Elements of design: the knowledge on which we build

A. P. MacGowan

Bristol Centre for Antimicrobial Research & Evaluation, University of Bristol and North Bristol NHS Trust, Department of Medical Microbiology, Southmead Hospital, Bristol, UK

ABSTRACT

The time the free drug serum concentration of antibiotic remains above the pathogen MIC (T > MIC) determines bacteriological efficacy and emergence or selection of resistance for penicillin and amoxicillin with or without clavulanate. Multiple studies in animal and in-vitro models now support this conclusion. The size of the T > MIC (the pharmacokinetic/-dynamic target) is > 40–50% to maximise antibacterial effect and pathogen eradication for *Streptococcus pneumoniae* and probably also *Haemophilus influenzae*. The size of the T > MIC for optimal antibacterial effect is changed by host immune status but not by bacterial inoculum or mechanism of resistance. There is good animal evidence to support the prediction that, as long as the target T > MIC is achieved, strains of *S. pneumoniae* with amoxicillin MICs of 0.016 mg/L will respond to amoxicillin in the same way as those with MICs of 1–2 mg/L. Emergence of resistance to amoxicillin/clavulanate in *S. pneumoniae* is related to low T > MIC (< 20%) and also to the degree of population heterogeneity to amoxicillin. Selection of resistant strains of *S. pneumoniae* is also related to T > MIC. Monte Carlo simulations based on the pharmacokinetics of amoxicillin with or without clavulanate in humans are needed to best predict the likely efficacy of different amoxicillin dosing regimens. This approach adequately allows the considerable pharmacokinetic variability in amoxicillin handling by infected patients to be accounted for as well as differences in pathogen β-lactam susceptibility.

Keywords Pharmacodynamics, pharmacokinetics, amoxicillin/clavulanate

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GENERAL PHARMACODYNAMIC PROPERTIES OF β-LACTAMS

The pharmacodynamic characteristics of β-lactam antibiotics are now well established. These are: (i) nonconcentration or time-dependent killing at drug concentrations in the therapeutic range; (ii) minimal-to-moderate persistent antibiotic effects; (iii) the pharmacokinetic goal of dosing being to maximize the duration of drug exposure; and (iv) the dominant pharmacodynamic index being the time that the free drug concentration remains above some threshold concentration—usually the time above the pathogen MIC (T > MIC) [1].

Corresponding author and reprint requests: A. P. MacGowan, Bristol Centre for Antimicrobial Research & Evaluation, University of Bristol and North Bristol NHS Trust, Department of Medical Microbiology, Southmead Hospital, Bristol, BS10 5NB, UK
Tel: +44 117959 5651/2
Fax: +44 117959 3154
E-mail: alasdair.macgowan@north-bristol.swest.nhs.uk

In-vivo, using a neutropenic murine lung or thigh infection model with over 200 β-lactam dose–response studies and a range of pathogens, it has been possible to show that the T > MIC for a defined antibacterial effect endpoint varies within the β-lactam family and between pathogen groups. The mean T > MIC to produce a 24-h bacteriostatic effect was 20–26% for carbapenems, 29–34% for penicillins and 35–55% for cephalosporins [2]. In addition, the T > MIC for a static effect was lower against staphylococci (24 ± 9%) than streptococci (41 ± 12%). More recently, it has been proposed that β-lactams could be reclassified according to their pharmacodynamic properties [3].

PHARMACODYNAMIC PROPERTIES OF PENICILLINS AGAINST *STREPTOCOCCUS PNEUMONIAE*

Penicillins such as phenoxymethyl and benzyl penicillin, ampicillin and amoxicillin with or

without clavulanate show nonconcentration-dependent killing of *Streptococcus pneumoniae*, with significant bacterial killing over 24 h [4,5]. In-vitro, penicillin exhibits a postantibiotic effect against *S. pneumoniae*; however, it has not been possible to reproduce a postantibiotic effect in a mouse thigh model in-vivo. This may be related to the pharmacokinetics of penicillin in mice. In contrast, ampicillin has a postantibiotic effect of 1–4 h in-vivo, which increases with increasing duration of drug exposure [6,7].

Studies in animal pharmacodynamic models have confirmed that for *S. pneumoniae* treated with penicillin the T > MIC is the dominant pharmacodynamic index [8,9]. As well as defining the dominant pharmacodynamic index, however, it is necessary to determine the size of the index for best effect, because this then becomes the pharmacodynamic target of therapy (Fig. 1). The process in Fig. 1 is now the paradigm used to develop or assess antimicrobials for clinical use. A knowledge of the dominant pharmacodynamic index defines the target that can be used in Monte Carlo simulations and to set doses for clinical studies. Subsequently, data from clinical trials can be used to confirm the size of the pharmacodynamic index required for clinical or bacteriological cure in humans as well as to provide further and more clinically relevant clinical data for confirmatory and comparative Monte Carlo simulations.

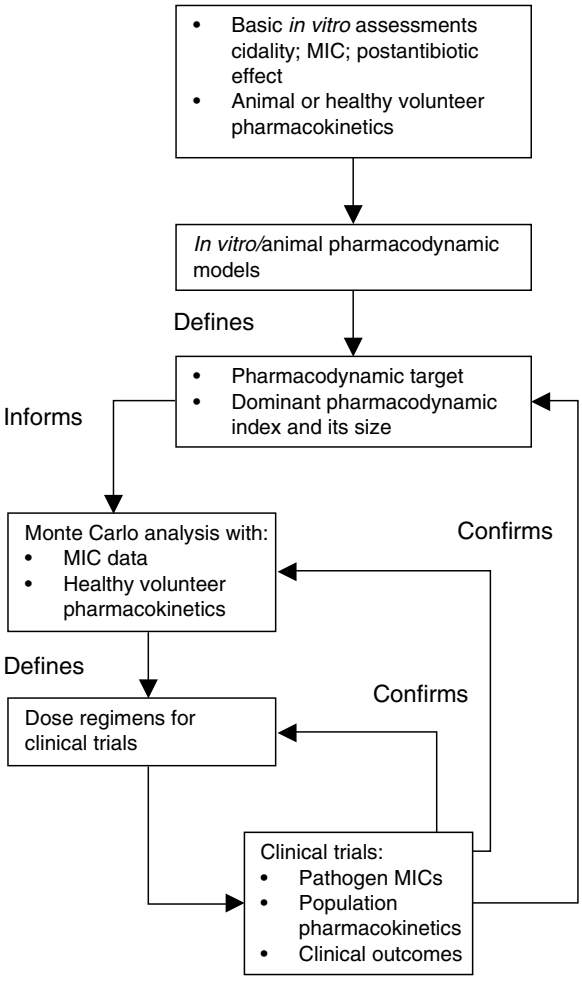

Fig. 1. Defining the pharmacodynamic target for therapy.

A number of factors are known to affect the size of the pharmacodynamic index. These are: (i) mechanism of antibiotic resistance, if present; (ii) bacterial inoculum; (iii) species treated; (iv) host immune status; and (v) choice of antibacterial endpoint (i.e. bacteriostatic or bactericidal).

Although the mechanism of resistance seems to be important in treating *S. pneumoniae* with some drug classes, this is not so for penicillins [10]. Furthermore, for both penicillin-susceptible (MIC 0.016 mg/L) and penicillin-nonsusceptible (MIC 1.0 mg/L) *S. pneumoniae*, the antibacterial effect of penicillin in neutropenic lung or thigh infection and in-vitro models was the same for similar T > MIC values [9,11]. In addition, low (10^6 CFU/mL) or high (10^8 CFU/mL) inocula of *S. pneumoniae* used in an in-vitro pharmacokinetic model had no effect on the amoxicillin/clavulanate T > MIC required to produce the EC_{50} or 80% of the maximum response [12].

There is little information on the effect of bacterial species on the T > MIC required for penicillins. In-vivo animal data in a neutropenic rat pneumonia model and similar work performed in an in-vitro pharmacokinetic model indicate that the amoxicillin/clavulanate T > MICs for bacteriostatic effects, or 80% or 95% of the maximum effect, are similar for *S. pneumoniae* and *Haemophilus influenzae* (G. Woodnutt, personal communication) [12].

In contrast, host immune status has a significant effect on the size of the pharmacodynamic index. The absence of white blood cells in a murine *S. pneumoniae* infection model increases the T > MIC to produce a 2-log bacterial kill from 25% to 35% [13]. Similar effects also occur for *S. pneumoniae* treated with clindamycin or doxycycline in murine models [14]. If, instead of immunocompromising the infected animal, the immunity is augmented by the use of specific

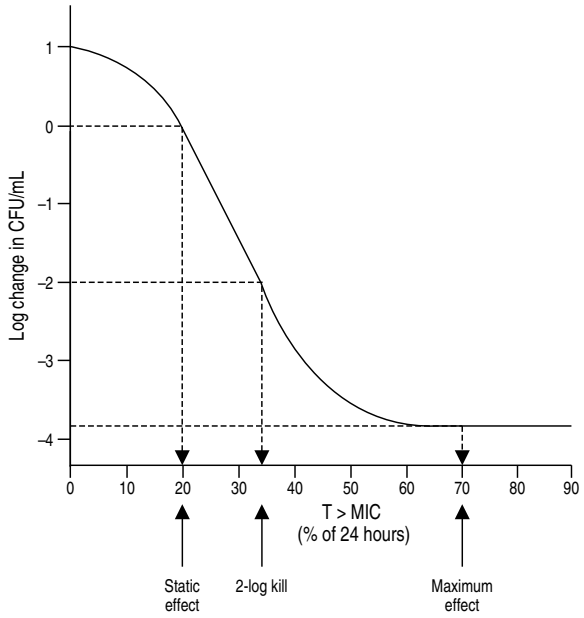

Fig. 2. Theoretical plot of relationship between the β-lactam T > MIC and eradication of a potential pathogen.

antibodies to *S. pneumoniae*, the minimal protective dose of amoxicillin and the T > MIC are significantly reduced [15,16].

The most significant factor in determining the size of the pharmacodynamic index is the choice of antibacterial endpoint. The β-lactam T > MIC will be larger for a 2-log bacterial kill than for a 24-h static effect, while the T > MIC for a 4- or 6-log kill over 24 h will be greater still (Fig. 2). Most of the information on the size of the T > MIC for the antibacterial effect of penicillin or amoxicillin with or without clavulanate comes from neutropenic animal pneumonia or thigh infection models. The T > MIC for a bacteriostatic effect after 6 or 24 h of treatment ranges from 16% to 40%, while the maximum effect occurs at 40–80%. Such experiments have been performed in a wide range of models, such as murine lung and thigh, rat pneumonia, and rabbit tissue cage experiments [8,9,17,18]. Data from in-vitro models are also in agreement because the T > MIC for amoxicillin/clavulanate against *S. pneumoniae* is 5–15% for a static effect and 41–46% for a 90% maximal effect [19].

In a neutropenic rat model of *H. influenzae* pneumonia, the T > MIC for an amoxicillin/clavulanate static effect was 23% and for a 95% maximal effect was 32%. Values derived from in-vitro models were similar, with an 80% maximal effect occurring at 20–48% (G. Woodnutt and V. Berry, personal communication) [19]. Lowden *et al.* reported somewhat different findings in their in-vitro model, with a T > MIC of < 50% being insufficient for amoxicillin/clavulanate to eradicate *H. influenzae* within 24 h, but a T > MIC of 73–79% producing the best pathogen eradication [20].

By analogy with the antibacterial effect of fluoroquinolones in pneumococcal respiratory infection, it would appear that the size of the pharmacodynamic index that best correlates with clinical outcome in mild-to-moderate pneumonia is that required to produce a 24-h bacteriostatic effect or a 1-log bacterial kill in a neutropenic system [21,22]. This would therefore imply that a T > MIC > 40% should be bacteriologically effective in pneumococcal infection in humans and perhaps also for *H. influenzae*.

There is some evidence in human respiratory tract infection to relate the T > MIC to bacteriological outcome [23]. Craig and Andes, however, reported a retrospective analysis of pooled data from clinical studies of acute otitis media treated with β-lactams, macrolides and trimethoprim-sulfamethoxazole. The correlation with in-vitro and animal data was not precise since these human data implied added benefit in terms of bacteriological cure, because the T > MIC exceeded 40%. More human data for penicillins are clearly needed.

THERAPEUTIC IMPLICATIONS

An unbound drug serum concentration present for > 40–50% of the dosing interval has been taken as predictive of bacteriological efficacy for β-lactams [24]. The implications of this are illustrated in Table 1, which shows the percentage of the dosing interval that average serum amoxicillin concentrations, after oral administration in humans, will remain above the MICs of *S. pneumoniae* ranging from ≤ 0.06 to ≥ 16 mg/L. Unsurprisingly, as the dose of amoxicillin increases, the MIC of potentially treatable *S. pneumoniae* strains also increases. The implication of Table 1 is that *S. pneumoniae* strains with MICs of 2 mg/L to amoxicillin would respond to amoxicillin at a dose of 1000 mg three times daily in a similar way to a strain with an amoxicillin MIC of ≤ 0.06 mg/L. An extremely detailed study of pneumonia in immunocompetent rabbits indicated that there was no

Table 1. T > MIC (%) for various amoxicillin doses for a range of *S. pneumoniae* MICs in the range ≤ 0.06 to ≥ 16 mg/L

Amoxicillin dose (mg)	Frequency	T > MIC (%) for each MIC assuming 8-hourly dosing								
		≤ 0.06	0.12	0.25	0.5	1	2	4	8	≥ 16
125	three times daily	100	86	71	54	35	11	0	0	0
250	three times daily	100	100	84	65	46	23	4	0	0
500	three times daily	100	100	100	74	57	40	24	0	0
875	twice daily	100	100	94	63	50	41	27	12	1
1000	three times daily	100	100	100	100	84	60	33	11	1

Dashed line denotes division of MICs into those for which, on average, the T > MIC is ≥ 40%.

Table 2. T > MIC (% of dose interval) for amoxicillin at doses of 15 mg/kg three times daily or 25 mg/kg twice daily for MIC values of 0.5, 1 and 2 mg/L after 3 days of therapy [26]

MIC (mg/L)	Dose	T > MIC	
		Mean	Range
0.5	15 mg/kg three times daily	89	57–100
	25 mg/kg twice daily	76	40–100
1	15 mg/kg three times daily	79	49–106
	25 mg/kg twice daily	65	37–100
2	15 mg/kg three times daily	62	13–98
	25 mg/kg twice daily	51	12–100

difference in terms of mortality, histology, macroscopic appearance, pneumococcal concentrations in tissues or computed tomography scan pneumonia appearances when penicillin-susceptible (amoxicillin MIC ≤ 0.06 mg/L) and -resistant (amoxicillin MIC 2 mg/L) *S. pneumoniae* was treated with amoxicillin at concentrations designed to be equivalent to 1000 mg three times daily in humans [25]. Obviously, some caution must be exercised in interpreting data of this kind, because the mean concentrations do not provide any information on the likely pharmacokinetic variability that will be observed in infected patients.

The degree of pharmacokinetic variability with amoxicillin has recently been illustrated in a study of 66 children under 60 months of age admitted to hospital in Brazil with nonsevere pneumonia [26]. The T > MIC on day 3 of therapy with either a 15 mg/kg three times daily or 25 mg/kg twice daily dose schedule for strains with MIC values of 0.5, 1 or 2 mg/L are shown in Table 2. The range of T > MIC values for any given MIC and dose schedule may vary considerably, particularly when MICs are high. This pharmacokinetic variability has recently been postulated as a cause of breakthrough *S. pneumoniae* bacteraemia in patients receiving coamoxiclav (27). The use of Monte Carlo simulations in predicting the likely antibacterial effect of different dosing regimens allows this variability to be taken into account. As yet, no simulations have been performed to model the relative efficacies of different amoxicillin doses against *S. pneumoniae*.

PHARMACODYNAMICS OF EMERGENCE OF RESISTANCE

Emergence of resistance to amoxicillin in *S. pneumoniae* and the ability of penicillin to select resistant strains have been investigated using in-vitro models, and in mouse and rabbit infection models [28,29]. The T > MIC and heterogeneity of the *S. pneumoniae* population seem to have an impact on the ability of amoxicillin/clavulanate to produce amoxicillin-resistant *S. pneumoniae* (Table 3) [28]. The strain that was more heterogeneously resistant to amoxicillin/clavulanate, as indicated by its ability to grow on plates with higher antibiotic concentrations, before exposure to antibiotic was also more likely to produce resistant populations when compared with the nonheterogeneous strain. However, resistance emerged only at low T > MIC drug exposure [28]. Knudsen *et al.* employed a mixture of *S. pneumoniae* with MICs of 0.016, 0.25 and 4 mg/L in mouse thigh, peritonitis, rabbit tissue cage and in-vitro models [29]. Maximum efficacy was observed, as expected, at a T > MIC of > 40–50% and also at a peak/MIC ratio of > 10. In all the model systems, more resistant strains were selected from the mixtures by insufficient penicillin therapy; that is, when the T > MICs were low. This correlates well with the clinical experience in which carriage of penicillin-nonsusceptible *S. pneumoniae* in the

Table 3. Effect of the T > MIC and bacterial population heterogeneity on emergence of resistance in *S. pneumoniae* when exposed to amoxicillin/clavulanate [28]

	T > MIC (%)	Log CFU/mL on agar containing amoxicillin/clavulanate at:			
		0 MIC	0.5 MIC	1 MIC	2 MIC
Non-heterogeneous strain					
Pre-exposure	–	6.2 ± 0.2	< 2	< 2	< 2
24-h exposure	13	4.8 ± 0.2	< 2	< 2	< 2
	47	< 2	< 2	< 2	< 2
	73	< 2	< 2	< 2	< 2
Heterogeneous strain					
Pre-exposure	–	6.1 ± 0.2	4.3 ± 1.8	< 2	< 2
24-h exposure	0	8.1 ± 0.2	6.4 ± 1.4	4.7 ± 0.5	< 2
	9	4.7 ± 0.3	4.5 ± 0.4	3.5 ± 0.4	< 2
	52	2.4 ± 0.4	< 2	< 2	< 2

nasopharynx is associated with prior β-lactam use (either penicillins or cephalosporins) [30]. It has been suggested that longer, low-dose β-lactam therapy may be worse in this regard [31]. Indeed, a prospective, randomised study of short-course, high-dose amoxicillin (90 mg/kg/day twice daily for 5 days) compared with long-course, lower-dose treatment (40 mg/kg/day twice daily for 10 days) in children with respiratory tract infection indicated that those who received high doses for a shorter time were less likely to carry penicillin-nonsusceptible *S. pneumoniae* after they had completed therapy [32].

CONCLUSIONS

The present knowledge base allows for a good understanding of the factors important in ensuring good therapeutic outcomes when penicillins are used to treat *S. pneumoniae* lower respiratory tract infection. Exploitation of this knowledge should allow for the use of amoxicillin/clavulanate dosing regimens that are satisfactory to treat a wide range of *S. pneumoniae* strains—including those presently classified as penicillin-nonsusceptible. Short, high-dose regimens should also help reduce the risks of emergence of resistance.

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