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Impact of Ibuprofen Therapy in the Outcome of Experimental Pneumococcal Acute Otitis Media Treated With Amoxicillin or Erythromycin

GEMA DEL PRADO, CARMINA MARTÍNEZ-MARÍN, LORENA HUELVES, MATILDE GRACIA, VIOLETA RODRÍGUEZ-CERRATO, RICARDO FERNÁNDEZ-ROBLAS, CARMEN PONTE, CARLOS CENJOR, AND FRANCISCO SORIANO

Unit for Research in Medical Microbiology and Antimicrobial Chemotherapy [G.P., C.M.-M., L.H., M.G., V.R.-C., R.F.-R., C.P., F.S.] and Service of Otolaryngology [C.C.], Fundación Jiménez Díaz-Capio, 28040 Madrid, Spain

ABSTRACT: The impact of ibuprofen combined with amoxicillin or erythromycin for therapy of penicillin-resistant pneumococcal acute otitis media (AOM) was evaluated in a gerbil model. Ibuprofen (at 2.5 or 7.5 mg/kg, orally) and/or amoxicillin or erythromycin (5 mg/kg each, s.c.) were administered at 5 h (early therapy, as single-dose regimen) or at 18 h (delayed therapy, five doses) postinoculation (PI). Each antibiotic alone and combined with ibuprofen was more effective administered as early regimen than as delayed treatment when evaluating the presence of otorrhea, otoscopic aspect, culture-positive and bacterial counts in middle ear (ME) samples, and loss of body weight. There was a trend for a better bacteriological outcome in animals receiving amoxicillin or erythromycin and ibuprofen, especially with the high dose. Such a dose of ibuprofen, associated with each antibiotic regimen, also preserved the animal well-being, avoiding a great weight loss in comparison to those receiving the antibiotic alone but a statistically significant difference was only observed for animals receiving delayed therapy with erythromycin and high-dose ibuprofen. In conclusion, ibuprofen combined with antibiotics seemed to improve the outcome of this experimental pneumococcal AOM. **(*Pediatr Res* 60: 555–559, 2006)**

AOM is one of the most frequently diagnosed infectious diseases and the most common reason for antibiotic prescriptions written for children in many countries (1). Most experts recommend empirical antimicrobial treatment of AOM in children to eradicate the pathogen and the use of an anti-inflammatory or analgesic drug such as ibuprofen or acetaminophen to relieve the symptoms (2,3). Acetaminophen acts as a weak inhibitor of the synthesis of prostaglandins inhibiting cyclooxygenase (COX)-2–dependent pathways (4). In contrast, ibuprofen inhibits both COX-1 and COX-2 having more potent analgesic and anti-inflammatory effects (5). We previously demonstrated using an experimental AOM model caused by *Streptococcus pneumoniae* that acetaminophen combined with amoxicillin/clavulanic acid or erythromycin had neither a synergistic nor an antagonistic effect on the antipneumococcal activity (6). Therefore, use of acetaminophen to alleviate ear pain and decrease body temperature (3) may be advocated alone or combined with an antibiotic in the management of AOM. We selected ibuprofen, a potent anti-inflammatory drug, to determine its effect when combined with antimicrobial treatment on the outcome of penicillin-resistant pneumococcal AOM in a gerbil model.

METHODS

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

Pharmacological compounds. Ibuprofen sodium salt and erythromycin (Sigma Chemical Co., St. Louis, MO) and amoxicillin trihydrate (Glaxo-SmithKline, Worthing, UK) were used for *in vitro* studies. For *in vivo* (therapeutic) use, Junifen, an ibuprofen suspension (Boots Healthcare International, Nottingham, UK), was used diluted with sterile distilled water to the desired concentrations. Commercial vials [Clamoxyl (amoxicillin), Glaxo-SmithKline, S.A., Madrid, 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. MIC and minimum bactericidal concentration values were determined for amoxicillin, erythromycin, and ibuprofen by a microdilution broth method following the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) procedures (7,8). Checkerboard broth microdilution method for antibiotic and ibuprofen synergy testing was used following recommended methods (9). A fractional inhibitory concentration index value of ≤0.5 was defined as synergism, >0.5–4 as indifference, and >4 as antagonism. All *in vitro* experiments were done in triplicate.

Animals. Eight- to 9-wk-old adult female Mongolian gerbils (*Meriones unguiculatus*) that weighed 49 ± 5 g each were purchased from the Centre 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 12-h light/dark cycles. For invasive procedures, animals were intramuscularly anesthetized with 50 mg/kg ketamine (Ketolar; Parke-Davis, Barcelona, Spain) and 13 mg/kg xylazine (Rompun; Bayer, Leverkusen, Germany).

The study was performed in accordance with prevailing regulations regarding the care and use of laboratory animals in the European Community

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Correspondence: Francisco Soriano, M.D., Ph.D., Unit for Research in Medical Microbiology and Antimicrobial Chemotherapy, Fundación Jiménez Díaz-Capio, Avenida de Reyes Católicos 2, 28040 Madrid, Spain; e-mail: fsoriano@fjd.es.

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Abbreviations: AOM, acute otitis media; **BHIE,** brain-heart infusion broth enriched with 5% horse serum; **CFU,** colony-forming unit; **ME,** middle ear; **MEWF,** middle ear washing fluid; **MIC,** minimum inhibitory concentration; **PI,** postinoculation

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Experimental otitis. An overnight culture of the organism was kept in aliquots at −70°C, and on each day of experimentation, a freshly thawed aliquot of *S. pneumoniae* was incubated for 4 h at 35°C in brain-heart infusion broth (Oxoid, Ltd., Basingstoke, UK) enriched with 5% horse serum (BHIE) (bioMérieux, Marcy-L'Étoile, France). Animals were inoculated bilaterally, as previously reported (10), with $\sim 10^6$ colony-forming units (CFU) of *S. pneumoniae* per 20 μL, which were introduced directly in the 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. The 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. Three animals (six ears) were inoculated with 20 μL of BHIE per ear to find out the possible role of broth as inductor of otitis media.

To explore 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 5 mg/kg and was administered s.c. in 500 μL. Ibuprofen was administered orally at 2.5 or 7.5 mg/kg 30 min before each antibiotic dose in experiments carried out in parallel. Different times of treatment initiation (at 5 and 18 h PI.) and number of doses (one or five at 3-h intervals) were evaluated. The first regimen (early therapy) comprised 60 animals per antibiotic distributed in six equal groups [untreated controls, ibuprofen 2.5 mg/kg, ibuprofen 7.5 mg/kg, antibiotic (amoxicillin or erythromycin), ibuprofen 2.5 mg/kg plus antibiotic, and ibuprofen 7.5 mg/kg plus antibiotic] treated in one single dose 5 h PI. The second regimen (delayed therapy) comprised six identical groups but treated in five doses (at 3-h intervals) starting 18 h PI. All therapeutic groups received the same volume administered orally and s.c. per animal with the drug dilution or alternatively with apyrogenated sterile distilled water.

Treated and control animals were studied longitudinally for the presence of otorrhea (24 h PI), change in weight and otoscopic aspect, and composition of ME fluid (48 h PI). The ME samples were obtained from both ears 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 $\log_{10}$ 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 (×1000) microscope. The number of cells in 20 fields was calculated and expressed as mean [standard deviation (SD)] per field.

Statistical analysis. To compare the otoscopic appearance, the χ^2 test for trends was used. For other qualitative variables, the Fisher exact test was used. Continuous variables were expressed as means (SD). The Kruskal-Wallis test was used to compare the loss of body weight, the reduction of $\log_{10}$ CFU/20 μL, and polymorphonuclear leukocyte counts in ME fluid among groups. 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). A p value of ≤0.05 was considered statistically significant.

RESULTS

In vitro studies. The MIC/minimum bactericidal concentration values were 1/1, 0.12/0.12, and 1024/2048 μg/mL for amoxicillin, erythromycin, and ibuprofen, respectively. The combination of ibuprofen with either amoxicillin or erythromycin was neither synergistic nor antagonist but indifferent (fractional inhibitory concentration index = 0.56–1.03).

Experimental otitis and therapeutic efficacy. Animals inoculated with BHIE showed no clinical or abnormal otoscopic signs, and at day 2 PI the MEWF cultures were negative. The blood cultures were positive in three of the four animals inoculated with the organism to explore the presence of bacteremia.

Table 1 presents the comparative findings of untreated control animals and the early treated animals with low- and high-dose ibuprofen alone and combined with amoxicillin. Early treatment with amoxicillin with and without ibuprofen significantly improved all the parameters related with outcome as compared to those observed in untreated controls and those receiving ibuprofen alone. Moreover, ibuprofen (especially in the high-dose group) associated with amoxicillin showed improved results compared to those of the antibiotic alone, although the difference was not statistically significant. All antibiotic-treated animals had significantly lower amounts of polymorphonuclear leukocytes per field in their MEWF (range, 0.81 ± 0.48–1.64 ± 1.06) compared to those animals not treated with antibiotic (range, 4.06 ± 5.1–6.19 ± 0.35).

Table 2 presents the comparative therapeutic findings of untreated controls and animals receiving delayed therapy with amoxicillin and/or combined with ibuprofen at low and high doses, administered as five doses 18 h PI. In 70–100% of animals receiving delayed therapy, otorrhea and AOM were observed. All animals receiving amoxicillin with or without ibuprofen showed significantly lower bacterial counts in the ME compared to untreated controls. In fact, animals receiving amoxicillin combined with ibuprofen showed a more pronounced reduction in bacterial counts even than those receiving the antibiotic alone. Animals receiving amoxicillin and the high dose of ibuprofen showed the lowest weight loss compared to any other group. All antibiotic-treated animals had lower amounts of polymorphonuclear leukocytes per field in

Table 1. *Outcome of AOM caused by S. pneumoniae early (5 h PI) treated with one single dose of ibuprofen (IBU) and amoxicillin (AMX)*

Group	Otorrhea (%)	No. of ears with AOM (%)	No. of ME culture-positive samples (%)	Bacterial counts $\log_{10}$ CFU/20 μL, mean ± SD	% Body weight loss, mean ± SD
Untreated controls	100	100	100	2.66 ± 0.65	12.94 ± 3.16
IBU 2.5 mg/kg	100	90	95	2.69 ± 0.72	10.76 ± 5.38
IBU 7.5 mg/kg	85	90	95	2.90 ± 0.86	12.35 ± 3.19
AMX 5 mg/kg	65*	55†	40†	1.21 ± 0.84†	3.41 ± 3.47†
AMX 5 mg/kg + IBU 2.5 mg/kg	50†	50†	35†	1.11 ± 1.00†	1.69 ± 2.67†
AMX 5 mg/kg + IBU 7.5 mg/kg	35†	55†	20†	0.85 ± 0.61†	0.92 ± 3.35†

* Significant difference ($p \leq 0.05$) from values of untreated controls and low dose of ibuprofen alone.

† Significant difference ($p \leq 0.05$) from values of non–amoxicillin-treated animals

Table 2. *Outcome of AOM caused by S. pneumoniae treated in delayed form (18 h PI) with five doses of ibuprofen (IBU) and amoxicillin (AMX)*

Group	No. of ME culture-positive samples	Bacterial counts $\log_{10}$ CFU/20 μL, mean ± SD	% Body weight loss, mean ± SD
Untreated controls	100	3.19 ± 0.73	11.40 ± 2.02
IBU 2.5 mg/kg	85	2.62 ± 1.11	9.35 ± 3.79
IBU 7.5 mg/kg	95	2.59 ± 0.97	13.18 ± 2.50
AMX 5 mg/kg	95	2.25 ± 0.78*	8.68 ± 2.04†
AMX 5 mg/kg + IBU 2.5 mg/kg	85	1.82 ± 0.92‡	9.57 ± 3.76
AMX 5 mg/kg + IBU 7.5 mg/kg	80	1.79 ± 0.93‡	6.79 ± 2.46†

* Significant difference ($p \leq 0.05$) from values of untreated controls.
† Significant difference ($p \leq 0.05$) from values of untreated controls and high dose of ibuprofen.
‡ Significant difference ($p \leq 0.05$) from all other non–amoxicillin-treated groups.

Table 3. *Outcome of AOM caused by S. pneumoniae early (5 h PI) treated with one single dose of ibuprofen (IBU) and erythromycin (ERY)*

Group	Otorrhea, %	No. ears with AOM (%)	No. of ME culture-positive samples (%)	Bacterial counts $\log_{10}$ CFU/20 μL, mean ± SD	% Body weight loss, mean ± SD
Untreated controls	95	90	100	3.42 ± 0.60	11.85 ± 3.57
IBU 2.5 mg/kg	100	100	95	3.17 ± 0.89	13.73 ± 3.60
IBU 7.5 mg/kg	100	100	100	3.65 ± 0.50	14.71 ± 1.26
ERY 5 mg/kg	65*	35*	65*	1.81 ± 1.35*	6.61 ± 4.87*
ERY 5 mg/kg + IBU 2.5 mg/kg	75*	40*	75*	1.87 ± 1.15*	6.01 ± 3.27*
ERY 5 mg/kg + IBU 7.5 mg/kg	70*	15*	70*	1.39 ± 0.93*	5.08 ± 2.81*

* Significant difference ($p \leq 0.05$) from values of non–erythromycin-treated animals.

Table 4. *Outcome of AOM caused by S. pneumoniae treated in delayed form (18 h PI) with five doses of ibuprofen (IBU) and erythromycin (ERY)*

Group	No. of ME culture-positive samples (%)	Bacterial counts $\log_{10}$ CFU/20 μL, mean ± SD	% Body weight loss, mean ± SD
Untreated controls	95	2.89 ± 0.94	11.67 ± 2.82
IBU 2.5 mg/kg	80	2.59 ± 1.25	10.13 ± 4.03
IBU 7.5 mg/kg	100	2.50 ± 0.91	8.67 ± 3.08
ERY 5 mg/kg	95	2.22 ± 0.58	8.23 ± 1.7†
ERY 5 mg/kg + IBU 2.5 mg/kg	80	1.65 ± 0.87*	8.18 ± 3.2†
ERY 5 mg/kg + IBU 7.5 mg/kg	75	1.80 ± 0.98*	5.60 ± 2.58‡

* Significant difference ($p \leq 0.05$) from non–erythromycin-treated animals.
† Significant difference ($p \leq 0.05$) from values of untreated controls and low dose of ibuprofen alone.
‡ Significant difference ($p \leq 0.05$) from all groups, except erythromycin plus low dose of ibuprofen.

the MEWF than animals not treated with antibiotic, but the difference was not statistically significant.

Table 3 presents the comparative findings of untreated control animals and the early treated animals with low- and high-dose ibuprofen alone and combined with erythromycin. Erythromycin therapy significantly improved all the parameters related with outcome *versus* the other comparative groups. Particularly, the regimen containing high-dose ibuprofen and erythromycin was more effective for most parameters tested than any other group, although the differences were not statistically significant. All antibiotic-treated animals had significantly lower amounts of polymorphonuclear leukocytes per field in the MEWF (range, 2.06 ± 2.04–2.92 ± 3.41) than those of animals not treated with antibiotic (range, 4.46 ± 3.84–6.65 ± 4.62).

Table 4 presents the findings of untreated controls and delayed treatment animals receiving erythromycin and/or ibuprofen administered in five doses every 3 h starting at 18 h PI. Again, as therapy was initiated late, most animals (80–100%) had both otorrhea and AOM by otoscopic examination. Animals receiving erythromycin with or without ibuprofen showed lower bacterial counts in the ME fluid than the comparative groups, but the difference was only significant for those receiving the antibiotic along with ibuprofen. Also

animals receiving erythromycin had less weight loss than those not receiving the antibiotic. Furthermore, the percentage of weight loss of animals receiving erythromycin with the high-dose ibuprofen was significantly lower than that of animals receiving erythromycin alone. All antibiotic-treated animals had lower amounts of polymorphonuclear leukocytes per field in the MEWF than those of not treated with antibiotic, but again the differences were not statistically significant.

DISCUSSION

In the gerbil model of AOM, it is possible to induce a true bacterial infection as proved by otoscopic examination and the presence of polymorphonuclear leukocytes in the ME fluid. On the other hand, this model also allows the comparison of the bacteriologic efficacy of antibiotics, which, if they are effective, clear bacteria from the ME faster than spontaneous bacterial clearance in nontreated animals (6,10–13).

As the pneumococcal strain used was resistant to penicillin but susceptible to amoxicillin (MIC = 1 μg/mL), a single dose of this antibiotic administered at 5 h PI was moderately effective in the treatment of AOM in this model. This result may be explained by its favorable pharmacokinetics in both serum (10,11) and ME fluid (12) after an early administration of 5 mg/kg amoxicillin. The amoxicillin ME fluid concentration and half-life have been found to be less advantageous when administration of the antibiotic was delayed (12). These data can explain, in part, the better outcome achieved when the antibiotic was administered early compared to delayed despite giving five doses. These results are also consistent with previous data showing decreased amoxicillin effectiveness when administered 21 h PI compared to early administration (12). It is interesting to point out that most parameters of efficacy (presence of otorrhea, number of ME culture-positive samples, bacterial counts, and body weight) were more favorable in animals treated early with single doses of amoxicillin and ibuprofen, especially when the latter was used at high dose, although these differences were not statistically significant when compared to those receiving the antibiotic alone.

After the delayed treatment, bacterial counts were also lower in animals treated with amoxicillin combined with any dose of ibuprofen than in those treated with the antibiotic alone. Furthermore, animals receiving amoxicillin and high-dose ibuprofen showed the lowest weight loss, although no significant difference was achieved compared to those receiving the antibiotic alone. These results clearly favor the use of ibuprofen combined with amoxicillin, in early or delayed administration, in the treatment of experimental AOM caused by this pneumococcal strain.

Early and delayed therapy with erythromycin, with and without ibuprofen, was effective in this experimental model. We have previously shown a favorable pharmacokinetic profile in both serum and ME fluid after early administration of 5 mg/kg erythromycin. However, the half-life in the ME fluid is shorter with delayed administration, which correlates with the poorer outcome of AOM treated with delayed administration of erythromycin, an observation also previously published (13). Animals treated with erythromycin and the high dose of ibuprofen, administered in early or delayed form, had lower bacterial counts in the ME fluid than those receiving the antibiotic alone, although the difference was not statistically significant. Regarding weight loss, animals treated with erythromycin and the high dose of ibuprofen lost less weight than those receiving the antibiotic alone, but the difference was statistically significant only for those receiving the treatment in delayed form. These results also favor the use of ibuprofen combined with erythromycin, in early or delayed administration, in the treatment of experimental AOM caused by this pneumococcal strain.

The benefit observed in animals treated with antibiotics combined with ibuprofen can be partly explained by the effect of ibuprofen in preventing animal deterioration, maintaining almost normal food and water intake, and hence, losing less body weight. This effect was statistically significant only for delayed administration of erythromycin, but it is possible that significant differences could be achieved if a greater number of animals had been used, but this was precluded by ethical reasons.

In animals treated with antibiotics and ibuprofen, there was also a tendency for a better bacteriological outcome, which may not be explained by a direct antimicrobial activity of ibuprofen against the organism (MIC = 1024 μg/mL) because ibuprofen serum concentration has been reported to be between 6 and 14 μg/mL in gerbils receiving similar doses of this drug (14). Additionally, we did not demonstrate any antibacterial synergism to explain these results because we only found indifference between antibiotics and ibuprofen using a checkerboard method. The effect of anti-inflammatory drugs in the clinical efficacy of antibiotics has been debated in many infections, particularly in bacterial meningitis (15,16). If ibuprofen had a relevant anti-inflammatory effect in the ME, the antibiotic concentration would be diminished as its effectiveness. As it has been suggested that the effect of ibuprofen may be dose dependent with a proinflammatory effect at low dose (17), we tested two different (low and high) doses, and a clear trend for benefit was always observed. Ibuprofen increases leukocyte phagocytosis and degranulation (18), and such features may explain, at least in part, its positive effect in the bacteriological outcome when combined with active antibiotics. A randomized, double-blind, controlled trial of cefaclor plus ibuprofen, acetaminophen, or placebo in children with AOM showed the efficacy of ibuprofen for pain relief (2), but no bacteriological analysis was provided in such study.

In summary, ibuprofen seems to be useful combined with antibiotics for the treatment of an experimental pneumococcal AOM, an effect not observed using acetaminophen (6). Early therapy clearly correlated with improved effectiveness; therefore, if antibiotics are indicated, they should be administered, combined with ibuprofen, as early as possible. This therapeutic approach, which has been proposed by some authors (19,20), may lead to achieving maximal effectiveness and preventing serious complications (21).

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REFERENCES

1. McCaig LF, Hughes JM 1995 Trends in antimicrobial drug prescribing among office-based physicians in the United States. JAMA 273:214–219
2. Bertin L, Pons G, d'Athis P, Duhamel JF, Maudelonde C, Lasfargues G, Guillot M, Marsac A, Debregeas B, Olive G 1996 A randomized, double-blind, multicentre controlled trial of ibuprofen versus acetaminophen and placebo for symptoms of acute otitis media in children. Fundam Clin Pharmacol 10:387–392
3. American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media 2004 Diagnosis and management of acute otitis media. Pediatrics 113:1451–1465
4. Graham GG, Scott KF 2005 Mechanism of action of paracetamol. Am J Ther 12:46–55
5. Hersh EV, Lally ET, Moore PA 2005 Update on cyclooxygenase inhibitors: has a third COX isoform entered the fray? Curr Med Res Opin 21:1217–1226
6. Ponte C, Parra A, Cenjor C, García-Olmos M, Giménez MJ, Aguilar L, Carcas A, Soriano F 2003 Does acetaminophen interfere in the antibiotic treatment of acute otitis media caused by a penicillin-resistant pneumococcus strain? A gerbil model. Pediatr Res 54:913–918
7. National Committee for Clinical Laboratory Standards 2003 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Sixth Edition: Approved Standard M7-A6. Wayne, PA, National Committee for Clinical Laboratory Standards
8. National Committee for Clinical Laboratory Standards 1999 Methods for Determining Bactericidal Activity of Antimicrobial Agents, Approved guideline. Document M26-A. Wayne, PA, National Committee for Clinical Laboratory Standards
9. Eliopoulos G, Moellering R 1996 Antimicrobial combinations. In: Lorian V (ed) Antibiotics in Laboratory Medicine. Williams & Wilkins, Baltimore, pp 330–396
10. Cenjor C, Ponte C, Parra A, Nieto E, Garcia-Calvo G, Gimenez MJ, Aguilar L, Soriano F 1998 In vivo efficacies of amoxicillin and cefuroxime against penicillin-resistant *Streptococcus pneumoniae* in a gerbil model of acute otitis media. Antimicrob Agents Chemother 42:1361–1364
11. Parra A, Ponte C, Cenjor C, Garcia-Calvo G, Gimenez MJ, Aguilar L, Soriano F 2002 Optimal dose of amoxicillin in treatment of otitis media caused by a penicillin-resistant pneumococcus strain in the gerbil model. Antimicrob Agents Chemother 46:859–862
12. Parra A, Ponte C, Cenjor C, Martínez-Marín C, Soriano F and the Spanish Pneumococcal Infection Study Network 2004 Effect of antibiotic treatment delay on therapeutic outcome of experimental acute otitis media caused by *Streptococcus pneumoniae* strains with different susceptibilities to amoxicillin. Antimicrob Agents Chemother 48:860–866
13. Martinez-Marín C, Huelves L, del Prado G, García-Cía JI, Cenjor C, Ponte C, Granizo JJ, Soriano F 2005 Effect of erythromycin treatment delay on therapeutic outcome of experimental acute otitis media caused by Streptococcus pneumoniae. J Antimicrob Chemother 56:783–786
14. Jungkeit MC, Chole RA 1991 Ibuprofen inhibits localized bone resorption in the middle ear. Calcif Tissue Int 48:267–271
15. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM, Whitley RJ 2004 Practice guidelines for the management of bacterial meningitis. Clin Infect Dis 39:1267–1284
16. Tuomanen E, Hengstler B, Rich R, Bray MA, Zak O, Tomasz A 1987 Nonsteroidal anti-inflammatory agents in the therapy for experimental pneumococcal meningitis. J Infect Dis 155:985–990
17. Rinaldo JE, Pennock B 1986 Effects of ibuprofen on endotoxin-induced alveolitis: biphasic dose response and dissociation between inflammation and hypoxemia. Am J Med Sci 291:29–38
18. Nickerson SC, Paape MJ, Harmon RJ, Ziv G 1986 Mammary leukocyte response to drug therapy. J Dairy Sci 69:1733–1742
19. Little P, Gould C, Williamson I, Moore M, Warner G, Dunleavey J 2001 Pragmatic randomised controlled trial of two prescribing strategies for childhood acute otitis media. BMJ 322:336–342
20. Handley JO Otitis media. N Engl J Med 2002;347:1169–1174
21. Van Zuijlen DA, Schilder AG, Van Balen FA, Hoes AW 2001 National differences in incidence of acute mastoiditis: relationship to prescribing patterns of antibiotics for acute otitis media? Pediatr Infect Dis J 20:140–144