MINIREVIEW
Pharmacokinetic and Pharmacodynamic Requirements for Antibiotic Therapy of Experimental Endocarditis

ANNE-CLAUDE CREMIEUX* AND CLAUDE CARBON
Service de Médecine Interne, Institut National de la Santé et de la Recherche Médicale U13, Hôpital Bichat-Claude Bernard, Paris, France

INTRODUCTION
Bacterial endocarditis is a deep, difficult-to-cure infection. Although most organisms are highly susceptible to many antibiotics, therapy must be maintained for weeks in order to eradicate the infection. Moreover, recent clinical observations with teicoplanin have reemphasized that treatment failure can occur, even with very susceptible organisms, after conventional durations of therapy (35). The difficulty encountered in sterilizing vegetations is usually explained by (i) the poor penetration of antibiotics into infected vegetations, (ii) the altered metabolic state of the organisms within the vegetation, and (iii) the absence of a host defense cellular response inside the vegetation which could cooperate with the antibiotic action. All three explanations were evoked very early in the antibiotic era, and after 50 years of antibiotic use, it is still difficult to distinguish between myth and reality. In this minireview we analyze what experimental models of endocarditis have taught us about (i) the pharmacokinetics of antibiotic permeation of fibrin vegetations and (ii) the specificity of pharmacodynamics in vegetations. We also describe the clinical consequences of these two parameters on the antibiotic dosing regimen and the length of therapy.

PHARMACOKINETICS OF ANTIBiotic PENETRATION INTO FIBRIN VEGETATIONS
The organisms responsible for infective endocarditis are separated from the bloodstream by layers of fibrin and platelets, the elements which compose vegetations (1, 2, 22). Very early on, investigators were intrigued by the potential barrier to chemotherapeutic agents posed by fibrin (31). As early as 1938, Friedman (31), who studied the diffusion of various solutes through the fibrin membrane in vitro, stated that, to be effective in endocarditis, a chemotherapeutic agent should “not be neutralized by the serum proteins, must be able to permeate the fibrin mass which surrounds the organisms, and must be able to remain in the bloodstream long enough and at sufficiently high concentrations so that it can permeate the fibrin in a quantity sufficient for bactericidal purposes.” A few years later, Nathanson and Liebhold (47) tried to explain the inability of sulfonamides to cure endocarditis. They demonstrated that, unlike penicillin, sulfonamides are unable to diffuse throughout the fibrin plates. They thus suggested that the diffusion of antibiotics into vegetations could be prevented by a fibrin barrier which could protect organisms from antibacterial agents. Even if the ineffectiveness of sulfonamides was also due to their bacteriostatic action, in contrast to the bactericidal action of penicillin, as emphasized later (37), the concept of a heterogeneous diffusion of antibiotics into fibrin was born and was often raised in the literature to explain differences between the in vitro and the in vivo activities of antibiotics (7, 38) or the necessity for long-term therapy (11). However, this heterogeneous permeation was still not demonstrated in vivo.

Two in vivo experimental models have subsequently been used to study the pharmacokinetics of antibiotic penetration into fibrin. The first model, subcutaneous fibrin clots, was designed by Weinstein et al. (59) to reproduce lesions similar to cardiac vegetations in order to determine the factors that influence the diffusion of antibiotics. These clots are artificially formed in vitro from pure fibrinogen and are secondarily implanted subcutaneously into rabbits. Studies of the diffusion of antibiotics into fibrin clots by Weinstein et al. (59), Barza and colleagues (4, 5), and others have generally shown that penetration into fibrin clots from plasma is slow and delayed, with the extent of the delay depending on the percentage of binding to serum proteins. The peak level in the clot was inferior to the peak level in plasma. Intermittent injections resulted in markedly higher peak levels than did continuous infusion (42). However, this fibrin clot model has three major pitfalls. (i) Clots, once they are implanted in vivo, are damaged by local fibrinolysis within less than 48 h. Thus, it is not possible to study organized lesions similar to the vegetations that occur in endocarditis. (ii) The subcutaneous location of the fibrin clots exposes them to only a small fraction of the cardiac output, thereby delaying a steady state between the plasma and the fibrin clots. Indeed, later studies have shown that antibiotic behavior in fibrin clots is very different from that seen in experimentally produced vegetations in vivo (45). (iii) As emphasized by the authors themselves (5), “clots were treated as if homogeneous in their antibiotic content,” since antibiotic concentrations were measured in dissolved clots.

With the description of a simple and reproducible model of experimental endocarditis in rabbits (32), measurement of concentrations in experimental cardiac vegetations after antibiotic injection into the animals was possible. These mean antibiotic concentrations measured in homogenates could also be correlated to the antibacterial activity of the drug. With this model, it has been shown (14, 34, 46) that equilibration between antibiotic concentrations in the serum and the vegetation is rapid and almost complete, with a similar elimination half-life in both compartments, and that the concentration in infected vegetations was higher than that in noninfected ones or in cardiac muscle. Thus, the
vegetations could be much higher than those that are active in vitro (38, 40), especially for a cephalosporin such as ceftriaxone (40). One explanation could be the hypothesis proposed by Nathanson and Liebhold (47), i.e., the heterogeneous diffusion of the antibiotic into large vegetations. To investigate this hypothesis, we devised an autoradiographic method to evaluate the pattern of diffusion of labeled compounds into infected cardiac vegetations in an animal model of streptococcal endocarditis (18). Even if indirect signs of the presence of antibiotics inside vegetations can be found by electron microscopic examination of the bacteria or by antibiotic microbiological assay of the fragment of the core of a vegetation, or even if it can be speculated by computer analysis (6, 44, 48), autoradiography remains the sole method that is able to give a precise and quantified image of the antibiotic's distribution pattern. Indeed, in 1954, when Ullberg (56) demonstrated only a very weak penetration of penicillin into experimentally induced abscesses, he stressed the potential role of autoradiography in examining cardiac vegetations. Unfortunately, a convenient experimental model was not available at that time.

Now that the autoradiographic distribution patterns of more than 10 compounds belonging to different groups of antibiotics have been investigated, three different diffusion patterns can be grossly described. The first one is that obtained with 14C-teicoplanin, which remains concentrated at the periphery of the vegetation and does not diffuse into the core of the vegetation (18). The second pattern of diffusion was observed with 14C-ceftriaxone (19), which progressively diffuses into the vegetation, but a high concentration gradient persists between the periphery and the core when autoradiography is performed one elimination half-life after the first determination. To a lesser degree, this concentration gradient is observed with 14C-penicillin (18). For these two groups of antibiotics, a decrease of the concentration in homogenates by conventional methods gives a false indication of the actual level of activity at the core of the lesion. The third most frequent pattern was a homogeneous diffusion throughout the whole lesion; it was observed with tobramycin, pefloxacin, temafloxacin, sparfloxacin (17, 19a), and daptomycin (unpublished data). Autoradiography also enabled the simultaneous investigation of the distribution patterns of amoxicillin and clavulanic acid labeled with different isotopes. Both compounds exhibited similar homogeneous profiles of permeation into vegetations (44a). Although the compounds were injected at a dose ratio of 4:1 for amoxicillin/clavulanic acid, 30 min later the concentration ratio of amoxicillin/clavulanic acid was 2:1 in all areas of the vegetation. Thus, diffusion of amoxicillin and clavulanic acid into vegetations should not constitute an impediment to the expression of the in vivo synergistic effect of the combination, at least if appropriate doses of the two drugs are used, as was recently shown for methicillin-resistant *Staphylococcus aureus* experimental endocarditis (29). For antibiotics which diffuse homogeneously, the concentrations determined in homogenates may be representative of the real levels that are active in vivo. Pharmacokinetic analysis at different sampling times after drug injection into the animal are even more feasible by the latter method for economic reasons. Indeed, two time points are the maximum that can reasonably be measured by the autoradiographic method. Moreover, as a rapid exchange occurs between plasma and vegetation, levels of antibiotic in serum may provide adequate indirect information on antibiotic levels in cardiac vegetation (33, 40).

Although it seems logical to hypothesize that to sterilize a vegetation the antibiotic should be able to diffuse throughout the infective lesion, the predictive value of the therapeutic efficacy of the autoradiographic diffusion pattern of antibiotics into vegetation is still not known. The concentration gradient between the periphery and the core of the vegetation observed with 14C-ceftriaxone (19) and, to a lesser degree, 14C-penicillin (18) could explain the need for local concentrations to significantly exceed the MIC and/or MBC for the offending organism (40), as assessed by determination of the drug concentration in a homogenate of the vegetation. The failure of teicoplanin to diffuse into the core of the vegetation could be one explanation for the failures observed with this antibiotic in the therapy of human staphylococcal endocarditis (35). However, this would be difficult to demonstrate in vivo by a comparative therapeutic study of two antibiotics with different patterns of diffusion, because other factors, such as the killing rate of the antibiotic, the possible inactivation of the antibiotic by local physicochemical conditions, and the metabolic state of the bacteria, must be taken into account, as discussed below. In addition to the distribution pattern, the concentration of antibiotic in contact with the bacteria is also an important consideration. Finally, it must be emphasized that these autoradiographic patterns of diffusion were determined after a single-dose infusion of labeled antibiotics. However, because no cumulative effect of repeated doses was demonstrated in pharmacokinetic studies by conventional methods (14, 40), it seems unlikely that repeated infusions of labeled antibiotics, which are hardly feasible, could modify those patterns.

At present, there is no way to predict the autoradiographic diffusion pattern of an antibiotic in fibrin vegetations because the parameters governing this diffusion have not yet been clearly defined. High levels of binding to serum proteins, a trait common to teicoplanin and ceftriaxone, may not be the sole factor, because teicoplanin, which is also highly bound, diffuses homogeneously. More than the extent of serum protein binding, the diffusion pattern of antibiotics into the fibrin vegetation could depend on the relative number and affinity of binding sites in serum (on albumin or α1-acid glycoprotein) and the fibrin matrix. Unfortunately, these last two parameters are difficult to measure without altering the physiologic characteristics of the infected vegetations.

**PHARMACODYNAMIC FACTORS: WHAT IS THE SPECIFICity OF ENDOCARDITIS?**

In severe infections, the kinetics of the bactericidal effect and the duration of the persistent suppression of bacterial growth once the antibiotic concentration has fallen to a subinhibitory local level (called the post antibiotic effect [PAE]) govern antibiotic efficacy as well as the dose and the dosing interval, at least when these antibiotics are used as single drugs (21, 57). For severe infections, it can be said (21, 57) that, in monotherapy, antibiotics with slow bactericidal killing rates (i.e., those with a time-dependent effect) and no PAE, such as β-lactams, must be administered at short intervals in order to maintain local inhibitory levels throughout the entire dosing interval. In contrast, antibiotics with a rapid killing rate (i.e., with a concentration-dependent effect) and a PAE, such as aminoglycosides and fluoroquinolones
have on some organisms, can be administered at longer dosing intervals.

Most of these conclusions regarding the relation between the pharmacodynamics of antibiotics and optimal dosage have been verified in experimental bacterial endocarditis (9, 36, 39, 50, 51, 55).

Some pharmacodynamic factors are, however, specific for endocarditis. This specificity comes from the particular conditions of activity faced by antibiotics within vegetations. Experimental endocarditis is considered a rigorous test of antibiotic efficacy. This is due to the large number of bacteria in vegetations, the inactive metabolic state of the bacteria, and for some bacterial species, the local secretion of exopolysaccharides (23, 30). Durack and Beeson (23) were the first to show that bacteria deep inside vegetations had reduced metabolic activity, as assessed by measurement of L-[3H]alanine incorporation after incubation of the infected vegetation in vitro. These resting-phase colonies could explain relapses after therapy and the necessity of long-term therapy. In an ultrastructural study of nutritionally variant streptococci in experimental vegetations (30), we showed that, during the course of endocarditis, nutritionally variant streptococci develop aberrant morphological features. These ultrastructural abnormalities, which involve shape, septum formation, and cell wall thickness, were not observed during the first week; but they appeared 10 days after the initiation of endocarditis. These abnormalities, which suggest metabolic modifications caused by nutritional limitations, appeared simultaneously with a dramatic increase in bacterial density and the development of a thick exopolysaccharide layer. All these factors could compromise the local antibacterial activity of some antibiotics, especially β-lactams (8, 17, 20). Indeed, evaluation of the bactericidal activity of β-lactam antibiotics on slowly growing bacteria cultured in a chemostat (17) have shown that, for β-lactam drugs, with the exception of penems and carbapenems, the killing rate is proportional to the growth rate. Moreover, the combination of dextranase, an enzyme that hydrolyzes exopolysaccharide, and penicillin increases the efficacy of the latter compound in experimental streptococcal endocarditis, suggesting that exopolysaccharide could impair the activity of penicillin (20). These factors, in addition to the heterogeneous diffusion of the antibiotics inside the vegetation, can increase the local effective level of antibiotics and thus modify the dose and the dosing interval free of regrowth compared with those of other infectious processes. Also, the time required to sterilize the lesions might be increased, with a consequent need to lengthen the duration of treatment.

Several studies have shown that, in endocarditis, unlike pneumoniitis or thigh infections (43), maintenance of a β-lactam concentration just above the MIC in plasma was not sufficient to ensure sterilization of vegetations. In experimental staphylococcal endocarditis (33), a continuous infusion of methicillin, which produced a level in serum (and, thus, a level in the vegetation (34)) that exceeded the MBC throughout the entire treatment period, was less effective than administration of the same dose every 4 or 8 h, which enhanced local peak concentrations. However, above this active peak concentration, the predominant parameter of efficacy becomes the interval between doses; even though it produces the highest peak concentrations, the 12-h regimen was the least effective. Similar results were obtained with cephalosporins in experimental Escherichia coli endocarditis (40). After a 4-day therapeutic regimen of ceftriaxone, a cephalosporin with a long elimination half-life that is administered once daily, a linear relationship was described between the peak antibiotic level in serum or vegetation (in a range of 142× to 600× the MBC in vegetations) and bacterial titers in vegetations. In all cases, the trough levels in serum were constantly at least 10 times greater than the MBC. A significant antibacterial effect in treated animals compared with the effect in control animals was observed only when the vegetation concentration/MBC ratio was equal to or greater than 220× the MBC. Ex vivo studies in which vegetations sampled from infected animals were incubated in rabbit serum containing different concentrations of ceftriaxone confirmed the need for high levels of the drug in serum to obtain a complete antibacterial effect in the lesions. This is compatible with the high concentration gradient found autoradiographically. Unlike ceftriaxone, cefmenoxime, which exhibits a short elimination half-life in rabbits (1.3 h versus 3 h for ceftriaxone), is ineffective when it is given once daily, despite a high peak level (104× the MBC in vegetations), and becomes effective when it is administered at the same daily dose divided into two injections, despite lower local peak levels (49). Thus, once the effective local level is attained, the duration of contact between the antibiotic and bacteria, and not the concentration itself, becomes the predominant parameter of efficacy. Along the same line of thinking, Cometta et al. (12a) have shown that increasing the dose of continuously infused benzathine penicillin G in order to obtain levels in serum of more than 20, 40, and 500 to 1,000 times the MIC did not enhance the in vivo killing in experimental streptococcal endocarditis, a pattern consistent with penicillin’s low concentration gradient of diffusion into vegetations and the absence of a dose effect on the in vitro killing rate. Because the therapeutic index for β-lactams is usually high, concentrations far above the MIC are easily obtained in humans. One exception is represented by β-lactamase inhibitors. In experimental endocarditis caused by an E. coli strain that produces SHV-2-like β-lactamase (25), the combination of sublactam and ceftriaxone was effective when the peak level of sublactam in serum was 124 µg/ml and the trough level in serum was constantly greater than 4 µg/ml (the concentration necessary to restore the activity of ceftriaxone in vitro). The same regimen was ineffective against Klebsiella pneumoniae that produces TEM-3 β-lactamase endocarditis (10), probably because of the high level of β-lactamase activity inside the vegetation, which increased the local active concentration. This is an in vivo translation of the inoculum effect.

The in vivo translation of PAE can also be altered in endocarditis. Like quinolones, imipenem exhibits a PAE on Pseudomonas aeruginosa in vitro. However, in contrast to ciprofloxacin (39), the efficacy of imipenem against experimental P. aeruginosa endocarditis is compromized when the interval between doses is increased from 3 to 6 h (38). This could be due to the absence of an in vivo PAE of imipenem because of poor diffusion of the drug inside the lesion. In fact, the PAE, at least in vitro, is a function of the concentration of drug and the duration of exposure (58). This could also be due to imipenem’s slow bactericidal rate. It is, in fact, often difficult to distinguish whether the profound bactericidal effect or the PAE as assessed in vitro is responsible for the absence of regrowth between doses in vivo. It is also important that, in the study mentioned above (38), the MIC and MBC of imipenem for the test strain were 1.9 and 14.9 µg/ml, respectively, while the peak vegetation concentration/MBC ratio was only about 2, suggesting that bactericidal concentrations were probably not reached locally.

Also, for antibiotics other than β-lactams, the metabolic state of the bacteria and the pattern of drug diffusion into the
vegetations are probably important in determining the dosage requirement in endocarditis. Kill rates of glycopeptides, like those of β-lactams, are not concentration dependent, so they are slowly bactericidal. Their efficacies are thus more dependent on the time during which concentrations are above the inhibitory level rather than on peak concentrations (12, 13). Because therapeutic failures have been reported in endocarditis with teicoplanin trough levels in serum of about 5 × the MIC for S. aureus, the dose was increased in order to maintain a trough concentration at about 10 × the MICs for most strains of staphylococci. At this dose, therapeutic failures have been still observed (35). These therapeutic failures could reflect insufficient doses and/or poor diffusion of teicoplanin into the core vegetation (18). The decreased activity, at least in vitro, of teicoplanin in the presence of a high inoculum (28) as well as the deleterious influence on the antimicrobial effect of a polysaccharide, like slime secreted by Staphylococcus epidermidis (27), can also contribute to these failures.

A few experimental reports on E. coli endocarditis have shown that aminoglycosides can be efficacious in vivo, with local levels not higher than the MIC at the peak obtained with a once-daily regimen (26). This is consistent with the high killing rate of aminoglycosides and their PAEs and, as was also noted with quinolones, their activities against some resting organisms.

Because long-term aminoglycoside therapy is toxic for patients, aminoglycosides are not used as monotherapy in patients with endocarditis. Moreover, clinical data and even some experimental data (3) suggest that monotherapy could be poorly effective even against endocarditis caused by some gram-negative bacilli. The explanation could be the regrowth of less susceptible organisms during therapy. Thus, the experimental data concerning the optimal dosage of aminoglycosides in endocarditis are more relevant for illustrating the influence of pharmacodynamic parameters, under stringent experimental conditions, than for therapeutic use against endocarditis, at least as monotherapy. The data obtained with combination therapies, especially with aminoglycosides, were recently been reviewed in detail (24) and therefore will not be discussed further in the present minireview.

Caution should also be taken for therapeutic extrapolation of data concerning quinolones. Indeed, even though results of experimental studies have suggested that resistant mutants are frequently encountered during monotherapy, despite the high density of organisms in vegetations (41, 52), the experimental model of endocarditis can underestimate this problem. A recent clinical report (54) has reinforced this idea, and quinolones are still investigational for therapy of human endocarditis.

In conclusion, both the diffusion pattern into fibrin and pharmacodynamics govern the efficacies and optimal dosage regimens of antibiotics against endocarditis. The experimental animal model of endocarditis provides a rigorous test of antibiotic activity. Infected vegetations represent a particular infection site which is somewhat different from other foci because of the heterogeneous distribution of some antibiotics, the high density of bacteria, and the reduced metabolic activities of microorganisms. All the factors discussed above may explain the need for high urinary doses or the lack of high in vivo PAE observed with some drugs. However, it must be stressed that these factors can be encountered in infections other than endocarditis. Fibrin is a regular component of the inflammatory process which accompanies many infections. Ultrastructural alterations of organisms suggesting reduced metabolic activity have been described in chronic refractory infections, such as experimental osteomyelitis (16). Glycocalyx-enclosed biofilms of bacteria have also been identified in experimental or clinical infections that arise from osteomyelitis or contaminated prostheses (15). Thus, the results obtained in experimental endocarditis, which define the pharmacokinetic and pharmacodynamic requirements essential for effective antibiotic therapy, may be useful in determining the dosages for other difficult-to-treat infections. Therapeutic studies in animal models have many practical and scientific advantages over clinical studies done in humans. Clinical studies are indeed difficult, because endocarditis is an infrequent and heterogeneous disease. Many factors besides pharmacologic ones can influence the overall outcome. Careful interpretation of experimental therapeutic results has been shown to be reliable in predicting drug efficacy in humans (53). The experimental model of endocarditis has led to optimization of the dosages of old antibiotics and should be further applied to investigations of new compounds.

REFERENCES


