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SUSCEPTIBILITY

In Vitro Susceptibility of *Neisseria gonorrhoeae* Strains to Mupirocin, an Antibiotic Reformulated for Parenteral Administration in Nanoliposomes

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ABSTRACT *Neisseria gonorrhoeae* is an urgent antibiotic-resistant threat. This study determined the MICs of mupirocin to be 0.0039 to 0.0625 μg/ml for 94 *N. gonorrhoeae* strains. Cross-resistance with other antibiotics was not detected. Mupirocin, which is currently limited to topical administration, demonstrated activity by injection when delivered in nanoliposomes. The nanoliposomal formulation of mupirocin is a potential treatment for drug-resistant *N. gonorrhoeae*.

KEYWORDS antibiotic resistance, antimicrobial activity, antimicrobial resistance, cross-resistance, liposomes, Nano-mupirocin

Neisseria gonorrhoeae is the causative agent of gonorrhea, a sexually transmitted disease that can cause discharge and inflammation in the urethra, cervix, pharynx, or rectum. The World Health Organization (WHO) estimated that there are 78 million new gonococcal infections per year in the world (1, 2). About 30% of all gonorrhea cases in the United States are caused by strains that are resistant to at least one antibiotic (2). Of special worldwide concern is the emergence of cephalosporin-resistant *N. gonorrhoeae* strains that are also resistant to several other antibiotics and thus represent a challenge for devising effective treatment regimens. Currently, in most countries, the injectable extended-spectrum cephalosporin (ESC) ceftriaxone is the only remaining empirical monotherapy for gonorrhea. However, gonococcal *in vitro* resistance and/or treatment failures to the last-line oral ESC cefixime (and, more rarely, to ceftriaxone) have been verified in many countries. Consequently, dual antimicrobial therapy, mainly ceftriaxone injection plus azithromycin, is recommended (3).

Mupirocin, an antibiotic with a unique mechanism of action (inhibition of isoleucyl-tRNA synthetase [4]), is limited to topical use due to its rapid systemic elimination and high protein binding (5). Mupirocin loaded in nanoliposomes, termed Nano-mupirocin, enabled mupirocin efficacy after intravenous (i.v.) administration (6). Following i.v. administration of 40 mg/kg Nano-mupirocin to mice, the plasma maximum concentration of drug (C_{max}) was 771 μg/ml, and the area under the concentration-time curve (AUC) obtained was 1,763 μg · h/ml. The AUC after administration of the free drug was 100 times lower. The free drug was rapidly eliminated from the circulation (last quantifiable time point, 30 min after injection) compared to prolonged exposure after the administration of Nano-mupirocin (quantifiable concentrations up to the last time point tested). A similar pharmacokinetic (PK) pattern of prolonged exposure to Nano-mupirocin was also shown after i.v. administration to rabbits. Nano-mupirocin is also suitable for intramuscular injection, as was demonstrated in a pharmacokinetic study

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TABLE 1 Summary of the MIC testing at Southern Research[a]

Compound	MIC (μg/ml)			EUCAST resistance breakpoint (μg/ml)[b]	No. of isolates with MIC above the breakpoint	No. of isolates tested
	MIC_{90}	MIC_{50}	Range			
Mupirocin	0.031	0.031[c]	0.0039 to 0.0625	NA	NA	94
Azithromycin	8	0.5	0.0156 to >16	0.5	21	96
Cefixime	0.25	0.125	0.0039 to 0.5	0.125	31	95
Ceftriaxone	0.063	0.031	0.0039 to 0.125	0.125	0	94
Ciprofloxacin	16	16[c]	<0.0156 to >32	0.06	71	96
Penicillin	2	1	0.0156 to 8	1	43	95
Tetracycline	2	2[c]	0.0156 to 16	1	55	96

[a]The numbers of isolates used to calculate individual MIC_{90} and MIC_{50} values are indicated for each compound. The individual MIC values are found in the supplemental material. NA, not available.

[b]A concentration of an antibiotic that defines whether a species of bacteria is susceptible or resistant to the antibiotic. If the MIC is less than or equal to the susceptibility breakpoint, the bacteria is considered susceptible to the antibiotic. If the MIC is greater than this value, the bacteria is considered intermediate or resistant to the antibiotic (11).

[c]No difference between MIC_{90} and MIC_{50} due to sharp susceptibility breakpoints across all isolates.

(our unpublished data), and therefore may be considered for the parenteral treatment of resistant *N. gonorrhoeae* strains.

Here, the efficacy of mupirocin against a panel of clinical isolates of *N. gonorrhoeae* was tested and directly compared with the efficacies of six control antibiotics (azithromycin, cefixime, ceftriaxone, ciprofloxacin, penicillin, and tetracycline). Mupirocin was active against all isolates tested, with an MIC_{90} value of 0.031 μg/ml. Moreover, an isolate resistant to both cefixime and ceftriaxone (H041) was sensitive to mupirocin.

Bacterial strains and antimicrobial susceptibility testing. The MICs of mupirocin and control antibiotics against clinical isolates of *N. gonorrhoeae* were determined by the agar dilution method according to guidelines established by the Clinical and Laboratory Standards Institute (7). The first part of the study was performed at Southern Research (SR). For this part, 96 *N. gonorrhoeae* isolates were received from the Centers for Disease Control and Prevention (CDC). The second part of the study was performed at Uniformed Services University (USU). In this study, 3 *N. gonorrhoeae* isolates, MS11, H041, and NG886, which have different antibiotic resistance profiles, were used. Strain MS11 is a laboratory isolate that was originally isolated from a cervical infection (8), strain H041 (WHO X) is a clinical isolate obtained from a pharyngeal infection from Kyoto, Japan, in 2009 (9), and strain NG886 was obtained from Eurofin.

Mupirocin and control antibiotics. Mupirocin was obtained from Teva (Hungary). Ciprofloxacin, azithromycin, ceftriaxone, cefixime, penicillin, and tetracycline were obtained from Sigma-Aldrich (USA).

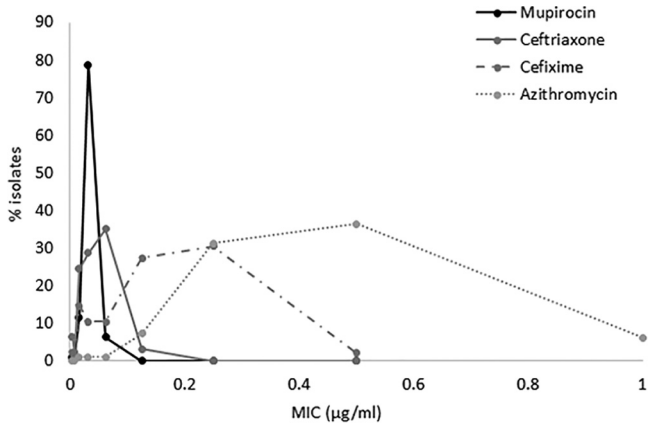

FIG 1 Percentages of *N. gonorrhoeae* isolates with MIC values at the low range (≤1 μg/ml) for mupirocin and three antibiotics used to treat gonorrhea. The individual MIC values are found in the supplemental material.

TABLE 2 MICs of mupirocin against resistant isolates tested at USUHS

Strain	Mupirocin MIC (μg/ml)	Resistance profile
MS11	0.05	Resistant to macrolide antibiotics
NG886	0.013	Resistant to tetracycline, penicillin, and ciprofloxacin
H041	0.05	Resistant to ceftriaxone, cefixime, other beta-lactam antibiotics, fluoroquinolones, macrolide antibiotics, and tetracycline

Mupirocin susceptibility and cross-resistance to existing antimicrobials. Table 1 presents the MIC data obtained for the first part of the study performed at SR. Mupirocin exhibited strong antibacterial activity against the test isolates, having an MIC_{90} value of 0.031 μg/ml. The MIC of mupirocin was in the range of 0.0039 to 0.0625 μg/ml, which is narrower than that obtained for the other compounds tested. Figure 1 shows the distribution of MICs for mupirocin, ceftriaxone, cefixime, and azithromycin against isolates that have an MIC below 1 μg/ml. The sharp peak of mupirocin at an MIC of 0.031 μg/ml accounts for most of the test isolates (79%). With the exception of ceftriaxone, all other compounds resulted in MICs above their susceptibility breakpoint for certain isolates. The activity of mupirocin against these isolates shows that there is no cross-resistance with mupirocin. The second part of the study performed at Uniformed Services University of the Health Sciences (USUHS) tested the MIC of mupirocin against 3 isolates with different resistance profiles (Table 2). Notably, strain H041, which is resistant to both cefixime and ceftriaxone, was sensitive to mupirocin (MIC, 0.05 μg/ml).

Mupirocin is an antibiotic having a unique mode of action that is not shared by any other therapeutically available antibiotics. Its use is currently limited to topical application due to its rapid elimination following injection and high protein binding. By formulating mupirocin in PEGylated nanoliposomes to obtain Nano-mupirocin (10), efficacy after parenteral administration and an improved pharmacokinetic profile were demonstrated. The plasma levels found in mice after i.v. administration of 40 mg/kg were above the MIC_{90} at all time points tested (2.0 μg/ml, 24 h after injection). The exposure parameters of C_{max} and AUC were ~26,000 and 59,000 times the MIC, respectively (6). Nano-mupirocin may therefore enable highly efficacious *in vivo* antibacterial activity of mupirocin against resistant *N. gonorrhoeae* strains.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02377-17.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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