

Effects of EDP-420 on penicillin-resistant and quinolone- and penicillin-resistant pneumococci in the rabbit meningitis model

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Objectives: To test the efficacy of EDP-420, a new ketolide, in experimental pneumococcal meningitis and to determine its penetration into the CSF.

Methods: The experimental rabbit model was used in this study and EDP-420 was tested against a penicillin-resistant and a penicillin- and quinolone-resistant mutant. EDP-420 was also tested against both strains in time-killing assays over 8 h *in vitro*.

Results: In experimental meningitis, EDP-420 produced a bactericidal activity comparable to the standard regimen based on a combination of vancomycin with ceftriaxone against a penicillin-resistant *Streptococcus pneumoniae* and a penicillin- and quinolone-resistant *S. pneumoniae* isolate. The penetration of EDP-420 into inflamed meninges was 38% after an iv injection of 10 mg/kg. The bactericidal activity of EDP-420 was also confirmed in *in vitro* time-killing assays.

Conclusions: EDP-420 is an efficacious alternative treatment in pneumococcal meningitis, especially when resistant strains are suspected.

Keywords: pneumococcal meningitis, ketolide, resistance

Introduction

The continuous spread of penicillin-resistant pneumococci has significantly complicated the treatment of pneumococcal infections.¹ The epidemiological situation is clearly deteriorating worldwide. Based on a recent surveillance study (PROTEKT) including 69 centres in 25 countries, the overall penicillin resistance ranged from 25% to 53%. The highest resistance rates were reported in Asia (53.4%), followed by France (46.2%) and Spain (42.1%), whereas in the Netherlands all strains were susceptible to penicillin.² In addition, resistance to erythromycin A was even higher (from 31.1% to 79.6%) with the highest rates observed in Asia. In that study, all strains were susceptible to telithromycin. These data emphasize the need for alternative regimens for pneumococcal diseases. In a more recent study in the USA, telithromycin retained activity against erythromycin-resistant [mainly *mef(A)*-positive] strains.³

Based on actual guidelines, the empirical treatment of pneumococcal meningitis consists of a combination of a

third-generation cephalosporin with vancomycin, especially when resistant strains are suspected.⁴ However, the constantly increasing number of pneumococcal isolates resistant to cephalosporins (cefuroxime, cefixime and cefpodoxime with resistance rates ranging from 62% to 68% in Asia) might jeopardize the use of cephalosporins in the empirical treatment of pneumococcal meningitis.² Furthermore, the unreliable penetration of vancomycin in case of adjunctive steroid therapy might compromise the efficacy of the empirical treatment.⁵

Little is known about the role of macrolides or ketolides in the treatment of meningitis. To our knowledge, no data are available about the role of telithromycin in pneumococcal meningitis.

The aim of this study was to test EDP-420, a new class of bicyclic ketolide (a bridged bicyclic macrolide) with excellent activity against pneumococci, in an experimental meningitis model using a penicillin-resistant and a penicillin- and quinolone-resistant *Streptococcus pneumoniae* isolate.

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Materials and methods

Pneumococcal strain

The pneumococcal strain WB4 (MIC of penicillin is 4 mg/L, serogroup 6) was isolated from the blood of a patient at the Inselspital in Bern and was provided by the Institute for Infectious Diseases at the University of Bern. This strain was grown in Mueller–Hinton broth until an approximate density of 10^8 cfu/mL and was then diluted to $\sim 10^6$ cfu/mL for *in vivo* experiments. MICs were determined in liquid culture and growth was controlled after 6, 12 and 24 h because of the spontaneous autolysis of pneumococci. The MICs of ceftriaxone, vancomycin and EDP-420 were 0.5, 0.12 and 0.06 mg/L, respectively. The quinolone-resistant strain was obtained by sequential exposure of the strain WB4 to trovafloxacin. The MICs after this exposure were 4 mg/L for penicillin, 0.5 mg/L for ceftriaxone, 0.12–0.25 mg/L for vancomycin, 4 mg/L for trovafloxacin, 32 mg/L for ciprofloxacin and 0.06 mg/L for EDP-420.

Experimental meningitis model

The experimental rabbit meningitis model described by Dacey and Sande⁶ was used in this project. The experimental protocols were approved by the federal veterinary office of the county of Bern.

Pathogen-free New Zealand rabbits weighing 2.5–3 kg were provided by the Zentraltierställe der Medizinischen Fakultät der Universität Bern, where all the experiments were performed.

One day before an experiment, rabbits were anaesthetized by intramuscular injection of a combination of ketamine and xylazine to fit prostheses on their calvarium to facilitate subsequent placement within a stereotactic frame. On the day of the experiment, rabbits received 1.75 g/kg ethylcarbamate (urethane) subcutaneously and then 10 mg/kg pentobarbital iv to induce deep anaesthesia. The animals were fixed in stereotactic frames and a 3.5 inch (25 G) spinal needle was introduced into the cisterna magna. Following the withdrawal of 0.2 mL of CSF, pneumococci (1×10^5 cfu in 0.2 mL of saline solution) were injected into the subarachnoid space. After inoculation, the animals were brought back to the cages for the night. The next day, the rabbits were fitted again in the frames using the techniques and anaesthesia described above. A catheter was fixed in the femoral artery for serum sampling. A spinal needle was fixed again in the subarachnoid space. Antibiotics were injected intravenously in standard doses described in the literature^{7,8} (100 mg/kg ceftriaxone, 20 mg/kg vancomycin).

EDP-420 initially was administered at a lower dose (10 mg/kg) to determine the penetration into inflamed meninges in order to estimate a proper dose for the larger efficacy study. A higher dose of 30 mg/kg was selected as the estimated dose for efficacy based on EDP-420 pharmacokinetics and pharmacodynamics described by Maglio⁹ in mice. Vancomycin was given at 0 and 4 h and ceftriaxone at 0 h according to their pharmacokinetic properties. CSF (0.2 mL) was sampled at 0, 1, 2, 4, 5, 6 and 8 h after initiation of therapy. Blood samples were collected at 0.25, 0.5, 1, 2, 3, 4, 4.25, 4.5, 5, 6, 7 and 8 h after initiation of therapy. Each group included untreated controls which received comparable volumes of saline.

Determination of antibiotic levels and cfu titres

EDP-420 concentration in serum and CSF was determined by LC/MS/MS (performed by Enanta Pharmaceuticals, Inc.). cfu were measured by serial dilution of CSF, plating on agar plates with 5% sheep blood and incubation overnight at 37°C in 5% CO₂.

Statistical analysis

The Student *t*-test and one-way analysis of variance (Newman–Keuls's multiple comparisons test) were used for parametric data. Comparison of positive and negative cultures was analysed by the two-tailed Fisher exact test. A *P* value of <0.05 was considered significant.

Results

The serum and CSF kinetics of EDP-420 in infected rabbits at 10 mg/kg is presented in Figure 1 and Table 1. After an iv injection to infected rabbits, EDP-420 serum level peaked at 2.59 mg/L at 0.25 h and decreased rapidly to 0.928 mg/L 2 h after an injection. During the following 6 h, EDP-420 levels decreased more slowly with levels of 0.453 mg/L at the end of the treatment period at 8 h. In the corresponding CSF, EDP-420 concentration increased during the first 2 h and then remained stable until the end of the 8 h treatment period with an average C_{max} of 0.676 mg/L and an average concentration of 0.479 mg/L at 8 h. The AUC_{0–8} values in CSF and serum were 2.48 and 6.53 mg·h/L, respectively. The CSF/serum ratios were 0.28 ± 27 and 0.38 ± 38 for C_{max} and AUC_{0–8}, respectively.

When 10 mg/kg was given intravenously to non-infected rabbits, there was almost no penetration (only 1%) across the non-inflamed meninges whereas the serum concentration remained relatively similar to the infected rabbits at the same dose (Table 1). However, at the same dose in infected animals, the concentrations were ~25% and 35% higher for C_{max} and AUC than in non-infected animals.

An EDP-420 dose of 30 mg/kg given intravenously to infected rabbits resulted in a proportional increase in the serum level from 10 to 30 mg/kg (Figure 2). However, the CSF penetration did not increase at this dose with a C_{max} of 0.607 mg/L and an AUC_{0–8} of 2.75 mg·h/L which are at a similar level to the 10 mg/kg dose (Figure 3 and Table 2).

As known from a previous study,⁷ vancomycin, used in standard doses (20 mg/kg every 4 h, 2 doses) in rabbits, produced CSF levels between 1.74 and 4.0 mg/L and ceftriaxone (100 mg/kg) led to CSF levels between 3.5 and 5.5 mg/L.⁸ During the entire treatment period, EDP-420 CSF levels remained above the MIC leading to CSF/MIC ratios between 5 and 8.

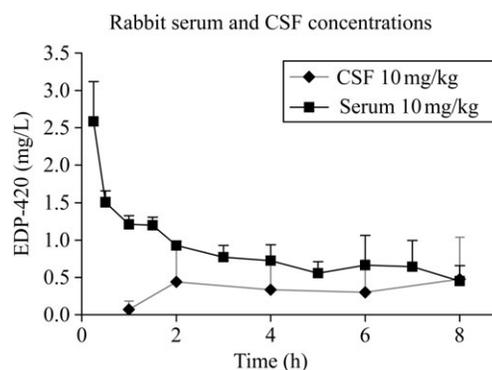


Figure 1. Serum and CSF concentration of EDP-420 at a dose of 10 mg/kg in rabbits infected with penicillin-resistant *S. pneumoniae* WB4. Filled squares represent serum levels and filled diamonds represent CSF levels.

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Table 1. EDP-420 kinetic parameters at 10 mg/kg iv in uninfected rabbits and rabbits infected with penicillin-resistant *S. pneumoniae* WB4

	Uninfected rabbits			Infected rabbits		
	C_{max} (mg/L)	AUC_{0-8} (mg·h/L)	$t_{1/2}$ (h)	C_{max} (mg/L)	AUC_{0-8} (mg·h/L)	$t_{1/2}$ (h)
CSF	0.0276 ± 0.0136	0.0806 ± 0.025	ND ^a	0.677 ± 0.617	2.48 ± 2.37	ND ^a
Serum	2.32 ± 0.905	6.16 ± 0.821	5.15 ± 2.65	2.59 ± 0.533	6.53 ± 1.13	8.48 ± 6.79
CSF/serum ratio	0.014 ± 0.011	0.013 ± 0.006		0.28 ± 0.27	0.38 ± 0.38	

^aCannot be determined, concentration still high at 8 h.

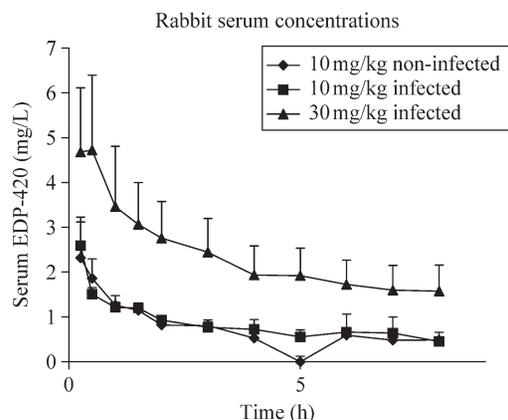


Figure 2. Serum concentration of EDP-420 at dosages of 10 and 30 mg/kg in non-infected and infected rabbits. Filled diamonds represent serum levels of 10 mg/kg in non-infected rabbits, and filled squares and filled triangles represent serum levels of 10 and 30 mg/kg in infected rabbits, respectively.

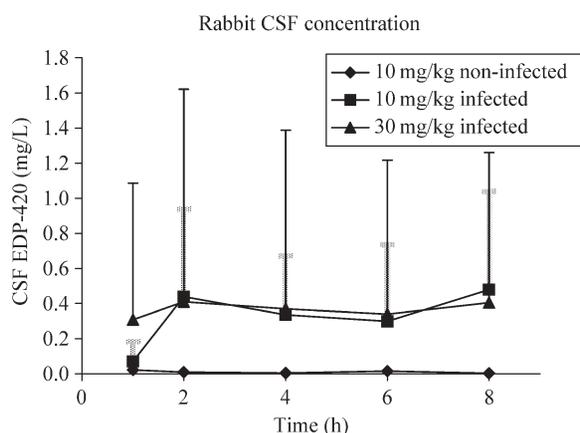


Figure 3. CSF concentration of EDP-420 at dosages of 10 and 30 mg/kg in non-infected and infected rabbits. Filled diamonds represent CSF levels of 10 mg/kg dose in non-infected rabbits, and filled squares and filled triangles represent CSF levels of 10 and 30 mg/kg doses in infected rabbits, respectively.

The antibacterial efficacy of EDP-420 against the two pneumococcal strains is presented in Table 3. In the untreated controls, the growth of bacteria was negligible, $<1 \log_{10}$ cfu/mL over 8 h. Before the initiation of treatment, the bacterial titre was significantly higher in the EDP-420 group (6.91 ± 0.41 EDP-420 versus 5.88 ± 0.70 for the standard regimen, $P < 0.004$). Against the penicillin-resistant strain, EDP-420 was

Table 2. EDP-420 kinetic parameters at 30 mg/kg iv in rabbits infected with penicillin- and quinolone-resistant *S. pneumoniae* WB4

	C_{max} (mg/L)	AUC_{0-8} (mg·h/L)	$t_{1/2}$ (h)
CSF	0.607 ± 1.20	2.75 ± 7.17	ND ^a
Serum	5.08 ± 1.59	18.6 ± 5.45	5.87 ± 2.07
CSF/serum ratio	0.12 ± 0.20	0.16 ± 0.36	

^aCannot be determined, concentration still high at 8 h.

bactericidal ($-0.61 \pm 0.10 \Delta \log_{10}$ cfu/mL·h) with a decrease of the viable cell count of $4.80 \log_{10}$ at the end of the treatment period, managing to sterilize the CSF of 3 out of 10 rabbits. The standard regimen based on a combination of vancomycin with ceftriaxone produced a similar antibacterial activity ($-0.57 \pm 0.13 \Delta \log_{10}$ cfu/mL·h and $-4.52 \pm 1.30 \log_{10}$ cfu/mL after 8 h). At the end of the experimental period, the CSF in 5 out of 10 rabbits was sterile in the comparator regimen. Using the penicillin- and quinolone-resistant strain, the initial titre was similar in all groups, ranging from 5.41 to $6.20 \log_{10}$ cfu/mL. In the untreated controls, the bacterial growth was slightly more pronounced after 8 h ($+1.36 \log_{10}$ cfu/mL compared with $+0.55 \log_{10}$ cfu/mL for the penicillin-resistant strain). Both treatment groups produced similar antibacterial activity (Table 3) and sterilized the CSF of 5 out of 10 rabbits.

In the *in vitro* time-killing assays, EDP-420 was tested only against the penicillin-resistant strain. With concentrations 5 and $10\times$ above the MIC, EDP-420 showed bactericidal activity, reducing the bacterial titre by 4.5 and $5.5 \log_{10}$ cfu/mL, respectively after 8 h (Figure 4).

Discussion

S. pneumoniae is one of the leading causes of severe infections, and is responsible for 500 000 cases of pneumonia, 55 000 cases of bacteraemia and 6000 cases of meningitis each year in the USA.¹ However, the introduction of the protein-polysaccharide conjugate vaccine in 2000 in the USA led to a significant decrease of invasive pneumococcal infections, especially in children under 2 years of age.¹⁰ Since the first report of penicillin-resistant pneumococcal isolates in Papua New Guinea and Australia, increasing resistance rates have been reported worldwide, especially in several countries in Asia.¹¹ In some tissues

Table 3. EDP-420 and ceftriaxone in combination with vancomycin against penicillin-resistant and penicillin- and quinolone-resistant *S. pneumoniae* in experimental meningitis

Groups (rabbits <i>n</i> = 10)	Bacterial strain	Dose (mg/kg)	Inoculum (log ₁₀ cfu/mL)	Killing rates/h (Δlog ₁₀ cfu/mL·h)	Killing rates/8 h (Δlog ₁₀ cfu/mL·8 h)
Controls	PenR	0	6.56 ± 0.49	+0.06 ± 0.06	+0.55 ± 0.45
EDP-420	PenR	30	6.91 ± 0.41	-0.61 ± 0.10 ^a	-4.80 ± 0.59 ^a
CRO + VAN	PenR	CRO (100) VAN (2 × 20)	5.88 ± 0.70	-0.57 ± 0.13 ^a	-4.52 ± 1.30 ^a
Controls	PenR + QuR	CRO (100) VAN (2 × 20)	5.41 ± 0.90	+0.21 ± 0.17	+1.35 ± 0.75
EDP-420	PenR + QuR	30	6.20 ± 0.55	-0.57 ± 0.11 ^b	-4.83 ± 0.86 ^b
CRO + VAN	PenR + QuR	CRO (100) VAN (2 × 20)	5.90 ± 0.51	-0.60 ± 0.13 ^b	-4.96 ± 1.15 ^b

CRO, ceftriaxone; VAN, vancomycin; PenR, penicillin-resistant *S. pneumoniae* WB4; PenR + QuR, *S. pneumoniae* WB4 following sequential exposure to sub-MIC of trovafloxacin.

^a*P* not significant for EDP-420 versus CRO + VAN.

^b*P* not significant for EDP-420 versus CRO + VAN.

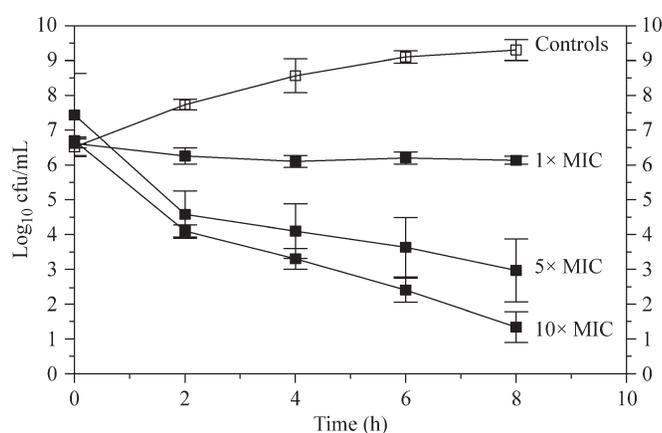


Figure 4. *In vitro* kill kinetics of EDP-420 against penicillin-resistant *S. pneumoniae* WB. Empty squares represent untreated controls and filled squares represent 1 ×, 5 × and 10 × MIC of EDP-420, respectively.

where the antibiotic penetration is limited, as is the case in meningitis, an inadequate treatment might have devastating consequences, underlining the need for new alternative treatments. Based on the recent PROTEKT surveillance study,¹¹ all 3362 tested pneumococcal strains were susceptible to telithromycin, the only ketolide available on the market.

EDP-420 is a new 6,11-bridged bicyclic macrolide antibiotic belonging to the new bicyclolide family. It is highly effective against resistant respiratory pathogens including erythromycin-resistant *S. pneumoniae* as well as MLS-inducible resistant *Staphylococcus aureus* and methicillin-susceptible *S. aureus*.¹² As with all macrolides and ketolides, the mechanism of action of EDP-420 is based on inhibition of protein synthesis by direct binding to the 50S subunit of bacterial ribosomes and prevention of translation and ribosome assembly.¹³ The good tissue penetration and the excellent pharmacokinetic features (i.e. a long half-life) seem to be promising properties of EDP-420 which should be explored further as a potential new treatment regimen for pneumococcal meningitis, especially when caused by resistant strains.

The penetration of EDP-420 into inflamed meninges was around 38% at 10 mg/kg. However, the penetration into the

meninges seemed saturated at 30 mg/kg (Tables 1 and 2). Further experiments with different doses may be needed to determine the real penetration of EDP-420 into inflamed meninges and its potential for use in humans.

The very low MIC (0.06 mg/L) against penicillin-resistant pneumococci and the bactericidal activity of EDP-420, demonstrated in time-killing assays *in vitro* (Figure 4), are two prerequisites qualifying EDP-420 as a new efficacious agent for pneumococcal infections. In our experimental meningitis model, EDP-420 at 1 × 30 mg/kg had similar efficacy to the standard regimen of a combination of ceftriaxone (1 × 100 mg/kg) with vancomycin (2 × 20 mg/kg) used in this animal model of meningitis. Even against erythromycin-resistant strains with MICs between 0.003 and 0.006 mg/L based on a recent study, EDP-420 is expected to be effective.¹⁴

In summary, its excellent efficacy *in vivo* and its good penetration into the CSF warrant further studies of EDP-420 as a potential candidate for the treatment of CNS pneumococcal infections, especially against resistant strains. We are aware that the penicillin-resistant pneumococcal strain used in this study is not ideal and a macrolide-resistant strain would have been more appropriate. On the other hand, this penicillin-resistant strain has been used in multiple studies allowing a precise comparison of the efficacies of the different regimens.

Further efficacy and pharmacokinetics–pharmacodynamics studies are needed. To our knowledge, this preliminary study shows, for the first time, the efficacy of a bicyclolide class antibiotic in experimental meningitis.

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Transparency declarations

A. S., P. G., F. A., M. C. and P. C. do not have conflicts of interest of a financial nature with the Enanta Company. L. J., P. N., D. W., G. W. and Ly T. P. are employees of the Enanta Company and measured the antibiotic levels but were not involved in the redaction of the manuscript.

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