ears</th>
<th>Otoscopic examination (%)</th>
<th>Otorrhoea (%)</th>
<th>% Body weight loss (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>30 (100)</td>
<td>0 (0.0)</td>
<td>23 (76.7)</td>
<td>11.2 (3.8)</td>
</tr>
<tr>
<td>A/C</td>
<td>1.5 d</td>
<td>24</td>
<td>2 (8.3)</td>
<td>22 (91.7)</td>
<td>2.1 (3.1)</td>
</tr>
<tr>
<td>A/C</td>
<td>2.5 d</td>
<td>24</td>
<td>0 (0.0)</td>
<td>24 (100)</td>
<td>5.3 (3.6)</td>
</tr>
<tr>
<td>A/C</td>
<td>10 d</td>
<td>24</td>
<td>0 (0.0)</td>
<td>24 (100)</td>
<td>3.3 (2.8)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2.5</td>
<td>24</td>
<td>2 (8.3)</td>
<td>22 (91.7)</td>
<td>5.5 (2.8)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10</td>
<td>24</td>
<td>0 (0.0)</td>
<td>24 (100)</td>
<td>2.7 (2.1)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>20</td>
<td>24</td>
<td>1 (4.2)</td>
<td>23 (95.8)</td>
<td>3.0 (5.0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50</td>
<td>24</td>
<td>0 (0.0)</td>
<td>24 (100)</td>
<td>3.0 (5.0)</td>
</tr>
</tbody>
</table>

a All parameters were referred at day 2 except otorrhoea (day 1).
b Acute otitis media.
c Otitis media with effusion.
d Referred to amoxicillin.
e Differences statistically significant vs. controls.
f Differences statistically significant vs. amoxicillin/clavulanic 1.5 mg/kg, erythromycin 20 and 50 mg/kg.

Table 3

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dose (mg/kg)</th>
<th>Serum C 15 (mg/l) b</th>
<th>MEF c Concentration (mg/l)</th>
<th>Penetration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/C</td>
<td>1.50.3</td>
<td>2.58 ± 0.330/0.52 ± 0.05</td>
<td>1.170/0.14</td>
<td>45.32/6.9</td>
</tr>
<tr>
<td>A/C + ACE c</td>
<td>50</td>
<td>–</td>
<td>1.210/1.12</td>
<td>46.9/2.1</td>
</tr>
<tr>
<td>A/C</td>
<td>2.50.5</td>
<td>5.49 ± 0.480/0.86 ± 0.16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A/C</td>
<td>10</td>
<td>23.92 ± 2.76/2.96 ± 1.39</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2.5</td>
<td>0.81 ± 0.14</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10</td>
<td>1.77 ± 0.43</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>20</td>
<td>4.87 ± 1.72</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50</td>
<td>5.66 ± 0.74</td>
<td>2.30</td>
<td>40.6</td>
</tr>
<tr>
<td>Erythromycin + ACE c</td>
<td>50</td>
<td>–</td>
<td>2.48</td>
<td>43.8</td>
</tr>
</tbody>
</table>

a Middle ear fluid.
b Values are means ± S.D. of six animals measured 15 min after administration for each antibiotic and dose.
c Acetaminophen.
of otorrhoea (0–20.8%). Non-significant lower numbers of polymorphonuclear cells were found in the ME of animals treated with antibiotics compared with untreated controls.

All antibiotic-treated groups had lost less weight by day 2 compared with the control group (P < 0.001) (Table 2).

3.3. Pharmacokinetic and pharmacodynamic data

Serum levels of acetaminophen after administration of 50 mg/kg were 39.6 ± 8.2, and 24.7 ± 4.4 μg/ml at 15 and 30 min, respectively. Table 3 shows serum and ME pharmacokinetic data. The ME antibiotic penetration (expressed as percentage in relation to serum concentration at 15 min) was similar for both antibiotics. The concomitant administration of acetaminophen did not influence the antibiotic concentration in ME fluid.

Table 4 shows the pharmacodynamic analysis in relation to the organisms inoculated. The favourable therapeutic and bacteriological results achieved with amoxicillin/clavulanic acid were related to serum ΔT > MIC higher than 11% of the dosing interval and ME inhibitory quotients ≥1.17 for both pathogens. Erythromycin changed the initial AOM pattern to OME by eradicating the organisms inoculated. The favourable therapeutic and bacteriological efficacy obtained by both drugs at any dose achieved a sterile OME in children with approximately 90% of cases of post-treatment OME resolved within 3 months [20].

From the bacteriological perspective, amoxicillin/clavulanic acid was able to eradicate both organisms in the mixed infection but the macrolide did not eradicate H. influenzae, even at high doses. It has been speculated that β-lactams, due to their mechanism of action, could induce greater inflammatory response than macrolides [21,22]. This inflammatory response could be responsible for the higher ME concentrations (achieving H. influenzae peri-MIC concentrations) that produces bacterial eradication. In the previously reported model [6], the increase in amoxicillin/clavulanic acid ME concentrations by the increased inflammation induced by S. pneumoniae allowed the β-lactam to eradicate H. influenzae even at low doses.

In this AOM model, amoxicillin/clavulanic acid administered at 1.5 mg/kg (referred as amoxicillin) achieved ME concentrations higher than those obtained after the administration of doses three times higher (5 mg/kg) in an OME model (1.17 μg/ml versus 0.95 μg/ml with 45% versus 10% penetration in relation to serum concentrations) [7]. This was not the case for the macrolide, since the penetration of erythromycin into the ME in this AOM (mixed) model was not higher than the one achieved in an OME model caused by H. influenzae alone (40.6% versus 43.6%).

### Table 4

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dose (mg/kg)</th>
<th>Serum C&lt;sub&gt;90&lt;/sub&gt;/MIC</th>
<th>H. influenzae</th>
<th>ME&lt;sub&gt;90&lt;/sub&gt; concentration/MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/C</td>
<td>2.5</td>
<td>2.58</td>
<td>2.58</td>
<td>54 (11.2)</td>
</tr>
<tr>
<td>A/C</td>
<td>5.49</td>
<td>5.49</td>
<td>72 (15.0)</td>
<td>72 (15.0)</td>
</tr>
<tr>
<td>A/C</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.92</td>
<td>23.92</td>
<td>114 (23.7)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2.5</td>
<td>6.75</td>
<td>0.20</td>
<td>96 (20.0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10</td>
<td>14.75</td>
<td>0.44</td>
<td>120 (25.0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>20</td>
<td>40.58</td>
<td>1.22</td>
<td>264 (55.0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50</td>
<td>47.17</td>
<td>1.41</td>
<td>276 (57.5)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50 + ACE&lt;sub&gt;b&lt;/sub&gt;</td>
<td>50</td>
<td>47.17</td>
<td>1.41</td>
</tr>
</tbody>
</table>

<sup>a</sup> Middle ear fluid  
<sup>b</sup> Referred to amoxicillin  
<sup>c</sup> Acetaminophen
increase the dose in infections caused by critical factor for amoxicillin/clavulanic acid, necessitating activity such as acetaminophen, although some pharmacokinetic interactions between antibiotics and non-steroidal anti-inflammatory drugs have been described in experimental models and exceptionally in humans [23-26].

The results of this study suggest that bacterial eradication in otitis is dependent on two facts: the antibiotic concentration in ME and the MIC of the infecting organisms. With respect to antibiotic concentrations in ME fluid, the penetration of the β-lactam is dependent on the inflammatory response that the infecting organism induces, and this is the critical factor for amoxicillin/clavulanic acid, necessitating an increase the dose in infections caused by H. influenzae that induces OME. With respect to the MIC for the infecting pathogen, in Spain, MIC90 of amoxicillin/clavulanic acid for both organisms are within the susceptibility range (≥2 mg/l) while for erythromycin values are ≥64 and 8 mg/l for S. pneumoniae and H. influenzae, respectively [17,18]. Therefore, although erythromycin has the advantage of a high penetration in ME regardless of the inflammatory situation, in Spain, MIC90 values of erythromycin compromise its therapeutic efficacy in otitis media; susceptibility and not antibiotic penetration being the critical factor for efficacy. For this reason it is important to know local patterns of resistance when choosing an antibiotic for empirical treatment of otitis media. Acetaminophen did not prevent the appearance of post-inflammatory condition with persistence of H. influenzae not eradicated by erythromycin sub-MIC concentrations in ME fluid. None of these conditions were modified by the concomitant administration of an analgesic drug with weak anti-inflammatory activity such as acetaminophen, although some pharmacokinetic interactions between antibiotics and non-steroidal anti-inflammatory drugs have been described in experimental models and exceptionally in humans [23-26].

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