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Antimicrobial practice

Monitoring serum concentrations for once-daily netilmicin dosing regimens

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A once-daily dosing regimen for aminoglycosides is less expensive, at least as effective and possibly less toxic than multiple-daily dosing regimens. Once-daily dosing might also allow the frequency of measuring the serum concentrations of these antibiotics to be reduced since two of the major objectives of monitoring, high peak and low trough concentrations, are more likely to be achieved with this regimen. A novel strategy for monitoring serum concentrations which relies on a single sample obtained 8 h after a dose, as opposed to both trough and peak samples, is evaluated here. Serum kinetics of netilmicin were studied prospectively in 51 adult patients with initial serum creatinine concentrations of < 130 μmol/L who were treated with a median daily dosage of 400 mg. Concentrations measured 8 h after administration were within the target range of 1·5–6 mg/L in 113 of 134 dosing intervals studied. Concentrations above and below this range correlated significantly with higher and lower 24-h trough concentrations and areas under the curve respectively. There was also a significant correlation between 8-h netilmicin concentrations and nephrotoxicity ($P < 0·05$); a relative increase of $\geqslant 25\%$ in the serum creatinine concentration or an absolute increase of > 25 μmol/L was detected in 0 of 7 patients with an 8-h concentration of $< 1·5$ mg/L, in 3 of 33 patients (9·1%) with an 8-h concentration of 1·5–6 mg/L and in 4 of 11 patients (36%) with an 8-h concentration of > 6 mg/L. The results of this study suggest that adequate information about serum netilmicin concentrations in patients receiving a once-daily dose may be derived from a sample obtained 8 h after administration.

Introduction

For many years the total daily dosage of an aminoglycoside has been administered in two or three divided doses. More recently, however, increasing numbers of experimental and clinical studies have demonstrated that a once-daily dosing regimen might be at least as effective and possibly less toxic (Bennett *et al.*, 1979; Powell *et al.*, 1983; Craig, Redington & Ebert, 1991; Gilbert, 1991). Such a significant modification of the dosing regimen might also necessitate changes in current thinking regarding the monitoring of the serum concentrations of these agents. Measuring both the peak and trough concentrations has been advocated by both pharmacologists and clinicians in order to ensure that therapeutic dosages are administered (Zaske *et al.*, 1982; Wenk, Vozeh & Follath, 1984). It is essential to achieve high peak concentrations since the bactericidal activities of the aminoglycosides are concentration-dependent and the ratio

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of the peak concentration to the MIC correlates closely with therapeutic outcome (Blaser *et al.*, 1987; Moore, Lietman & Smith, 1987). Monitoring trough concentrations is also undertaken to avoid drug accumulation which is usually the result of reduced renal elimination; elevated trough concentrations have been associated with aminoglycoside toxicity (Wenk *et al.*, 1984).

To date, there have been no studies which have considered the issue of monitoring serum concentrations specifically in relation to once-daily dosing regimens. In two recent publications, it was proposed that aminoglycoside dosages in patients receiving single daily doses should be reduced when the 24-h trough concentrations exceeded 2 mg/L (Parker & Davey, 1993; Prins *et al.*, 1993). However, while a threshold concentration of this magnitude has been used routinely for multidose regimens, there is little evidence to suggest that it is equally appropriate for once-daily regimens. Indeed, such an approach would result in very high areas under the concentration *vs* time curve (AUC) in patients with impaired renal function.

Monitoring both peak and trough serum concentrations is expensive and labour-intensive. In addition, it is often difficult in clinical practice to obtain samples which have been timed accurately, a factor which is particularly critical in relation to the peak concentration (Blaser *et al.*, 1985). Once-daily dosing of aminoglycosides may allow the frequency of monitoring to be reduced. Administration of the total daily dosage in one short infusion leads to peak concentrations which are at least two-fold greater than those obtained when multiple-daily doses are administered i.e. the goal of a high peak concentration to MIC ratio is more likely to be achieved; increasing the dosing interval to 24 h also reduces the likelihood of drug accumulation. None the less, the need for monitoring is not totally removed and remains necessary in order to detect significant drug accumulation in patients with impaired renal function. Moreover, the peak concentrations and AUCs might be significantly different in heterogeneous groups of patients receiving standard aminoglycoside dosages because of larger inter-individual variations in the distribution and elimination of these agents (Zaske *et al.*, 1982; Wenk *et al.*, 1984). Savings in both manpower and laboratory costs would be realized if serum monitoring required the collection of only a single serum sample during a dosing interval. We have assessed the feasibility of such a strategy during the course of a prospective clinical trial with netilmicin.

Patients and methods

Netilmicin serum concentrations were studied in 51 adult patients with intial serum creatinine concentrations of < 130 μmol/L. Serum creatinine concentrations were measured before starting therapy, on alternate days during the course of therapy and following completion of therapy; the concentrations determined before and after treatment were used to calculate changes during the intervening period. A clinically significant increase in the creatinine concentration was suggestive of nephrotoxicity which was defined in both relative (an increase of $> 25\%$) and absolute (an increase of > 25 μmol/L) terms. Blood samples for the determination of netilmicin concentrations were obtained on three occasions during a 24-h dosing interval — immediately after a 60-min infusion and 8 and 24 h after starting the infusion. For each patient, this was undertaken on the day after therapy was initiated and every second or third day thereafter until treatment was terminated. Patients initially received dosages of 6 mg/kg

body weight in a single daily dose; these were subsequently adjusted, if necessary, in order to achieve 8-h serum concentrations of between 1·5 and 6 mg/L.

Netilmicin serum concentrations were determined by a fluorescence polarization assay (TDx, Abbott Laboratories). The coefficient of variation of the assay was < 10% for concentrations of > 1 mg/L (Joos, Lüthy & Blaser, 1989) and the results (means ± S.D.) for spiked specimens containing 0·5, 0·25 and 0 mg/L were 0·52 ± 0·03, 0·31 ± 0·04 and 0·07 ± 0·06 mg/L, respectively. Measurements of < 0·3 mg/L were taken as 0·2 mg/L for the purpose of calculating the AUC and the mean trough concentrations. The AUC was calculated according to the trapezoidal rule.

All statistical calculations were performed with the computer program StatView II (Abacus Concepts Inc., Berkeley, CA, USA). The significance of correlations between 8-h concentrations of < 1·5 mg/L and low 24-h trough concentrations (< 0·5 mg/L) and between 8-h concentrations of > 6 mg/L and high 24-h trough concentrations (≥ 0·5 mg/L) was calculated by the two-tailed Chi-square test. The statistical significance of differences between AUCs was analyzed by the two-tailed Kruskal-Wallis test and the significance of an increased incidence of nephrotoxicity in relation to 8-h concentrations of > 6 mg/L was analyzed by the Chi-square test with continuity correction. For a statistical analysis of trough and peak concentrations and AUCs, box-plots were constructed. Diagnostic sensitivity was calculated by dividing the number of patients in whom nephrotoxicity was diagnosed correctly according to the criteria used by the total number of patients who actually developed nephrotoxicity, Diagnostic specificity was calculated by dividing the number of patients without nephrotoxicity according to the diagnostic criteria by the total number of patients who did not develop nephrotoxicity.

Results

The daily dosages administered to patients ranged from 150–600 mg with a median of 400 mg. A total of 134 intervals between doses were monitored. Peak and trough concentrations were categorized in relation to the 8-h determinations. In the box-plots (Figures 1–3), the boxes define the boundaries within which the 25th, 50th and 75th percentiles fall while the 10th and 90th percentiles are represented by the lower and upper stems respectively; the notches represent the 95% confidence bands about the median. The box-plots in Figures 1 and 2 show the frequency distributions of the netilmicin trough and peak concentrations respectively. The 8-h concentrations were < 1·5 mg/L in 9 of the 134 (6·7%) intervals and > 6 mg/L in 12 of the 134 (9%) intervals (Table I). There was no correlation between peak and 8-h concentrations. However, there was a statistically significant correlation between 8-h concentrations of < 1·5 mg/L and low trough concentrations, all 9 of which were < 0·5 mg/L ($P < 0·05$), and between 8-h concentrations of > 6 mg/L and high trough concentrations, 11 of 12 of which were ≥ 0·5 mg/L ($P < 0·001$). The three categories of 8-h concentrations (< 1·5 mg/L, 1·5–6·0 mg/L and > 6·0 mg/L) also differed significantly in terms of the AUC ($P < 0·001$) (Figure 3 and Table I).

The netilmicin dosages administered to seven patients were increased from a mean of 360 mg to a mean of 500 mg when the initial 8-h concentrations were found to be < 1·5 mg/L. In five of these seven patients, the effects of the increases were evaluated at the next dosing interval when it was observed that the mean peak serum concentration and mean AUC had increased from 16 to 27 mg/L and from 80 to 139 mg.h/L respectively.

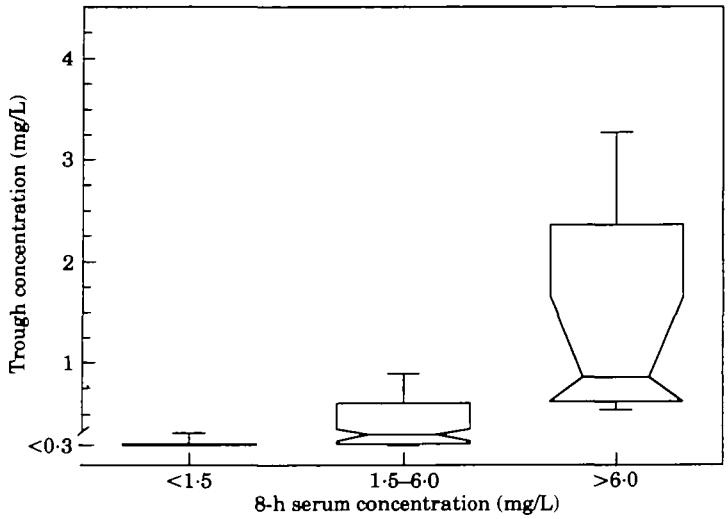

Figure 1. Box-plots displaying 24-h netilmicin trough concentrations for seven patients with 8-h serum concentrations of < 1·5 mg/L, 33 patients with 8-h concentrations of 1·5–6 mg/L and 11 patients with 8-h concentrations of > 6 mg/L.

For the purpose of assessing nephrotoxicity, patients were categorized as follows: those with at least one 8-h concentration which was < 1·5 mg/L; those for whom all 8-h determinations fell between 1·5 and 6 mg/L; and those with at least one 8-h concentration of > 6 mg/L (Table II). The incidences of nephrotoxicity in the three groups were 0%, 9·1% and 36% respectively, regardless of whether the relative or absolute endpoint was used. The incidence of nephrotoxicity in patients in the third category (those with at least one 8-h concentration of > 6 mg/L) was significantly higher than the incidences in the other two categories ($P < 0·05$).

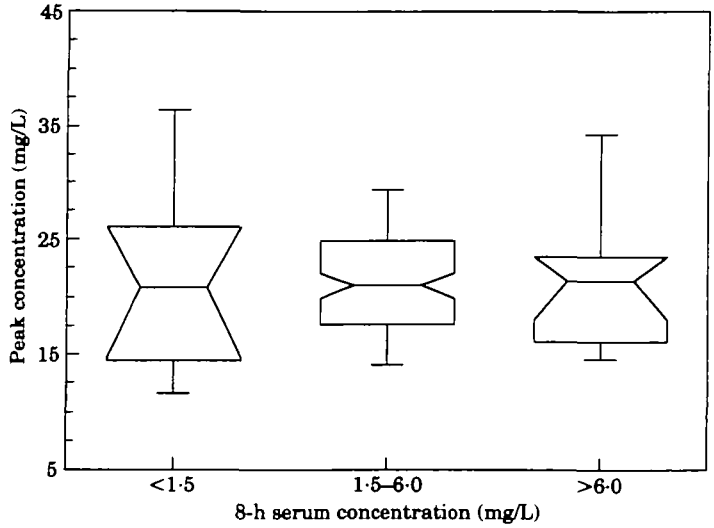

Figure 2. Box-plots displaying netilmicin peak concentrations for seven patients with 8-h serum concentrations of < 1·5 mg/L, 33 patients with 8-h concentrations of 1·5–6 mg/L and 11 patients with 8-h concentrations of > 6 mg/L.

Table I. Peak and trough netilmicin serum concentrations and AUCs in relation to 8-h serum concentrations

Variable	8-h netilmicin serum concentration (mg/L) < 1·5	1·5–6·0	> 6·0
No. (%) of monitored dosage intervals	9 (6·7)	113 (84·3)	12 (9·0)
Mean (±S.D.) dosages (mg/day)	391 (±55)	388 (±96)	357 (±94)
Mean (±S.D.) peak concentrations (mg/L)	21·5 (±8·8)	21·3 (±6·3)	21·7 (±6·6)
Mean (±S.D.) trough concentrations (mg/L)	0·2 (±0·1)[a]	0·4 (±0·3)	1·5 (±1·3)[b]
Mean (±S.D.) AUC (h.mg/L)[c]	99 (±34)	127 (±30)	184 (±40)

[a]Significantly lower ($P < 0·05$) than the other two categories.
[b]Significantly higher ($P < 0·001$) than the other two categories.
[c]$P < 0·001$ for differences between categories.

The sensitivities and specificities for the various criteria which were considered as potential diagnostic markers of nephrotoxicity were 0·57 and 0·84 respectively for 8-h netilmicin concentrations of > 6 mg/L, 0·71 and 0·64 respectively for 24-h trough concentrations of > 0·5 mg/L, 0·43 and 0·91 for 24-h trough concentrations of > 1 mg/L, 0·43 and 0·98 respectively for 24-h trough concentrations of > 2 mg/L and 0·29 and 0·43 for peak concentrations of > 24 mg/L.

Discussion

It is presently standard practice to monitor the serum concentrations of patients receiving aminoglycosides both before (trough) and after (peak) a dose. In this study,

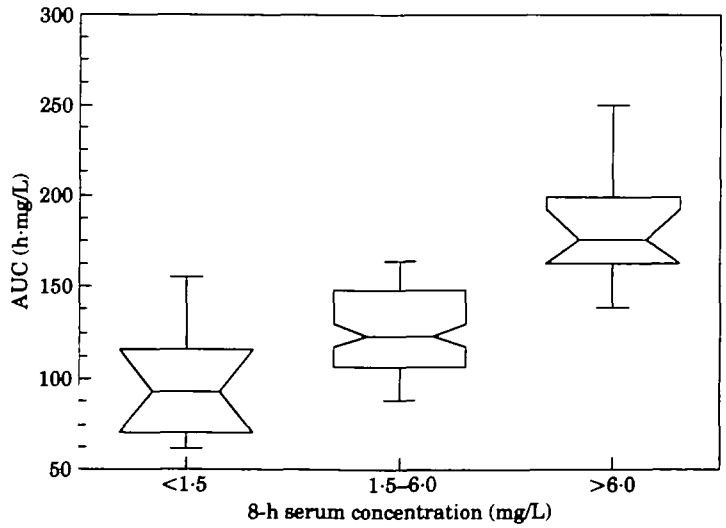

Figure 3. Box-plots displaying areas under the concentration *vs* time curve (AUC) for netilmicin in seven patients with 8-h serum concentrations of < 1·5 mg/L, 33 patients with 8-h concentrations of 1·5–6 mg/L and 11 patients with 8-h concentrations of > 6 mg/L.

Table II. Increase in serum creatinine concentrations during therapy in relation to 8-h netilmicin serum concentrations

	8-h netilmicin serum concentration (mg/L)		
	< 1·5	1·5–6·0	> 6·0
No. of patients	7	33	11
Mean (±S.D.) pre-treatment serum creatinine concentration (μmol/L)	84·4 (±17·3)	93 (±18·5)	102 (±17·8)
Mean (±S.D.) post-treatment serum creatinine concentration (μmol/L)	90·4 (±21·3)	90·5 (±20·4)	142 (±98)
No. (%) of patients whose serum creatinine concentrations increased by > 25 μmol/L	0 (0)	3 (9·1)	4 (36·4)*
No. (%) of patients whose serum creatinine concentrations increased by > 25%	0 (0)	3 (9·1)	4 (36·4)*

* $P < 0·05$ compared with the other cateories.

we have assessed the suitability of measuring the serum concentration in a single sample obtained 8 h after the start of a 1-h infusion in patients with an initial serum creatinine concentration of < 130 μmol/L who were receiving a once-daily dose of netilmicin. The target concentrations for this sample were defined as 1·5–6 mg/L; this range is three-fold higher than that which is currently considered acceptable at our institution for a trough concentration in patients receiving a thrice-daily dosing regimen. The 8-h concentrations determined in the present study were within the target range for the majority (84%) of patients. Concentrations which were above and below this target were predictive of high and low trough concentrations and AUCs respectively. An 8-h concentration which exceeded 6 mg/L was associated with a 4·8-fold increase in the risk of nephrotoxicity; the sensitivity of this criterion was greater than those for 24-h trough concentrations of > 1 or > 2 mg/L and for peak concentrations of > 24 mg/L and the specificity was greater than those for 24-h trough concentrations of > 0·5 mg/L and for peak concentrations of > 24 mg/L.

The results reported here suggest that the proposed target range is sufficiently broad to obviate the need for frequent changes in dosages but is also sufficiently narrow to enable dosing regimens which provide either very low or very high serum concentrations to be identified. However, we are not suggesting that achieving uniform serum kinetics should be the ultimate goal of monitoring treatment with aminoglycosides. Indeed, it might be of greater benefit to some patients to be assigned aminoglycoside regimens which give rise to serum concentrations which are higher or lower than those which are usually obtained with standard dosing regimens; for example, higher serum concentrations might be therapeutically advantageous in patients with infections which are associated with particularly poor prognoses, such as those caused by *Pseudomonas aeruginosa*, despite the increased risk of toxicity. Investigations which have demonstrated the importance of a high peak to MIC ratio for the aminoglycosides (Blaser *et al.* 1987; Moore *et al.*, 1987) and experimental studies which suggest that antibiotic concentrations should exceed the MIC, except for the duration of the post-antibiotic effect (Vogelman *et al.*, 1988), support the recommendation that, when devising

aminoglycoside dosing guidelines, account must also be taken of the susceptibility of the pathogen.

Currently-available commercial assays are designed to yield reliable results which fall within the range of the peak and trough concentrations encountered in most patients receiving multiple daily doses of the aminoglycosides. Peak serum samples obtained from patients receiving once-daily doses must be first diluted in order to ensure that concentrations fall within this range, a procedure which affects the accuracy of the assay. The accurate determination of trough concentrations requires assay techniques with very low limits of sensitivity; most existing assays are not sufficiently precise to give reliable results for the low concentrations which are usually present in 24-h trough samples. Serum concentrations measured 8 h after the onset of short infusions, on the other hand, fall within the ranges of reproducibility and accuracy of most commercial assays. As this probably applies equally to specimens taken either a few hours earlier or later, the choice of 8 h may, therefore, seem arbitrary. However, if samples are obtained during the second half of the 24-h dosing interval, it is essential to ensure that the assay result is available in sufficient time to allow the dosage of the subsequent dose to be adjusted if necessary. Such samples may provide only limited information since half-lives increase towards the end of a 24-h interval because of redistribution from deep compartments (γ-phase). Consequently, a two-compartment model will not reliably fit the serum kinetics for all patients over the entire 24-h period (Wenk *et al.*, 1984). It may be difficult, therefore, to adjust dosages which are based on concentrations determined during the γ-phase. In addition, serum concentrations in samples which were obtained during the second half of the 24-h interval and which fall below the limit of sensitivity of the assay do not allow patients with normal rates of elimination to be reliably distinguished from those with very rapid elimination rates, such as children and patients with burns. Viscoli *et al.* (1991) studied serum concentrations in children receiving single daily doses of amikacin (20 mg/kg) and recorded 6-h concentrations of < 3 mg/L in 13 of 16 cases, despite mean peak concentations of 73 mg/L. The detection of 8-h concentrations which are low makes it possible to increase the dosages in patients with correspondingly low AUCs; in the present study, this facilitated dosage increases in seven of the 51 patients. Experimental data suggest that high peak concentrations and AUCs are associated with superior response rates (Blaser *et al.*, 1987; Moore *et al.*, 1987; Vogelman *et al.*, 1988; Craig *et al.*, 1991).

Virtually every aspect of the aminoglycosides has been extensively investigated to date. More than 36,000 papers have been indexed under 'aminoglycosides' or 'antibiotics-aminoglycosides' since 1966 in MEDLINE, the bibliographical database of the National Library of Medicine, Washington, USA. However, whether traditional, multiple-daily dosing regimens lead to suboptimal therapy compared with a once-daily regimen is an issue which remains to be resolved. Even greater controversy surrounds the monitoring of serum concentrations.

A recent re-evaluation of the therapeutic range for the aminoglycosides was critical of the scientific basis for current recommendations (McCormack & Jewesson, 1992). It is unlikely, however, that, within the next few years, sufficient experimental and clinical data will have been accumulated to resolve the questions of optimal timing and concentration ranges in relation to the monitoring of serum concentrations in patients receiving once-daily doses of aminoglycosides, irrespective of whether their renal function is normal or not. The present study has successfully demonstrated that there is a significant association between nephrotoxicity and 8-h serum concentrations, the criterion proposed here for monitoring treatment with the aminoglycosides. However,

we were unable to confirm that this parameter can also be used to ensure that serum concentrations which optimize the therapeutic efficacy of the aminoglycosides are more likely to be achieved: indeed, obtaining sufficient evidence for this in prospective, ethically non-controversial clinical trials will be a considerable challenge.

In conclusion, the administration of the total daily dosage of netilmicin in one short infusion yielded high peak concentrations and concomitant increases in the ratio of the peak concentration to the MIC of the pathogen. Furthermore, increasing the dosing interval to 24 h minimized the potential for drug accumulation. The determination of 8-h concentrations in patients receiving once-daily dosing regimens has been shown to be useful in identifying patients with either low AUCs or increased risks of nephrotoxicity.

References

Bennett, W. M., Plamp, C. E., Gilbert, D. N., Parker, R. A. & Porter, G. (1979). The influence of dosage regimen on experimental gentamicin nephrotoxicity: dissociation of peak serum levels from renal failure. *Journal of Infectious Diseases* **140,** 576–80.

Blaser, J., Simmen, H. P., Gonzenbach, H. R., Sonnabend, W. & Lüthy, R. (1985) Aminoglycoside monitoring: timing of 'peak' levels is critical. *Therapeutic Drug Monitoring* **7,** 303–7.

Blaser, J., Stone, B. B., Groner, M. C. & Zinner, S. H. (1987). Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. *Antimicrobial Agents and Chemotherapy* **31,** 1054–60.

Craig, W. A., Redington, J. & Ebert, S. C. (1991). Pharmacodynamics of amikacin *in vitro* and in mouse thigh and lung infections. *Journal of Antimicrobial Chemotherapy* **27,** *Suppl. C,* 29–40.

Gilbert, D. N. (1991). Once-daily aminoglycoside therapy. *Antimicrobial Agents and Chemotherapy* **35,** 399–405.

Joos, B., Lüthy, R. & Blaser, J. (1989). Long term accuracy of fluorescence polarization immunoassays for gentamicin, tobramycin, netilmicin and vancomycin. *Journal of Antimicrobial Chemotherapy* **24,** 797–803.

McCormack, J. P. & Jewesson, P. J. (1992). A critical reevaluation of the "therapeutic range" of aminoglycosides. *Clinical Infectious Diseases* **14,** 320–39.

Moore, R. D., Lietman, P. S. & Smith, C. R. (1987). Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. *Journal of Infectious Diseases* **155,** 93–9.

Parker, S. E. & Davey, P. G. (1993). Practicalities of once-daily aminoglycoside dosing. *Journal of Antimicrobial Chemotherapy* **31,** 4–8.

Powell, S. H., Thompson, W. L., Luthe, M. A., Stern, R. C., Grossniklaus, D. A., Bloxham, D. D. *et al.* (1983). Once-daily vs. continuous aminoglycoside dosing: efficacy and toxicity in animal and clinical studies of gentamicin, netilmicin, and tobramycin. *Journal of Infectious Diseases* **147,** 918–32.

Prins, J. M., Büller, H. R., Kuijper, E. J., Tange, R. A. & Speelman, P. (1993). Once versus thrice daily gentamicin in patients with serious infections. *Lancet* **341,** 335–9.

Viscoli, C., Dudley, M., Ferrea, G., Boni, L., Castagnola, E., Barretta, M. A. *et al.* (1991). Serum concentrations and safety of single daily dosing of amikacin in children undergoing bone marrow transplantation. *Journal of Antimicrobial Chemotherapy* **27,** *Suppl. C.,* 113–20.

Vogelman, B., Gudmundsson, S., Leggett, J., Turnidge, J., Ebert, S. & Craig, W. A. (1988). Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. *Journal of Infectious Diseases* **158,** 831–47.

Wenk, M., Vozeh, S. & Follath, F. (1984). Serum level monitoring of antibacterial drugs. A review. *Clinical Pharmacokinetics* **9,** 475–92.

Zaske, D. E., Cipolle, R. J., Rotschafer, J. C., Solem, L. D., Mosier, N. R. & Strate, R. G. (1982). Gentamicin pharmacokinetics in 1640 patients: method for control of serum concentration. *Antimicrobial Agents and Chemotherapy* **21,** 407–11.

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