

In Vitro Activity of Ro 15-8074, a New Oral Cephalosporin, against *Neisseria gonorrhoeae*

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Ro 15-8074, a new cephalosporin the pivaloyloxymethylester of which (Ro 15-8075) is orally absorbable, showed greater in vitro activity than cefaclor against 48 *Neisseria gonorrhoeae* strains, including 25 penicillinase-producing strains. Unlike cefaclor, Ro 15-8074 was unaffected by increase in inoculum size, and it exhibited a remarkable stability against gonococcal β -lactamase hydrolysis.

The emergence of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strains showing high resistance to penicillin has prompted the search for effective alternative drugs against these isolates. Although a number of new β -lactam antibiotics have been reported to show promising in vitro and in vivo activities (2, 3, 6, 8, 9), few can be administered orally. Of these drugs, cefaclor and amoxicillin-clavulanic acid (Augmentin) have been reported to be active against PPNG strains both in vitro and in vivo (4, 10). In this study, therefore, we evaluated the in vitro activity of Ro 15-8074 in comparison with those of cefaclor and amoxicillin-clavulanic acid against *N. gonorrhoeae* strains and also tested their hydrolytic stability to gonococcal β -lactamase. Ro 15-8074 is an aminothiazolyl-2-methoxyiminoacetamido-3-desacetoxy cephalosporanic acid, and its pivaloyloxymethylester, Ro 15-8075, is orally absorbable. After absorption, the ester group of Ro 15-8075 is enzymatically split off the cephalosporin ring, giving rise to the active cephalosporanic acid Ro 15-8074 (Fig. 1).

The 48 *N. gonorrhoeae* strains tested were clinical isolates

(land), and Ro 15-8074 (Hoffmann-La Roche Inc., Basel, Switzerland), which was supplied as the sodium salt suitable for in vitro testing.

MICs were determined by the agar dilution method with GC agar base (Oxoid Ltd., London, England) supplemented with 1% hemoglobin and 1% defined GC supplement (Oxoid). Inocula were prepared by suspending a few colonies of each test and control strains (*N. gonorrhoeae* strains 76-073389 and 77-083718 from the Centers for Disease Control, Atlanta, Ga.) grown overnight on GC medium into tryptic soy broth until the turbidity matched that of a 0.5 McFarland standard. This suspension was further diluted to give a final inoculum of 10^3 CFU per spot and inoculated onto the test and control (without antibiotic) plates with a Dynatech multipoint inoculator. When the effect of inoculum size was tested, an inoculum of 10^6 CFU was applied to each spot. After the inocula had dried, the plates were incubated in a 5% CO₂ incubator for 24 h and the MICs were recorded as the lowest concentration of antibiotic which inhibited visible growth.

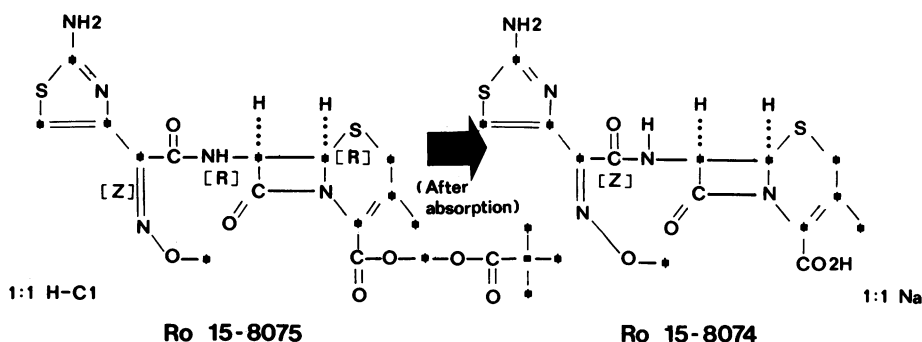


FIG. 1. Structure of Ro 15-8074.

obtained in Hong Kong over the past 2 years. Of these strains, 25 were penicillinase producers, as determined by the chromogenic cephalosporin method (7). All strains were lyophilized until ready for drug susceptibility testing. The antibiotics tested were: cefaclor (Eli Lilly & Co., Indianapolis, Ind.); a mixture of amoxicillin and clavulanic acid (Augmentin, ratio, 2:1; Beecham Laboratories, Surrey, Eng-

Gonococcal β -lactamase was extracted from a PPNG strain, and enzyme activity was assayed by the spectrophotometric method as detailed elsewhere (P. C. L. Wong, W. Y. H. Ho, and W. W. S. Ng, Sex. Transm. Dis., in press). Hydrolysis of cefaclor was monitored at a wave length of 263 nm, and that of Ro 15-8074 was tested over the range of 240 to 300 nm, since the hydrolysis of penicillins and other cephalosporins showed a decrease in absorbance in this region.

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TABLE 1. Susceptibility of *N. gonorrhoeae* strains to Ro 15-8074 and three other oral antibiotics

Antibiotic	MIC (mg/liter) for ^a :					
	PPNG strains (n = 25)			Non-PPNG strains (n = 23)		
	Range	50%	90%	Range	50%	90%
Amoxicillin	8-64	16	64	0.06-4	0.5	2.0
Augmentin ^b	0.25-4	0.5	4.0	0.125-2	0.5	2.0
Cefaclor	0.06-1	0.5	0.5	0.06-2	0.25	1.0
Ro 15-8074	0.0075-0.06	0.0075	0.03	0.0075-0.125	0.015	0.06

^a 50% and 90%, MIC for 50 and 90% of the strains, respectively.

^b Combination of amoxicillin and clavulanic acid; ratio, 2:1.

The MICs required to inhibit 50 and 90% of the strains and the MIC ranges of the drugs tested against both PPNG and non-PPNG strains are shown in Table 1. All PPNG strains showed high resistance to amoxicillin, requiring MICs of ≥ 8 mg/liter. In comparison, none of the non-PPNG strains required MICs of amoxicillin > 4 mg/liter. Although strains of PPNG showed resistance to amoxicillin, the activity of this antibiotic was greatly enhanced when it was combined with the β -lactamase inhibitor clavulanic acid. This was evident by the 16- to 32-fold reduction in MICs when these strains were tested against amoxicillin-clavulanic acid. The presence of clavulanic acid had no such effect on the MICs of amoxicillin against non-PPNG strains. With the exception of amoxicillin, which clearly differentiated the PPNG from the non-PPNG strains, the distribution of MICs for the other antibiotics did not show marked differences between these two types of strains. Against both PPNG and non-PPNG strains, Ro 15-8074 was the most active antibiotic; its activity is approximately 16 times more effective than that of cefaclor, inhibiting all the isolates at a concentration of 0.125 mg/liter. The effect of increase in inoculum size was determined in 10 randomly selected PPNG strains and 10 non-PPNG strains. When the inocula of these strains were raised from 10^3 to 10^6 CFU per spot, the MICs of Ro 15-8074 were not affected significantly, and in no instance was there a difference of greater than fourfold in MICs between the two inoculum sizes (results not shown).

The stability of Ro 15-8074, cefaclor, and amoxicillin to enzymic hydrolysis was tested with other antibiotic substrates against an extract prepared from a PPNG strain. The relative rates of hydrolysis of the different antibiotics as compared with that of penicillin, which was expressed as 100%, are presented in Table 2. Both cefaclor and amoxicillin were hydrolyzed by the enzyme at 43 and 71% of the rate of penicillin, respectively. No hydrolytic activity of the

TABLE 2. Relative rates of hydrolysis of Ro 15-8074, cefaclor, amoxicillin, and other antibiotics against gonococcal β -lactamase

Substrate	Relative activity (%) ^a
Benzylpenicillin	100
Ampicillin	87
Amoxicillin	71
Cephaloridine	43
Cefaclor	43
Ro 15-8074	0 ^b

^a Enzyme activity relative to the hydrolysis of benzylpenicillin, which was expressed as 100%.

^b No decrease in absorbance at a wavelength of 240, 250, or 270 nm was detected.

enzyme was detectable, however, when it was tested against Ro 15-8074. The hydrolysis observed with cefaclor and amoxicillin was inhibited when the enzyme was preincubated for 10 min with 50 μ M clavulanic acid before the addition of these drugs.

The results of this study show that Ro 15-8074 has a high in vitro activity against *N. gonorrhoeae* strains, irrespective of whether they produce penicillinase. Its activity is at least 16 times more effective than that of cefaclor, the other oral cephalosporin compared. Unlike the MICs of cefaclor, which were reported to be affected by an increase in inoculum size (1, 5), the MICs of Ro 15-8074 were unaffected when the inoculum of gonococcal strains was raised to 10^6 CFU. In view of the good in vitro activity of Ro 15-8074 against both PPNG and non-PPNG strains, its stability to gonococcal β -lactamase, and the fact that peak levels of Ro 15-8074 in serum after oral administration of 1 g of Ro 15-8074 is 5 mg/liter (data on file, Hoffmann-La Roche Inc.), which is approximately 50 times the concentration required to inhibit all the *N. gonorrhoeae* strains tested in our laboratory, this new oral cephalosporin warrants further investigation.

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