

## Evaluation of fosfomycin alone and in combination with ceftriaxone or vancomycin in an experimental model of meningitis caused by two strains of cephalosporin-resistant *Streptococcus pneumoniae*

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**Objectives:** To study the *in vitro* and *in vivo* efficacy of fosfomycin, alone and in combination with ceftriaxone or vancomycin, against two strains of *Streptococcus pneumoniae*: HUB 2349 (fosfomycin and ceftriaxone, MICs 16 and 2 mg/L) and ATCC 51916 (MICs 4 and 32 mg/L).

**Methods:** Pharmacokinetics/pharmacodynamics data were collected from the study of eight infected animals after a single intravenous dose of 300 mg/kg of fosfomycin. Time–kill curves were plotted using CSF antibiotic concentrations achievable clinically. In the rabbit model, we studied the efficacy and effects on inflammation of treatment with fosfomycin 1200 mg/kg/day, ceftriaxone 100 mg/kg/day and vancomycin 30 mg/kg/day, over 26 h.

**Results:** Fosfomycin peak level in serum was  $324.48 \pm 102.1$  mg/L at 0.5 h; CSF penetration was 49.2%. Time–kill curves showed that fosfomycin was bactericidal against the ATCC 51916 strain and that the addition of fosfomycin to ceftriaxone or vancomycin was synergic against the HUB 2349 strain. Resistance to fosfomycin was detected both when fosfomycin was studied alone and in combination. In the rabbit model, fosfomycin showed bactericidal activity only against the ATCC 51916 strain. Combinations of fosfomycin with ceftriaxone or vancomycin were bactericidal against both strains; they improved efficacy and decreased CSF inflammatory parameters over monotherapies, without showing statistical differences in comparison with the combination of ceftriaxone and vancomycin.

**Conclusions:** Fosfomycin in combination with ceftriaxone or vancomycin appeared to be effective for the treatment of experimental cephalosporin-resistant pneumococcal meningitis. These combinations are possible alternatives in cases of allergy or intolerance to first-line drugs or in rare meningitis caused by highly cephalosporin-resistant pneumococci.

Keywords: pneumococcal meningitis, cephalosporins, *Streptococcus pneumoniae*, resistance

### Introduction

The worldwide increase of penicillin- and cephalosporin-resistant isolates of *Streptococcus pneumoniae* has prompted changes in the treatment of pneumococcal meningitis. However, controlled studies have not yet established the best antibiotic schedule for resistant cases. Most authorities, based on experimental and clinical data, recommend the combination of a third-generation

cephalosporin such as ceftriaxone or cefotaxime with vancomycin as an empirical first-line therapy.<sup>1–3</sup> Some non- $\beta$ -lactam antibiotics with anti-pneumococcal activity, such as rifampicin or quinolones, have proven to be effective in the rabbit model of meningitis and have been suggested as possible alternatives, especially for patients with penicillin allergy or for patients infected with strains showing very high cephalosporin resistance.<sup>4–7</sup> In this setting, other antimicrobial agents with

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anti-pneumococcal activity, alone or in combination, merit further evaluation.

Fosfomycin is a broad-spectrum antibiotic with low molecular weight and negligible protein binding activity which acts by inhibiting the first step of cell wall synthesis.<sup>8</sup> It exhibits *in vitro* activity against a large number of Gram-positive and Gram-negative bacteria, including both penicillin-susceptible and penicillin-resistant pneumococci.<sup>9–11</sup> It has a large volume of distribution in human tissues and penetrates well into the CSF of patients with meningitis.<sup>9,12</sup> Owing to these properties, fosfomycin has been suggested as an alternative for treating CNS infections.<sup>9,11</sup> However, few experimental studies have addressed this issue. Fosfomycin has been used successfully in a rabbit model of meningitis caused by *Escherichia coli* and *Staphylococcus aureus*,<sup>13</sup> but emergence of resistance has been reported during *in vitro* studies when high concentrations were tested.<sup>8</sup> Nau *et al.* studied the activity of fosfomycin in a rabbit model of pneumococcal meningitis, using a fully susceptible pneumococcal strain.<sup>14</sup> They found that fosfomycin was bactericidal only at very high concentrations and the bactericidal rate at all fosfomycin doses tested was always lower than that of ceftriaxone.

The aim of this study was to determine the *in vitro* and *in vivo* efficacy of fosfomycin, alone and in combination with ceftriaxone or vancomycin, against two strains of drug-resistant *S. pneumoniae* with different patterns of antimicrobial susceptibility. The combination of ceftriaxone with vancomycin was used for comparisons.

## Materials and methods

### Bacterial strains

Two strains of *S. pneumoniae* belonging to serotype 23F, isolated originally from patients with meningitis, were used. The HUB 2349 strain is a penicillin- and cephalosporin-resistant pneumococcus. The ATCC 51916 strain (Tennessee<sup>23F-4</sup> clone) is an intermediate penicillin-resistant and highly cephalosporin-resistant pneumococcus.<sup>15,16</sup> Both strains are susceptible to fosfomycin, according to the currently accepted breakpoints.<sup>17</sup> The NCCLS (now CLSI) guidelines do not list breakpoints for fosfomycin against *S. pneumoniae*.

MICs and MBCs were determined using the Etest and the microdilution method following the CLSI guidelines.<sup>18</sup> A final concentration of 25 mg/L glucose-6-phosphate was added to the medium for all *in vitro* fosfomycin susceptibility tests.<sup>17</sup> MIC/MBCs (in mg/L) for the HUB 2349 strain were penicillin, 4/4; ceftriaxone, 2/4; fosfomycin, 16/64; and vancomycin, 0.25/0.5; for the ATCC 51916 strain they were penicillin, 0.12/0.25; ceftriaxone, 32/32; fosfomycin, 4/32; and vancomycin, 0.25/0.25.

### *In vitro* studies

Time-kill curves were derived using glass tubes containing a final volume of 10 mL of cation-adjusted Mueller-Hinton broth supplemented with 5% lysed horse blood and a final inoculum of  $5 \times 10^5$  to  $1 \times 10^6$  cfu/mL. Antibiotic concentrations achievable in CSF, ranging from 1/4 to 4 × MIC of antibiotics alone, were studied, as were concentrations of 1/4, 1/2 and 1 × MIC of each drug in combination. In addition, ceftriaxone concentrations of 1/16 and 1/8 × MIC were studied against the ATCC 51916 strain. Samples were removed at 0, 6 and 24 h of incubation. The detection limit was 10 cfu/mL. Bactericidal effect was defined as a decrease in the initial inoculum of

$\geq 3$  log cfu/mL. Synergy of a combination was defined as a  $>2$  log cfu/mL reduction over the most active agent alone, with one of the drugs at subinhibitory concentration. Additive effects and indifferent effects were, respectively, defined as a reduction of between 1 and 2 log cfu/mL and of  $\pm 1$  log cfu/mL compared with the most active single antibiotic.<sup>19</sup> Emergence of resistance was studied at 24 h using the Etest method.

### Meningitis model

The experimental protocol was approved by the Ethics Committee for Animal Experiments at the University of Barcelona. The rabbit model of meningitis described originally by Dacey and Sande was modified slightly.<sup>20</sup> Young female New Zealand White rabbits were anaesthetized intramuscularly (im) with 35 mg/kg of ketamine (Ketolar; Parke-Davis, Prat de Ll., Spain) and 5 mg/kg of xylazine (Rompum; Bayer AG, Leverkusen, Germany). Meningitis was induced using an intracisternal injection of 250  $\mu$ L of a saline suspension containing  $10^6$  cfu/mL of inoculum. In rabbits infected with the HUB 2349 strain, therapy was started 18 h post-inoculation. Owing to the slow progression of meningitis caused by the strain, therapy was initiated 40 h after inoculation in rabbits infected with the ATCC 51916 strain.<sup>21</sup> Rabbits were anaesthetized using urethane (Sigma Chemical Company, St Louis, MO, USA) at 1.75 g/kg subcutaneously and thiopental sodium (Tiopental; B. Braun Medical S.A., Rubí, Spain) at 5 mg/kg intravenously (iv). A blood sample was collected to assess secondary bacteraemia. Animals were placed in the stereotactic frame and a baseline CSF sample was taken (0 h). Antibiotic iv therapy ( $n \geq 8$  rabbits/group) was then administered for 26 h using one of the following therapy schedules: ceftriaxone at 100 mg/kg given once daily, fosfomycin at 300 mg/kg every 6 h, vancomycin at 15 mg/kg every 12 h, fosfomycin plus ceftriaxone, fosfomycin plus vancomycin, and ceftriaxone plus vancomycin. Doses of ceftriaxone and vancomycin were the same as those used in previous experiments.<sup>6,22</sup> Fosfomycin dosage was chosen in an attempt to obtain CSF levels similar to those achievable in humans with meningitis and taking into account the study by Nau *et al.*<sup>14</sup> Untreated controls ( $n \geq 10$ ) received saline. CSF samples were taken at 2, 6, 24 and 26 h of therapy. Hydration was ensured throughout the experiment. Mortality was assessed at 24 h. Surviving animals were euthanized using a lethal dose of thiopental sodium at the end of each experiment.

### Sample processing

CSF samples were used to determine bacterial counts, lactate and protein concentrations, and antibiotic levels at peak and trough time points. The lowest bacterial concentration detectable was 10 cfu/mL. For purposes of analysis, a value of 0.99 log cfu/mL was assigned to the first sterile culture, and a value of 0 log cfu/mL was assigned to the subsequent ones. Changes in bacterial counts ( $\Delta$ log cfu/mL) were calculated as the difference between bacterial concentrations at the start of therapy and at 2, 6, 24 and 26 h. Therapeutic failure was defined as an increase in bacterial concentration of at least 1 log cfu/mL compared with a previous count. A therapy was considered bactericidal when a reduction of 3 log cfu/mL was achieved. Samples were centrifuged at 5000 g for 10 min, and the supernatants were stored at  $-70^\circ\text{C}$ . Lactate concentrations were measured using a Lactate PAP kit (Biomérieux S.A., Marcy l'Etoile, France). Protein concentrations were determined using the Bradford method (Bio-Rad Protein Assay, Bio-Rad Laboratories, Munich, Germany).

### Pharmacokinetics

Pharmacokinetics data were compiled from a study of eight infected animals after a single iv dose of 300 mg/kg of fosfomycin,

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100 mg/kg of ceftriaxone or 15 mg/kg of vancomycin. Blood and CSF samples were taken at different time points, according to each therapy. A computer-assisted method (PK functions for Microsoft Excel; J. I. Usansky, A. Desai and D. Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA 92606, USA) was used to determine the following parameters in serum and CSF: maximum concentration ( $C_{max}$ ), area under the concentration–time curve over 24 h ( $AUC_{0-24}$ ) and CSF penetration as the comparison of areas under the curve ( $AUC_{CSF\ 24}/AUC_{serum\ 24}$ ).

### Antibiotic assays

Ceftriaxone and fosfomycin concentrations were measured using the agar disc diffusion method, using *Bacillus subtilis* ATCC 12432 and *Proteus vulgaris* ATCC 21100, respectively, as assay organisms. Vancomycin levels were determined using fluorescent polarization immunoassay (FPIA).

### Statistical analysis

Comparisons between groups were performed using the ANOVA test (followed by the Tunkel multiple-comparison test) if data were normally distributed and the Kruskal–Wallis test if data were not normally distributed. For analyses, inflammatory data comparisons between two groups were performed using the Mann–Whitney test. A *P* value of <0.05 was considered significant.

## Results

### In vitro studies

Although samples were removed at 0, 6 and 24 h of incubation, viable plate counts were considered only at 0 and 6 h because *S. pneumoniae* frequently underwent autolysis at 24 h (we observed spontaneous autolysis at 24 h in all killing curves with a bacterial growth >6.5 log cfu/mL at 6 h). A bactericidal effect was reached only with vancomycin at achievable concentrations in CSF (0.25–1 mg/L) against both strains and with

fosfomycin at high concentration (32 mg/L) against ATCC 51916 (MICs: ceftriaxone, 32 mg/L; fosfomycin, 4 mg/L). Synergy and additive effect were observed when fosfomycin was combined with ceftriaxone against HUB 2349 (MICs: ceftriaxone, 2 mg/L; fosfomycin, 16 mg/L). An indifferent effect was noted in all combinations of fosfomycin and ceftriaxone against ATCC 51916. The addition of fosfomycin to vancomycin was synergic against HUB 2349 and showed an additive effect against ATCC 51916. Resistant mutants were demonstrated readily for both strains after 24 h of exposure to fosfomycin alone and in combination, with a 4- to 256-fold increase in MIC values.

### Pharmacokinetics

Fosfomycin peak level in serum was  $324.48 \pm 102.1$  mg/L at 0.5 h and in CSF was  $35.07 \pm 8.59$  mg/L at 1 h. The maximum ceftriaxone concentration found in serum was  $28.82 \pm 9.13$  mg/L at 0.5 h and in CSF was  $3.65 \pm 1.96$  mg/L at 2 h. Vancomycin peak level in serum was  $38.96 \pm 6.54$  mg/L at 0.5 h and in CSF was  $0.91 \pm 0.34$  mg/L at 2 h. CSF penetration was 49.2, 28.4 and 14.6% for fosfomycin, ceftriaxone and vancomycin, respectively.

### Pharmacodynamics

The true pharmacodynamic parameter related to MIC for each antibiotic is included in Table 1.

### Experimental meningitis caused by the HUB 2349 strain

Secondary bacteraemia at 0 h in different groups ranged from 75 to 100%. Mortality at 24 h was 30% in the control group and 0% in all the therapy groups. Table 1 shows CSF bacterial counts at 0 h and CSF bacterial reduction at 24 h with the different schedules. Antibiotic levels in the CSF of rabbits infected with the HUB 2349 strain are summarized in Table 2. Lactate and protein concentrations in CSF at 24 h are summarized in Table 3. Fosfomycin alone failed in the two animals that presented the lowest CSF levels (concentrations of 40.54 and 39.09 mg/L at 26 h) and

**Table 1.** Initial bacterial concentrations and bacterial killing rates in CSF of rabbits with pneumococcal meningitis caused by the HUB2349 strain (MICs: FOF, 16 mg/L; CRO, 2 mg/L) and the ATCC 51916 strain (MICs: FOF, 4 mg/L; CRO, 32 mg/L)

Therapy group (dose in mg/kg/day)	HUB 2349 strain				ATCC 51916 strain			
	initial titres (log cfu/mL)	bacterial decreases ( $\Delta$ log cfu/mL) at 24 h	no. of animals with sterile CSF cultures at 24 h/total	PD parameter related to MIC <sup>a</sup>	initial titres (log cfu/mL)	bacterial decreases ( $\Delta$ log cfu/mL) at 24 h	no. of animals with sterile CSF cultures at 24 h/total	PD parameter related to MIC <sup>a</sup>
FOF 1200	4.55 ± 0.40	-2.46 ± 1.77	5/9	3.65	5.16 ± 0.89	-4.29 ± 0.86 <sup>†</sup>	9/9	11.96
CRO 100	4.55 ± 0.47	-3.38 ± 1.38	8/9	5.94%	5.23 ± 0.88	-0.75 ± 1.72	0/8	0%
VAN 30	4.45 ± 0.55	-3.85 ± 0.73	10/10	37.82 h	5.00 ± 0.76	-3.44 ± 1.47 <sup>†</sup>	6/8	37.82 h
FOF + CRO	4.89 ± 1.02	-4.52 ± 0.84*	8/8		4.91 ± 0.78	-4.78 ± 0.73 <sup>†</sup>	8/8	
FOF + VAN	4.59 ± 0.66	-4.30 ± 0.97*	8/8		4.85 ± 0.59	-4.23 ± 0.63 <sup>†</sup>	8/8	
CRO + VAN	4.48 ± 0.57	-4.24 ± 0.74*	8/8		5.17 ± 1.14	-4.25 ± 1.17 <sup>†</sup>	8/8	
Control	4.59 ± 1.04	0.97 ± 1.94	0/10		4.78 ± 0.84	1.12 ± 2.09	0/12	

FOF, fosfomycin; CRO, ceftriaxone; VAN, vancomycin.

Data are expressed as means ± SD.

<sup>a</sup>PD parameters were  $C_{max}/MIC$  for fosfomycin;  $t > MIC$  for ceftriaxone; and  $AUC/MIC$  for vancomycin.

\**P* < 0.05 against FOF monotherapy (ANOVA test).

<sup>†</sup>*P* < 0.05 against CRO monotherapy (ANOVA test).

**Table 2.** Mean ( $\pm$  SD) antibiotic concentrations (mg/L) in CSF of rabbits with pneumococcal meningitis caused by two tested strains of *Streptococcus pneumoniae*

Antibiotics (doses in mg/kg/day) <sup>a</sup>	HUB 2349 strain				ATCC 51916 strain			
	2 h	6 h	24 h	26 h	2 h	6 h	24 h	26 h
CRO 100	3.30 $\pm$ 1.54	2.30 $\pm$ 0.98	0.64 $\pm$ 0.12	3.23 $\pm$ 0.97	4.61 $\pm$ 2.74	1.76 $\pm$ 0.89	0.30 $\pm$ 0.02 <sup>b</sup>	4.84 $\pm$ 3.06
FOF 1200	58.40 $\pm$ 21.81	36.64 $\pm$ 15.58	37.05 $\pm$ 23.18	66.27 $\pm$ 29.63	47.82 $\pm$ 16.86	31.52 $\pm$ 15.13	30.46 $\pm$ 11.58	50.95 $\pm$ 21.45
VAN 30	1.93 $\pm$ 1.06	1.21 $\pm$ 0.49	0.80 $\pm$ 0.29	1.89 $\pm$ 1.11	1.01 $\pm$ 0.59	0.52 $\pm$ 0.16	0.39 $\pm$ 0.25	1.44 $\pm$ 1.27

CRO, ceftriaxone; FOF, fosfomicin; VAN, vancomycin.

<sup>a</sup>Ceftriaxone was administered twice (at 0 and 24 h), vancomycin three times (at 0, 12 and 24 h) and fosfomicin every 6 h [at 0, 6, 12 (double dose) and 24 h].

<sup>b</sup>Trough concentrations were detected in samples from two animals only. The remainder of the samples were below the level of detection (0.25 mg/L).

**Table 3.** CSF lactate (mmol/L) and protein (g/L) concentrations at 24 h in rabbits with pneumococcal meningitis caused by two strains of *Streptococcus pneumoniae*

Therapy group (dose in mg/kg/day)	HUB 2349 strain		ATCC 51916 strain	
	CSF lactate levels	CSF protein levels	CSF lactate levels	CSF protein levels
FOF 1200	4.37 $\pm$ 1.17	1.92 $\pm$ 0.87	4.41 $\pm$ 2.63	1.79 $\pm$ 0.68
CRO 100	3.20 $\pm$ 0.50	1.98 $\pm$ 1.17	6.87 $\pm$ 2.73	2.60 $\pm$ 1.00
VAN 30	3.37 $\pm$ 0.82	2.24 $\pm$ 0.99	3.64 $\pm$ 2.81 <sup>†</sup>	1.94 $\pm$ 1.12
FOF + CRO	3.15 $\pm$ 0.73	1.67 $\pm$ 0.71	3.93 $\pm$ 2.70 <sup>†</sup>	1.98 $\pm$ 1.30
FOF + VAN	2.92 $\pm$ 0.93	1.55 $\pm$ 0.85	2.56 $\pm$ 0.72* <sup>†</sup>	1.59 $\pm$ 0.87
CRO + VAN	2.93 $\pm$ 0.99	1.94 $\pm$ 0.80	2.39 $\pm$ 1.14* <sup>†</sup>	1.73 $\pm$ 0.94
Control	6.90 $\pm$ 5.84	2.53 $\pm$ 1.08	11.74 $\pm$ 10.34	3.45 $\pm$ 3.09

FOF, fosfomicin; CRO, ceftriaxone; VAN, vancomycin.

Data are expressed as means  $\pm$  SD.

\* $P < 0.05$  against CRO monotherapy (Mann–Whitney test).

<sup>†</sup> $P < 0.05$  against the control group (Mann–Whitney test).

in two others that had high CSF levels but a 10-fold increase in fosfomicin MIC value at 24 h. The remainder of the fosfomicin-treated animals (5/9) had CSF cultures below the level of detection after 24 h of therapy. Ceftriaxone was bactericidal in eight out of nine animals, but failed in the remaining one. Vancomycin was bactericidal from 6 h and no re-growth was observed. The addition of fosfomicin to ceftriaxone or vancomycin improved the efficacy of both antibiotics tested alone, being statistically significant compared with fosfomicin monotherapy ( $P < 0.05$ ), and decreased lactate and protein levels (though the differences were not statistically significant). In fosfomicin combinations, all CSF cultures were below the level of detection from 24 h and no resistant mutants were observed. The efficacy of fosfomicin combinations was similar to that provided by the combination of ceftriaxone with vancomycin.

#### Experimental meningitis caused by the ATCC 51916 strain

Secondary bacteraemia at 0 h in different groups ranged from 62.5 to 100%. Mortality at 24 h was 58.3% in control animals and 0% in all therapeutic regimens. Initial CSF bacterial concentrations and bacterial decreases at 24 h of different regimens are summarized in Table 1. CSF drug levels of rabbits with meningitis caused by the ATCC 51916 strain are shown in Table 2. CSF inflammatory parameters at 24 h are shown in Table 3. Fosfomicin was almost bactericidal from 6 h and no re-growth

was observed. Ceftriaxone showed a bacteriostatic effect, and therapeutic failure occurred in three out of eight animals. Vancomycin reached a bactericidal effect at 24 h but therapeutic failure occurred in 1 out of 10 animals that presented the lowest CSF vancomycin levels (0.03 mg/L). All regimens were significantly more effective than ceftriaxone alone at 24 h ( $P < 0.05$ ). The use of fosfomicin in combination with ceftriaxone or vancomycin improved activities and decreased CSF levels of lactate and protein in comparison with antibiotics tested alone (no statistical differences). All fosfomicin-treated animals had CSF cultures below the level of detection from 24 h and no emergence of resistance was noted. The efficacy of fosfomicin combinations was similar to that provided by the combination of ceftriaxone with vancomycin.

#### Discussion

The potential interest in the use of fosfomicin in the therapy of CNS infections is based on its *in vitro* activity against most bacterial pathogens that causes meningitis and on its good penetration across the blood–brain barrier. Our findings of ~50% penetration of fosfomicin into CSF add evidence to previous reports of its favourable CSF kinetics.<sup>12,23</sup> Using a fosfomicin dose of 300 mg/kg/6 h, we attained peak CSF levels, ranging from 35 to 60 mg/L, similar to those observed in humans

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receiving high iv doses. In a study of 12 patients with pneumococcal meningitis, iv administration of 16 g daily led to a CSF fosfomycin level of 52.2 mg/L within 3 days of treatment.<sup>23</sup> In another series of 10 patients with bacterial meningitis, mean peak CSF levels of 31 mg/L on the second day and 37.2 mg/L on the fifth day were achieved using a dose of 200 mg/kg/day.<sup>12</sup> Furthermore, even in the presence of an intact blood-brain barrier, fosfomycin penetrates into the CSF to some extent.<sup>24</sup> These kinetic characteristics presumably mean that its penetration would not be affected by the concomitant use of dexamethasone.

In this experiment we used two penicillin-resistant pneumococcal strains belonging to the 23F serotype, with different phenotypes. The susceptibility pattern of the HUB 2349 strain is common among penicillin-resistant pneumococci causing meningitis, whereas that of the ATCC 51916 strain is very rare. The use of both strains helped us to better explore the possibilities of fosfomycin use alone and in combination.

In the *in vitro* studies, at levels achievable in human CSF, fosfomycin was bactericidal against ATCC 51916 (MIC/MBC to fosfomycin of 4/32 mg/L) but was unable to produce a bactericidal effect against HUB 2349 (MIC/MBC to fosfomycin of 16/64 mg/L). Fosfomycin showed bactericidal activity only against ATCC 51916 in the rabbit model as well.

The pharmacodynamics of fosfomycin in the CNS is not well known. It has been suggested that high  $C_{max}$  values are not essential as the drug exerts a non-concentration-dependent *in vitro* bacterial growth inhibition of *S. aureus*.<sup>11</sup> However, in the rabbit meningitis model Nau *et al.* observed that a CSF concentration of at least 10 times the MIC of the tested strain was necessary to produce a rapid bacterial killing of *S. pneumoniae*.<sup>14</sup> This is in accordance with our study, where a bactericidal effect was obtained *in vitro* but in the animal model only when fosfomycin concentrations were eight times the MIC value, a condition unattainable with the HUB 2349 strain. Whatever parameter best predicts fosfomycin efficacy, the MIC of the causative strain is of paramount importance. It is widely accepted that most pneumococci are susceptible to fosfomycin, regardless of their susceptibility to penicillin; however, for penicillin-susceptible strains fosfomycin MICs tend to be lower (from 1 to 8 mg/L) than for penicillin-resistant strains (from 8 to 64 mg/L).<sup>9,10,14</sup> In addition, fosfomycin MBCs are usually 2- to 4-fold higher than MICs. Our data suggest that for meningeal infections fosfomycin breakpoints should be revised and that strains with MICs  $\geq 16$  mg/L should be regarded as non-susceptible.

The efficacy shown by ceftriaxone and vancomycin as single agents, both *in vitro* and in the rabbit model, was in accordance with previous reported studies.<sup>6,22,25</sup> Failures were rare but occurred with either agent once CSF levels declined in comparison with the average from the other animals. Furthermore, in the particular case of meningitis caused by ATCC 51916, ceftriaxone did not show bactericidal effect.<sup>26</sup>

Several investigators have reported *in vitro* synergy between  $\beta$ -lactams and fosfomycin against penicillin-resistant pneumococcal strains.<sup>27,28</sup> *In vivo*, using an experimental fibrin clot pneumococcal infection model in rabbits, Chavanet *et al.* demonstrated that combinations of fosfomycin with ceftriaxone increased bacterial reduction and delayed re-growth.<sup>29</sup> In the rabbit model of meningitis caused by a fully susceptible *S. pneumoniae* type 3 strain, the combination of the two antibiotics produced an additive effect.<sup>14</sup> In our study, the combination of fosfomycin with ceftriaxone was synergic only against the

strain with higher MIC to fosfomycin *in vitro*, whereas in the animal model the combination improved the antibacterial activity against both strains.

Little information is available on the activity of combinations of fosfomycin with vancomycin against pneumococci. In an *in vitro* study of 26 pneumococcal strains, bactericidal activity of vancomycin was unaffected by the addition of fosfomycin.<sup>25</sup> In our study, the efficacy of this combination was similar to that observed for the combination of fosfomycin with ceftriaxone: *in vitro* synergy against the strain with higher MIC of fosfomycin and improvement of antibacterial activity against both strains in rabbits.

The combination of fosfomycin with ceftriaxone was slightly more effective than the other combined therapies, including the reference combination of ceftriaxone with vancomycin, although the differences were not significant.

The specific mechanisms of action of different antibiotics may induce differences in the release of pneumococcal cell wall components. Among antibiotics acting against the bacterial cell wall we have found only that CSF lactate and protein levels with ceftriaxone are statistically higher than those observed with combinations of fosfomycin with vancomycin ( $P = 0.000$ ) and ceftriaxone with vancomycin ( $P = 0.001$ ) in groups of rabbits infected with the ATCC 51916 strain. This increase of CSF lactate and protein levels may be attributable to the lack of activity that ceftriaxone has against this strain.

Development of resistance to fosfomycin was a frequent phenomenon *in vitro* when fosfomycin was studied alone and in combination against both strains. In the animal model, the use of fosfomycin in combination prevented the emergence of drug-resistant populations. The discordance between the emergence of resistant mutants *in vitro* and in the animal model has been observed previously with other drugs such as rifampicin.<sup>30</sup> The emergence of resistant mutants during therapy precludes the use of both rifampicin and fosfomycin as single agents in clinical practice. The possible role of fosfomycin combinations described in our study parallels to some extent that of rifampicin combinations: both may be used as alternatives to the standard ceftriaxone with vancomycin combination therapy in patients with allergy or intolerance to the reference drugs or in rare cases of infection with pneumococcal strains showing very high cephalosporin resistance.

In conclusion, the anti-pneumococcal activity of fosfomycin, at CSF achievable concentrations, is greatly affected by the MIC of the infecting strain. This, coupled with the frequent emergence of resistance during therapy, precludes its use as single agent. However, combinations of fosfomycin with ceftriaxone and vancomycin improve the activity of either drug used alone and are as effective as the reference combination of ceftriaxone with vancomycin. Accordingly, fosfomycin combinations might be considered as alternatives in cases of allergy or intolerance to first-line drugs or in rare cases of meningitis caused by pneumococcal strains showing very high resistance to cephalosporin.

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## Transparency declarations

None to declare.

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