Proof of concept: performance testing in models

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ABSTRACT

Pharmacokinetic (PK) and pharmacodynamic (PD) principles that predict antimicrobial efficacy can be used to set targets for antimicrobial design and optimisation. Although current formulations of amoxicillin and amoxicillin/clavulanate have retained their efficacy against many, but not all, penicillin-nonsusceptible Streptococcus pneumoniae, additional coverage is required to address the growing problem of drug-resistant strains. Accordingly, two new oral formulations of amoxicillin/clavulanate, a paediatric formulation at 90/6.4 mg/kg/day and a pharmacokinetically enhanced formulation at 2000/125 mg twice daily for adults, were designed using PK/PD principles. These principles indicate that for amoxicillin and amoxicillin/clavulanate, a time above MIC of 35–40% of the dosing interval is predictive of high bacterial efficacy. In line with PK/PD predictions, simulation of human pharmacokinetics in in-vitro kinetic models and in a rat model of pneumonia, amoxicillin/clavulanate 2000/125 mg twice daily was highly effective against S. pneumoniae strains with amoxicillin MICs of 4 or 8 mg/L. Against strains with amoxicillin MICs of 4 mg/L, amoxicillin/clavulanate 2000/125 mg twice daily was significantly more effective than the conventional 875/125 mg twice daily formulation, azithromycin and levofloxacin, even though all levofloxacin MICs were ≤1 mg/L. Following infection with S. pneumoniae strains with amoxicillin MICs of 8 mg/L, the amoxicillin/clavulanate 2000/125 mg twice daily formulation was more effective than the conventional amoxicillin/clavulanate formulations of 875/125 mg twice daily and three times daily and 1000/125 mg three times daily, and had similar or better efficacy than azithromycin and levofloxacin, depending on the strain. These data indicate the potential benefit of therapy with amoxicillin/clavulanate 2000/125 mg twice daily compared with conventional formulations and other marketed antimicrobials in the treatment of respiratory tract infection.

Keywords Amoxicillin/clavulanate, pharmacokinetics, pharmacodynamics, animal model, drug development

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INTRODUCTION

The increasing frequency of antimicrobial resistance in respiratory tract pathogens provides a major challenge for the successful treatment of pneumonia, sinusitis and other respiratory tract infections. Amoxicillin/clavulanate at doses of 500/125 mg three times daily, 875/125 mg twice or three times daily, or 1000/125 mg twice or three times daily has proven to be a useful drug for eradicating many of these resistant bacteria in a variety of infections [1,2]. An array of studies over the past decade has demonstrated that the success of a specific dosing regimen is dependent on the relationship between the extent of drug exposure and the degree of drug susceptibility of the infecting pathogen [3–5]. For drugs such as amoxicillin and amoxicillin/clavulanate, the important determinant is the duration of time that serum concentrations exceed the MIC [6]. In general, for amoxicillin ± clavulanate, serum concentrations should exceed the MIC for 35–40% of the dosing interval to ensure antimicrobial efficacy.

Over the past few years there has been a gradual increase in the number of strains of
**Streptococcus pneumoniae** with amoxicillin MICs of 4 and even 8 mg/L [7]. Two new oral formulations of amoxicillin/clavulanate, a paediatric formulation of 90/6.4 mg/kg/day in two divided doses and a pharmacokinetically enhanced formulation of 2000/125 mg twice daily for adults, were developed to provide high drug concentrations that persist long enough to ensure eradication of these high MIC strains [8,9]. The paediatric formulation consists of a doubling of the amoxicillin concentration used in conventional amoxicillin/clavulanate in children. The pharmacokinetically enhanced formulation consists of a bilayer tablet containing a layer of immediate-release amoxicillin/clavulanate and a layer of sustained-release amoxicillin. Two tablets provide a unit dose of 2000/125 mg of amoxicillin/clavulanate that is administered twice daily. Simulation of the high amoxicillin concentrations produced by this formulation in animal and in-vitro infection models should provide useful data on the potential of this new formulation for successfully treating high MIC organisms in patients. This brief review will (i) summarise the results of several in-vitro and animal model studies using strains of *S. pneumoniae* and (ii) correlate outcome with the duration of time for which drug concentrations exceed the MIC of these pathogens.

**IN-VITRO MODELS**

In-vitro kinetic models have proven to be very useful for determining the activity of human simulated serum concentrations against a variety of micro-organisms. Löwdin, Cars and Odenholt [10,11] have examined the activity of the new pharmacokinetically enhanced formulation of amoxicillin/clavulanate against both *S. pneumoniae* and *Haemophilus influenzae*. Their kinetic model uses a filter to prevent elimination of the bacteria when antibiotic concentrations are reduced by dilution with fresh media. They studied 12-hourly dosing of the new formulation for 24 h against four strains of *S. pneumoniae* that had amoxicillin MICs of 1, 2, 4 and 8 mg/L. The starting inoculum was approximately $10^6$ colony-forming units (CFU)/mL. The new formulation completely eradicated the strains with MICs of 1 and 2 mg/L, however regrowth followed the initial killing of the organisms with MICs of 4 and 8 mg/L. At 24 h the new formulation reduced the CFUs of the organism with a MIC of 4 mg/L by several logs, but was only bacteriostatic against the most resistant strain.

In their studies with two strains of *H. influenzae*, Löwdin, Cars and Odenholt [10] compared the activity of pharmacokinetically enhanced amoxicillin/clavulanate 2000/125 mg twice daily with that of conventional amoxicillin/clavulanate 500/125 mg three times daily and 875/125 mg twice daily. They observed that three times daily administration of 500/125 mg of the drug tended to give a greater bactericidal effect than observed with 875/125 mg twice daily. On the other hand, the pharmacokinetically enhanced formulation produced a statistically higher bactericidal effect than with the other two dosing regimens, and resulted in complete killing of both strains by 24 h.

MacGowan and associates [12,13] have used a slightly different in-vitro kinetic model to study the activity of various antimicrobials against strains of *S. pneumoniae*. The model is a dilution kinetic model that can dilute both the drug and the organism being studied. Their studies on the activity of twice-daily dosing of pharmacokinetically enhanced amoxicillin/clavulanate used seven strains of *S. pneumoniae* with MICs of 4–8 mg/L at two different inocula ($10^6$ and $10^8$ CFU/mL). They observed rapid bactericidal activity with all strains at both inocula. At the high inoculum, however, one strain with an MIC of 8 mg/L was associated with regrowth after 12 h that was due to the emergence of a more resistant organism. A summary of the results at 2, 4, 6, 12 and 24 h with all seven organisms at the high inocula is illustrated in Figure 1. It is important to remember that these in-vitro kinetic models measure antimicrobial activity without the influence of host immune factors, such as polymorphonuclear leukocytes and complement. In-vitro studies using polymorphonuclear leukocytes and complement have demonstrated increased bactericidal activity of the aminopenicillins against *S. pneumoniae* [14].

**ANIMAL INFECTION MODELS**

A variety of animal studies have already evaluated the efficacy of amoxicillin and/or amoxicillin/clavulanate at 500/125 mg and 1000/125 mg three times daily and 875/125 mg twice daily,
and paediatric formulations of 45/6.4 and 90/6.4 mg/kg/day in two divided doses [6,15–17]. Human pharmacokinetics were simulated in these studies either by computer-coordinated drug infusion through intravenous catheters or by inducing renal impairment with the prior administration of uranyl nitrate [18]. These studies suggested that the lower doses were effective against pneumococci with MICs up to 2 mg/L. The higher doses, such as 1000/125 mg three times daily and 90/6.4 mg/kg/day in two divided doses, were active against organisms with MICs up to 4 mg/L. Berry [19] also compared the activity of pharmacokinetically enhanced amoxicillin/clavulanate with more traditional dosing regimens and other drugs in a rat pneumonia model infected with strains of *S. pneumoniae* with MICs of 4 and 8 mg/L. These studies involved six of the seven strains studied by Noel, MacGowan and Bowker in the in-vitro kinetic model [13]. Infection in this model is induced by direct intrabronchial instillation of a 100 μL bacterial inoculum in cooled molten agar. Drug dosing was started 24 h post infection and was continued for 3 days. The drugs and doses simulated through computer-controlled intravenous infusion included pharmacokinetically enhanced amoxicillin/clavulanate at 2000/125 mg twice daily, conventional immediate-release amoxicillin/clavulanate at 875/125 and 1000/125 mg three times daily and 875/125 mg twice daily, levofloxacin at 500 mg once daily, and azithromycin at 1000 mg initially followed by 500 mg once daily. The serum concentrations obtained in this animal model were very similar to those obtained in humans (see Figure 2 for pharmacokinetically enhanced amoxicillin/clavulanate). Animals were euthanized at 14 h after cessation of therapy. The lungs were removed and homogenised, and serial dilutions were plated for CFU determinations.

Bacterial numbers in the control animals varied from 5.7 to 7.1 log_{10} CFU/lungs with a mean value of 6.3 log_{10} CFU/lungs. The difference between controls and the animals treated with different dosage regimens of conventional and pharmacokinetically enhanced amoxicillin/clavulanate are illustrated in Fig. 3(a) for pneumococcal strains with MICs of 4 mg/L and Fig. 3(b) for strains with MICs of 8 mg/L. The variation in bacterial numbers among the different animals was relatively small as standard deviations ranged from 0.6 to 1.4 log_{10} CFU/lungs.

With the three strains of *S. pneumoniae* with MICs of 4 mg/L, pharmacokinetically enhanced amoxicillin/clavulanate at 2000/125 mg twice daily and conventional amoxicillin/clavulanate at 875/125 and 1000/125 mg three times daily gave significantly better results than conventional amoxicillin/clavulanate at 875/125 mg twice daily. With the three strains that had MICs of 8 mg/L, only the pharmacokinetically enhanced formulation of amoxicillin/clavulanate markedly reduced
the number of organisms remaining in the lungs after 3 days of therapy.

A comparison of the efficacy of the pharmacokinetically enhanced amoxicillin/clavulanate at 2000/125 mg twice daily with levofloxacin at 500 mg once daily and azithromycin at 1000 mg followed by 500 mg once daily are shown in Fig. 4(a,b) for strains with MICs of 4 mg/L; (b) S. pneumoniae strains with MICs of 8 mg/L. Data obtained from reference 19.

degree of killing was rather modest for the three strains with amoxicillin MICs of 4 mg/L. Pharmacokinetically enhanced amoxicillin/clavulanate was statistically more effective than levofloxacin against these strains, and was statistically more effective against one of the three pneumococcal strains with the highest amoxicillin MICs.

**CORRELATION OF OUTCOME WITH TIME ABOVE MIC**

Previous animal studies that have used dose fractionation to reduce the interrelationship between different pharmacokinetic/pharmacodynamic (PK/PD) parameters, have clearly
demonstrated that time above MIC (T > MIC) is the important PK/PD parameter for determining the efficacy of β-lactam antimicrobials [20–22]. Specific studies with amoxicillin and amoxicillin/clavulanate have suggested that maximal activity is observed when serum concentrations exceed the MIC for 35–40% of the dosing interval [6,15,16]. Very similar values have been observed using both thigh and lung infection models [23]. Fig. 5 illustrates the relationship between T > MIC and the reduction in $\log_{10}$ CFU/lungs observed with the different formulations of amoxicillin/clavulanate against the various strains shown in Fig. 3(a,b). The curved line represents the best-fit line by nonlinear regression using a maximum effect model [24]. The correlation of activity with T > MIC was very strong, with 84% of the variation being attributed to T > MIC. It appears that with the strains studied, killing of 2.5–3 $\log_{10}$ CFU/lungs requires a T > MIC of 35%, and that maximum killing occurs when amoxicillin/clavulanate serum concentrations are above the MIC for 40% of the dosing interval. Noel, MacGowan and Bowker [13] also looked at the relationship between T > MIC and killing of these same pneumococci in their in-vitro kinetic model. They not only evaluated pharmacokinetically enhanced amoxicillin/clavulanate at 2000/125 mg twice daily, but also determined the extent of killing at doses that were half and a quarter of the standard dose. They also observed that maximum killing of these pneumococci occurred when concentrations in their in-vitro model exceeded the MIC for 40% of the dosing interval.

CONCLUSIONS

These studies provide further support to the PK/PD principles that indicate that a T > MIC of 35–40% of the dosing interval for amoxicillin and amoxicillin/clavulanate is predictive of high bactericidal efficacy. Pharmacokinetically enhanced amoxicillin/clavulanate was developed using these principles to increase efficacy against strains of $S. pneumoniae$ with amoxicillin ± clavulanate MICs of 4 and 8 mg/L. The in-vitro kinetic and animal model studies reviewed suggest that this formulation will be very effective in patients. The final proof of these PD principles can only be demonstrated in prospective comparative clinical trials.

REFERENCES

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