Does Acetaminophen Interfere in the Antibiotic Treatment of Acute Otitis Media Caused by a Penicillin-Resistant Pneumococcus Strain? A Gerbil Model

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ABSTRACT

The possible interference of acetaminophen, combined with antibiotics, in the treatment of acute otitis media (AOM) caused by a penicillin-resistant (minimal inhibitory concentration [MIC], 2 μg/mL), amoxicillin/clavulanic acid– and erythromycin-sensitive pneumococcus was evaluated in a gerbil model. Animals were challenged with 5×10^6 bacteria in each ear through transbullar instillation. Acetaminophen was administered s.c. at 50 mg/kg 30 min before each antibiotic dose. Amoxicillin/clavulanic acid and erythromycin (2.5 and 10 mg/kg) were administered s.c. at 2, 10, and 18 h after inoculation. Samples were obtained from the middle ear (ME) on day 2 after inoculation for bacterial count. The overall results showed no difference between animals that received acetaminophen, with or without antibiotics, and those that did not receive acetaminophen. The antibiotic concentrations in the ME were practically identical in both groups of animals, so acetaminophen did not interfere with the pharmacokinetics of antibiotics in the ME. However, both antibiotics significantly reduced the number of culture-positive and the bacterial concentration in ME samples when compared with antibiotic-untreated animals. Both antibiotics, whether combined with acetaminophen or not, lowered the number of AOM to <25%, but >75% of animals presented otitis media with effusion, and no differences were shown between groups. A high rate of bacterial eradication from the ME corre-lated with antibiotic serum concentrations being over the MIC of the infecting organism for only >15% of the dose interval and with an ME concentration exceeding the MIC by a factor of 1.7. In this experimental model, acetaminophen had neither a synergistic nor an antagonistic effect on the antibiotics tested. (*Pediatr Res* 54: 913–918, 2003)

Abbreviations

AOM, acute otitis media
BHIE, brain-heart infusion broth enriched with 5% horse serum
C$_{15 min}$, concentration at 15 min after antibiotic administration
CFU, colony-forming units
MBC, minimal bactericidal concentration
ME, middle ear
MEF, middle ear fluid without washing
MEWF, middle ear washing fluid
MIC, minimal inhibitory concentration
MIC$_{50}$, minimal concentration inhibiting 50% of the strains tested
MIC$_{90}$, minimal concentration inhibiting 90% of the strains tested
OME, otitis media with effusion

Acute otitis media (AOM) is one of the most frequently diagnosed infectious diseases of childhood, and the number of office visits for this pathology is increasing in many countries

(1). One of the most common approaches to treating AOM in children is to administer an antibiotic to eradicate the pathogen, as well as an anti-inflammatory or analgesic drug such as acetaminophen (2, 3). Anti-inflammatory activity is mainly exerted through the inhibition of COX-1 and COX-2 isoenzymes that catalyze the rate-limiting step of prostaglandin synthesis (4). Recent data suggest that the little anti-inflammatory activity of acetaminophen is due to its activity against COX-3 (that shows <20% activity of COX-1 and COX-2 and is present at approximately 25% of the level of COX-1) (4). However, other agents to which COX-1, COX-2,

and COX-3 are sensitive as ibuprofen (4), given at low doses for antipyresis, have shown to be proinflammatory (increasing neutrophil influx and cytokine levels) to a level that may have adverse clinical translations in *Streptococcus pneumoniae* infections (5).

The organisms most frequently involved in AOM are *S. pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*; therefore, antibiotics active *in vitro* against these bacteria are recommended (6). Amoxicillin/clavulanic acid is active against all three organisms and is one of the most frequently prescribed antimicrobial drugs in Spain (7). In countries where macrolide-resistant pneumococci are not prevalent, erythromycin or the new macrolides are also recommended (6, 8). Although most antibiotic treatments usually resolve the AOM, up to 70% of children develop otitis media with effusion (OME) (9, 10). The reason for this complication is not known, but some antibiotics may, by inducing a rapid bacterial lysis, contribute to an inflammatory response that could be responsible for the discharge (11).

The aim of this work was, first, to determine the possible interference of acetaminophen, combined with antibiotics, in the clinical and bacteriologic efficacy in AOM, in the antibiotic pharmacokinetic parameters, and in the development of OME. The second objective of this study was to find out whether antibiotics with different mechanisms of action and immuno-modulatory properties (a β-lactam and a macrolide) induce, after clearing the pathogen, different rates of OME.

METHODS

Bacteria. A strain of *S. pneumoniae* serotype 23 F (minimal inhibitory concentration [MIC] for benzylpenicillin = 2 μg/mL) isolated from a bacteremic patient was used.

Pharmacologic compounds. Propacetamol chlorohydrate (Pro-efferalgan), a pro-drug of acetaminophen, was purchased from Upsa Laboratories (Agent-Lot et Garonne, France). Amoxicillin trihydrate, lithium clavulanate (SmithKline Beecham Pharmaceuticals, Worthing, England), and erythromycin (Sigma Chemical Co., St. Louis, MO, U.S.A.) were used for *in vitro* studies. For *in vivo* (therapeutic) use, the contents of commercial vials [Augmentin: amoxicillin/clavulanic acid, 5/1 (SmithKline Beecham S.A., Toledo, Spain) and Pantomicina: erythromycin lactobionate (Abbott Laboratories, S.A., Madrid, Spain)] were reconstituted in nonpyrogenic sterile distilled water to the desired concentrations.

In vitro *studies.* MICs and minimal bactericidal concentrations (MBCs) were determined by a microdilution broth method following National Committee for Clinical Laboratory Standards procedures (12, 13). The median of three separate determinations was used as the MIC and MBC values of each antibiotic.

Animals. Eight- to 9-wk-old adult female Mongolian gerbils (*Meriones unguiculatus*) that weighed 49 $\pm$ 5 g each were purchased from the Center d'Élevage R. Janvier (Le Genest, St. Isle, France). They were given free access to food and water and were housed in a protected unit with a slight negative pressure and with a 12-h light and 12-h dark cycle. For invasive procedures, animals were anesthetized with 50 mg/kg

body wt ketamine (Ketolar; Parke-Davis, Barcelona, Spain) and 13 mg/kg of xylazine (Rompun; Bayer, Leverkusen, Germany) by intramuscular route.

The study was performed in accordance with prevailing regulations regarding the care and use of laboratory animals in the European Community (14) and approved by the Review Board for Animal Experimentation of Fundación Jiménez Díaz.

Experimental otitis. On the day of experimentation, *S. pneumoniae* was incubated for 4 h at 35°C in a 5% CO_2 atmosphere in brain-heart infusion broth (Oxoid, Ltd, Basingstoke, Hampshire, England) enriched with 5% horse serum (BHIE; bioMérieux, Marcy-L'Étoile, France). Animals were inoculated bilaterally, as previously reported (15), with ~5 $\times$ 10^6 CFU of *S. pneumoniae* per 20 μL, which was introduced directly in the middle ear (ME) bulla. The number of viable bacteria in the different inocula was determined by colony counting. The tympanic membrane was left intact and swelled without rupture during the inoculation. A normal tympanic aspect and correct inoculation were verified by an otolaryngologist by direct otomicroscopy using an operating microscope. AOM was defined as otorrhea through a perforation in the tympanic membrane and/or inflammatory signs with changes in the membrane's normal yellowish-pink appearance to a gray, 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 or ME fluid with or without signs of negative ME pressure. Three animals (six ears) were inoculated with 20 μL of BHIE/ear to find out the possible role of broth as inductor of otitis media. For exploring the presence of bacteremia in infected animals, blood cultures were performed in four animals 20 h after bacterial inoculation.

Treatment regimen and efficacy studies. Each antibiotic was tested at two doses (2.5 and 10 mg $\cdot$ kg^{-1} $\cdot$ dose^{-1}) and was administered s.c. in 500 μL at 2, 10, and 18 h after inoculation. Antibiotics were administered alone or combined with 50 mg/kg of propacetamol administered s.c. 30 min before each antibiotic dose, in experiments carried out in parallel. Two groups of antibiotic-untreated animals received apyrogenated sterile distilled water instead of antibiotic, one of which also received propacetamol. Groups of 12 animals per treatment and control groups were included. Treated and control animals were studied longitudinally for otorrhea, weight, and behavior. The otoscopic aspect and ME samples were obtained from both ears on day 2 after inoculation by washing the ME fossa with 20 μL of saline injected and withdrawn *via* the epitympanic membrane with a 0.33-mm needle to determine bacterial counts in ME washing fluid (MEWF). Shortly after sampling, aliquots of serial 10-fold dilutions in saline were plated onto sheep blood agar and incubated for 24 h at 35°C in a 5% CO_2 atmosphere. Bacterial counts are expressed as log$_{10}$' colony-forming units (CFU)/20 μL; the lowest detectable bacterial count was 4 CFU/20 μL (0.60 log$_{10}$ CFU/20 μL). For evaluating the presence of polymorphonuclear leukocytes, 3 μL of MEWF was extended over a 6-cm^2 slide surface to be Gram stained and observed under a high-power ($\times$1000) mi-

croscope. The number of cells in 10 fields was calculated and expressed as mean (SD) per field.

Pharmacokinetic studies. Serum levels of acetaminophen were determined in healthy animals 15 and 30 min after s.c. administration of 50 mg/kg of propacetamol, by Fluorescence Polarization Immunoassay in an Axsym autoanalyser (Abbott Científica, Madrid, Spain). Groups of six animals per sample time were killed with CO_2 and were exsanguinated by intra-cardiac 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 s.c. injection of the same doses used for the treatment. Groups of six animals per dose and blood sample time (15, 30, 60, and 120 min after drug administration) were killed as described for acetaminophen.

Antibiotic concentrations in ME fluid without washing (MEF) were also determined in groups of 10 animals bilaterally inoculated under the same conditions as previously indicated in the experimental otitis model. Single doses of 2.5 mg/kg of either antibiotic, alone and combined with propacetamol, were administered s.c. 46 h after bacterial inoculation. MEF samples were obtained 90 min later, *via* the epitympanic membrane, with a 0.33-mm needle. Aliquots of MEF samples having >2 μL of exudate were pooled and frozen at $-70°$C until determination of antibiotic levels. This procedure permits collection of MEF samples at a time when effusion is consistently abundant with high bacterial density and without otorrhea.

Antibiotic concentrations were determined by microbiological assay with *Micrococcus luteus* ATCC 9341 and *Klebsiella pneumoniae* NCTC 11228. Standard curves for determination of antibiotic concentrations were obtained from standard solutions prepared in pooled gerbils' sera for serum levels and in 0.1 mol/L phosphate buffer (pH 6.0) for MEF. The variability of the assay for individual samples was <10%. Antibiotic concentration-time curves for each antimicrobial were analyzed by a noncompartmental approach using the Win-Nonlin program (Pharsight, Mountainview, CA, U.S.A.). Half-life was calculated by the expression $t_{1/2} = (\ln 2)/\text{Kel}$, where Kel is the apparent elimination rate constant calculated by least square regression from at least three time-point values. Time over MIC was calculated graphically from the semilogarithmic representation of the concentration-time curve and the regression line representing the Kel. The relationship between the maximum antibiotic serum concentration obtained [concentration at 15 min after antibiotic administration ($C_{15\ min}$)] and the MIC of the infecting microorganism [serum inhibitory quotient ($C_{15\ min}$/MIC)] and the relationship between the MEF antibiotic concentration after 90 min of drug administration and the MIC of the infecting microorganism (MEF inhibitory quotient: MEF level/MIC) were calculated.

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. For detecting differences in eradication rates in each group, the Fisher's exact test was used, with Bonferroni correction for multiple comparisons. Otorrhea at day 1 was analyzed using the χ^2 test and the Fisher's exact test for paired comparisons with the Bonferroni correction for multiple comparisons. Bac-

terial counts were expressed in untreated and treated animals as arithmetic means in $\log_{10}$ CFU per 20 μL of MEWF, culture-negative samples being included in the calculation of means assuming a value at the detection limit. ANCOVA was used to compare the reduction of $\log_{10}$ CFU and loss of body weight at day 2 in each group controlled for the $\log_{10}$ CFU or weight basal values and for the differences by groups. ANOVA was used to compare MEWF volume and polymorphonuclear leukocyte counts. 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.

RESULTS

In vitro *studies.* The MIC/MBC of the antibiotics (referred to as amoxicillin in the case of amoxicillin/clavulanic acid) for the pneumococcal strain used was 1/1 and 0.12/0.12 μg/mL for amoxicillin and erythromycin, respectively.

Experimental otitis. Animals inoculated with BHIE showed neither clinical nor otoscopic signs of disease and at day 2; the MEWF was culture-negative. After *S. pneumoniae* inoculation ($6.54 \pm 0.27\ \log_{10}$ CFU), bilateral AOM was obtained in 98% of all ears from animals that did not receive antibiotics at day 2, with 100% of culture-positive MEWF samples. Most MEWF specimens contained between 7.5 and 10.8 polymorphonuclear cells per field with intra- and extracellular organisms. Animals showed lethargy, with an important weight loss and 66.6% of otorrheic ears. There were no differences between animals that received acetaminophen and those that did not receive it. Blood cultures were positive for the organisms in three of the four inoculated animals from which blood samples were taken.

Therapeutic efficacy in experimental otitis. All animals that received antibiotics at any dose were active and showed normal behavior. Table 1 presents the comparative therapeutic results for controls and antibiotic-treated animals. Both antibiotics, at any dose, with or without acetaminophen, significantly reduced the number of culture-positive MEWF specimens and the number of organisms when compared with antibiotic-untreated animals ($p < 0.001$). No statistical differences were found between antibiotic-treated groups (combined or not with acetaminophen).

Table 2 presents the data from the otoscopic examination, otorrhea, volume of MEWF recovered, presence of polymorphonuclear cells, and weight loss. All animals that received antibiotics had low percentages of AOM, and few had positive cultures. Most antibiotic-treated animals developed posttreatment OME, and no differences were detected between doses or antibiotics (with or without acetaminophen). All antibiotic-treated animals showed lower percentages of otorrhea compared with untreated controls, although differences were significant only for both doses of amoxicillin/clavulanic acid ($p < 0.05$). In addition, animals treated with the high dose of amoxicillin/clavulanic acid had significantly less otorrhea than animals treated with the low dose of erythromycin ($p < 0.001$). The presence of otorrhea correlated directly with AOM, with no differences being shown between animals that received

Table 1. *Outcome on day 2 of AOM caused by penicillin-resistant S. pneumoniae treated with acetaminophen, amoxicillin/clavulanic acid, and erythromycin*

Group	No. of animals	No. (%) of animals with culture-positive ME samples			No. (%) of ME culture-positive samples	Bacterial counts $\log_{10}$ CFU* [mean (SD)]
		Bilateral	Unilateral	Total		
Untreated controls	12	12 (100)	0 (0.0)	12 (100)	24 (100)	3.23 (1.01)
ACE, 50 mg/kg	12	12 (100)	0 (0.0)	12 (100)	24 (100)	3.14 (0.93)
A/C, 2.5 mg/kg†	12	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)‡	<0.60‡
A/C, 2.5 mg/kg + ACE, 50 mg/kg	12	0 (0.0)	1 (8.3)	1 (8.3)	1 (4.2)‡	0.64 (0.25)‡
A/C, 10 mg/kg	12	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)‡	<0.60‡
A/C, 10 mg/kg + ACE, 50 mg/kg	12	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)‡	<0.60‡
ERY, 2.5 mg/kg	12	0 (0.0)	1 (8.3)	1 (8.3)	1 (4.2)‡	0.66 (0.36)‡
ERY, 2.5 mg/kg + ACE, 50 mg/kg	12	1 (8.3)	2 (16.7)	3 (25.0)	4 (16.7)‡	0.82 (0.64)‡
ERY, 10 mg/kg	12	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)‡	<0.60‡
ERY, 10 mg/kg + ACE, 50 mg/kg	12	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)‡	<0.60‡

ACE, acetaminophen; A/C, amoxicillin/clavulanic acid; ERY, erythromycin.

* Values are the mean $\log_{10}$ CFU in 20 μL of washing fluid. Data for culture-negative samples were included in the calculation of means by assuming a value at the detection limit (0.60 $\log_{10}$ CFU).

† Referred as amoxicillin.

‡ Differences between values for treated with antibiotics *vs* those for antibiotic-untreated animals were statistically significant ($p < 0.0001$).

Table 2. *Relationship between otoscopic examination, presence of otorrhea, volume (μL), and polymorphonuclear leukocytes of middle ear washing fluid recovered, and variation in body weight in an experimental otitis media caused by S. pneumoniae*

Group	Otoscopic examination (%)		Otorrhea (%)	Volume [mean (SD)]	PMN [mean (SD)]	% Body weight loss [mean (SD)]
	AOM	OME				
Untreated controls	23 (95.8)	1 (4.2)	62.5	21.8 (6.9)	7.5 (10.7)	−11.6 (2.1)
ACE, 50 mg/kg	24 (100)	0 (0.0)	70.8	22.2 (10.1)	10.8 (11.7)	−10.5 (2.1)
A/C, 2.5 mg/kg†	0 (0.0)	24 (100)	12.5‡	21.1 (8.9)	1.8 (2.4)‡	−2.5 (2.6)‡
A/C, 2.5 mg/kg + ACE, 50 mg/kg	4 (16.7)	20 (83.3)	12.5‡	28.8 (13.4)	1.6 (1.7)‡	−2.8 (2.9)‡
A/C, 10 mg/kg	1 (4.2)	23 (95.8)	0.0‡§	25.1 (8.7)	2.8 (1.5)‡	−4.6 (2.2)‡
A/C, 10 mg/kg + ACE, 50 mg/kg	0 (0.0)	24 (100)	0.0‡§	24.9 (11.2)	1.9 (2.2)‡	−2.6 (3.7)‡
ERY, 2.5 mg/kg	6 (25.0)	18 (75.0)	58.3	21.4 (4.7)	1.4 (1.7)‡	−3.6 (3.3)‡
ERY, 2.5 mg/kg + ACE, 50 mg/kg	4 (16.7)	20 (83.3)	29.2	23.0 (5.9)	1.6 (2.6)‡	−3.7 (3.3)‡
ERY, 10 mg/kg	0 (0.0)	24 (100)	25.0	21.2 (7.7)	1.9 (1.6)‡	−3.7 (2.4)‡
ERY, 10 mg/kg + ACE, 50 mg/kg	2 (8.3)	22 (91.7)	33.3	23.5 (8.9)	1.9 (2.4)‡	−3.4 (3.1)‡

PMN, poly morphonuclear leukocytes.

* Data referred at day 2 except otorrhea (day 1).

† Referred as amoxicillin.

‡ Differences between values for treated with antibiotics *vs.* those for antibiotic-untreated animals were statistically significant ($p < 0.01$ for otorrhea and $p < 0.05$ for PMN and weight loss).

§ Statistically significant differences versus ERY 2.5 mg/kg.

acetaminophen and those that did not receive it. As far as the volume of MEWF is concerned, there were no differences between groups. All antibiotic-treated animals had lower amounts of polymorphonuclear cells in the MEWF compared with controls ($p < 0.05$), with no differences between groups. With regard to loss of body weight (as recorded on day 2), all antibiotic-treated animals lost less weight than the antibiotic-untreated controls ($p < 0.001$) without differences being shown between groups.

Pharmacokinetic and pharmacodynamic data. Serum levels of acetaminophen after administration of 50 mg/kg were 39.6 ± 8.2 μg/mL and 24.7 ± 4.4 μg/mL at 15 and 30 min, respectively. Table 3 presents the serum and MEF (with or without acetaminophen) pharmacokinetic data for amoxicillin/clavulanic acid and erythromycin. Serum half-lives for amoxicillin/clavulanic acid were shorter than for erythromycin. ME antibiotic concentrations were lower for erythromycin than for amoxicillin/clavulanic acid. Nevertheless, penetration of amoxicillin/clavulanic acid (approximately 31/25%) was lower than that of erythromycin (approximately 53%) in relation to

serum concentrations at 15 min. The administration of acetaminophen in addition to the 2.5 mg/kg dose of either amoxicillin/clavulanic acid or erythromycin did not influence the antibiotic concentration in the ME (as compared with animals that received only antibiotic).

Table 4 presents the pharmacodynamic analysis in relation to the organism inoculated and the percentage of bacterial eradication. Serum inhibitory quotients ($C_{15\ min}$/MIC) were >5 and time above MIC >70 min (*i.e.* 15–25% of the dose interval) for both antibiotics, and these values correlated with bacterial eradication (>83%). The MEF inhibitory quotients (antibiotic concentration in the MEF/MIC) for the lower doses of both antibiotics were higher than 1.

DISCUSSION

For the present study, we selected a penicillin-resistant, erythromycin-sensitive pneumococcal strain serotype 23F, because these characteristics are representative of the strains commonly found to be responsible for AOM in children (7,

Table 3. *Pharmacokinetic data for A/C and ERY in serum and MEF in relation to S. pneumoniae susceptibility*

| | Serum | | MEF | |
| | C$_{15\,min}$ (mean ± SD; μg/mL) | Half-life (min) | Concentration (μg/mL)* | Penetration %† |
Antibiotic [dose (mg/kg)]				
A/C (2.5/0.5)	5.49 ± 0.48/0.86 ± 0.16	22.8/25.2	1.70/0.23	30.9/26.7
A/C (2.5/0.5) + ACE (50)	–	–	1.70/0.22	30.9/24.4
A/C (10/2)	23.92 ± 2.76/2.98 ± 1.39	21.6/16.8	–	–
ERY (2.5)	0.81 ± 0.14	27.6	0.44	54.3
ERY (2.5) + ACE (50)	–	–	0.41	50.6
ERY (10)	1.77 ± 0.43	28.2	–	–

* Antibiotic concentration in MEF 90 min after drug administration.

† In relation to the serum concentration at 15 min.

Table 4. *Pharmacodynamic data for A/C* and ERY in serum and MEF in relation to S. pneumoniae susceptibility*

| | Serum | | | |
Antibiotic [dose (mg/kg)]	C$_{15min}$/MIC	t > MIC min (% dose interval)	MEF concentration†/MIC	Eradication %
A/C (2.5)	5.49	72 (15.00)	1.70	100
A/C (2.5) + ACE (50)	–	–	1.70	95.8
A/C (10)	23.92	114 (23.75)	–	100
A/C (10) + ACE (50)	–	–	–	100
ERY (2.5)	6.75	96 (20.00)	3.66	95.8
ERY (2.5) + ACE (50)	–	–	3.42	83.3
ERY (10)	14.75	120 (25.00)	–	100
ERY (10) + ACE (50)	–	–	–	100

* Referred as amoxicillin.

† Antibiotic concentration in MEF 90 min after drug administration.

16). The MIC for erythromycin represents MIC$_{50}$ in Spain (17), although significantly higher erythromycin resistance prevalence is found in patients with otitis (47%), with an MIC$_{90}$ >64 μg/mL as a result of the prevalence of the MLS$_B$ constitutive phenotype (17).

Low antibiotic doses were chosen to obtain in gerbils serum levels similar to those obtained in children after standard oral doses of both antibiotics (18). For investigating whether higher doses could induce different rates of OME, a second regimen of each antibiotic was also used, so as to attain in gerbils serum concentrations similar to those attainable in children treated with high doses (18–20).

Amoxicillin/clavulanic acid and erythromycin both were efficacious, at any dose, in eradicating this penicillin-resistant, erythromycin-sensitive pneumococcus from the ME and in avoiding the occurrence of AOM as compared with controls. In addition, antibiotic-treated animals were active and had low loss of body weight as compared with controls. Such efficacy was obtained with antibiotic serum concentrations exceeding the MIC of the pathogen by >70 min (*i.e.* from 15% to 25% of the dosing interval), a value lower than that previously suggested (21). However, these data corroborated the results obtained by our group in a previous animal model of AOM caused by penicillin-resistant pneumococci treated with low doses of amoxicillin (22). The concentrations of both amoxicillin/clavulanic acid and erythromycin in the ME, as well as the percentages of penetration, were very similar to rates reported in children with AOM (18–20). The inhibitory quotient, calculated taking into account the ME concentrations (antibiotic concentration in MEF/MIC) was >1.6, thus indicating that the antibiotic concentration in the infectious foci

was higher than the MIC of the offending penicillin-resistant, erythromycin-sensitive pneumococcus. In this study, as has been demonstrated previously (23), the main pharmacodynamic parameter predicting antibiotic efficacy in otitis media is the MEF inhibitory quotient. Clinical and bacteriologic success was obtained when the antibiotic concentration in the MEF exceeded the MIC of the pathogen, which may, to some extent, be independent of serum levels (23, 24).

Most animals treated with antibiotics developed OME, regardless of whether they were treated with amoxicillin/clavulanic acid or with erythromycin. The immunomodulatory effect of erythromycin is well known (25, 26), and because its mechanism of action is different from that of β-lactam antibiotics, macrolides could induce a lower inflammatory response than β-lactams do (27–29). The hypothesis of this study was that such differences could affect the rate of OME, but it has not been corroborated in this experimental study, as had occurred in a previous study carried out using clarithromycin in the chinchilla model (30).

The administration of acetaminophen concomitantly with either amoxicillin/clavulanic acid or erythromycin did not affect the antibiotic concentration in the ME; therefore, we did not study the possible interference in serum pharmacokinetics. Some pharmacokinetic interactions between antibiotics and nonsteroidal anti-inflammatory or antipyretic drugs have been described in experimental models but very rarely in humans (31–33). Carsenti-Etesse *et al.* (34) reported some interference of acetaminophen in the bactericidal activity of cefixime against *S. pneumoniae*. In our experimental model, no significant differences were found in clinical, otoscopic, or bacteriologic parameters between antibiotic-untreated animals that

received acetaminophen and those that did not receive it, and this could be related to the weak anti-inflammatory activity of the compound (35). Furthermore, there were no significant differences in antibiotic efficacy between animals that did and did not receive acetaminophen. Nevertheless, this may be a power issue because in the lower erythromycin dose group, worse rates (without significant statistical differences) are found in the presence of acetaminophen (16.7% *versus* 4.2%), and the only failure obtained with the low dose of amoxicillin/clavulanic acid received acetaminophen concomitantly. At higher doses, antimicrobial effects may be too potent to detect any possible interference. Further studies are warranted because low doses are not uncommon as a result of poor compliance or to a decreased susceptibility of the infecting organism. In addition, acetaminophen did not prevent the appearance of OME, suggesting that no clear benefit may be expected from combining antibiotics with acetaminophen. Nonetheless, because acetaminophen may alleviate ear pain and decrease body temperature (35), its use, alone or combined with an antibiotic, may be advocated.

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