The comparative in-vitro activity of roxithromycin and other antibiotics against *Bordetella pertussis*

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In spite of vaccination programmes, whooping cough epidemics continue to occur. The disease affects all age groups, although its severity is greatest in the young, with infants being particularly vulnerable. Erythromycin is generally accepted as the drug of choice both for treatment and for prophylaxis during epidemics. Roxithromycin is a macrolide with pharmacokinetic advantages over erythromycin; it is well absorbed, produces high serum concentrations, has a long half-life and penetrates respiratory secretions well. There are no accepted standards for testing the sensitivity of *Bordetella pertussis* to antibiotics, and reports of the activity of roxithromycin and erythromycin are variable. Using Isosensitest agar supplemented with 5% horse blood and an inoculum of 10⁴ cfu, 88 strains of *B. pertussis* were tested for their sensitivity to roxithromycin, erythromycin, rifampicin and trimethoprim/sulphamethoxazole. The range of MICs was 0.12–0.5 mg/L for both roxithromycin and erythromycin. Roxithromycin was bactericidal, with an MBC of 1 mg/L (as compared with 0.5 mg/L for erythromycin). Since roxithromycin is well tolerated by children when used for respiratory tract infections, the good in-vitro activity against *B. pertussis*, combined with its favourable pharmacokinetics, suggest it may be a good candidate for use in the treatment and prophylaxis of whooping cough.

Introduction

Pertussis, or whooping cough, is an acute and highly infectious respiratory disease caused by the Gram-negative coccobacillus *Bordetella pertussis*. Despite routine childhood immunization programmes, epidemics of whooping cough still occur in many countries, frequently with a 3–4 year cycle.¹⁴

Erythromycin is established as the antibiotic of choice both for treatment of whooping cough and in prophylaxis for contacts with the disease.⁵,⁶ However, in spite of good activity *in vitro*, failure to eradicate the organism and to prevent relapses is common. Some of these failures have been attributed to the poor absorption of some formulations of erythromycin. The estolate derivative produces higher serum concentrations than most formulations and appears to be associated with the best results.⁵

Roxithromycin is a macrolide with in-vitro activity similar to that of erythromycin.⁷ Roxithromycin has a longer half-life than erythromycin, produces higher serum concentrations and penetrates tissues and mammalian cells to a greater extent.⁸,⁹ It produces fewer gastrointestinal adverse effects than erythromycin, and is well tolerated by children.¹⁰,¹¹

There have been few published reports on the susceptibility of *B. pertussis* to roxithromycin *in vitro*, but all have shown that roxithromycin has good activity. MIC ranges of 0.03–0.25 mg/L, ² ≤ 0.06–0.4 mg/L,¹² and ≤ 0.008–0.03 mg/L¹³ have been reported. The methodology used for susceptibility testing of slow-growing fastidious organisms, such as *B. pertussis*, has not been standardized, so it is difficult to compare results obtained in different studies.¹⁴

The favourable pharmacokinetics and improved tolerance of roxithromycin relative to erythromycin suggest that it may be of value in the treatment and control of outbreaks of pertussis. We have compared the activity of roxithromycin against a number of isolates of *B. pertussis* with those of erythromycin, rifampicin and trimethoprim/sulphamethoxazole (TMP/SMZ) *in vitro*.
Materials and methods

Bacterial strains
Hospital and community laboratories throughout New Zealand are requested to refer *B. pertussis* isolates to the Communicable Disease Centre at the Institute of Environmental Science and Research. Eighty-eight such isolates, referred between 1991 and 1995, were selected for this study. All isolates were identified by standard microbiological procedures and were stored at −70°C in glycerol broth soon after receipt. The isolates were serotyped using antisera prepared in-house or obtained from the National Institute for Biological Standards and Control, UK. Slide agglutination and antiserum preparation were as described by Preston. Control strains of *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) were included for susceptibility tests.

Antibiotics
Roxithromycin was obtained from Hoechst Marion Roussel (Pennant Hills, Australia), erythromycin from Pharmacia Upjohn (Kalamazoo, MI, USA), rifampicin from Gruppo Lepetit (Milan, Italy) and trimethoprim (TMP) and sulphamethoxazole (SMZ) from Sigma Chemical Co. (St Louis, MO, USA). Antibiotic stock solutions were prepared according to NCCLS and manufacturers’ guidelines, and dilutions incorporated into agar supplemented as detailed below.

Susceptibility tests
An agar dilution method following NCCLS guidelines was used. Roxithromycin, erythromycin and rifampicin were tested in Isosensitest agar (Oxoid, Basingstoke, UK) supplemented with 5% horse blood as recommended by Hoppe & Eichhorn. Tests with TMP/SMZ were performed in Isosensitest agar supplemented with 5% lysed horse blood.

The *B. pertussis* isolates were thawed, subcultured on Isosensitest agar supplemented with 5% horse blood and incubated at 35°C with high humidity (95% relative humidity) for 72 h. The isolates were subcultured on to a second supplemented Isosensitest agar plate and incubated for a further 48 h. The inoculum was prepared from these plates by suspending colonies in Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA) and adjusting the turbidity to a McFarland standard 0.5 to give an approximate final inoculum of $10^4$ cfu/spot which was applied to the plates using a multipoint inoculator (HI Clements Pty, Sydney, Australia). Viable counts were performed to determine the exact inoculum used for four of the isolates. The plates were incubated at 35°C with high humidity for 48 h. Appropriate antibiotic-free control plates were inoculated as controls. MIC endpoints were read as recommended by NCCLS and determinations were performed in duplicate on all 88 isolates.

MBCs were determined for 19 randomly selected isolates using the method of Hoppe & Eichhorn. Briefly, agar plugs were excised from plates at the MIC and the next four higher concentrations. The plugs were incubated in 3 mL of Isosensitest broth supplemented with 5% horse blood for 24 h at 35°C. The broths were then subcultured on to supplemented Isosensitest agar and incubated for 48 h. The MBC was defined as the lowest concentration showing complete absence of growth.

Interpretative MIC criteria
Interpretative MIC criteria indicating susceptible, intermediate resistance and resistance towards erythromycin, rifampicin and TMP/SMZ were taken from the NCCLS recommendations. In the absence of NCCLS recommendations for roxithromycin, the susceptibility breakpoints selected were those recommended by Kurzynski et al. and Olsson-Liljequist et al. These values are listed in Table I.

Results

Characteristics of *B. pertussis* isolates
Of the 88 isolates, 83 were serotype 1.3, four were serotype 1.2 and one was serotype 1.2.3. There were 39 isolates from patients aged under 1 year, six from patients aged between 1 and <2 years, 39 from patients aged between 2 and <15 years, and four from patients aged 15–30 years.

Table I. Interpretative MIC susceptibility criteria (mg/L) for roxithromycin, erythromycin, rifampicin and trimethoprim/sulphamethoxazole (TMP/SMZ)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Susceptible</th>
<th>Intermediate resistant</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roxithromycin</td>
<td>≤1.0</td>
<td>2.0–4.0</td>
<td>≥8.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤0.5</td>
<td>1.0–4.0</td>
<td>≥8.0</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>≤1.0</td>
<td>2.0</td>
<td>≥4.0</td>
</tr>
<tr>
<td>TMP/SMZ</td>
<td>≤2.0/38</td>
<td></td>
<td>≥4.0/76</td>
</tr>
</tbody>
</table>
Roxithromycin activity against *B. pertussis*

**Susceptibility of *B. pertussis* isolates**

Quantitative viable counts showed that the inoculum used was $2 \times 10^4$ cfu for the four isolates tested; results for the control strains were within the NCCLS acceptable ranges.\(^{17}\)

The MIC ranges, MIC\(_{50}\), MIC\(_{90}\) and MBCs of the four antimicrobial agents against the 88 *B. pertussis* isolates are shown in Table II. The activity of roxithromycin was similar to that of erythromycin, both having MICs of 0.12–0.5 mg/L. Erythromycin MIC\(_{50}\) and MIC\(_{90}\) (0.25 mg/L) were slightly lower than those of roxithromycin (0.5 mg/L). Both compounds were bactericidal with MBCs twice the MIC\(_{90}\). All strains were fully sensitive to erythromycin, roxithromycin and TMP/SMZ but only 69 (78.4\%) were fully sensitive to rifampicin; the remaining strains were of intermediate susceptibility. MBCs of rifampicin (4 mg/L) were higher than those of the other three compounds tested.

The distribution of MICs for the four compounds tested showed no clear differences between strains tested between 1991 and 1995 (Table III).

**Discussion**

There are few reports on the susceptibility of *B. pertussis* to roxithromycin. While these showed that it has good activity, some reported that it was slightly less active than erythromycin,\(^{7,12}\) while others\(^{13}\) found that it was equally active. Hoppe & Haug\(^{14}\) reviewed the data on the susceptibility of *B. pertussis* to a range of antibiotics; they found considerable variation in the methods and a wide range of results for erythromycin. They recommended the use of Isosensitest agar, a standardized medium that is widely used for sensitivity testing with many bacterial species, rather than media for isolating *B. pertussis*. Supplementation of Isosensitest agar with 5\% whole or

### Table II. Susceptibility of 88 *B. pertussis* isolates: MICs and MBCs (mg/L)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC range</th>
<th>MIC(_{50})</th>
<th>MIC(_{90})</th>
<th>% Susceptible(^a)</th>
<th>MBC(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roxithromycin</td>
<td>0.12–0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.12–0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>100</td>
<td>0.5</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.25–2.0</td>
<td>1.0</td>
<td>2.0</td>
<td>78.4</td>
<td>4.0</td>
</tr>
<tr>
<td>TMP/SMZ</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>100</td>
<td>0.5/10</td>
</tr>
</tbody>
</table>

\(^a\)Percentage of strains susceptible using criteria specified in Table I.

\(^b\)MBC was determined for 19 isolates.

TMP/SMZ, trimethoprim/sulphamethoxazole.

### Table III. Distribution of MICs (mg/L) for *B. pertussis* strains isolated between 1991 and 1995

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 39)</td>
<td>(n = 14)</td>
<td>(n = 11)</td>
<td>(n = 7)</td>
<td>(n = 17)</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>MIC range</td>
<td>0.12–0.5</td>
<td>0.25–0.5</td>
<td>0.25–0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>MIC(_{50})</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>MIC(_{90})</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>MIC range</td>
<td>0.12–0.25</td>
<td>0.25</td>
<td>0.12–0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>MIC(_{50})</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>MIC(_{90})</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>MIC range</td>
<td>0.5–2.0</td>
<td>0.5–2.0</td>
<td>1.0–2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MIC(_{50})</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MIC(_{90})</td>
<td>2.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>TMP/SMZ</td>
<td>MIC range</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
</tr>
<tr>
<td>MIC(_{50})</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
</tr>
<tr>
<td>MIC(_{90})</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
<td>0.12/2.5</td>
</tr>
</tbody>
</table>

TMP/SMZ, trimethoprim/sulphamethoxazole.
lysed blood supports the growth of B. pertussis. They also suggested that the incubation period should be <48 h to avoid degradation of antibiotics, and that an inoculum of 10⁵ cfu should be used.

We have used the technique described by Hoppe & Hau,⁴ and found that the activity of roxithromycin was very close to that of erythromycin against 88 strains of B. pertussis isolated between 1991 and 1995. The range of MICs was identical for the two compounds (0.12–0.5 mg/L), although erythromycin had slightly lower MIC₅₀, MIC₉₀, and MBCs. Both compounds were bactericidal, roxithromycin at a concentration (1 mg/L) that is readily achieved in vivo.¹⁹,²⁰ The MIC₉₀ of roxithromycin obtained in our study, 0.5 mg/L, was comparable to those reported by Hardy et al.⁷ and Kurzynski et al.¹² However, a roxithromycin MIC₉₀ of 0.03 mg/L was reported by Hoppe & Eichhorn.¹³

Frequently, the complex media used for isolating B. pertussis have been used for sensitivity testing; for example, Hardy et al.⁷ used Bordet–Gengou agar supplemented with 15% sheep blood and incubated for 72 h, while Kurzynski et al.¹² used the same medium but supplemented with 20% sheep blood and incubated for 48 h. Kurzynski et al.¹² used a relatively high inoculum, 2–6–2 × 10⁵ cfu, while Hardy et al.⁷ used the standard inoculum 10⁴ cfu. Both groups found that roxithromycin was slightly less active than erythromycin, although 90% of the 18 strains tested by Hardy et al.⁷ and 90% of the 75 strains tested by Kurzynski et al.¹² were inhibited by 0.25 and 0.5 mg/L, respectively. Given the good bioavailability of roxithromycin, these concentrations are readily achievable in most tissues and body fluids.¹⁹,²⁰

Erythromycin and the newer macrolides have greater activity than most antibiotics against B. pertussis.¹⁴,²¹ β-Lactam antibiotics vary considerably in their activity against B. pertussis, some penicillins having activity at relatively low concentrations,¹⁴ whereas cephalosporins, including a wide range of newer third-generation cephalosporins, have virtually no activity.²² and have been used as selective agents in the isolation of B. pertussis. Tetracycline and rifampicin have good in-vitro activity¹⁴ but there is some evidence that resistance to these agents may be increasing;¹² of the 88 strains tested here, some had reduced susceptibility to rifampicin. The quinolones mostly show good in-vitro activity, but they are not generally recommended for routine administration to children.

Most countries have an immunization programme for pertussis and although this has markedly lowered the incidence of and mortality from whooping cough, the disease has not been eliminated and epidemics continue to occur. Immunity is not complete and decreases with age,⁵ so adults can become infected from children and then in turn be the source of fresh infection.²³,²⁴ Although the disease is relatively mild in adults and immunized older children, infants are at greater risk, and both morbidity and mortality are highest in the very young unvaccinated infant.³,⁵,²³

Erythromycin has been shown to have some benefit in the treatment of whooping cough, generally shortening the course of the infection and helping to reduce the spread of the disease.⁵,⁶ However, the clinical course is unaffected if drugs are administered after the onset of the paroxysmal stage of the disease, although the organism may still be eliminated from the respiratory tract, reducing the possibility of spread.⁵ In a review of the use of erythromycin in the control and prevention of whooping cough, it has been stressed that the role of antibiotic therapy or prophylaxis is to support vaccination as the primary method of controlling epidemics.³ Some apparent failures of erythromycin prophylaxis have been attributed to the drug being given to contacts too late.⁶,²⁵

A prerequisite in the control of pertussis is that the drug must penetrate respiratory tract secretions in significant concentrations, as the organism is localized on the ciliated epithelial cells of the respiratory tract.²¹ The ability of roxithromycin to penetrate respiratory and, in particular, bronchial tissues and secretions to a greater degree than erythromycin⁹,¹⁹ could prove of value in the treatment of pertussis. Roxithromycin is well absorbed, producing high serum concentrations and a prolonged half-life; a 300 mg dose results in serum concentrations of 5–10 mg/L with a t½ of 19 h, allowing the possibility of once- or twice-daily dosing. Once- or twice-daily dosing is also facilitated by the pronounced post-antibiotic effect of roxithromycin against some respiratory pathogens (Streptococcus pneumoniae, Streptococcus pyogenes and Haemophilus influenzae).²⁵ However, the post-antibiotic effect of roxithromycin on B. pertussis has not been investigated.

Roxithromycin has been shown to be well-tolerated in infants and children when used to treat respiratory tract infections.⁰,¹¹ The good in-vitro activity demonstrated in this study against B. pertussis, together with the favourable pharmacokinetic properties of roxithromycin, suggest that it may have potential for prophylactic use in whooping cough epidemics.

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References

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