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Leading article

Pneumococcal macrolide resistance—myth or reality?

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Streptococcus pneumoniae is one of the most common community-acquired respiratory tract pathogens, causing as many as ⩾20% of all cases of community-acquired pneumonia (CAP) annually.[1] Over the years a number of agents have been utilized as first-line treatment options for this organism. Invariably, as various agents have been used extensively, in-vitro antimicrobial resistance has developed. Penicillin-resistant pneumococci (PRP) have become highly prevalent in many countries over the past 10–15 years and are continuing to spread in many regions of the USA.[2–5] This (and other) trends in resistance, as well as the recognition of the importance of atypical pathogens, resulted in CAP outpatient treatment guidelines being released in 1993 that recommended that the macrolides be utilized as first-line treatment for patients <60 years of age with no comorbidity.[6] This line of thinking has recently been reinforced by the Infectious Diseases Society of America with their 1998 CAP treatment guidelines.[7] As a result of these recommendations together with, resistance trends, and the advantages of the new azalide azithromycin and the macrolide clarithromycin having antimicrobial activity against all relevant community-acquired respiratory pathogens, these agents have been heavily prescribed over the past few years.[8]

As a result of this new prescribing focus, reports are now describing increasing macrolide resistance in pneumococci.[2,3] A study by Doern *et al.*[3] of 1527 isolates of pneumococci recovered from outpatients during the winter months of 1994 and 1995 demonstrated that 10% were resistant by NCCLS standards [9,10] to erythromycin, clarithromycin (⩾1.0 mg/L) and azithromycin (⩾2.0 mg/L). A separate study by Ballow *et al.*[2] investigated the erythromycin susceptibility of *S. pneumoniae*, including strains of varying penicillin susceptibilities. The investigators studied 2537 clinical isolates from institutions throughout the USA. Of these, 21% overall were erythromycin resistant. When the isolates with varying penicillin sensitivities were studied, it was demonstrated that the rates of macrolide sensitivity in these strains had also decreased. Isolates with intermediate penicillin susceptibility were only sensitive to erythromycin 63% of the time and only 40% of those with high-level penicillin resistance were erythromycin sensitive.

This spread of macrolide resistance, as is the case for other agents, results not only from the appropriate use of these agents but also from inappropriate prescribing habits.[11] It was recently demonstrated by Gonzales *et al.*[12] that, based on a national survey of ambulatory care physicians, office visits for colds, upper respiratory tract infections and acute bronchitis resulted in 12 million antibiotic prescriptions (accounting for 21% of all antibiotic prescriptions for adults) in 1992. As antibiotics are unnecessary for viral infections and it is becoming commonplace to offer only symptomatic relief to acute bronchitis patients, this report represents massive over-prescribing in the clinical setting. Curtailment of erythromycin over-prescribing has been demonstrated recently in Finland to decrease macrolide resistance in Group A streptococci.[13] Curiously, although this drop in resistance coincided with a decrease in erythromycin use there was an increase in use of azithromycin and the other macrolides clarithromycin and roxithromycin. This observation could, therefore, possibly result from poor compliance with erythromycin and better compliance with the newer agents, resulting in more effective eradication of the organisms, rather than encouraging resistance.[14]

As a result of these in-vitro reports, many prescribers are changing their choice of antibiotics for pneumococcal, as well as for community-acquired respiratory tract infections in general, away from macrolides. However, the practice of just changing one drug for another without also altering poor prescribing habits merely results in different resistance issues.

Despite this trend of decreased macrolide use, the

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question that still needs to be addressed is whether these alarming in-vitro results correlate with a negative impact on clinical efficacy *in vivo*. There has always been controversy concerning the relevance of laboratory sensitivity standards in the clinical setting without taking into consideration both the pharmacokinetics and pharmacodynamics of the drug *in vitro*. An example of this would be the NCCLS guidelines indicating that a strain of *Pseudomonas aeruginosa* with a gentamicin MIC of 4 mg/L should be considered sensitive.[9,10] Clinically, owing to the need to provide a peak serum concentration to MIC ratio of at least 10:1 for aminoglycosides, target peak serum gentamicin concentrations would have to be at least 40 mg/L, which is not realistic (and perhaps not safe) even with od dosing schemes.[15]

In-vitro resistance mechanisms

Pneumococcal macrolide resistance manifests itself through mechanisms classified within MLS resistance, since they generally affect the macrolides, lincosamides and streptogramins. The mechanisms involve alteration of the ribosomal target site, production of inactivating enzymes, and the production and utilization of active efflux mechanisms. Two of these three mechanisms are responsible for the vast majority of the macrolide resistance that is now appearing *in vitro*.[16–18] The first of these is the production of a ribosomal methylase which alters the ribosomal target site of the macrolides. This mechanism is coded for by the *ermB* gene (also known as *ermAM*) and confers broad MLS resistance. The second mechanism is the production of an efflux mechanism which is encoded by the *mefE* gene. Unlike the *ermB*-induced ribosomal modification, this efflux mechanism is macrolide specific and does not affect the lincosamides or streptogramins.[18] Both of these mechanisms are transmissable to other isolates, usually via transposons.[17] The more prevalent of these two mechanisms is currently the *ermB* mechanism: this is irregardless of the penicillin susceptibility of the isolate. A study by Shortridge *et al.*[17] investigated the macrolide resistance mechanisms associated with 60 clinical pneumococcal isolates with varying degrees of penicillin susceptibility from four New York City medical centres. They discovered that the *ermB* mechanism was present in 22% and 38% of the pneumococcal isolates that had intermediate and high-level penicillin resistance, respectively. In contrast, the efflux mechanism was present in 8%, 11% and 19% of the isolates that were sensitive, intermediate or high-level penicillin-resistant, respectively. Another study, by Marchese *et al.*[19] of penicillin-resistant *S. pneumoniae* isolates from central and northern Italy, noted that 82.6% of the isolates that were macrolide-resistant possessed the *ermB* gene. The remaining isolates were macrolide-resistant as a result of the efflux mechanism. Finally, a study of 117 pneumococcal isolates that were fully susceptible to penicillin (MIC $\leqslant$ 0.06 mg/L) but resistant to macrolides, showed that *ermB* encoded for the resistance in 88% of isolates compared with 12% owing to the efflux mechanism.[18]

The MIC_{90}s for these macrolide-resistant pneumococci vary in the literature. NCCLS standards define pneumococcal resistance as $\geqslant$1 mg/L for macrolides such as erythromycin and clarithromycin and $\geqslant$2 mg/L for the azalide azithromycin.[9,10] Reports describe MICs for resistant pneumococci that are as low as 3 mg/L and as high as 256 mg/L. The majority of these isolates, however, fall in one of two groups: the first have MICs between 4 and 16 mg/L and the others have MICs >128 mg/L.[2,17] This high degree of variability is not unique for either the pneumococcus or the macrolides. Though not unique, it is important to make sure it is real, as different laboratory techniques have produced variable MIC results. As an example, Gerardo and colleagues.[20] demonstrated that when pneumococcal isolates were tested for susceptibility to azithromycin and clarithromycin by broth microdilution into an ambient environment as compared with when they were later tested by the Etest method in a 5% CO_2 environment, MIC values for the same isolate varied by $\geqslant$2 dilutions. This variability is secondary, at least in part, to CO_2 lowering the pH of the environment and falsely elevating the MICs of the basic macrolide compounds.

In-vivo data

Despite the growing in-vitro resistance trends described above, there is a paucity of data indicating that these resistance trends are translating into in-vivo clinical failures. Rather, it appears that the opposite is true. There are no studies specifically investigating the clinical activity of these drugs against pneumococcal isolates with varying degrees of resistance, but the suggestion that there is a lack of translation between increasing in-vitro resistance and in-vivo failure comes from there being no clinical data to counter it. In contrast, all of the recently released studies that have utilized macrolides such as erythromycin, clarithromycin, roxithromycin, dirithromycin and azithromycin as comparators in clinical trials have demonstrated equivalent high levels of activity against *S. pneumoniae* infections of the upper and lower respiratory tree.[21–25]

Pharmacology is the answer

The clinical efficacy reports suggest that there is more going on *in vivo* than in-vitro susceptibility tests can portray. One piece of evidence to support this is the demonstration that when 50% human serum is added to in-vitro media, the MIC_{90} for pneumococcal isolates drops one to two dilutions for clarithromycin and two to six dilutions for azithromycin, owing to its buffering effects.[21] How this may affect the sensitivity testing of otherwise resistant

isolates is unknown but can be speculated to make them more sensitive.

The second factors in this controversy are the normal host-defence mechanisms and immune responses, together with the pharmacokinetics and dynamics of the drug at the infection/bacterial site also plays an important role. The pharmacodynamic properties of the macrolides and azalides vary. Classic macrolides such as erythromycin and clarithromycin are dependent on the time the drug concentration is above the MIC for the organism for their optimal activity. In contrast, for the azalide, azithromycin, optimal activity is dependent on maximizing the area under the concentration–time above the MIC curve (AUIC).[26] The dosing regimens of these agents are designed to maximize these pharmacodynamic parameters.

Once absorbed, macrolides and azalides are avidly taken up by white blood cells that are chemotactically attracted to the infection site.[27–29] These cells then not only release drug at the infection site but also, after phagocytosis of the pathogen, expose it to high intracellular drug concentrations. Peripheral blood neutrophils and monocytes have both been demonstrated to contain azithromycin concentrations of $\geqslant$10 mg/L, 12 days after the start of either the fifth day (500 mg on day 1250 mg daily on days 2–5) or third day (500 mg daily) of azithromycin dosing regimens.[28,30,31] This delivery of drug by WBCs, passive diffusion via serum equilibrium and release by local fibroblasts results in high, prolonged drug concentration at the site of infection. Foulds *et al.*[32] demonstrated that tissue concentrations were above the MIC_{90} for all relevant community-acquired respiratory pathogens, including pneumococci, for at least 8 days after a single 500 mg po dose of azithromycin. Similar data were also obtained by Baldwin and coworkers.[33] Macrolides, although retained at the infection site and within WBCs more transiently, still achieve peak infection site concentrations (17 mg/L) considerably above the MIC_{90} for relevant community pathogens.[34]

Both infection site penetration and retention maximize the pharmacodynamics of macrolides and azalides. Recent reports concerning lack of penetration of azalides into lung tissue are significant, although the studies have certain design limitations.[35,36] Both studies demonstrated high macrolide epithelial lining fluid concentrations but minimal, or no, azalide tissue penetration. The key limitation of these studies is that they were conducted in healthy volunteers. Whereas macrolide penetration into tissues is as much serum driven as WBC driven, azalide penetration is in large part dependent on inflammation.[28,29] Once WBCs are present, azalide concentrations rise dramatically.[28,29] Although the study investigators did not interpret the data as such, one common and flawed interpretation of these results is that azalide resistance is induced by the sub-MIC concentrations at the infection site. This has recently been shown to be unlikely by Hyatt *et al.*[37] who exposed pneumococcal isolates to sub-inhibitory concentrations of cefaclor, ampicillin and azithromycin for five passages or until antibiotic resistance emerged or the inoculum was eradicated. The investigators demonstrated that azithromycin was the only compound of the three that did not induce resistance and concluded that the azithromycin resistance trends being reported were more likely to be due to inappropriate antibiotic overuse rather than in-vivo exposure to sub-inhibitory concentrations.

The proclivity of the azalides for WBC lysosome penetration and retention may also theoretically give them an advantage over the macrolides against all intermediately resistant (MIC 1.0 mg/L) and some fully resistant (MIC $\geqslant$ 2.0 mg/L) pneumococcal isolates. As was indicated above, peak intracellular azithromycin concentrations within phagocytic cells reach at least 80 mg/L and these concentrations are still 20–40 mg/L 12 days later (see Figure 1).[14,15,28,30,38] As it is the phagocytes that take up and clear the bacteria from the infection site or the blood, it is evident that azithromycin's pharmacokinetics should enable the drug to be effective in an infection, even with resistant pneumococci, as long as the MICs do not rise much above 16–32 mg/L. These values could be used as the breakpoint, whether the dynamics of azithromycin are dependent on maximizing the AUC above the MIC or the time above MIC. This concept is not foreign, as azithromycin has shown good intracellular and clinical activity against *Mycobacterium avium* complex (MAC) despite its MIC_{90}s ranging from 8 to 16 mg/L.[39] It could also be theorized that azithromycin's pharmacokinetics and dynamics may lead it to induce resistance to a lesser degree than other compounds, including the macrolides.

Although macrolides do penetrate WBCs, their penetration and retention is much less and of shorter duration (intracellular $T_{1/2}$ = 5 min), than that of azithromycin.[38] Peak concentrations are approximately eight times the serum concentrations, and unlike azithromycin, do not increase as exposure times increase.[38] This results from

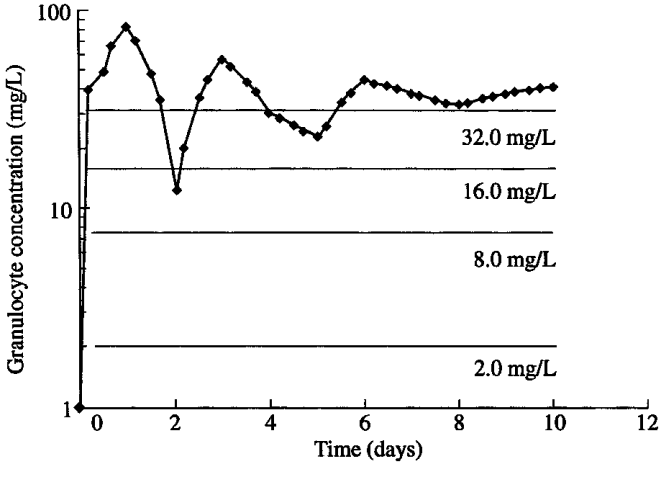

Figure 1. Simulation of granulocyte concentrations for 10 days after the start of azithromycin 500 mg q24h $\times$ 3 doses. Horizontal lines represent potential pathogen MICs.[27,30]

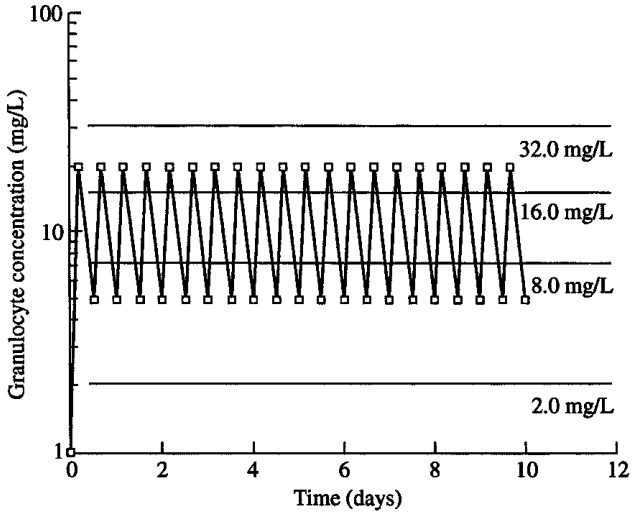

Figure 2. Simulation of granulocyte concentrations for 10 days after the start of clarithromycin 500 mg q12h × 20 doses. Horizontal lines represent potential pathogen MICs.[38]

their monobasic chemical nature and their ability to convert rapidly back to a nonionized state and exit the WBC.[38] As peak and trough serum concentrations are approximately 2.5 and 0.6 mg/L, respectively, when clarithromycin 500 mg is dosed every 12 h, this translates into peak phagocyte concentrations of approximately 20 mg/L and trough concentrations of about 5 mg/L.[27,40] As is evident from Figure 2, when a standard course of clarithromycin is given, although it will be active against sensitive pneumococcal isolates, it is less likely to be able to optimize its pharmacodynamic properties against bacterial isolates with MIC values >8 mg/L.

Summary

There is no doubt that owing to the prolific use of the macrolides and azithromycin over the past several years, resistance has developed and is increasing in incidence. I believe we should re-evaluate the use of these antibiotics for our patients and consider parameters other than the negative in-vitro results. Firstly, microbiology laboratories should return to the habit of providing the clinician with MIC values for pathogenic isolates rather than generic susceptibility reports ((S)usceptible, (I)ntermediate, (R)esistant) that are based on standard disc diffusion testing. Although agar dilution MIC testing is a bulky and labour intensive practice, it provides the best data when conducted in the appropriate environment.

Secondly, and more importantly, these MIC values need to be compared with in-vivo antibiotic pharmacokinetics and pharmacodynamics. Although it is possible to compare MIC values directly with serum concentrations of β-lactams and aminoglycosides, this is not a valid practice for azithromycin or the macrolides. MICs of azithromycin and the macrolides must be compared with the infection site and phagocytic cell concentrations to determine the utility, or lack thereof, of one of these agents. Whereas azithromycin cellular penetration allows maximal pharmacodynamics potentially even against moderately or highly resistant pneumococci, the macrolides do so less optimally. Although there are no reports of widespread clinical failures resulting from macrolide/azalide resistance in pneumococci, it is expected that such reports will appear once the isolates become consistently highly resistant. This is likely to affect the macrolides, erythromycin and clarithromycin, before the azalide, azithromycin owing to the differences in pharmacokinetics of these drugs. Until then, it will be important to determine the MICs of not just one macrolide, but of all macrolides and azalides for the isolates. This will allow the clinician to make a pharmacokinetically and pharmacodynamically sound choice. By choosing clinical MIC breakpoints of 4–8 mg/L for oral macrolides and ⩽32 mg/L for oral azithromycin, rather than the present standard breakpoints, the clinician can make a macrolide/azalide choice that will optimize the pharmacodynamics of the drug against the isolated pathogen and result in the best possible clinical outcome. Once data concerning the cellular penetration of intravenous formulations of these drugs becomes available, it will be possible to develop clinical breakpoints for these formulations as well.

Only through utilizing good antibiotic prescribing practices and by using the drugs appropriately when they are used, can resistance trends be stemmed. In this way, not only does a clinician treat the patient more effectively, but they also extend the antibiotic's useful life.

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