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Review

Determinants of efficacy and toxicity of aminoglycosides

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The relative efficacy of different aminoglycosides or of different dosage schedules of the same aminoglycoside should be quantitated and related to relative toxicity. Quantitative experimental indicators of efficacy should not only include MIC and MBC, but also the postantibiotic effect *in vitro* and *in vivo*, the emergence of resistance in in-vitro and in-vivo models, and the relationship between plasma concentration profiles and efficacy. Parameters of clinical efficacy are to be related to pharmacokinetic parameters such as the ratio between the peak serum concentration and the MIC.

Toxicity in clinical trials should be assessed by the most sensitive methods available. Experimental and clinical studies have shown cortical uptake to be a sensitive indicator of renal toxicity. As far as ototoxicity is concerned endolymph and perilymph pharmacokinetics are not clearly related. Clinical ototoxicity should be assessed by sensitive methods, such as high frequency tone audiometry. Finally, risk factors for nephrotoxicity and ototoxicity (e.g., duration of treatment, associated nephrotoxic drugs, dehydration) should be assessed in the evaluation of clinical trials.

Introduction

As there seems to be no qualitative difference between the various aminoglycosides with respect to their antibacterial efficacy, much emphasis is laid on their relative toxicity when it comes to the choice of a particular aminoglycoside. However, the criteria to be used to compare quantitatively the toxic features of aminoglycosides, especially under clinical conditions, have not been established systematically. Moreover, if quantified at all, they should be evaluated in relation to the efficacy. This review is intended to discuss accepted methods to quantify efficacy and toxicity, in a way to optimize the

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proper choice of an aminoglycoside. It is concluded that data are still insufficient to allow for a definite choice. For instance, any modification in the dose or dosage regimen of a drug that lessens toxicity may also reduce the drug's therapeutic efficacy. This is especially true for the aminoglycosides since their therapeutic index is narrower than that observed with β-lactam antibiotics, for example. Although some members of the aminoglycoside family have been available since the early days of antibiotic therapy, it is only during the last decade that new insights have been provided into the pharmacodynamic activity of these drugs. Those new insights are the focus of discussion in this review.

Efficacy

The time-honoured method of assessing the antibacterial activity of the aminoglycosides is by measurement of the MIC and the MBC. Although these values are influenced by the concentration of divalent cations, the pH of the medium, and the inoculum (Reller *et al.*, 1974; Thrupp, 1986), standardized techniques provide reasonable accuracy for these measurements. The activity of the aminoglycosides in human serum is virtually identical to that in broth with similar cation content, and this provides the rationale for using serum bactericidal activity as an indication of efficacy (Stratton & Reller, 1977; Van der Auwera & Klastersky, 1987). Parameters such as the MIC and MBC give only a partial view of the full pharmacodynamic activity of these drugs. The MBC does not provide the bactericidal rate or show whether the latter is enhanced by increasing concentrations. Furthermore, the MIC does not reveal whether drugs exhibit persistent suppression of bacterial growth after antibiotic exposure, a phenomenon referred to as the postantibiotic effect or PAE (Vogelman & Craig, 1985; Craig & Gudmundsson, 1986). In fact, the rate of bactericidal activity with increasing concentrations and the presence or absence of a postantibiotic effect (PAE) better describe the time course of antimicrobial activity than MIC or MBC.

Aminoglycoside antibiotics differ from β-lactams in that their bactericidal activity is rapid, and concentration-dependent over a much wider range of concentrations. This has been shown to be so *in vitro* (Shah, Junghanns & Stille, 1976; Van der Auwera & Klastersky, 1987) as well as *in vivo* (Vogelman & Craig, 1986; Flueckiger, Feller & Gerber, 1986). On the other hand, MacArthur *et al.* (1984) have suggested that killing by aminoglycosides is concentration-dependent only during the first hour and that this stage is followed by a fixed limited bactericidal rate which is not enhanced by increasing concentrations. This may be so, but studies with repeated doses of aminoglycosides in neutropenic mice have demonstrated concentration-dependent killing for several hours at least (Gudmundsson, Turnidge & Craig, 1982; Vogelman *et al.*, 1988*b*).

Aminoglycoside antibiotics produce PAEs against staphylococci and various Gram-negative bacilli (Vogelman & Craig, 1985; Craig & Gudmundsson, 1986; Van der Auwera & Klastersky, 1987). In general, the duration of the PAE increases with longer exposure times or higher drug concentrations up to a maximal response. Although the rapid and relatively complete bactericidal activity of aminoglycosides against many bacteria makes it difficult to quantitate a maximal response, PAEs of 1–3 h have consistently been observed *in vitro*, both in broth and in human serum. *In vivo*, even longer PAEs (4–8 h) have been observed with *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Craig & Gudmundsson, 1986; Vogelman *et al.*, 1988*a*). These pharmacodynamic characteristics of the aminoglycosides, namely con-

centration-dependent bactericidal activity and the presence of prolonged postantibiotic suppression of bacterial growth, provide the therapeutic rationale for less frequent dosing of these drugs than in current clinical practice. The administration of large intermittent doses would produce high peak levels which might maximize the rate of killing, and the PAE would maintain efficacy when serum and tissue levels fell below the minimal effective concentration. Blaser, Stone & Zinner (1985) used an in-vitro model, simulating human pharmacokinetics, to examine the efficacy of several aminoglycoside dosage regimens against staphylococci and Gram-negative bacilli. When combined with ceftazidime, netilmicin was as effective in this model when given as a single daily dose as when administered in three daily doses that provided 50% more total dosage per day (Blaser *et al.*, 1985). Emergence of resistance did not occur during the combination therapy. Netilmicin alone as a once-daily administration was also superior to either a continuous infusion or an 8-h regimen of the same total dose per 24 h, in terms of faster killing and greater reduction of bacterial numbers. Regimens that did not provide peak levels of at least ten times the MIC were associated with the selection of resistant subpopulations (Blaser *et al.*, 1987). The emergence of aminoglycoside-resistant subpopulations during therapy has also been observed *in vivo* in normal and neutropenic mice (Gerber *et al.*, 1982; Pechère et al., 1986). These observations provide another reason for maximizing the peak level by intermittent dosing.

There are a variety of animal studies that have compared the efficacy of continuous infusion or frequent dosing with infrequent (as long as once daily) administration of the same amount of aminoglycoside. No difference in outcome was observed against *P. aeruginosa* in rabbit endocarditis (Powell *et al.*, 1983), chronic pneumonia in rats (Powell *et al.*, 1983), acute pneumonia in guinea pigs (Kapusnik *et al.*, 1988), peritonitis in rats (Mordenti, Quintiliani & Nightingale, 1985), and thigh infection in neutropenic mice (Gerber *et al.*, 1983). Infrequent dosing was more effective than continuous infusion or frequent bolus administration in acute pseudomonas pneumonia in guinea pigs (Powell *et al.*, 1983) and in staphylococcal endocarditis in rabbits, in combination with nafcillin (Kapusnik & Sande, 1986). On the other hand, 12- and 24-h dosages of tobramycin were less effective than 4- and 8-h dosing regimens in a klebsiella pneumonia model in mice (Pechère, Letarte & Pechère, 1987). Similar findings were observed with 4-h and 24-h dosing of tobramycin in pseudomonas pneumonia in neutropenic guinea pigs (Kapusnik *et al.*, 1988). Conclusions drawn from experimental studies focusing on dosage intervals are limited by the fact that in small animals the drugs are eliminated at a much faster rate than in man. In simulated human pharmacokinetics, in both normal and granulocytopenic mice, it was demonstrated that a simulation of once daily administration to man of netilmicin (in combination with β-lactam drugs) was superior or at least equal to a simulation of a thrice-daily regimen (Kozak *et al.*, 1985; Gerber *et al.*, 1986).

Four human trials have found no statistical difference in outcome between once daily and 8-hourly administration or continuous infusion (Powell *et al.*, 1983; Nordström *et al.*, 1985) and between continuous infusion and 6-hourly dosing (Feld *et al.*, 1977; Feld *et al.*, 1984). However, none of these animal and human studies was designed to correlate specific pharmacokinetic parameters with efficacy. Several clinical studies involving patients with Gram-negative bacillary infections have demonstrated that peak serum bactericidal titres of at least 8 (Klastersky, Meunier-Carpentier & Prevost, 1977; Sculier & Klastersky, 1984), or peak serum concentrations greater than 5 mg/l for gentamicin and tobramycin and greater than 20 mg/l for amikacin (Noone *et al.*, 1974;

Moore, Smith & Lietman, 1984) were associated with a significantly improved clinical response. Since bactericidal titres will be strongly correlated with both antibiotic concentration and sensitivity of causative micro-organisms, they should be expected to correlate more strongly with clinical outcome than serum concentrations alone. Indeed, in a reanalysis of their data, Moore, Lietman & Smith (1987) observed that the ratio of the peak concentration to the MIC was the major determinant of the clinical response to aminoglycoside therapy. At maximum peak concentration: MIC ratios of 6:1 or less the average response rate was less than 70%, as opposed to a clinical response rate of approximately 90% at ratios of 8:1 or higher.

Since each of the studies mentioned above used a narrow range of doses, administered primarily at one dosing frequency, it cannot be determined whether the peak drug level is the critical parameter or whether a higher peak concentration simply reflects a larger area under the concentration-versus-time curve (AUC) and persistence of inhibitory or bactericidal levels for a greater proportion of the dosage interval. By using a large number of dosing regimens in the neutropenic mouse-thigh model, Vogelman *et al.* (1988*b*) were able to reduce much of the interdependence among pharmacokinetic parameters and determine the parameter that correlated best with efficacy. Their studies demonstrated that the AUC or the total dose, rather than the peak level or the duration of inhibitory serum levels, was the major determinant of the efficacy of aminoglycosides against Gram-negative bacilli. However, efficacy was reduced when the dosing interval was longer than the time period during which serum levels exceeded the MIC plus the duration of the postantibiotic effect, both as determined *in vitro*.

In conclusion, the pharmacodynamic characteristics of the aminoglycosides allow for equal efficacy with a variety of different dosing regimens. Although the AUC or total dose appears to be the primary determinant of efficacy, peak levels greater than ten times the MIC may be advantageous in preventing the selection of aminoglycoside resistant subpopulations. The studies cited provide support for a once-daily aminoglycoside dosing regimen, but there are still insufficient data on the effectiveness of such a regimen in human infections.

Toxicity

Although the toxicity of aminoglycosides is distinguished as nephrotoxicity on the one hand, and ototoxicity and vestibulotoxicity on the other, these two aspects are clearly interwoven, in so far as the risk for ototoxicity increases with impaired renal function. Insights into ototoxicity, and especially vestibulotoxicity, are still less developed than those into renal toxicity, mainly because the site of the toxic lesion is less accessible. Nephrotoxicity certainly limits the clinical utility of aminoglycosides (Lietman, 1985). Therefore, the identification of factors associated with a greater incidence of renal damage is critical. Potential risk factors can be classified as those related to the drug and its administration and those related to the clinical condition of the patient (Table I).

Aminoglycosides are highly polar drugs which cross biological membranes poorly or not at all, but they are filtered by the glomerulus. After entering the luminal fluid of the proximal renal tubule a small portion of the total filtered drug is reabsorbed and stored in the proximal tubular cells. After charge-mediated binding, the drug is taken up into the cell in small invaginations of the cell membrane, by a mechanism called 'carrier mediated pinocytosis' (Collier, Lietman & Mitch, 1979). Within one hour after injec-

tion, the drug is translocated into apical cytoplasmic vacuoles (Silverblatt & Kuehn, 1979). These endocytic vesicles fuse with lysosomes, sequestering the aminoglycosides inside those organelles in an unchanged form. Since pinocytosis is a continuing phenomenon aminoglycosides tend to accumulate extensively inside the lysosomes. The lysosomes have been identified as the first organelles to show alterations during aminoglycoside treatment (De Broe *et al.*, 1984). Once trapped in the lysosomes of proximal tubular cells aminoglycosides inhibit lysosomal phospholipases and sphingomyelinase (Laurent *et al.*, 1982; Giuliano *et al.*, 1984), resulting in lysosomal phospholipidosis, with a non-specific accumulation of polar phospholipids in myeloid bodies. Once a threshold in cortical drug concentration is reached, the lysosomal phospholipidosis progresses and the overloaded lysosomes continue to swell even in the absence of any further drug administration. This may result in the loss of integrity of the restricting membranes of lysosomes and release of large amounts of aminoglycosides, lysosomal enzymes and phospholipids into the cytosol. The extralysosomal aminoglycosides can gain access to other organelles, disturbing their functional integrity which may lead to cell death (Tulkens *et al.*, 1984).

The nephrotoxicity of aminoglycosides is determined by two major variables: (a) the intrinsic potential of the drug to damage subcellular structures and (b) the amount of drug accumulation in the renal cortex (Kaloyanides & Pastoriza-Munoz, 1980).

Any factor that increases the renal uptake of aminoglycosides is a risk factor for nephrotoxicity. The duration of exposure of the proximal tubular cells to aminoglycosides is a critical factor since it determines the extent of drug uptake. Persistent exposure undoubtedly results in increased renal drug levels. Dosage regimen is another important determinant of the extent of cortical aminoglycoside concentrations. Experimental nephrotoxicity caused by gentamicin is more severe when the total daily dose is divided or given by continuous infusion than when it is given as a single bolus (Frame *et al.*, 1977; Reiner, Bloxham & Thompson, 1978; Powell *et al.*, 1983). The reason for this may be found in the renal cortical uptake-storage kinetics of various aminoglycosides (Giuliano *et al.*, 1986). In the rat, steady-state elevations of serum gentamicin and netilmicin are associated with a nonlinear increase in renal cortical levels, strongly

Table I. Potential risk factors for aminoglycoside nephrotoxicity (De Broe, 1985)

Drug-related	Patient related
Dose duration dosage regimen	Age
Prior aminoglycoside treatment	Prior renal insufficiency Hepatic insufficiency
Choice of drug	'Critically ill' patient Sodium-volume depletion
Associated drugs diuretics cyclosporin cisplatin amphotericin	Other causes

suggesting saturable uptake while cortical uptake of tobramycin is linearly related to serum concentrations. These findings imply for gentamicin and netilmicin that the fraction of drug taken up by proximal tubule cells is higher when the same amount of drug is given by continuous infusion than by intermittent or single injections. Cortical uptake of tobramycin, in the rat, is independent of the dosage schedule, agreeing with the linear uptake kinetics of this aminoglycoside.

In humans, in a study in renal cancer patients who underwent nephrectomy, a critical effect of dosage schedule on renal uptake of gentamicin and netilmicin was found. In those patients a single injection resulted in 30% and 50% lower cortical drug concentrations of netilmicin and gentamicin, respectively, than the administration of the same amount of drug by a 24-h continuous infusion (Verpooten *et al.*, 1989). Although an impressive number of comparative studies of aminoglycoside nephrotoxicity in humans are available (Whelton, 1985), it is difficult to draw firm conclusions from these studies because of the multiple clinical variables and because most of the studies failed to achieve critical methodological standards. Very often detection of clinical nephrotoxicity was based on proving decreased glomerular filtration in the individual patient. This led to high threshold values, resulting in underestimation of minor damage. In comparative clinical studies, statistical analysis of parameters of glomerular filtration (e.g., serum creatinine) in different groups of patients would be much more sensitive in detecting minor differences between different aminoglycosides. In a short-term prospective, controlled, randomized, comparative study in humans using clinical dosage regimens, gentamicin, tobramycin and netilmicin could not be distinguished on the basis of either tissue accumulation or early drug-induced cellular effects (De Broe *et al.*, 1983). Advanced age has been suggested as a risk factor for aminoglycoside nephrotoxicity, as aging is accompanied by a decreased capacity in renal function and a marked decline of the regenerative response to drug-induced cell injury.

Pre-existing renal disease does not appear to be a risk factor for the induction of further renal impairment by aminoglycosides. Adjusting the dose or extending the interval between doses is not very difficult and can be accomplished reliably with the use of simple nomograms or with computer programs. Although monitoring of serum levels remains mandatory, gross deviations from desired plasma concentrations are rarely observed when dose adjustment procedures are being used. Since in most clinical trials this precaution has been taken, it may have contributed to the apparent lack of risk in renal failure patients. Moreover, it was shown experimentally that in rats with chronic renal failure less aminoglycoside is accumulated than in normal rats at comparable serum concentrations (Verpooten *et al.*, 1986; Pattyn *et al.*, 1988).

Finally, a prospective clinical study has shown that liver disease is associated with the development of nephrotoxicity. Hepatic insufficiency leading to intra-renal vasoconstriction, reduced renal blood flow and stimulation of the renin-angiotensin system is the proposed pathophysiological mechanism (Moore *et al.*, 1984).

The assessment of ototoxicity by conventional means, in clinical studies on aminoglycoside treated patients as well as in clinical practice, appears clearly inadequate. Careful clinical monitoring of hearing and vestibular functions is mandatory, but irreversible anatomical damage may have occurred before symptoms appear. Moreover, problems may arise for the first time days or even weeks after the treatment has ended. Conventional audiograms also lack sensitivity, since they omit the higher frequencies, the first to be affected by aminoglycosides (Tange *et al.*, 1982). Three new approaches have recently been introduced to improve early detection, but none is

perfect. Brain stem evoked response audiometry (BERA) is a non-invasive, objective and sensitive method by which minute cochlear lesions might be recognized at a reversible stage (Pechère & Bernard, 1982). As BERA requires no active participation from the patient, it can be used in neonates, infants and comatose individuals. Limitations of the method include relatively high costs for performing the test, the need for a reference recording in the individual patients before therapy and the multiple interferences of anoxia, neurological disorders, intubation, agitation of the patient, etc. Also, the actual significance of altered recordings at the anatomical and physiological level remains uncertain, so that interpretation is difficult, notably in neonates. Electrocochleography is another objective technique but the need to put an electrode through the ear drum limits its clinical application. High frequency tone audiometry is indeed very promising for patients who can actively respond to this subjective method (Dreschler *et al.*, 1985; Tange, Dreschler & Van der Hulst, 1985). Ototoxic alterations can be shown while the patient does not yet complain of hearing loss or tinnitus (Jacobson, Downs & Fletcher, 1969). However, again reference testing has to be done before therapy when the patient is often too ill for reliable responses to be obtained. The same holds true for the methods available for monitoring the vestibular function.

The pharmacokinetics of aminoglycosides in the inner ear have recently been clarified. It was shown that the uptake of gentamicin, netilmicin and amikacin into different inner ear tissues did not correlate with the degree and the anatomical specificity of toxicity (Dulon *et al.*, 1986), indicating a very specific cellular target (Aran *et al.*, 1988). It was also observed that ototoxicity and nephrotoxicity induced by gentamicin did not correlate (Dulon *et al.*, 1988*a*). Moreover, it was also demonstrated that cochlear damage induced by gentamicin was not associated with an accumulation of the drug into the inner ear (Tran Ba Huy, Bernard & Schacht, 1986). The uptake of gentamicin by these tissues, however, is dose-dependent and manifests rapid saturation kinetics, followed by a concentration plateau and a very slow release. As a consequence, the prolonged exposure of the hair cells to the aminoglycoside probably accounts for the damage. Comparisons of different aminoglycosides with regard to their respective ototoxicity still poses problems, notably since the differences in nephrotoxicity are often not taken into account in animal studies. However, it has been shown experimentally that toxicity towards the cochlear and the vestibular portions may vary between drugs (Aran *et al.*, 1982). Streptomycin is essentially vestibulotoxic, while amikacin is exclusively cochleotoxic. Gentamicin and tobramycin attack both targets, although the latter is somehwat less vestibulotoxic. Dibekacin has similar vestibulotoxicity to tobramycin, but exhibits little cochleotoxicity; netilmicin shows no cochleotoxicity and little vestibulotoxicity (Aran *et al.*, 1982). The reasons for these quantitative differences remain unclear, as the structure of the two receptor organs is very similar, and the differential toxicity is not correlated with differential uptake of drug. In-vitro studies dealing with separate hair cells (Brownell *et al.*, 1985; Zenner, Zimmermann & Schmitt, 1985; Dulon *et al.*, 1988*b*), as well as uptake studies at the cellular level using immunohistology and autoradiography, indicate indeed that the aminoglycosides interfere specifically with the hair cells (Hayashida *et al.*, 1985; Aran *et al.*, 1988).

Very few reports have addressed the issue of the ototoxicity with regard to dosage schedules. These studies are limited by ethical problems when conducted on patients, and by dosing problems when carried out in experimental animals. Dosages used in animals are multiples of those used in humans in terms of mg/kg, and even in terms of

mg/body area, and hence are difficult to extrapolate to the clinical situation. In addition, different dosage schedules may have different impacts on renal function, which, in turn can impair the inner ear through the accumulation kinetics. These considerations might explain apparently conflicting experimental results. In rats monitored by brain stem evoked potentials, two or three daily doses appeared more vestibulo- and cochleotoxic than the same total dose given once a day (Pechère & Bernard, 1984). Similar results were found in guinea pigs (Brummett & Fox, 1982). In contrast, Davis *et al.* (1984), found experimentally that the magnitude of kanamycin ototoxicity was related to the total daily dose alone and not to the dosing schedule. With gentamicin, however, the dosage regimen does affect the uptake of drug by the inner ear. Less antibiotic penetrates into the cochlear tissues and fluids after a single intramuscular injection than after equal doses administered as a continuous infusion (Tran Ba Huy, Bernard & Schacht, 1986). Thus, it may well be that wider dosing intervals produce less inner ear damage even though, in the latter study, the uptake of gentamicin was not directly correlated with toxicity. In patients with cystic fibrosis no differences with regard to ototoxicity were seen when the same daily dose of tobramycin was given for about ten days either by continuous infusion or by once daily dosing (Powell *et al.*, 1983).

In humans, ototoxicity can be only poorly predicted from serum concentrations. Some older studies suggested a correlation between peak or trough serum aminoglycoside concentrations and ototoxicity. In a retrospective study on tuberculous patients, it was found that high trough levels of streptomycin predisposed to ototoxicity but those patients who had elevated trough levels were also older and had impaired renal function (Line, Poole & Waterworth, 1970). Nordström *et al.* (1973) showed a significant correlation between high trough levels of gentamicin and ototoxicity, in a well conducted prospective study, but toxicity also correlated with high total dosage, longer duration of therapy and, once again, with abnormal renal function. Black *et al.* (1976), in monitoring for ototoxicity patients treated with amikacin, found that 57% of patients with a peak serum concentrations exceeding 32 mg/l, and 55% of patients with trough concentrations exceeding 10 mg/l, developed cochlear damage. However, the group with high frequency hearing loss received a larger mean total dose and were treated for a longer period than the patients without audiometric changes. It is sometimes suggested that the area under the time concentration curve, rather than peak or trough values might be a major risk factor for ear damage (Barza & Lauermann, 1978), but there are no experimental or clinical studies substantiating this hypothesis. On the other hand, a specific threshold for peak or trough concentrations of aminoglycosides, above which ototoxicity occurs, can also not be identified: severe damage can occur in individuals with 'normal' serum concentrations and sometimes in a very unexpected and brutal fashion. The experimental and clinical studies with less frequent dosing schedules, in keeping the same daily dose, as described above, tend to obscure the correlations between serum levels and ototoxicity found previously. However, increased serum half-life, peak concentrations, trough concentrations or area under the time concentration curve may reflect impaired renal function, which is a recognized risk factor of ototoxicity.

In conclusion, there are experimental and clinical data that suggest that variation of dosage regimens may broaden the therapeutic index of aminoglycosides. Decreased dosage frequency may decrease nephro- as well as oto- and vestibulotoxicity of aminoglycosides, although tobramycin might be an exception to the rule. On the other

hand experimental data suggest that efficacy is at least not decreased by increasing the dosage interval. Clinical data are still insufficient to draw strong conclusions, either about the equal efficacy of decreased dosage frequency, or about nephro- and ototoxicity. Part of the lack of clinical data on toxicity may be the result of inadequate use of available methods, including statistical sophistication, to make satisfactory use of the data. Future clinical trials should determine multiple pharmacokinetic parameters in patients (e.g., peak level, ratio of peak to MIC, AUC, AUC above MIC, and duration of inhibitory serum levels) so that multiple variate analysis can identify the major parameters determining efficacy. These studies should also look closely for resistant subpopulations by direct plating of clinical specimens on aminoglycoside-containing plates. They should also concentrate on the use of refined clinical methods to detect toxicity.

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Editor-in-Chief's note. This review will be complemented by a forthcoming leading article, which will discuss further clinical comparisons of dosage regimens, assess in terms of these the present evidence supporting less frequent dosage and define the economic benefit of reducing the dosage frequency.

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