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Efficacy of Single-Dose Ceftriaxone in Experimental Otitis Media Induced by Penicillin- and Cephalosporin-Resistant *Streptococcus pneumoniae*

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We used a gerbil model of otitis media to assess the efficacy of single-dose ceftriaxone against three *Streptococcus pneumoniae* strains highly resistant to penicillin (MICs, 4 to 8 μg/ml) and with various susceptibilities to ceftriaxone (MICs, 0.5, 4, and 8 μg/ml). Middle ear infection was induced by bilateral transbullar challenge with 10^7 bacteria per ear. Antibiotic treatment was administered subcutaneously at 2 h postinfection. Infection status was checked 2 days later by counting the bacteria in middle ear and cerebrospinal fluid samples. With the cefriaxone-susceptible strain (MIC, 0.5 μg/ml), we tested doses of 5 to 100 mg/kg of body weight. With a dose of 50 mg/kg, treatment outcome was equivalent to that with amoxicillin, which was used as a reference (25 mg/kg, two injections); no bacteria were recovered from 82% of the middle ear samples, and the rate of cerebrospinal fluid culture positivity was significantly reduced to 6%, relative to 59% for the untreated controls. Similar efficacy was obtained with a dose of 100 mg/kg against the two ceftriaxone-resistant strains. Pharmacokinetic study indicates that the values of the parameters in plasma after the administration of a dose of 100 mg/kg (peak level of total drug, 268 ± 33 μg/ml; elimination half-life, 0.8 h; area under concentration-time curve, 488 $\mu g \cdot h \cdot ml^{-1}$) were still suboptimal compared with the values of the parameters measured in pediatric patients after intravenous or intramuscular administration of a dose of 50 mg/kg. Our results indicate the efficacy of ceftriaxone against experimental cephalosporin-resistant pneumococcal otitis and provide a basis for the clinical use of single-dose ceftriaxone against pneumococcal otitis media.

Otitis media is the most common bacterial infection and the most common single indication for antimicrobial chemotherapy in young children (28). *Streptococcus pneumoniae* accounts for 30 to 50% of cases of acute otitis media, and international reports indicate that a large proportion of isolates have acquired relative or high levels of resistance to penicillin. The growth of this phenomenon and the related increase in clinical failures after oral treatment of otitis media with beta-lactam antibiotics (8, 13, 19) have led to a search for alternatives.

The pharmacokinetic properties of ceftriaxone (CRO), together with its good antipneumococcal activity in vitro, form the basis for short courses of once-daily treatment and even single-dose therapy. The clinical efficacy of a single intramuscular injection of 50 mg of CRO per kg of body weight in pediatric patients with uncomplicated otitis media is equivalent to those of standard oral 7- or 10-day amoxicillin (22, 36), 10-day amoxicillin-clavulanate (37), and 10-day trimethoprim-sulfamethoxazole (4) therapy. The recent emergence of clinical cephalosporin-resistant *S. pneumoniae* strains, revealed by treatment failures in patients with pneumococcal meningitis (10–12, 17, 24, 31), although initially considered an epiphenomenon (15), now appears to be a continuum in penicillin-binding protein alterations in penicillin-resistant strains (5). This extension of resistance must be taken into account in the assessment of CRO therapy.

S. pneumoniae isolates with abnormal susceptibilities to beta-lactam agents belong to a limited list of serotypes which are those recovered from children with nasopharyngeal carriage and otitis media, namely, serotypes 6, 9, 14, 19, and 23 in most European countries and the United States (3). Most of the strains of these noninvasive serotypes, which are avirulent in immunocompetent mice, can induce otitis media in immunocompetent gerbils when a large inoculum is used (6). Pneumococcal otitis media in gerbils is frequently followed by meningeal complications, and this model can thus be used to evaluate antimicrobial efficacy in middle ear (ME) infections in terms of both the eradication of bacteria from the ME and the prevention of spread to the meninges. We studied the efficacy of single-dose CRO in this model using three highly penicillin-resistant *S. pneumoniae* strains with various susceptibilities to CRO. A pharmacokinetic study was performed for comparison with the pediatric setting.

(Part of this work has been presented at the 19th International Congress of Chemotherapy, Montreal, Quebec, Canada, 16 to 21 July 1995 [5a].)

MATERIALS AND METHODS

The study was performed in accordance with prevailing regulations regarding the care and use of laboratory animals in the European Community (14a).

Animals. Eight- to 9-week-old adult female Mongolian gerbils weighing 40 to 50 g each were purchased from Centre d'Elevage R. Janvier (Le Genest Saint Isle, France). They were given free access to food and water and were housed in a protected unit (Iffa Credo, l'Arbresle, France) with slight-negative-pressure, filtered air and a 12-h light and 12-h dark cycle. Prior to bacterial challenge and sampling, the animals were anesthetized with an intramuscular injection of 40 mg of ketamine (Ketalar; Parke-Davis, Courbevoie, France) per kg plus 13 mg of xylazine (Rompun; Bayer Pharma, Sens, France) per kg.

Experimental otitis. Animals were inoculated bilaterally with 20 μl of a bacterial suspension introduced directly into the ME by transbullar challenge (18, 21). The tympanic membrane was left intact and swelled without rupturing during inoculation. A normal tympanic aspect and correct inoculation were verified with an operating microscope.

Pharmacokinetic studies. Delivery of CRO to the infected ME was studied on

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TABLE 1. Characteristics of study strains

Strain	Serotype	MIC/MBC (μg/ml)		
		Penicillin	Amoxicillin	CRO
PRSp 15986	19	4–8/8–16	2–4/4–8	0.5/0.5
PCRSp 32870	19	4/4–8	4/4	4/4
PCRSp 40984	14	8/8	8/8	8/8

day 3 postinfection (p.i.) in animals with otitis media induced by inoculation of 40 to 100 CFU of an invasive serotype 3 *S. pneumoniae* strain per ear. This standard procedure (6) permits collection of ME samples without blood contamination at a time when effusion is consistently abundant and the bacterial density is about 10^7 CFU/ml. To construct concentration-time curves, animals received a single subcutaneous (s.c.) dose of 100 mg/kg and were killed 0.25, 0.5, 1, 2, 4, and 6 h later. Blood and ME samples were collected from three to six animals at each time point. The relationship between the dose and peak drug levels (C_{max}) in the ME effusion was determined in groups of six to seven animals injected s.c. with 10, 50, or 100 mg/kg and sampled 2 h later. The relationship between the dose and C_{max} in plasma was determined in groups of four to five animals injected s.c. with 10, 25, 50, or 100 mg/kg and sampled 30 min later.

Total CRO in the samples was assayed by high-performance liquid chromatography (HPLC). The HPLC technique was based on the recommendations of Trautmann and Haefelfinger (35) (ion-pair reversed phase chromatography, precipitation by acetonitrile, and a wavelength of 275 nm). The limit of sensitivity was 0.5 μg/ml of sample. The standard curve was linear up to 300 μg/ml. The between- and within-day coefficients of variation during the study were <5%. All samples were assayed in duplicate.

To evaluate the antimicrobial activity of drug in the samples, bioassays were also performed by the agar (pH 6.6) disk diffusion method with *Escherichia coli* ATCC 39118 as the test strain. Standard curves were constructed from serial twofold dilutions of CRO in pooled gerbil plasma and in protein-free phosphate buffer (pH 6.6). The bioactive equivalent concentration of drug in the samples was defined as the concentration in the protein-free standard which produces the same antibacterial effect. The limits of detection were 0.125 and 0.25 μg/ml in buffer and plasma, respectively. Standard curves were linear up to 64 μg/ml. The between- and within-day coefficients of variation during the study were <14%. Antibiotic levels in samples were calculated in triplicate.

CRO was assayed in pure plasma and in 5-fold-diluted ME washing fluid (which is diluted about 2.5-fold relative to the pure ME effusion). Pharmacokinetic parameters were computed with APIS software (23) by using a model of monoexponential decline with absorption phase.

Protein binding of CRO in gerbil plasma was determined with a disposable Centrifree ultrafiltration device (Amicon, Beverly, Mass.). Plasma samples of 500 μl containing CRO at concentrations ranging from 10 to 350 μg/ml were centrifuged at 1,500 × *g* for 15 min. The concentration of unbound CRO in the ultrafiltrate was determined by the HPLC method described above, and the percent protein binding was calculated with reference to the initial concentration in the sample.

Infecting organisms. We used three clinical isolates of *S. pneumoniae* provided by the Centre de Référence du Pneumocoque (P. Geslin, Créteil, France). One strain was highly penicillin resistant but susceptible to cephalosporins (strain PRSp 15986, serotype 19), and the other two strains were highly penicillin and cephalosporin resistant (strains PCRSp 32870 and PCRSp 40984; serotypes 19 and 14, respectively). MICs were determined in Mueller-Hinton infusion broth supplemented with 5% filtered horse serum (Diagnostics Pasteur) by the tube dilution method and with a final bacterial density of 10^6 CFU/ml. The MIC was defined as the lowest concentration of antibiotic at which no growth was visible to the naked eye. For the determination of the MBC, 0.01-ml aliquots from tubes with no visible growth were plated onto Columbia agar with 5% sheep blood (Bio-Merieux, Lyon, France), and the plates were incubated overnight at 37°C. The MBC was defined as the lowest concentration killing more than 99.99% of the inoculum. The characteristics of the study strains are given in Table 1.

Virulence was maintained by passage in mice. Aliquots of the pneumococcal suspension were stored at −80°C. On each day of experimentation a freshly thawed aliquot was incubated for 6 h at 37°C in brain heart infusion broth (Bio-Merieux) enriched with 5% horse serum. The number of viable bacteria in this culture was determined by the pour plate colony counting method after 10-fold serial dilution of the culture in saline. In the efficacy study with noninvasive strains, an inoculum of 10^6 to 10^7 CFU per ear was used for each strain.

Efficacy study. First, increasing single doses of CRO (5 to 100 mg/kg) were tested against strain PRSp 15986. The criteria for bacterial cure were considered in reference to 1-day effective amoxicillin treatment, which was previously determined for the same strain to be two injections of 25 mg/kg (6). Second, on the basis of the pharmacokinetic study results, the efficacy of a single 100-mg/kg dose was evaluated against strains PCRSp 32870 and PCRSp 40984.

CRO sodium salt (Produits Roche, Neuilly, France) and amoxicillin sodium salt (Clamoxyl; Beecham, Paris, France) were dissolved and diluted in sterile water. Treatment was initiated 2 h p.i. and was administered s.c. in a volume of 500 μl. A control group was included in each set of experiments, and the animals in the control group were treated s.c. with sterile water under conditions similar to those for treated animals. Treated and control animals were examined on day 2 p.i. for body weight changes, tympanic aspect, and the infection status of the ME effusion. ME samples were obtained by washing the ME fossa with 20 μl of saline (which was injected and withdrawn via the epitympanic membrane). Meningeal involvement was assessed by bacterial counts in cerebrospinal fluid (CSF) obtained by percutaneous intracysternal puncture.

Otoscopic examination. Prior to ME sampling, the presence of a retrotympanic exudate was checked for and the epitympanic membrane was examined for thickening, inflammation, and shape changes. Four grades were attributed to each membrane feature, as follows: for inflammation, 0 indicates none, 1 is mild vasodilation, 2 is frank hyperemia, and 3 is acute inflammation with abundant extravascular blood cells; for thickening, 0 is none, 1 is opalescence, 2 is opacity, and 3 is opacity plus graining; for shape, 0 is normally concave, 1 is flat, 2 is convex, and 3 is bulging.

Bacterial counts. Shortly after sampling, 100-μl aliquots of serial 10-fold dilutions in saline were plated onto sheep blood agar and the plates were incubated for 18 h at 37°C. Bacterial counts are expressed in $\log_{10}$ CFU per 20 μl of washing fluid; the lowest detectable bacterial count was 0.34 $\log_{10}$ CFU. Culture-negative samples were included in the calculation of means by assuming a value at the detection limit. In CSF, the lowest detectable bacterial count ranged from 1.5 to 2 $\log_{10}$ CFU/ml according to the volume obtained.

Statistical analysis. Data are expressed as means ± standard deviations. For the efficacy study, means were compared between groups by analysis of variance or nonparametric tests, as appropriate. Parameters expressed as frequencies were compared between groups by using Fisher's exact χ^2 test. Relationships between quantitative parameters were sought by using the linear regression *r* coefficient or the Spearman rank correlation test. *P* values of 0.05 or less were considered significant.

RESULTS

Pharmacokinetic data. HPLC determination of concentration-time curves in plasma and ME effusions after the administration of a single dose of 100 mg of CRO per kg are represented in Fig. 1. The C_{max} in plasma 0.5 h after drug administration was 268 ± 33 μg/ml, and the half-life was 0.77 h ($k_a = 5.63 \cdot h^{-1}$, $k_e = 0.896 \cdot h^{-1}$), the area under the concentration-time curve (AUC) from time zero to infinity ($AUC_{0-\infty}$) was 488 μg · h/ml, and drug clearance was 0.205 ml/g · h^{-1}. In the ME effusion the C_{max} was 43 ± 17 μg/ml 2 h after administration, and the elimination half-life was 2.34 h ($k_a = 1.21 \cdot h^{-1}$, $k_e = 0.296 \cdot h^{-1}$), $AUC_{0-\infty}$ was evaluated to

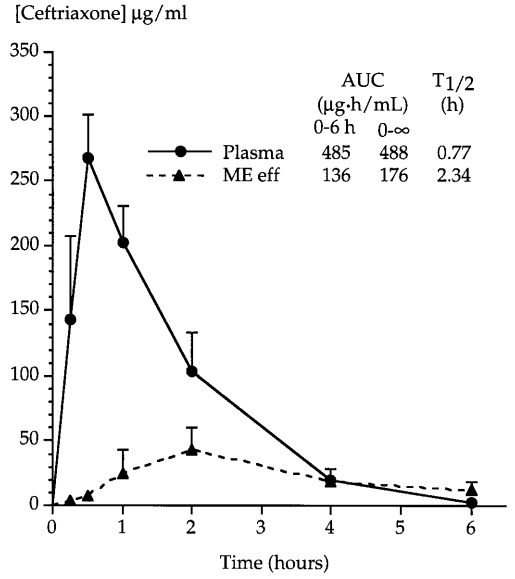

FIG. 1. Total CRO concentration-time curves determined by HPLC in plasma and ME effusions (ME eff) of gerbils with pneumococcal otitis after the administration of a single s.c. dose of 100 mg/kg. $T_{1/2}$, half-life.

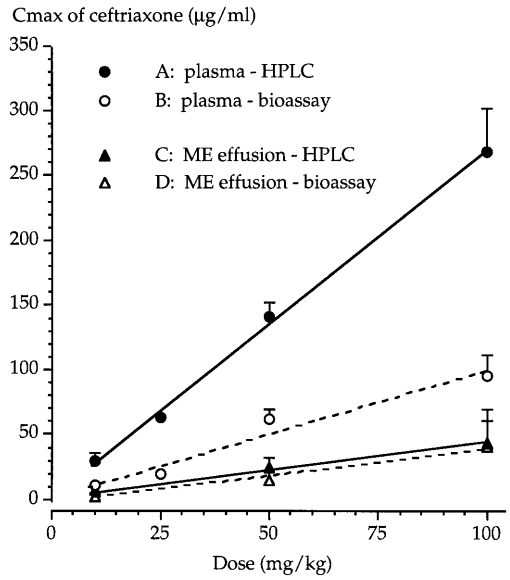

FIG. 2. Relationships between s.c. dose and C_{max} of ceftriaxone in plasma and ME effusions of gerbils with pneumococcal otitis. Means ± standard deviations are plotted for both HPLC and bioassay (with a standard curve obtained without protein) determinations. The parameters of the linear regressions calculated without an intercept for n values were as follows: A, $C_{max} = 2.69 \times$ dose ($r = 0.993$; $n = 24$); B, $C_{max} = 0.97 \times$ dose ($r = 0.984$; $n = 24$); C, $C_{max} = 0.44 \times$ dose ($r = 0.993$; $n = 39$); D, $C_{max} = 0.38 \times$ dose ($r = 0.828$; $n = 33$).

be 176 μg · h/ml, representing a 36% penetration rate of total drug into the ME. During the period of observation, i.e., 0 to 6 h, the penetration rate was 28%. The relationship between peak concentrations and CRO doses (10 to 100 mg/kg) was linear for both plasma and ME effusions (Fig. 2). In plasma, the total concentrations determined by the bioassay were not significantly different from the values determined by HPLC, confirming the report of Scully et al. (30). The bioactive equivalent concentrations represented, on average, 40% of the total drug concentrations. In the ME effusion, the concentrations determined by bioassays were a mean of 86% of the levels determined by HPLC. Within the range of the total concentrations in plasma studied, i.e., 10 to 350 μg/ml, the protein binding of CRO decreased from 90 to 72%.

The choice of the 100-mg/kg dose in the second part of the study with *S. pneumoniae* strains resistant to cephalosporins was based on the results of the pharmacokinetic study and comparison with the few available data from studies with pediatric subjects (the data are summarized in Table 2). The maximum total drug concentration achieved in gerbil plasma after a single s.c. injection was in the range of values reported in the pediatric setting after a 50-mg/kg intravenous infusion (1, 14, 25, 29, 32); the values measured after intramuscular administration at the same dose were lower (9), but the AUC values were similar, in keeping with the total bioavailability of ceftriaxone administered intramuscularly (30). However, the clearance of ceftriaxone from the plasma of gerbils was about five times greater than that from the plasma of children, and the 100-mg/kg s.c. dose resulted in suboptimal drug kinetics in plasma compared with those determined after intravenous or intramuscular administration of 50 mg/kg to children.

Efficacy study. (i) Single-dose CRO against strain PRSp 15986. In the control and treatment groups, animals inoculated with PRSp 15986 showed a significant body weight loss on day 2 p.i., which correlated strongly with the bacterial counts in ME ($P < 10^{-4}$) (Table 3). Otoscopic examination showed the presence of a retrotympanic exudate in all inoculated ears. In animals in the untreated control group, the modal score of epitympanic membrane inflammation (50.7% of ears) was grade 2 (frank hyperemia). This changed to grades 0 and 1 (absent or mild vasodilation) in the treatment groups, but there was no specific relationship with bacterial clearance. Membrane thickening ran parallel to the degree of inflammation. Membrane shape changes were observed in 65.7% of ears in the untreated control group and were equally distributed among grades 1, 2, and 3; the degree of alteration correlated with CFU counts ($P < 0.002$). These alterations were only occasionally observed in the treatment groups.

At the same time p.i. (day 2), mean CFU counts in the MEs of animals treated with 5 mg of CRO per kg were significantly decreased compared with those in the ME of the untreated controls (1.26 ± 1.32 versus 3.11 ± 1.30; $P < 10^{-4}$), but bacteria were still detected in 50% of the ME washings and the rate of CSF culture positivity was unchanged (50.0% versus 59.3% in controls). After the administration of doses of 10 and 25 mg/kg, mean counts in the MEs were close to the detection limit, but bacteria were still detected in more than 30% of the samples and CSF samples were frequently culture positive (50% after the administration of a dose of 25 mg/kg). After the administration of doses of 50 and 100 mg/kg, mean counts in the MEs were not significantly different from the detection limit, and bacteria were not detected in 82 and 86% of the samples, respectively, values which did not significantly differ from the 90% rate in amoxicillin-treated animals; the rate of CSF culture positivity was significantly reduced, to values similar to those observed with amoxicillin (5.6 and 7.1%, respectively, versus 6.3% for amoxicillin-treated animals).

(ii) CRO against strain PCRSp 32870 and PCRSp 40984. The treatment efficacy of a single 100-mg/kg dose of CRO against PCRSp 32870- and PCRSp 40984-induced otitis media is presented in Fig. 3. For comparison, data for PRSp 15986-infected animals are included. Body weight loss on day 2 p.i. was significantly reduced in all CRO-treated groups. Retrotympanic effusion was present in all inoculated ears. Similar to PRSp 15986-infected animals, the modal score of epitympanic membrane inflammation was grade 2 in PCRSp 32870-infected control animals (50.0% of ears) and grades 0 and 1 in animals in the CRO-treated group. The inflammation caused by PCRSp 40984 infection was more marked, with a modal score of grade 3 (57.1% of animals); however, CRO treatment limited the inflammation to grades 0 and 1, as was the case with the other strains. Membrane shape changes were less frequent in PCRSp 32870- and PCRSp 40984-infected ears (22.2 and 32.1%, respectively) than in PRSp 15986-infected ears (65.7%) and were not observed in CRO-treated animals.

Of the 14 control animals inoculated with PCRSp 40984, ME samples were sterile bilaterally in 2 animals and unilaterally in 8 animals on day 2 p.i. Consequently, the mean CFU counts in ME samples were significantly lower than those for the other strains ($P < 0.01$). For PCRSp 32870 and PCRSp

TABLE 2. Pharmacokinetics of CRO determined in plasma of pediatric patients[a]

Route of administration of 50-mg/kg dose	C_{max} (mg/liter)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (mg · h/liter)	CL (liter/kg/h)
Intravenous perfusion[b]	200–400	4–7	1,200–2,000	0.03–0.06
Intramuscular injection[c]	175 ± 34	9.0 ± 2.8	2,021 ± 870	0.03 ± 0.01

[a] $t_{1/2}$, half-life; CL, clearance; the other abbreviations were defined in the text.
[b] Pooled data from references 1, 14, 25, 29, and 32.
[c] Data are from reference 9.

TABLE 3. Outcome of ME infection induced by highly penicillin resistant *S. pneumoniae* PRSp 15986 treated with a single dose of CRO[a]

Drug and dose (mg/kg [no. of injections])	No. of animals	Relative body wt loss			% Culture-positive ME samples				Bacterial counts in ME samples ($\log_{10}$ CFU)[b]			Infection status of CSF			$\log_{10}$ CFU/ml	
		% of initial body wt	SD	P[c]	Bilateral	Unilateral	None	All inoculated MEs (P value[d])	Mean	SD	P[e]	No. of culture-positive CSF samples/total no. of observations	% of animals	P[d]	Mean	SD
Untreated controls	70	−11.9	3.5		82.9	15.7	1.4	90.7	3.11	1.30		35/59	59.3		4.2	1.7
Amoxicillin																
25 (2)	30	−5.2	3.9		0	20.0	80.0	10.0	0.44	0.26		1/16	6.3		4.2	
5 (1)	8	−7.7	5.1	NS[f]	37.5	25.0	37.5	50.0 (0.001)	1.26	1.32	0.012	4/8	50.0	0.028	5.5	2.5
10 (1)	24	−1.8	3.3	0.009	25.0	20.8	54.2	35.4 (0.002)	0.53	0.31	NS	3/22	13.6	NS	4.9	3.0
25 (1)	8	−7.3	5.6	NS	12.5	37.5	50.0	31.2 (0.047)	0.77	0.85	NS	4/8	50.0	0.028	5.1	1.6
50 (1)	14	−2.5	3.2	NS	0	35.7	64.3	17.9 (NS)	0.39	0.11	NS	1/14	7.1	NS	4.9	
100 (1)	18	−2.2	3.0	0.046	0	27.8	72.2	13.9 (NS)	0.42	0.21	NS	1/18	5.6	NS	3.5	

[a] Comparison on day 2 p.i. with the reference amoxicillin treatment.
[b] Values are the mean $\log_{10}$ CFU in 20 μl of washing fluid.
[c] P value versus amoxicillin-treated group by one-way analysis of variance plus the Bonferroni-Dunn test for five comparisons.
[d] P value versus amoxicillin-treated group by Fisher's exact χ^2 test.
[e] P value versus amoxicillin-treated group by the Mann-Whitney U test.
[f] NS, not significant.

40984, mean counts fell significantly after CRO treatment, to close to the detection limit, and bacteria were not detected in 90.0 and 92.9% of the samples, respectively. After CRO treatment, the rate of CSF culture positivity was markedly reduced: not significantly so for strain PCRSp 32870 (20.0 versus 66.7% without treatment; $P = 0.07$) and significantly so for strain PCRSp 40984 (7.1% versus 57.1% without treatment; $P < 0.01$).

DISCUSSION

The gerbil model of ME infection proved to be suitable for the experimental evaluation of antibiotic treatment of otitis media caused by penicillin-resistant strains of *S. pneumoniae* (6). Meningeal involvement following ME infection is frequent in this model (30 to 70% of animals) (7), in contrast to the clinical setting, but this factor provides a powerful additional criterion for assessing antibiotic efficacy in eradicating bacteria from the ME.

The pharmacokinetics of CRO in gerbils with pneumococcal otitis showed a linear relationship between injected doses and the peak level of total drug achieved in plasma and ME effusion. The penetration rate of total drug in ME was found to be 36% after the administration of a 100-mg/kg s.c. dose, ensuring C_{max}s of 268 and 43 μg/ml in plasma and ME effusion, respectively.

The high rate of CRO binding to plasma proteins, primarily albumin, has been extensively described as playing an important role in drug availability and activity. Stoeckel et al. (33) reported a fall in the level of CRO protein binding in adults of from 96 to 83%, with concentrations in plasma increasing from 0.5 to 300 μg/ml. Moine et al. (26) reported a decrease in the level of protein binding from 96 to 86% in mice when the total drug concentration in serum increased from 0.6 to 95 μg/ml. In gerbils the fraction of protein-bound CRO also decreased from 90 to 72% when the total drug concentration increased from 10 to 350 μg/ml. These similarities could be expected in view of albumin concentrations in serum of 25 to 45 and 25 to 48 g/liter in gerbils and mice, respectively. Routledge (27) reported an increase in the albumin concentration in serum from 35 g/liter in neonates to 45 g/liter in adults. In our gerbil model the bioactive equivalent concentration of drug was about 40% of the total drug in plasma. Similarly, in the mouse model (26) the bioactive equivalent concentration of drug determined by bioassay in serum containing 94.5 μg of total drug per ml was 46.5 μg/ml, i.e., 49%. These values, even if they are slightly overestimated because of methodological bias, are certainly higher than the proportion of free drug measured at equilibrium. Interpretation of this notion in terms of the pharmacokinetics of CRO is complex, but the data suggest that the amount of drug available for microbiological activity which is provided by the in vivo dynamic process of protein binding is underreflected by the concentration of free drug considered at a given time. Binding of CRO to protein in ME effusion has never been documented but probably varies according to the amount and nature of the inflammatory products. Under our experimental conditions the bioactive equivalent concentrations of CRO in ME effusion were close to the concentration of total drug.

The results obtained with increasing single doses of ceftriaxone against strain PRSp 15986 (highly resistant to penicillin and susceptible to ceftriaxone) showed a reduction in ME inflammation and a dose-related effect on bacterial clearance. Treatment with a single 5-mg/kg dose of ceftriaxone had a significant, although incomplete, antibacterial effect in the ME. At doses of 10 and 25 mg/kg, the efficacy of CRO in the ME

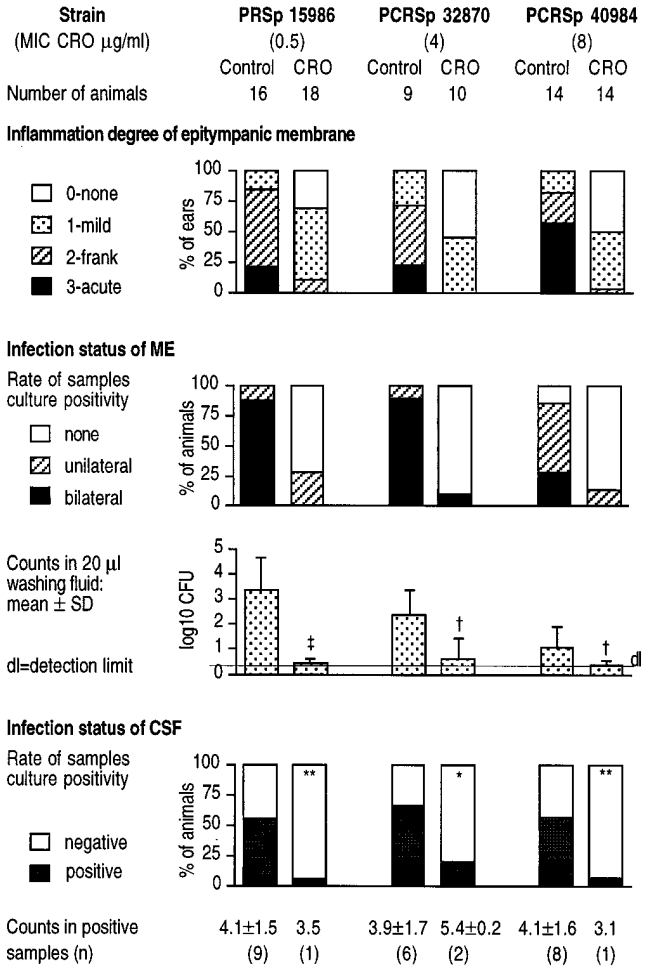

FIG. 3. Outcome of gerbil ME infection induced by penicillin-resistant *S. pneumoniae* strains with various susceptibilities to cephalosporins. The gerbils were treated with a single s.c. dose of 100 mg of ceftriaxone per kg. Data were obtained on day 2 p.i. †, $P < 10^{-3}$ versus untreated control group (Mann-Whitney U test); ‡, $P < 10^{-4}$ versus untreated control group (Mann-Whitney U test); *, $P = 0.07$ versus untreated control group (Fisher's exact χ^2 test). **, $P < 10^{-2}$ versus untreated control group (Fisher's exact χ^2 test).

was increased but was still incomplete. With doses of 50 mg/kg and higher, giving a peak level of total drug in plasma at least 270 times the MIC, a single dose of CRO was consistently effective. Prevention of meningeal involvement was markedly improved in groups treated with CRO doses of 50 mg/kg or more compared with that in groups treated with lower doses. On the basis of the pharmacokinetics of CRO in ME, the time during which the total drug concentration exceeded the MIC for strain PRSp 15986 (MIC, 0.5 μg/ml) was ≈11 h after administration of the 25-mg/kg dose and ≈13 h after administration of the 50-mg/kg dose. This small difference alone cannot explain the difference in efficacy between the two doses, particularly with regard to the prevention of meningeal involvement, and suggests that antibacterial efficacy is dependent on both the concentration and the exposure time.

With cephalosporin-resistant strains, a single dose of 100 mg/kg limited the inflammatory process, gave a rate of bacterial eradication equivalent to that with PRSp 15986, and also markedly reduced the frequency of meningeal involvement. With PCRSp 32870 (MIC, 4 μg/ml) and PCRSp 40984 (MIC, 8 μg/ml), a dose of 100 mg/kg gave peak levels of total drug in plasma/MIC ratios of about 70 and 35, respectively, and drug concentrations greater than the MIC in ME for about 8 to 9 and 6 to 7 h, respectively, which represent lower drug demands compared with those required for PRSp 15986. However, the influence of phenotypic properties on treatment outcome must be taken into account. The three study strains had equivalent in vitro tolerances to CRO (i.e., a decrease of 1 to 2 $\log_{10}$ CFU/ml after 6 h of antibiotic contact at 50-fold the MIC in broth with an initial inoculum of 10^8/ml), but PRSp 15986 was the only strain lacking autolytic properties (2), which could increase drug demand. For PCRSp 40984, the significantly lower level of infectivity associated with likely higher levels of ME inflammation could also have favored treatment efficacy as a result of a combined lower level of drug demand and a higher level of drug penetration. However, the rate of meningeal involvement with this strain was 57% in untreated control animals and was reduced to 7% by CRO treatment, suggesting rapid antibacterial efficacy in the ME focus.

The main finding of the present study was that a single 100-mg/kg dose of CRO that gave suboptimal pharmacokinetics compared with those determined in children at the recommended therapeutic intramuscular dose of 50 mg/kg was effective in the treatment of experimental pneumococcal otitis induced by strains resistant to both penicillin and cephalosporins. The distribution of CRO to noninflamed ME mucosae has been evaluated in patients receiving a single intramuscular dose of 1 g (16), but no data on drug delivery to the ME of children with acute otitis media are available. This means that our experimental findings cannot be directly extrapolated to the dose and treatment duration required in the pediatric setting. Nevertheless, our results suggest that the single dose to be used clinically for the reliable treatment of pneumococcal otitis should achieve over ≈12 to 13 h a concentration of total drug in the ME effusion that exceeds the highest MIC reported in epidemiological surveys. Thornsberry et al. (34) reported finding 8% of CRO-intermediate strains (MIC, 1 μg/ml; 5.6% of strains and CRO-resistant strains (MIC, >2 μg/ml; 2.3% of strains) among unselected pneumococcal isolates in the United States. In France, 24% of isolates are intermediate and less than 1% are resistant (20) to cefotaxime. These values suggest that, in order to limit selective pressure, CRO use should be restricted to bacteriologically documented otitis and patients infected with isolates at a high risk of penicillin resistant pneumococcal otitis.

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