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In Vitro Activity of the New Macrolide Antibiotic Roxithromycin (RU 28965) against Clinical Isolates of *Haemophilus influenzae*

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The in vitro activity of the new macrolide antibiotic roxithromycin (RU 28965) was compared with the activities of five other orally absorbable antimicrobial agents against 100 clinical isolates of *Haemophilus influenzae*. Roxithromycin MICs were generally twofold to fourfold higher than those of erythromycin; the MIC for 90% of the strains for roxithromycin was 8 μg/ml.

Erythromycin is a macrolide antibiotic which is useful for therapy of infections due to strains of *Legionella*, *Mycoplasma*, *Chlamydia*, and *Campylobacter*, as well as for certain respiratory tract infections in penicillin-allergic patients. Roxithromycin (formerly RU 28965) is a new ether oxime derivative of erythromycin (4) with improved pharmacokinetic properties (E. Bergogne-Bérézin, Abstr. 14th Int. Congr. Chemother. 1985, WS-11-6, p. 53) and greater inhibitory activity against *Legionella* spp. (R. N. Jones, Abstr. 14th ICC. 1985, WS-11-12, p. 54; A. Saito, Abstr. 14th ICC. 1985, WS-11-7, p. 54) and *Chlamydia trachomatis* (C. M. Khurana, and P. A. Deddish, Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 204, 1985). This report describes a comparison of the in vitro activity of roxithromycin with several other orally administered, non-beta-lactam antibiotics against 100 clinical isolates of *Haemophilus influenzae* (64 type b, 36 non-type-b, 35 beta-lactamase positive).

Roxithromycin was kindly provided by Hoechst-Roussel Pharmaceuticals Inc., Sommerville, N.J. Drugs tested for comparative purposes included erythromycin, erythromycin-sulfisoxazole (Pediazole) tested in a fixed ratio of 1:64 (P. N. Whitley and S. I. Pelton, Abstr. 21st ICAAC, abstr. no. 12, 1981), trimethoprim-sulfamethoxazole tested at 1:19 ratio, chloramphenicol, and tetracycline.

Microdilution MICs (6) were determined for all isolates and drugs by using Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with 10 μg of hematin (Sigma Chemical Co., St. Louis, MO.) per ml, 10 μg of beta-NAD (Sigma) per ml, and 5 mg of yeast extract (Difco) per ml, final pH 7.3. Thymidine phosphorylase (Burroughs-Wellcome Co., Research Triangle Park, N.C.) was added at a concentration of 0.1 IU/ml to wells which contained erythromycin-sulfisoxazole or trimethoprim-sulfamethoxazole to sharpen inhibition endpoints (9). Inocula were prepared so as to contain 2×10^5 to 5×10^5 CFU/ml for all tests. MICs were determined after 20 h of incubation of microdilution trays at 35°C in ambient air. In addition, agar dilution MICs (6) were determined on 22 isolates with roxithromycin and erythromycin, using the test medium described above with the addition of 1.7% agar (Difco), final pH 7.3. Inocula of 10^4 CFU per spot were applied to the agar surfaces of a duplicate set of drug-containing plates and incubated for 20 h at 35°C in both 5% CO_2 and ambient air environments. Control organisms used to verify potency of all drugs and adequacy of the susceptibility methods included *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213.

Roxithromycin demonstrated consistent, although modest, antimicrobial activity against both type b and non-type-b isolates of *H. influenzae* by microdilution testing (Table 1). Roxithromycin MICs were generally twofold to fourfold higher than those of erythromycin. The combination of erythromycin-sulfisoxazole proved active against all isolates, including three trimethoprim-sulfamethoxazole-resistant isolates (MIC, ≥2 + 38 μg/ml). With the exception of the aforementioned resistant strains, trimethoprim-sulfamethoxazole was highly active, with 90% of isolates inhibited at 0.03 to 0.06 μg/ml (based on the trimethoprim component of the combination). Chloramphenicol and tetracycline were active against most isolates, although seven tetracycline-resistant strains (MIC, ≥16 μg/ml) are included in Table 1.

Agar dilution MICs for both roxithromycin and erythromycin on the subset of 22 isolates were similar to the microdilution MICs when agar dilution plates were incubated in ambient air. However, when agar dilution plates were incubated in CO_2, MICs were twofold to fourfold higher with both drugs than for MICs determined by microdilution.

Despite the widespread use of erythromycin for therapy of a number of different infectious diseases, very few newer macrolide antibiotics have been described in recent years. Roxithromycin appears to possess improved antimicrobial activity against some organism groups (1, 3, 4). However, the principal advantage of roxithromycin is more likely to be its improved pharmacokinetics, including more predictable absorption, as well as higher, prolonged serum and tissue levels of the drug (Bergogne-Bérézin, 14th ICC; Jones, 14th ICC).

Otitis media due to *H. influenzae* is one infectious process which might be treated by using an oral macrolide antibiotic. However, erythromycin levels in middle ear fluids have been shown to be variable (2, 7, 8), with erythromycin estolate providing higher levels than those provided by erythromycin ethyl succinate (2, 7). Studies should be undertaken to determine the levels of roxithromycin which can be achieved in middle ear fluids.

The results reported in this study regarding the specific activity of roxithromycin against *H. influenzae* are slightly less favorable than those reported by Barlam and Neu (1) and similar to the findings of Jones et al. (4) with respect to the activity of roxithromycin being one-fourth to one-half that of erythromycin. Our observation of higher MICs with

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TABLE 1. Susceptibilities of 100 *H. influenzae* clinical isolates to the study drugs tested by microdilution

Isolates (no. tested)	Drugs	MIC (μg/ml)		
		50%	90%	Range
Type b strains (64)	Roxithromycin	4	8	0.5–16
	Erythromycin	1	2	0.125–8
	Erythromycin-sulfisoxazole[a]	0.25	1	0.06–2
	Chloramphenicol	0.5	0.5	0.125–8
	Tetracycline	≤0.25	0.5	≤0.25–16
	Trimethoprim-sulfamethoxazole[b]	0.06	0.06	0.007–4
Non-type-b strains (36)	Roxithromycin	8	8	2–16
	Erythromycin	2	4	0.5–4
	Erythromycin-sulfisoxazole[a]	1	1	0.125–2
	Chloramphenicol	0.5	0.5	0.25–8
	Tetracycline	≤0.25	4	≤0.25–16
	Trimethoprim-sulfamethoxazole[b]	0.03	0.03	0.007–0.25

[a] Based on erythromycin component at 1:64 ratio.
[b] Based on trimethoprim component at 1:19 ratio.

agar dilution tests incubated in CO_2 may relate to the acidifying properties of CO_2, resulting in diminished macrolide activity (5).

The majority of isolates included in this study were inhibited by trimethoprim-sulfamethoxazole, chloramphenicol, and tetracycline. However, strains resistant to trimethoprim-sulfamethoxazole (MIC, ≥2 + 38 μg/ml) and tetracycline (MIC, ≥16 μg/ml) were encountered in this study. They were inhibited by roxithromycin and erythromycin at levels typical for other strains. Similarly, several isolates demonstrated elevated chloramphenicol MICs of 4 to 8 μg/ml but did not show increased resistance to the macrolides.

Orally absorbable non-beta-lactam antibiotics which are effective against respiratory tract pathogens represent important facets of antimicrobial therapy. Roxithromycin is a promising macrolide antibiotic with pharmacokinetic properties which may be superior to those of erythromycin. The ultimate clinical usefulness of roxithromycin against *H. influenzae* infections would seem to rely heavily on its improved pharmacokinetics, since it demonstrates somewhat less inhibitory activity than erythromycin.

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