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Ketolide Treatment of *Haemophilus influenzae* Experimental Pneumonia

KERRYL E. PIPER, MARK S. ROUSE, JAMES M. STECKELBERG, WALTER R. WILSON, AND ROBIN PATEL*

Division of Infectious Diseases and Department of Internal Medicine, Mayo Clinic and Foundation, Rochester, Minnesota

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The MICs of HMR 3004 and HMR 3647 at which 90% of beta-lactamase-producing *Haemophilus influenzae* isolates were inhibited were 4 and 2 μg/ml, respectively. Both HMR 3004 and HMR 3647 were active against beta-lactamase-producing *H. influenzae* in a murine model of experimental pneumonia. As assessed by pulmonary clearance of *H. influenzae*, HMR 3004 was more effective ($P < 0.05$) than was azithromycin, ciprofloxacin, clarithromycin, erythromycin A, pristinamycin, or HMR 3647 in this model.

Haemophilus influenzae is an important cause of upper and lower respiratory tract infections in children and in adults, especially among adult patients with preexisting lung disease, smokers, and the elderly. Many *H. influenzae* isolates are resistant to ampicillin and erythromycin. For example, a U.S. surveillance study of antimicrobial resistance documented that only 67.2% of respiratory tract *H. influenzae* isolates were ampicillin susceptible (11). In a study of *H. influenzae* isolates from the United States, the erythromycin MIC at which 90% of the isolates were inhibited was 8 μg/ml (9).

HMR 3004 (previously RU 004) and HMR 3647 (previously RU 66647) belong to a new class of antimicrobial agents, the ketolides. Ketolides are semisynthetic derivatives of 14-membered-ring macrolide antibiotics and have activity in vitro against beta-lactamase-producing *H. influenzae* (4, 8). Ketolides differ from macrolides by harboring a 3-keto group instead of an L-cladinose group on the erythronolide A ring (3). The mechanism of action of ketolides is similar to that of macrolides, involving binding of the drug to the 50S ribosomal subunit and consequent inhibition of protein synthesis (8). Ketolides have also been shown to penetrate well into phagocytic cells (12).

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The purpose of this study was to compare the efficacy of treatment of beta-lactamase-producing *H. influenzae* experimental murine pneumonia with HMR 3004 or HMR 3647 to that of treatment with an azalide and three macrolide compounds. Amoxicillin and ciprofloxacin were included in this model as treatments known to be ineffective and effective, respectively.

HMR 3004, HMR 3647, and pristinamycin were obtained from Hoechst Marion Roussel (Romainville, France); clarithromycin was obtained from Abbott Laboratories (North Chicago, Ill.); azithromycin was obtained from Pfizer (Groton, Conn.); amoxicillin was obtained from SmithKline Beecham (Philadelphia, Pa.); ciprofloxacin was obtained from Bayer (West Haven, Conn.); and erythromycin lactobionate (erythromycin A) was obtained from Lederle (Wayne, N.J.).

MICs for 30 clinical *H. influenzae* isolates were determined by a standard microtube dilution assay, and MBCs were determined with a 0.1-ml subculture onto chocolate blood agar plates (Table 1) (10). (For testing of susceptibility to erythromycin A, the same guidelines were followed, although the methodology is not specifically outlined by the National Committee for Clinical Laboratory Standards [10].) All 30 isolates produced beta-lactamase as assessed by a nitrocefin disk (10). Based on in vitro studies, HMR 3004 and HMR 3647 showed comparable activities against beta-lactamase-producing *H. influenzae* isolates (Table 1). The in vitro activity of the two ketolides was comparable to that of azithromycin and superior to that of clarithromycin and erythromycin A, consistent with previous reports (Table 1) (2, 3). Additionally, the study *H. influenzae* isolates were uniformly susceptible to ciprofloxacin (Table 1). One isolate, with antimicrobial susceptibilities representative of the group, was chosen for in vivo study (Table 1).

Serum antimicrobial concentrations were determined in triplicate by a microbiological assay (13). Murine blood was collected by entering the retroorbital sinus with a microhematocrit tube; serum was separated by centrifugation at 16,000 × *g* for 10 min. All bioassays were performed on Mueller-Hinton agar seeded with *Micrococcus lutea* ATCC 9341 as the indicator organism with the exception of ciprofloxacin, for which *Klebsiella pneumoniae* ATCC 10031 was used as the indicator organism. Paper disks with 20 μl of serum were placed on the bioassay plates, which were then incubated for 16 to 18 h in room air at 30°C (for *M. lutea*) or 35°C (for *K. pneumoniae*). The zone sizes were measured with calipers, and concentrations were calculated against a five-point standard curve by linear regression.

Mean concentrations in the sera of five uninfected mice at timed intervals after a single dose of the study antimicrobial are shown in Table 2. Antimicrobial dosages for in vivo study were chosen to approximate expected peak concentrations in human serum after the administration of standard dosages. Peak levels in serum were achieved within 60 min for all antimicrobials studied with the exception of azithromycin. The mean peak concentration of azithromycin in serum was not detected in the 90-min time frame studied (Table 2); serum samples collected 2 and 3 h after azithromycin administration had mean drug concentrations of 2.2 and 1.5 μg/ml, respectively.

* Corresponding author. Mailing address: Division of Infectious Diseases, Department of Internal Medicine, Mayo Clinic and Foundation, Rochester, MN 55902. Phone: (507) 255-7762. Fax: (507) 255-7767. E-mail: patel.robin@mayo.edu.

TABLE 1. Susceptibilities of 30 clinical isolates of beta-lactamase-producing *H. influenzae*

Antimicrobial agent	MIC (μg/ml)				MBC (μg/ml)			
	Range	50%	90%	For study strain	Range	50%	90%	For study strain
Amoxicillin				8				64
Azithromycin	0.5–4	1	2	1	1–8	2	4	1
Ciprofloxacin	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125
Clarithromycin	2–32	8	16	8	8–64	32	32	16
Erythromycin A	2–16	8	16	8	4–32	16	32	16
HMR 3004	0.5–8	2	4	2	1–16	4	8	2
HMR 3647	0.5–4	1	2	1	1–16	2	4	1
Pristinamycin	0.25–8	1	4	1	2–16	4	8	4

Experimental bacterial pneumonia was established in Jackson Laboratories C57BL/6 mice (25 to 30 g) by a modification of a previously described technique (7). Anesthesia was induced by intraperitoneal administration of pentobarbital, 50 mg/kg of body weight. The trachea was cannulated with a blunt animal feeding needle (1.25-mm ball diameter), and 0.05 ml of a bacterial suspension containing 10^8 CFU of *H. influenzae* per ml was instilled into the lower respiratory tract of each mouse. Mice were maintained in a vertical position for 3 min and then returned to their cages. Antimicrobial therapy was initiated 6 h after the mice were infected and continued for four doses, at 6 h intervals. Four hours after the last treatment, the animals were sacrificed with a lethal dose of 100 mg of pentobarbital per kg, given intraperitoneally. The chest was opened, and the lungs were excised aseptically. The lungs were weighed and homogenized in a Stomacher 80 (Tekmar Co., Cincinnati, Ohio) with 2 ml of Todd-Hewitt broth; the homogenate was quantitatively cultured. Serial 10-fold dilutions in Todd-Hewitt broth were plated on chocolate blood agar plates (0.1-ml aliquot per plate) and incubated for 48 h at 35°C in 5% CO_2. Tissue culture results were expressed as CFU of bacteria per gram of lung homogenate (Table 3). All sterile cultures were assigned a $\log_{10}$ CFU per gram of lung of 1.70.

For in vivo studies, enteral suspensions of amoxicillin and ciprofloxacin hydrochloride were administered. Enteral suspensions of azithromycin, clarithromycin (clarithromycin and 14-hydroxyclarithromycin in a 3:1 ratio), and pristinamycin were prepared in 1/10 methanol to 9/10 phosphate-buffered saline (pH 7) and sonicated for 30 s. A parenteral preparation of erythromycin A was used. The ketolides, HMR 3004 and HMR 3647, were administered as an enteral suspension in carboxymethyl cellulose sonicated for 30 s. All enterally administered antibiotics were administered into the stomach via a bent feeding needle.

Statistical analysis was performed by rank sum analysis. All antimicrobial regimens tested were more effective ($P < 0.05$) than no treatment, with the exception of amoxicillin (P = not significant). Quantitative lung cultures from mice infected with *H. influenzae* showed azithromycin, ciprofloxacin, HMR 3004, and HMR 3647 to be significantly more effective ($P < 0.05$), as assessed by pulmonary clearance of *H. influenzae*, than amoxicillin, clarithromycin, erythromycin A, and pristinamycin. HMR 3004 was significantly more effective ($P < 0.05$ by Fisher's exact test) than all other antimicrobials studied as assessed by percent sterile tissues after treatment. Despite in vitro activity, pristinamycin was not significantly (P = not significant) different than amoxicillin treatment; this may be due to unfavorable pharmacokinetics (2).

In this study, in a murine model of beta-lactamase-producing *H. influenzae* pneumonia, HMR 3004 was significantly more active than HMR 3647 and azithromycin. This occurred despite a twofold-higher MIC and MBC of HMR 3004 than those of azithromycin and HMR 3647 for the study strain. This finding was unexpected; our study was not designed to explore in detail the reasons for the superior in vivo activity of HMR 3004. Our in vivo study used only a single *H. influenzae* strain, and our results await confirmation by others. One or more of the following may, however, be contributory to the results noted. It has been shown elsewhere that both HMR 3004 and HMR 3647 are bacteriostatic at or near the MICs for *H. influenzae* and that activity can be increased to a bactericidal level at higher concentrations (5, 6). Minor differences in bioavailability between the two ketolides may exist. In this regard, we noted slightly higher concentrations in serum of HMR 3004 than of HMR 3647 at 60 and 90 min after dosing (Table 2); this may have contributed to the superior in vivo activity of HMR 3004 compared to HMR 3647. Similarly, differential influences

TABLE 2. Mean serum concentrations (micrograms per milliliter) in five uninfected mice after a single dose of antimicrobial agent

Antimicrobial agent[a]	30 min	60 min	90 min
Amoxicillin (25 mg/kg p.o.)	12.0	3.8	1.3
Azithromycin (100 mg/kg p.o.)	0.8	1.2	1.8
Ciprofloxacin (100 mg/kg p.o.)	5.4	2.5	1.5
Clarithromycin (100 mg/kg p.o.)	3.2	3.9	2.8
Erythromycin A (100 mg/kg i.p.)	6.1	4.2	3.6
HMR 3004 (50 mg/kg p.o.)	3.2	4.8	4.5
HMR 3647 (50 mg/kg p.o.)	3.2	4.3	3.9
Pristinamycin (100 mg/kg p.o.)	2.2	0.8	0.8

[a] p.o., orally; i.p., intraperitoneally.

TABLE 3. Results of treatment of murine *H. influenzae* experimental pneumonia[a]

Treatment	No. sterile/total no. of animals	Median $\log_{10}$ CFU/g of lung	Range (25th–75th percentile)
None	0/35	9.05	8.65–9.57
Amoxicillin	0/15	8.56	7.28–9.70
Azithromycin	5/18	3.93	1.70–4.23
Ciprofloxacin	3/13	3.33	2.06–3.94
Clarithromycin	0/12	5.73	5.57–6.00
Erythromycin A	0/12	5.40	5.07–5.69
HMR 3004	9/14	1.70	1.70–3.75
HMR 3647	3/12	4.34	2.08–5.08
Pristinamycin	0/14	7.35	6.50–8.37

[a] There were two deaths among the ciprofloxacin- and clarithromycin-treated animals, one death among the HMR 3004- and pristinamycin-treated animals, and three deaths among the erythromycin- and HMR 3647-treated animals. Accidental gall bladder puncture occurred in one clarithromycin-treated animal at the time of sacrifice. These animals are not represented in the analysis shown.

of pH on the in vivo activities of HMR 3004, HMR 3647, and azithromycin and/or differences in intracellular accumulation, protein binding, and/or postantibiotic effect between these agents may have contributed to our findings (1, 5, 12). Similar considerations may explain the observed superior in vivo efficacy of HMR 3004 versus ciprofloxacin. Notably, other investigators have demonstrated that HMR 3004 exhibits activity similar to that of azithromycin and superior to those of erythromycin, clarithromycin, and pristinamycin in a murine *H. influenzae* septicemia model (2).

In conclusion, we have found that the ketolides HMR 3004 and HMR 3647 are active against *H. influenzae* in an experimental model of murine pneumonia. Our results indicate that further studies examining the activity of ketolides in the treatment of *H. influenzae* infections are warranted.

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