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Macrolides: pharmacokinetics and pharmacodynamics

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Abstract

Three pharmacokinetic/pharmacodynamic parameters—(i) the peak concentration to the minimum inhibitory concentration ratio (C_{max}/MIC); (ii) the area under the concentration–time curve to MIC ratio (AUC_{24h}/MIC); and (iii) the time the concentration exceeds the MIC ($T > MIC$)—are important predictors of the clinical efficacy of antibiotics. For antibiotics with pronounced concentration-dependent killing, such as the fluoroquinolones or the aminoglycosides, C_{max}/MIC and AUC_{24}/MIC are the main factors that establish efficacy. Antibiotics with a weak, or no, concentration dependency generally have their efficacy linked to $T > MIC$, and these include the β-lactams and the conventional macrolides. Antibiotics with weak concentration-dependent effects, but with prolonged persistent effects, such as tetracyclines and azithromycin, have their activity mostly related to the AUC_{24}/MIC. By applying these concepts to current antibiotics, and also to the development of novel agents, it is possible to optimise their dosages and administration schedules. This will maximise therapeutic efficacy, may prevent or delay the emergence of bacterial resistance to antibiotics, and can certainly minimise side-effects. © 2001 Published by Elsevier Science B.V. on behalf of the International Society of Chemotherapy.

Keywords: Pharmacokinetics; Pharmacodynamics; Macrolides; Azithromycin; Clinical efficacy

1. Introduction

Determination of the optimal dosing for antibiotics has clinical cure as its aim. Development of effective antibiotic treatment commences with the consideration of the basic chemistry of antibiotic molecules, continues with the evaluation of their microbiological activity (including bactericidal activity and spectrum of activity) and, through understanding of their pharmacokinetic (PK) and pharmacodynamic (PD) properties and application of the corresponding concepts, culminates in proven therapeutic effectiveness.

PK/PD are key in facilitating the translation of microbiological activity into clinical situations and to ensuring that the antibiotic achieves a successful outcome. Dosing regimens for antibiotics have not always been the most appropriate. For example, aminoglycosides have been dosed three times daily for many years, but we now know that this schedule was far from being optimal [1,2]. Conversely, the macrolides with relatively short half-lives have been often considered for twice- or even once-daily administration. Even a twice-daily dosing regimen has been suggested for fourth-generation cephalosporins, even though these drugs have very short half-lives and are, as we shall see, time-dependent [3,4]. In very general terms, PK/PD aims at avoiding these mistakes by determining, as early on as possible during the drug development process what is the optimal dosage and schedule of administration for the antibiotic under study.

PK/PD has often been considered as a 'black box', the intricacies of which could only be understood by highly specialised scientists and were of little interest to the clinician. However, beyond the esoterics of mathematical formulae and the corresponding analyses, the 'black box' of PK/PD is nothing more than trying to understand how the peak plasma concentration of a drug (C_{max}), its area under the plasma concentration–time curve (AUC_{24h}) and, in case of an antibiotic, its MIC relate to its clinical activity and toxicity (Fig. 1). In that sense, PK/PD is simply putting PK to work for what should be its aim, namely allowing for an efficient therapy.

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2. PK/PD: efficacy and safety

A critical point in antibiotic therapy is to balance the serum antibiotic concentrations, which vary over time, in order to achieve optimal bacterial eradication and minimal side-effects [5]. In vitro modelling, properly designed animal model experiments and PK information collected from clinical trials should, if combined and assessed globally, provide us with information about the characteristics of an antibiotic that are essential for therapeutic efficacy, and may be able to predict toxicity hazards. The advantages of introducing PK/PD concepts into drug development are: (i) the facilitation of early selection of leading antibiotic candidates; (ii) the rational selection of appropriate dosage regimens; and (iii) enhanced understanding of the clinical and microbiological outcomes. In the future, this will lead to more efficient antibiotic development programmes, whether based on efficacy or toxicity considerations.

An additional important role of PK/PD could also be in the prevention of the emergence of bacterial resistance. The fluoroquinolones are a good example of where underdosing of antibiotic has led to rapid development of resistance among bacteria [6–8]. It is still uncertain to what extent this problem also applies to other antibiotics, but the risk of resistance needs, nowadays, to be taken fully into account in the development and assessment of new molecules.

Thus, PK/PD considerations are certainly important in the development of novel antibiotics and should be borne in mind by the pharmaceutical industry. Recent successful examples are moxifloxacin and, to some extent, telithromycin, for which PK/PD parameters have been integrated very early on into the preclinical development and clinical evaluation [9]. It must be stressed that PK/PD concepts have received serious attention from regulatory bodies, such as the Food and Drugs Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA), for their analyses of new drugs. More recently and, at least, in the case of the EMEA, the same approach is being followed for the reappraisal of older drugs. Finally, and not the least, PK/PD concepts and their practical applications have become critical for clinicians to aid optimisation of therapy.

3. Concentration-dependent versus concentration-independent bacterial killing

Research undertaken over the last 15 years has allowed us to define the key PK/PD properties of the main classes of antibiotics that need to be taken into account for optimising their efficacy [5,10–15].

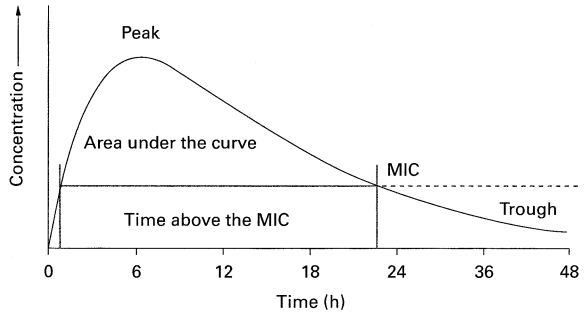

Fig. 1. Peak/MIC, AUC_{24h}/MIC and $T > MIC$, the three main pharmacokinetic/pharmacodynamic parameters governing antibiotic efficacy.

For a large series of antibiotics (e.g. aminoglycosides, fluoroquinolones, metronidazole, daptomycin, ketolides and amphotericin), the C_{max}/MIC and the AUC_{24h}/MIC ratios clearly play the most important roles (Table 1), probably because these drugs have a marked concentration-dependent killing effect. This is illustrated in Fig. 2, which shows the results of an in vitro study on *Listeria monocytogenes* [16]. One can see that a fluoroquinolone, like sparfloxacin, has a sharp concentration-dependent killing effect. Optimisation of dosage regimens for these antibiotics should centre on the AUC_{24}/MIC and C_{max}/MIC ratios. Optimising the first parameter means essentially adapting the total daily dosage (which will govern directly the AUC_{24h}). The second parameter will be dependent on the unit dose. For aminoglycosides, the C_{max}/MIC ratio (which should be higher than 8) will take precedence and be the parameter of choice because of toxicity considerations (toxicity is related to AUC_{24h}

Table 1
Pharmacokinetic/pharmacodynamic parameters correlating with efficacy, as observed in murine thigh and lung infections

$T > MIC$	AUC_{24h}/MIC and C_{max}/MIC
Penicillins	Aminoglycosides[a]
Cephalosporins	Fluoroquinolones[b]
Carbapenems	Metronidazole
Monobactams	Daptomycin
Tribactams	Ketolides
Macrolides	Azithromycin[c]
Clindamycin	Streptogramins
Oxazolidinones	Glycopeptides
Glycylcylines	Tetracyclines[c]

Adapted from WA Craig in [5,37,39] and various oral presentations.

[a] A high C_{max} (>8) and a correspondingly extended interval dosing (once daily) will be preferred for toxicological reasons (see Craig [1]).

[b] C_{max} should be >10 and AUC_{24h}/MIC >125 (although animal models using non-neutropenic animals suggest that a value of 30 could be sufficient).

[c] AUC_{24h}/MIC takes precedence over $T > MIC$, mainly because of marked persistent effects.

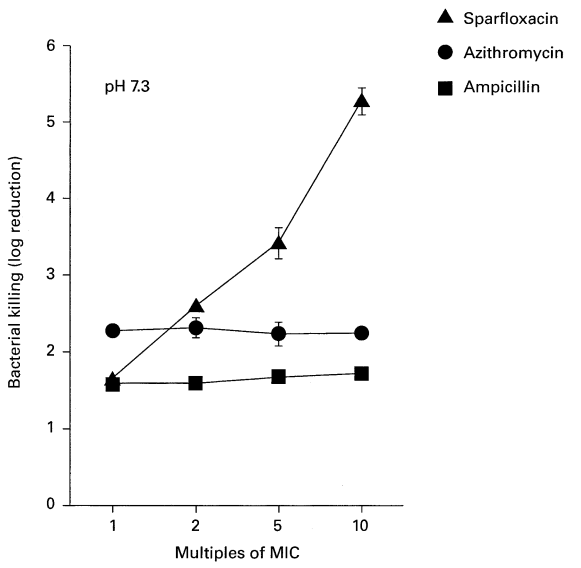

Fig. 2. The role of concentration dependence on bacterial killing: an example with *Listeria monocytogenes* [16].

and to repeated low trough concentrations). For fluoroquinolones, the combination of a C_{max}/MIC ratio of 10 and an $AUC_{24h}/MIC > 125$ is optimal.

Other antibiotics (β-lactams, vancomycin, erythromycin, clindamycin and tetracyclines) have little or no concentration-dependent effects on bacteria (see again the example with *L. monocytogenes* in Fig. 2). These agents do not need a high C_{max} and should have their dosages primarily optimised on the basis of $T > MIC$ (see, however, below other factors that may influence the final recommendation). At this point, this means essentially repeating the unit doses at intervals short enough to prevent the serum concentration falling below the MIC.

Macrolides are somewhat different from the other classes of antibacterial agents since they do not fall in a single category. $T > MIC$ is, indeed, the most important parameter for erythromycin. However, experimental studies show that both $T > MIC$ and AUC_{24h}/MIC influence the clinical efficacy of clarithromycin and azithromycin [17]. As we shall see, this probably reflects differences not only in half-life but also in tissue penetration and subsequent release of the antibiotic from there.

4. Bacterial kill kinetics and prolongation of effect

Another matter of importance in antibiotic action is the speed of bacterial killing. Aminoglycosides and fluoroquinolones are rapidly bactericidal. By contrast, β-lactams, vancomycin, macrolides, oxazolidinones, clindamycin, tetracyclines and flucytosine exhibit considerably slower killing. This makes it all the more necessary to optimise the time of exposure of the offending organism to these antibiotics [15].

Besides the direct effect of antimicrobial agents on bacteria, some classes of drugs also demonstrate a prolonged effect on bacterial growth. There are three types of such effect:

1. Post-antibiotic effect (PAE). This is a delay in bacterial regrowth following the removal of the agent after an initial challenge [18]. PAE has been demonstrated for a number of different antibiotic classes, but is particularly noticeable for aminoglycosides, rifampicin, fluoroquinolones, glycopeptides and tetracyclines [19–22].
2. Sub-MIC effects (SME). These effects, which are genuine, are nevertheless difficult to integrate in drug assessment and dosing policies since they would tend to favour suboptimal exposures (which clearly will cause a fast emergence of resistance). We probably should use them essentially as a test of the distribution of antibiotic susceptibility in bacterial subpopulations [23].
3. Post-antibiotic leucocyte enhancement effect (PALE). Pretreatment of bacteria with an antibiotic may make the bacteria more susceptible to phagocytosis and killing [24]. This effect has been demonstrated for fluoroquinolones, penems and macrolides [25–27].

The factors involved in the PAE may include the concentration of the antibiotic used, the time that the bacteria are in contact with the agent and its mode of action [28–30]. A PAE may be produced by non-lethal bacterial damage caused by an antibiotic through its persistence at the binding site [18]. Part of the PAE observed in vivo, however, is due to tissue binding of the antibiotics and to their subsequent release. It is noteworthy that PAEs tend to be considerably longer lasting in vivo than in vitro. It is interesting to note in this context that azithromycin, in contrast to the other macrolides, shows a marked PAE in vivo. A possible explanation for this is the high binding and probably slow leakage of azithromycin from phospholipids or other components within the cell to which it binds.

Generally speaking, antibiotics with important PAEs do not need to be administered as frequently as those with little or no PAE. In the case of azithromycin, as well as glycopeptides, tetracyclines, streptogramins and fluconazole, it means that the parameter $T > MIC$ becomes less important (even though these drugs are not concentration-dependent) and that the AUC_{24h}/MIC ratio may take precedence. This may clearly explain the efficacy of these drugs even when given only once or twice a day.

5. Achieving appropriate values for PK/PD parameters

Having determined which parameter is important for a given antibiotic in determining clinical efficacy, the

question naturally arises as how to obtain it. Considering first the C_{max}/MIC ratio, it is clear that this value will be directly dependent on the dose and inversely related to the volume of distribution. Taking aminoglycosides as an example, it becomes clear that the initial dose will be the critical point to take into consideration, which in itself explains the importance of the once-a-day schedule. The value of the AUC_{24h} is proportional to the total daily dose and, in case of oral antibiotics, to the bioavailability of the molecule. This parameter can be adjusted by increasing the amount of drug that is administered by, for example, changing from a daily administration of 200 mg to a dose of 600 mg once daily (Fig. 3). Alternatively, the same increase in AUC_{24h} can be obtained by giving 200 mg three times over the 24 h period (Fig. 3). A third option is to use an antibiotic with a low clearance rate, since the AUC_{24h} is inversely proportional to that clearance. Azithromycin, with its exceptionally long half-life, achieves this easily.

β-Lactams, which are largely dose-independent and show only weak or no persistent effects, clearly also need to be administered several times a day since this is the only logical way of achieving a high $T > MIC$. To achieve an increased $T > MIC$ by increasing the unit dose with a twice-daily schedule (or by changing from three-times-daily to twice-daily administration with a proportionally higher unit dose) is a brave attempt, but does not take into account the basic pharmacodynamic properties of this class of drugs.

6. Intracellular activity of antibiotics

Some bacteria (e.g. *Legionella* and *Chlamydia* species) are found within subcellular compartments, such as the phagosomes (Fig. 4). Others, such as *Staphylococcus aureus*, *Salmonella* species and *Mycobacterium leprae*, are mostly located within phagolysosomes. Some bacteria, including *Listeria* and *Shigella*, are detected in the cytosol because they are able to escape very quickly from phagosomes. Infections caused by these organisms are often difficult to treat, and the causative organism is rarely eradicated [31]. In this context, the question that is often asked is whether high intracellular penetration can be equated to activity. Clearly, antibiotics need to accumulate within the cells since low intracellular levels will mean no activity. We know that very large differences in intracellular concentrations exist between antibiotics. Fig. 5, for instance, shows that penicillin, sparfloxacin and azithromycin behave very differently in this respect. Azithromycin, being dibasic, achieves considerably higher cellular concentrations than penicillin or sparfloxacin. Yet, would high intracellular concentration, in itself, be enough? Taking again the example of *L. monocytogenes*, but considering its intracellular form, we have found that β-lactams (ampicillin in this case) and azithromcyin have essentially the same activity. Interestingly enough, the activity of sparfloxacin was the highest of all three drugs, when compared at equipotent extracellular concentration [16]. *L. monocytogenes* is normally present in the cytosol, but if macrophages are exposed to γ-inter-

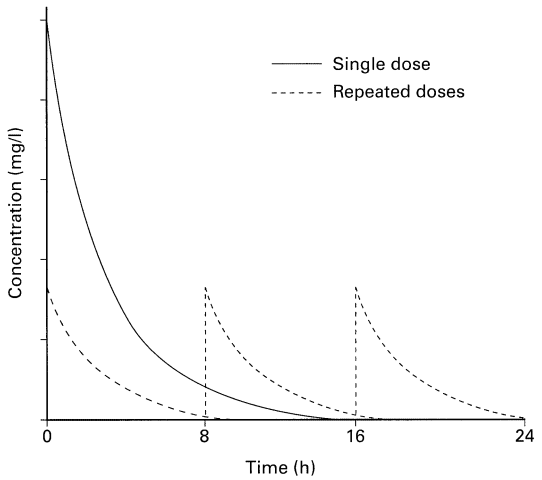

Fig. 3. Increasing AUC_{24h} by single dose increases or repeated single doses over 24 h. An increase of the AUC_{24h} can also be obtained by using a drug with a lower clearance (prolonged serum half-life; not shown).

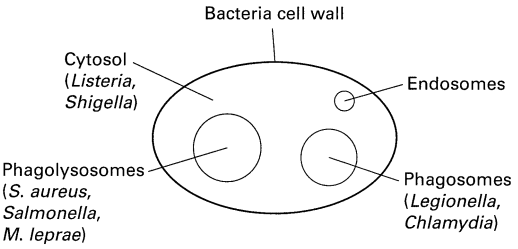

Fig. 4. Localisation of bacteria in the intracellular milieu.

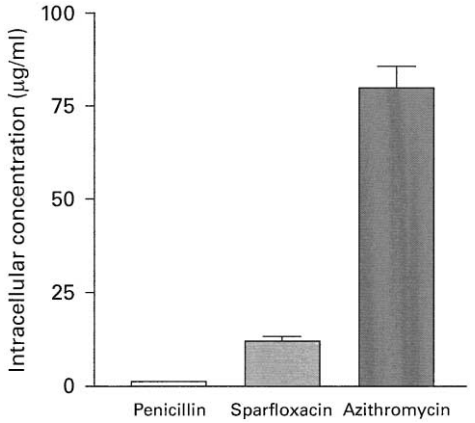

Fig. 5. Typical cellular accumulation of a β-lactam (penicillin), a fluoroquinolone (sparfloxacin) and azithromycin in macrophages. (Data from [16] and unpublished results).

feron, the organism is restricted to the phagosomes and its intracellular multiplication is prevented. After pre-exposure of cells to γ-interferon, the activity of ampicillin was virtually undetectable. By contrast, azithromycin activity was unaltered, and sparfloxacin activity was markedly enhanced [32]. These modulations of antibiotic activity could be due to alterations in bacterial susceptibility to antibiotics, modifications of antibiotic activity, or different antibiotic bioavailabilities in the cytosol and phagosomes. The global conclusion of this type of study is that high cellular penetration is an important parameter, but is by no means the only one. Modulating factors may actually either increase or decrease the activity of the intracellular antibiotics to the point where accumulation per se is no longer predictive of activity [33]. For instance, the absence of activity of certain antibiotics against intracellular pathogens may be related to changes in pH. Aminoglycosides, in particular, have low levels of activity within macrophages due to phagosome acidification [34]. Fluoroquinolones lack effective bactericidal activity against intracellular *Brucella*, perhaps also because their activity is lower at acidic pH [35]. However, this is no more than a partial explanation. Fluoroquinolones, indeed, are active against intracellular *S. aureus*, which are known to be present in the acidic phagolysosomes. Although it has been shown that fluoroquinolones loose 20–40 % of their activity at acidic pH [36], this was less than in the case of the macrolides, which loose about 90% of their activity for each drop of 1 U of pH. Acidic pH seems, therefore, to be more problematical for macrolides than for fluoroquinolones, but there are most likely multifactorial aspects to the bactericidal activity of antibiotics against intracellular organisms.

Thus, antibiotics not only need to penetrate cells and reach the locus where intracellular bacteria sojourn and thrive, but they also need to be bioactive in order to produce effective killing of these bacteria. Bacteria appear to be protected against various antibiotics within phagosomes and perhaps also other intracellular sites of multiplication or survival.

7. Pharmacokinetics and bacterial resistance

If antibiotics do not effectively and quickly kill bacteria, there is a possibility of selection of less sensitive subpopulations or, worse, of bacteria that have either become resistant by mutation or have acquired resistance mechanisms (often from the commensal flora). This can result in clinically meaningful failures. Macrolides, in general, are not bactericidal, but emergence of resistance has not been for long as critical an issue as it has been for fluoroquinolones. Yet, the situation has changed rapidly in recent years. The sharp rise in resistance of *S. pneumoniae*, and to a lesser extent *S. pyogenes*, to macrolides represents the chief reason some why in European countries these drugs are not now used for first-line treatment of respiratory infections. Animal studies suggest that $T > \text{MIC}$ for about 50% of the dosing interval is sufficient to achieve maximal bactericidal activity [37]. This is easily achieved with conventional dosages and susceptible organisms. At first sight, the recent emergence of resistance to macrolides may, therefore, not be directly related to a suboptimal dosing. Yet, suboptimal local concentrations (intracellular?) may have played an important role. Insufficient tissue penetration could indeed help less susceptible organisms to survive protected from the circulating antibiotic, before spreading again and reinitiating the infection. Many other reasons, more directly related to serum pharmacokinetics, have also been proposed [38–40]. Yet, the question may be raised as whether or not it is better to use antibiotics that accumulate within cells, even though their antibacterial activity may be slower than other antibiotics with a more rapid bactericidal action but lower accumulation.

8. The optimal dosing of a macrolide

For macrolides, it is necessary to consider whether serum levels or tissue concentrations of these antibiotics are of most importance. Serum concentrations of antibiotics are easily determinable, and rationally there must be a relationship between serum concentrations of an antibiotic and tissue levels, or the concentrations at the site of infection. The relationship is simple for β-lactams and aminoglycosides, which are mostly found in the extracellular compartments, but the situation, is clearly more complex for the macrolides. Of clear interest for these drugs is the relationship between the total amount of antibiotic in the circulation and the concentrations that can be achieved within tissues.

Azithromycin may be administered for 3 or 5 days, without any notable changes in efficacy [41]. This undoubtedly relates to its marked tissue penetration, which gives to the antibiotic an AUC_{24h}/MIC-related pattern of activity. In any case, however, the treatment needs to be limited in its duration, unless the severity of the infection dictates otherwise. The high tissue penetration of the drug has been shown to cause cell damage in the long term in animals [42]; these effects, however, have not been observed in humans treated at therapeutic doses for short periods.

As far as the other macrolides are concerned, the dosage depends on the plasma half-life and tissue retention. Clarithromycin and erythromycin clearly need to be given several times each day: in the case of erythromycin four times daily and for clarithromycin three times daily, as opposed to twice-daily recommended

dosing regimen. Roxithromycin, which has a relatively prolonged half-life but does not display the high tissue concentrations of azithromycin, still requires a twice-daily schedule (partly also because of its relatively high MICs compared with those of azithromycin and clarithromycin). The option to give more drug less often is, in our view, less appropriate. This will result in higher peak serum concentrations, which has implications for side-effects. A slow-release formulation of clarithromycin has been developed that is designed to mimic the PK behaviour of azithromycin, but it may be feared that a slow-release formulation may be dosed at too low a level. Indeed, macrolides such as clarithromycin and erythromycin definitely need to be administered using a schedule that allows their concentrations to remain above the MIC for most of the dosing interval.

Acknowledgements

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