Pharmacodynamics of Macrolides, Azalides, and Streptogramins: Effect on Extracellular Pathogens

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The efficacy of macrolides against extracellular pathogens depends on extracellular levels of free drug and the organisms' patterns of susceptibility to the macrolides. The effect of macrolides against most bacteria is considered time-dependent. The size of inoculum affects erythromycin's activity against streptococci and, moreover, against staphylococci. The optimal effect is observed at a pH of 8. A significant postantibiotic effect (PAE), lasting ~9 hours, has been shown with erythromycin and roxithromycin against gram-positive cocci. Azalides share the same properties. For the streptogramin synercid, a dose-dependent bactericidal activity within a range of low concentrations has been demonstrated. The serum area under the curve appeared to be the best predictor of in vivo effect on the mouse thigh model. Synercid also exhibited a prolonged PAE (~10 hours) against the main pathogens of its spectrum. A better knowledge of the pharmacodynamic properties of macrolides and streptogramins is essential for definition of proper dosing regimens.

Macrolide antibiotics have been considered for many years to be safe and valuable for the treatment of various infections of the upper and lower respiratory tracts and of skin and soft tissues, either as first-line therapy or as an alternative to penicillins for allergic patients. Moreover, in the past decade, interest in these agents has revived with the recognition and increasing prevalence of pathogens such as Legionella, Mycoplasma, Chlamydia, and Campylobacter species. Several new macrolide compounds—including the azalide azithromycin, a 15-membered molecule—with improved pharmacokinetics and more or less broader antibacterial activity than erythromycin have appeared in recent years and have been submitted to laboratory and clinical investigations [1, 2].

Two other types of compounds, the lincosamides and the streptogramins, although chemically unrelated but exhibiting similar modes of action and antibacterial spectrum, are classically considered together with the macrolides. Among lincosamides, lincomycin is now mainly of historical interest, and clindamycin remains of particular interest as therapy for anaerobic and some parasitic infections.

No specific studies have addressed the pharmacodynamic properties of these compounds. However, their mode of action, close to that of macrolides, makes it likely that their pharmacodynamic properties are similar. Synercid (formerly RP 59500), a new semisynthetic injectable streptogramin that is a combination of two derivatives of pristinamycin I (RP 57669, or quinupristin) and pristinamycin II (RP 54476, or dalfopristin), exhibits attractive antibacterial activity through the synergistic activity of its two components. This drug is currently undergoing phase III clinical trials. The potential interest in this compound concerns its activity against some gram-positive cocci otherwise resistant to macrolides [3]. There is a surprisingly limited amount of information regarding the pharmacodynamic properties of macrolides. The main reason is that erythromycin is a compound of longstanding use for which basic preclinical information was not required at the time it was marketed. The newest compounds have been more extensively studied in various more or less sophisticated in vitro and in vivo models.

This article reviews these investigations and focuses on the properties of clinical relevance toward extracellular pathogens. Some effects, such as those on intestinal motility or related to pH, will not be discussed.

Main Pharmacokinetic Properties

It is important to briefly review the main kinetic properties of macrolides and derivatives in order to put them in the perspective of their pharmacodynamic profiles. The major drawback of earlier macrolides was their poor intestinal absorption, in addition to large interindividual and intraindividual variations, short half-life, high degree of binding to serum proteins (mainly the α1 acid glycoprotein), and poor gastrointestinal tolerance.

Figure 1 shows the different compounds developed in past years that provided significant improvement over erythromycin, in terms of acidic instability and oral bioavailability [4]. The main kinetic properties of macrolides are depicted in Table 1, adapted from reports of Lode et al. [5] and Bechtel et al.
Figure 1. Macrolides developed in past years (adapted from [4]). The asterisk indicates various erythromycin derivatives named from A to F.

[6]. It is important to note wide variations in peak serum concentrations, elimination half-life \( t_{1/2} \) values, and area under the curve (AUC) values [5]. In addition, differences in terms of maximal concentration \( C_{\text{max}} \) have been described for the different erythromycin preparations [6].

All macrolides accumulate intracellularly, mainly in polymorphonuclear leukocytes and macrophages, and some have persistently high intracellular levels. It is therefore possible to distinguish three types of macrolides, in comparison with erythromycin: (1) azithromycin, which has low extracellular levels, high intracellular penetration, and long extracellular and intracellular elimination half-lives; (2) roxithromycin, which has relatively high serum levels and a shorter half-life than azithromycin; and (3) clarithromycin, which has all the properties of both compounds. Some older compounds such as spiramycin and josamycin are also in this intermediate position.

The pharmacokinetic properties of the two components of synercid, given iv in a 30/70 ratio at 5-, 10-, and 15-mg/kg doses, have been studied in healthy volunteers [7]. Mean \( C_{\text{max}} \) values (µg/mL) were 1.3, 2.4, and 3.3 for quinupristin and 5.1, 7.1, and 8.5 for dalfopristin. Elimination half-live \( t_{1/2} \) values ranged between 0.6 hour and 1 hour (quinupristin) and 0.3 hour and 0.4 hour (dalfopristin).

Plasma clearance was high for both compounds: 1 L/(h·kg) for quinupristin and 0.8 L/(h·kg) for dalfopristin. The plasma levels of dalfopristin active metabolite were 20%–45% of those of the parent drug, showing a trend to increase with the dose. Therefore, it is important to point out that both components exhibit different \( t_{1/2} \) values, which must be kept in mind in some difficult-to-treat situations; the optimal ratio between the two components must be maintained as long as possible at the site of infection during the interval between two doses.

### Table 1. Pharmacokinetics of macrolides and azalides.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Oral dose (mg)</th>
<th>( C_{\text{max}} ) (mg/L)</th>
<th>( t_{\text{max}} ) (h)</th>
<th>( t_{1/2} ) (h)</th>
<th>AUC (mg·L·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin base</td>
<td>500</td>
<td>0.3–1.9</td>
<td>3.7</td>
<td>2.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Erythromycin stearate</td>
<td>500</td>
<td>0.4–1.8</td>
<td>3</td>
<td>\ldots</td>
<td>\ldots</td>
</tr>
<tr>
<td>Erythromycin ethylsuccinate</td>
<td>500</td>
<td>1.5</td>
<td>2</td>
<td>\ldots</td>
<td>\ldots</td>
</tr>
<tr>
<td>Erythromycin estolate</td>
<td>500</td>
<td>4.2</td>
<td>3.5–4</td>
<td>\ldots</td>
<td>\ldots</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>300</td>
<td>10.8</td>
<td>1.6</td>
<td>11.9</td>
<td>116.9</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>400</td>
<td>2.1</td>
<td>1.7</td>
<td>4.7</td>
<td>17</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>500</td>
<td>0.4</td>
<td>2.0</td>
<td>14</td>
<td>4.5</td>
</tr>
<tr>
<td>Dalfopristin</td>
<td>600</td>
<td>0.32</td>
<td>4.2</td>
<td>28</td>
<td>1.4</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>6 MU</td>
<td>3.3</td>
<td>1</td>
<td>8</td>
<td>\ldots</td>
</tr>
<tr>
<td>Josamycin</td>
<td>500</td>
<td>1.2</td>
<td>1</td>
<td>2</td>
<td>\ldots</td>
</tr>
</tbody>
</table>

**NOTE.** This table is adapted from [5, 6]. AUC = area under the curve; \( C_{\text{max}} \) = maximal concentration; MU = million units; \( t_{1/2} \) = half-life; \( t_{\text{max}} \) = time after dosing for \( C_{\text{max}} \) to be reached.
Pharmacodynamic Properties

Modalities of the antibacterial effect. The efficacy of macrolides against extracellular pathogens depends on the extracellular concentrations of free drug and the level of susceptibility of the organisms. Against most of the bacteria of their clinical spectrum, macrolides exhibit a time-dependent bactericidal effect, mainly against streptococci. This means they are slowly bactericidal, with a poor influence of increasing concentrations on the rapidity and importance of killing.

Several factors influence the antibacterial effect [8]. The size of the inoculum affects the antistreptococcal effect and, moreover, the antistaphylococcal activity. In the presence of serum, the MIC of some macrolides (roxithromycin, rokitamycin) against Streptococcus pyogenes increases onefold to fourfold. In contrast, the MICs for Streptococcus pneumoniae do not change.

The optimal effect is observed at a pH of 8, with a significant decrease in efficacy at low pH values (<6). The pH, variability of activity, serum effect, and activity of the metabolites are among the most important factors to be controlled in the evaluation of antibacterial activity of macrolides. For instance, most of the studies on anaerobes have missed some of the effects of macrolides, since incubation with CO2 lowers the pH of the medium [8].

The two components of RP 59500 are bacteriostatic against macrolide, lincosamide, streptogramin B (MLS B)-susceptible and MR-MLS B-inducibly resistant Staphylococcus aureus, whereas the MLS B-constitutively resistant strains are resistant to quinupristin [3]. Synergy between antibiotics in vitro is a phenomenon found at concentrations close to the MIC, except for strains with constitutive resistance. High-level resistance to quinupristin does not significantly reduce the activity of the combination, and generally the fractional inhibitory concentration index is found to be <0.5. When studied in vivo in experimental murine models of septicemia due to S. aureus and S. pneumoniae, the combination exhibited the highest effect with quinupristin/dalfopristin ratios ranging from 16/84 to 92/8.

This wide range of ratios of both components has been confirmed in the rabbit endocarditis model, demonstrating the in vivo synergistic effect of both compounds, despite a different pattern of distribution of the two labelled components throughout the infected vegetations [9]. The bactericidal effect of RP 59500 against S. aureus appears to be time-dependent but not concentration-dependent, as neither quinupristin nor dalfopristin alone is bactericidal against the organism [10]. RP 59500 is more rapidly bactericidal against S. pneumoniae than against S. aureus (1 hour vs. 6 hours) [11] and is slowly active against Enterococcus faecium [8].

Postantibiotic effect. Postantibiotic effect (PAE) is a common feature of all macrolides. However, several factors may affect the in vitro PAE of these drugs [12]. The duration of this effect is longer with gram-positive cocci than with Haemophilus influenzae and longer with streptococci than with staphylococci. For instance, for S. pneumoniae, a 4.1-hour PAE has been observed with erythromycin, following a 2-hour exposure at a concentration of twice the MIC.

The specific macrolide used greatly influences the duration of PAE. For instance, in vitro PAEs of clarithromycin are longer than those of erythromycin at similar concentrations (5.5 hours vs. 4.2 hours for S. aureus) [13]. In addition, azithromycin has been shown to have longer in vitro PAEs than erythromycin against H. influenzae, but similar values were reported against S. aureus and S. pneumoniae (1.8 hours and ~2.3 hours, respectively) [14]. Increasing macrolide concentrations and exposure time prolong the duration of PAE to a point of maximum response [15], with an excellent linear relationship between the duration of PAE and the log of the concentration or exposure time. Azithromycin had a pronounced decrease in bactericidal activity when added during the PAE phase [16].

The PAE of RP 59500 has been evaluated against a variety of bacteria, mainly staphylococci, pneumococci, and streptococci. Whatever the criterion used to define this effect (time required either for the viable counts to increase by one log10 or for bacteria to regain their maximal rate of growth), PAE was constantly observed when concentrations greater than or equal to the MICs were used. The concentration of antibiotic was found to be a more important parameter than the time of exposure in determining the importance of PAE [17]. For instance, after 30 minutes of exposure to 5 µg/mL, the PAE was 5–7.5 hours for most staphylococci, 7–9 hours for S. pneumoniae, and >18 hours for S. pyogenes [18]. In the presence of human serum, the duration of PAE against staphylococci was increased [19].

The in vivo PAE is generally evaluated on the neutropenic mouse thigh-infection model. Clarithromycin and azithromycin have been shown to induce a longer in vivo PAE than erythromycin [13, 14]. It is interesting to note that with azithromycin against staphylococci, this difference was noted in vivo despite similar in vitro PAE values for azithromycin and erythromycin. In the same model, RP 59500 had 10-hour and 9-hour in vivo PAEs, respectively, after single doses of S. aureus and S. pneumoniae (despite short exposure times) [20]. Data obtained from other models, such as infected subcutaneous fibrin clots [21] and mouse pneumococcal pneumonia [22, 23], also established the reality of an in vivo PAE with macrolides and streptogramins.

Integration of pharmacokinetic/pharmacodynamic properties. The definition of E max , corresponding to the static dose following repeated injections of a compound at various intervals in the mouse thigh (and/or lung) infection model, provides information on the overall in vivo consequences of pharmacokinetic/pharmacodynamic parameters and allows quantification of efficacy and potency. The lowest E max values found with clarithromycin vs. erythromycin in this model (3.7 mg/[kg·d] vs. 101 mg/[kg·d]) against S. pneumoniae, when both drugs were given every 12 hours, reflect longer periods of time during which serum levels remained over the MIC in vivo [13].

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The more recent data on PAE for a variety of antimicrobials and microorganisms provide theoretical rationale for either intermittent or more-continuous dosing of antimicrobials, depending on the offending microorganism and the drug to be given. An intermittent dosing regimen would apply primarily to drug-organism combinations that exhibit a prolonged PAE. Conversely, more-continuous administration is required for drug-organism combinations lacking a PAE.

The thigh-infection and pneumonitis models in leukopenic mice are suitable for comparing the efficacy of the same amount of drug given by either more-continuous dosing regimens (every 1 or 2 hours) or by discontinuous dosing regimens (every 4 hours to once daily) [24]. For instance, with erythromycin, which exhibits a prolonged PAE against *S. pneumoniae*, no major difference was observed between hourly and 6-hourly dosing regimens. On the other hand, the more-continuous dosing regimen was significantly more effective for penicillin G, which does not exhibit an in vivo PAE against *S. pneumoniae* in this model. At very high doses of penicillin G, equal efficacy was observed, as serum levels above the MIC were constantly maintained with both regimens [25].

The mouse models of *S. pneumoniae* and *H. influenzae* infections have provided interesting data for better integration of pharmacokinetic/pharmacodynamic properties of macrolides. It has been shown that roxithromycin [26] and spiramycin [27] were more efficient than erythromycin in pneumococcal infection, through both higher tissue levels and longer t1/2β values. Similar results have been obtained with azithromycin vs. erythromycin in two models of acute (Swiss mice) or subacute (C57BL/6 mice) pneumococcal pneumonia [22]. Azithromycin demonstrated a sevenfold higher tissue penetration and a 15-fold longer lung half-life than erythromycin.

Eighteen hours after infection, the AUC for the lungs of Swiss mice was twice that for uninfected animals. This can be explained by the trapping of azithromycin in locally recruited macrophages. Neutropenic animals did not have these increased AUC values [28].

A comparison of five macrolides (erythromycin, roxithromycin, spiramycin, clarithromycin, and azithromycin) in these models has established, in standard experimental conditions, that the relative efficacy of macrolides in vivo is related not only to their penetration into the lung but also to their lung t1/2β, which reflects their potential uptake, sequestration, and durable delivery at the site of infection [28].

Similar results have been obtained with azithromycin/erythromycin in a mouse model of *H. influenzae* pulmonary infection [23]. This supports the notion that macrolides are targeted antibiotics and reinforces interest in compounds with extended half-life, such as azithromycin, which could demonstrate increased efficacy despite once-daily administration or allow reduction in the duration of therapy because of the persistence of high cellular levels.

With the mouse thigh-infection model, Craig and Ebert have shown that in the determination of the dose required to achieve 50% of Emax, the dosing interval of RP 59500 had little impact [20]. The AUC in serum was the pharmacokinetic parameter that correlated best with in vivo efficacy. Thus, despite a short half-life, in vivo efficacy of the compound can be maintained at wide dosing intervals through a prolonged in vivo PAE.

In order to determine the microbiological and pharmacokinetic parameters that best predicted the in vivo antistaphylococcal activity of the streptogramin RP 59500, Fantin et al. [29] utilized a rabbit model of aortic endocarditis. They evaluated the activity of three regimens of quinupristin/dalfopristin against five strains of *S. aureus* with various streptogramin B-type antibiotic resistance phenotypes and with susceptibility to streptogramin A-type antibiotics. Quinupristin/dalfopristin was as active as vancomycin against three strains that were susceptible to its streptogramin-B component quinupristin (including one strain that was inducibly resistant to erythromycin) but had significantly decreased activity against two strains that were resistant to quinupristin, in all quinupristin/dalfopristin regimens tested. The AUC for quinupristin-dalfopristin in plasma, divided by the MIC of quinupristin, was the only parameter retained by multilinear regression that predicted the in vivo activity of quinupristin/dalfopristin, a finding emphasizing the importance of determining the susceptibility to quinupristin in order to predict the in vivo activity of quinupristin/dalfopristin against *S. aureus* [29].

Entenza et al. [30] compared the therapeutic efficacy of RP 59500 and of vancomycin against rat experimental endocarditis due to either of two erythromycin-susceptible or two constitutively resistant isolates of methicillin-resistant *S. aureus*. Three-day therapy was initiated 12 hours following infection, and the drugs were delivered via a computerized pump, which permitted mimicking of the human serum kinetics produced by a b.i.d. iv injection of 7 mg of RP 59500/kg or 1 g of vancomycin.

Both antibiotics cured 70% of infections due to the erythromycin-susceptible isolates. Vancomycin was also effective against the constitutively resistant strains, but RP 59500 failed against these isolates. Further experiments proved that RP 59500 failures were related to the very short serum life-span of dalfopristin (≤2 hours, vs. ≥6 hours for quinupristin), since successful treatment was restored by artificially prolonging the dalfopristin life span to 6 hours. This observation might be relevant for humans, in whom the serum half-life of dalfopristin is also shorter than that of quinupristin.

Conclusions

Integration of pharmacokinetic/pharmacodynamic properties of macrolides, azalides, and streptogramins is an important step in the definition of optimal dosing regimens for humans. This preclinical evaluation allows definition of a range of doses to be used in different clinical indications to be tested in human trials. Against extracellular pathogens, the time during which concentrations of free extracellular drug are greater than the MIC is the major determinant of the efficacy of macrolides.
The role played by the progressive release of drug from phagocytes in the persistence of active extracellular concentrations is probably very important. Moreover, for compounds such as azithromycin with very low extracellular levels, further information is needed to definitely establish that cells are able to deliver a sufficient amount of drug to control disseminating infections due to extracellular microorganisms.

With RP 59500, the observed rapid bactericidal effect followed by a long PAE on streptococci and pneumococci (whatever their profile of susceptibility to macrolides) argues for long intervals between doses. A more cautious approach toward definition of optimal dosing regimens against staphylococci, according to their resistance phenotypes, is certainly needed.

References