# Kill Kinetics and Regrowth Patterns of *Escherichia coli* Exposed to Gentamicin Concentration-Time Profiles Simulating In Vivo Bolus and Infusion Dosing

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The relative influence of peak concentration ($C_{max}$) versus the area under the antibiotic concentration-time curve (AUC) on the bactericidal effect of gentamicin against *Escherichia coli* NCTC 10418 was studied. Bacteria in the lag phase were exposed to an in vitro gentamicin concentration series which mirrored the concentrations determined in patients after 80-mg intravenous bolus (1 min) and 80-mg intravenous infusion (30 min) doses. Bacterial viable cell counts and gentamicin concentrations were measured before and during antibiotic exposure. Both the $C_{max}$ and AUC were shown to be factors determining antibacterial activity; however, the $C_{max}$ was an independent determinant of effect. These findings indicate that bolus intravenous dosing with gentamicin could maximize bactericidal activity. Increased efficacy could result at any given daily antibiotic dose if delivered via bolus with long intervals (12 to 24 h) between doses if appropriate precautions to avoid toxicity are taken.

Antibiotic therapeutic effect depends on administration schedules (6, 7, 20) and the time course of antibiotic concentration in serum, including the area under the antibiotic concentration in plasma-time curve (AUC), the magnitude of the AUC above the MIC (AUC > MIC), the total time that the antibiotic concentration exceeds the MIC, and the maximum antibiotic concentration attained during a dosing interval ($C_{max}$) (2, 7–10, 13, 14, 22).

The issue of $C_{max}$ as opposed to AUC as a major determinant of antibiotic activity is of critical importance for dosage design; however, this issue has not yet been definitely resolved (16, 22). We report the results of studies designed to test the independent effects of $C_{max}$ and AUC of gentamicin on bactericidal effect.

Preliminary data were presented to the Australian Society of Clinical and Experimental Pharmacologists and Toxicologists, December 1991.

Pharmacokinetic studies involved 20 patients on chronic 8-h dosing regimens with gentamicin at 80 mg; the regimen consisted of a standard 30-min infusion or a bolus over 1 min. Blood samples were drawn predose and at 1, 20, and 30 min postdose. A 10-min postdose in vivo datum point was estimated from a first-order fit of the 0- to 30-min in vivo data.

*Escherichia coli* NCTC 10418 was used in in vitro bactericidal experiments. Both the MIC and the MBC of gentamicin for this organism are 0.5 mg/liter. This organism was cultured under standard conditions in the presence and absence of gentamicin (Delta West, Bentleigh, Australia) as detailed below. An overnight culture of *E. coli* in brain heart infusion broth (BHIB) (Oxoid, Basingstoke, England) was diluted to $10^7$ CFU/ml in 0.1% peptone water (Difco Laboratories, Detroit, Mich.). A 1-ml sample of the $10^7$-CFU/ml

culture was added to the experimental culture broth, resulting in an initial density of $10^6$ CFU/ml.

In vitro concentration-time modelling of clinical concentrations of gentamicin assumed linear extrapolation of in vivo data (Fig. 1). Bolus and infusion profiles based on these findings at two exposure levels are illustrated in Fig. 2 and detailed in Table 1. The lowest-dose bolus profile included a $C_{max}$ of 14.2 mg/liter, a postdistributional peak concentration of 4 mg/liter at 30 min, and a half-life of decline of 2 h. The infusion profile had a $C_{max}$ of 6.5 mg/liter, a postdistributional peak concentration of 4 mg/liter at 30 min, and half-life of decline of 2 h. Other profiles used were constructed by linear extrapolation of these profiles and involved bolus and infusion modelling with postdistributional peaks of 6 and 8 mg/liter (Table 1). Infusion regimens involved increments in gentamicin concentration at intervals of 7.5 min by the addition of BHIB from a prewarmed (37°C) stock solution containing gentamicin at a concentration of 400% of the target peak. During the first 30 min following peak exposure, dilutional volumes of prewarmed BHIB at 37°C were added at 10-min intervals, reducing the peak concentration by a cumulative multiple of 0.63 to yield a postdistributional peak concentration at 30 min (Fig. 2). Dilutional volumes were added at 12-min intervals for 2 h, reducing the postdistributional peak by a cumulative multiple of 0.5. Subsequently, dilutional volumes were added hourly for 10 h, mirroring a half-life of 2 h, and then the gentamicin concentration was halved at 24.5 h. Bolus profile modelling differed only in an initial increment in gentamicin concentration by the addition of BHIB containing gentamicin at a concentration 1,000% of the target peak (Fig. 2), and dilutional volumes were added at 10-min intervals so that the peak concentration was reduced by a cumulative multiple of 0.29 to yield the postdistributional peak concentration at 30 min. Subsequent dilution protocols were as for the infusion profile.

Samples for the determination of gentamicin concentra-
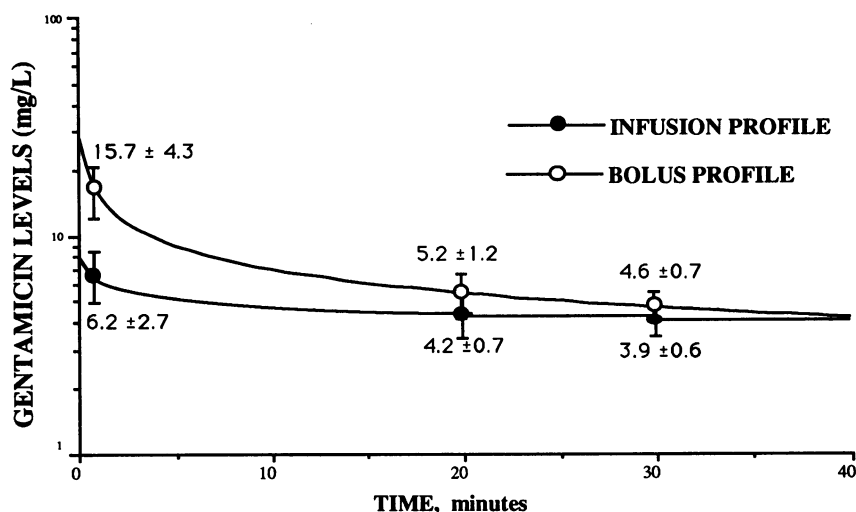
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FIG. 1. Comparison of aminoglycoside concentrations in sera of patients after an 80-mg intravenous bolus (1 min) and an 80-mg intravenous infusion (30 min) dose ($n = 20$).

tions and viable cell counts were taken at $-0.5$, $-0.25$, $0$, $0.17$, $0.33$, $0.5$, $2.5$, $4.5$, $6.5$, $8.5$, $10.5$, $24.5$, and $48.5$ h postdose for infusion regimen studies and at $0$, $0.17$, $0.33$, $0.5$, $2.5$, $4.5$, $6.5$, $8.5$, $10.5$, $24.5$, and $48.5$ h postdose for bolus regimen studies. Counts were determined from colony formation on the surface of blood agar plates (Oxoid), adjusted in all instances for net changes in incubation broth volume resulting from the addition of broth containing antibiotic, the addition of dilutional broth volumes, and the removal of broth for subculture and antibiotic assay. All results were compared with those for control cultures not exposed to gentamicin. All in vitro experiments involved five replicate studies.

Gentamicin assay concentrations were determined by a homogenous enzyme immunoassay technique (EMIT) (Syva, Palo Alto, Calif.). The threshold for detection is $0.6$ mg/liter because of limitations in machine process settings. The assay is linear over a range of $0.6$ to $10$ mg/liter ($r^2 = 0.99$). The interrun coefficient of variation is $3.4$ to $14.6\%$ over a range of $0.6$ to $10$ mg/liter ($n = 6$), and the intrarun coefficients of variation are $10.8\%$ at $0.6$ mg/liter, $3.3\%$ at $2$ mg/liter, $3.3\%$ at $6$ mg/liter, and $10.4\%$ at $10$ mg/liter ($n = 10$).

The AUC from $0$ to $48.5$ h was calculated by using the trapezoidal rule (11). The Student $t$ test was used for statistical analysis, with a significance level of $0.05$.

Serum profiles determined for patients are illustrated in Fig. 1. Differences between postbolus and postinfusion profiles were confined to postdose peak and the 30-min postdose period. The predose trough (bolus) = $0.8 \pm 0.4$ mg/liter, and the predose trough (infusion) = $0.9 \pm 0.3$ mg/liter (mean $\pm$ standard deviation [SD]; $n = 20$; $P = 0.24$). The postbolus (1-min) peak = $15.8 \pm 4.3$ mg/liter, and the postinfusion (1-min) peak = $6.2 \pm 2.7$ mg/liter (mean $\pm$ SD; $n = 20$; $P < 0.001$). The 20-min-postbolus level = $5.2 \pm 1.2$ mg/liter, and 20-min-postinfusion level = $4.2 \pm 0.7$ mg/liter (mean $\pm$ SD; $n = 20$; $P < 0.001$). The postbolus (30-min) peak = $4.6 \pm 0.7$ mg/liter, and the postinfusion (30-min) peak = $3.9 \pm 0.6$ mg/liter (mean $\pm$ SD; $n = 20$; $P < 0.001$). The calculated 10-min gentamicin concentration values were (postbolus) $10.5 \pm 2.6$ mg/liter and (postinfusion) $5.2 \pm 1.6$ mg/liter.

Bacterial growth in the absence of exposure to aminoglycoside showed a plateau at or near $10^9$ CFU/ml, whereas exposure to gentamicin caused early bactericidal effects apparent by 2 h, followed by a bacteriostatic phase measured to $\leq 11$ h and a recovery phase measured at $24.5$ and $48.5$ h [Fig. 2a(ii) and b(ii)]. Early bactericidal effect increased with the $C_{max}$ and AUC with both exposure levels [Fig. 2a(i) and b(i)]. Similarly, the duration of the bacteriostatic phase increased with the $C_{max}$ and AUC (Table 1).

There were significant quantitative between-schedule differences in the culture profiles at all three exposure levels (Table 1). The immediate (2.5-h) bacterial counts differed significantly between regimens with 4 mg/liter (postinfusion = $2.36 \pm 0.98$ $\log_{10}$ CFU/ml, postbolus = $0$ CFU/ml; $n = 5$; $P < 0.005$), as did the 10.5-h E. coli counts (postinfusion = $3.29 \pm 0.19$ $\log_{10}$ CFU/ml, postbolus = $0$ CFU/ml; $n = 5$; $P < 0.005$). The 24.5-h culture counts differed at all three dose levels: postinfusion with 4 mg/liter = $8.8 \pm 0.31$ $\log_{10}$ CFU/ml, postbolus with 4 mg/liter = $4.28 \pm 0.83$ $\log_{10}$ CFU/ml ($n = 5$; $P < 0.005$); postinfusion with 6 mg/liter = $7.87 \pm 0.29$ $\log_{10}$ CFU/ml, postbolus with 6 mg/liter = $3.57 \pm 1.12$ $\log_{10}$ CFU/ml ($n = 5$; $P < 0.005$); postinfusion with 8 mg/liter = $3.50 \pm 1.35$ $\log_{10}$ CFU/ml, postbolus with 8 mg/liter = $0$ CFU/ml ($n = 5$; $P < 0.005$). The 48.5-h growth differed significantly only with 8 mg/liter (postinfusion = $8.97 \pm 0.43$ $\log_{10}$ CFU/ml, postbolus = $0$ CFU/ml) (mean $\pm$ SD; $n = 5$; $P < 0.005$), reflecting persistent bactericidal effects associated with the bolus profile only at 8 mg/liter.

In the studies reported here, we have been able to demonstrate an independent effect of the $C_{max}$ which is additive to the AUC effect. We confirmed previous reports of a biphasic concentration-time plasma profile for gentamicin (1, 4, 5, 17, 19), specifically noting the significant difference in peak and minimal difference in the AUC between bolus and infusion regimens (19). We modelled in vitro exposures on these principles and noted extreme differences in outcomes between bolus and infusion profiles despite small ($\leq 5\%$) differences in the AUC. Following exposure to a bolus model incorporating a 30-min postdose maximum of 8 $\mu$g/ml [Fig. 2b(i)], there was evidence of complete bacterial kill in contrast to regrowth with the infusion profile. These results are consistent with the observations of other workers (3).

Resolution of the question of dependence of the antibacterial effect of antibiotics on the $C_{max}$ or AUC is fundamental to the design of dosing regimens. Specifically, significant
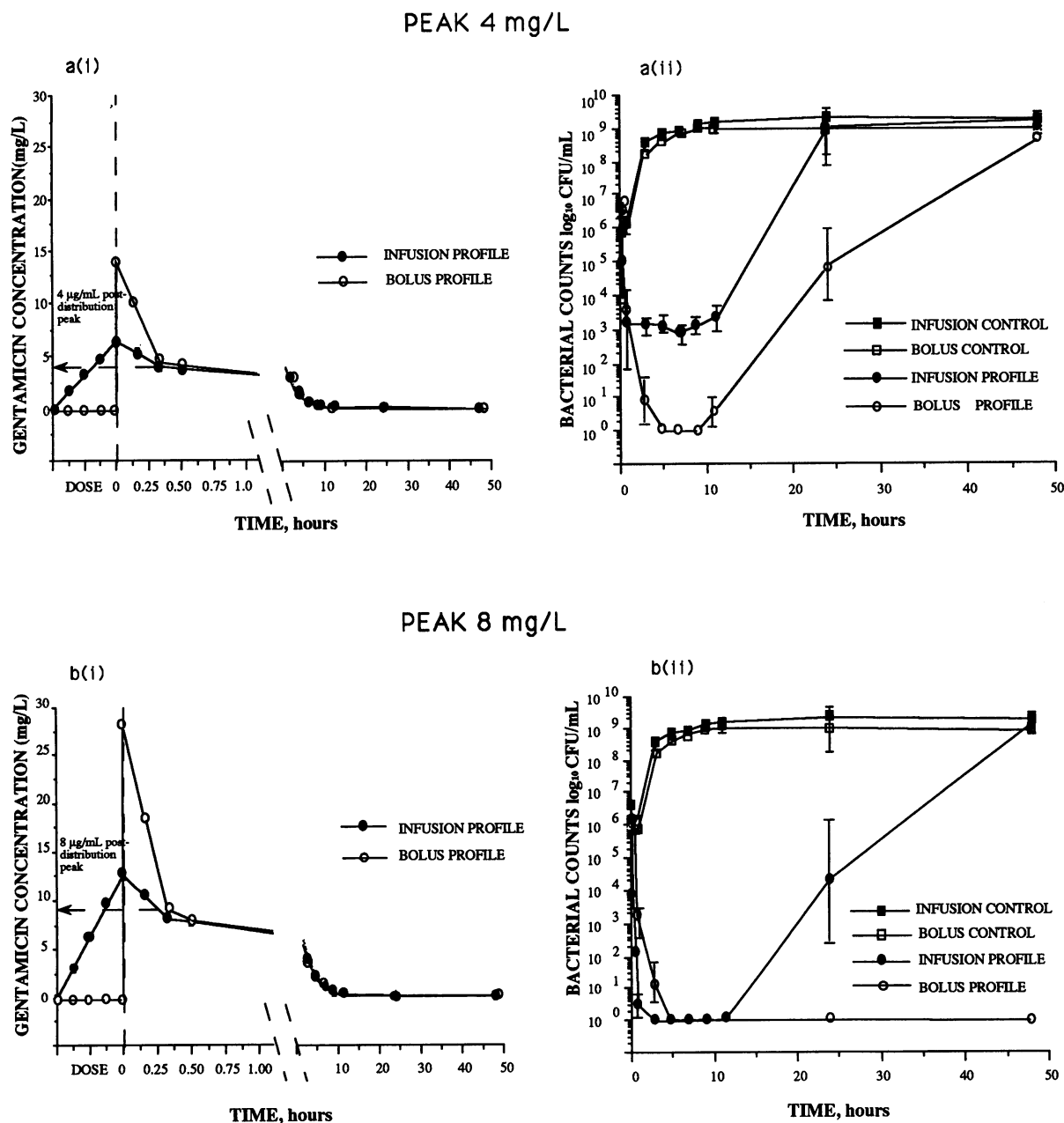
FIG. 2. Gentamicin concentration-time profiles (i) and time course of bacterial counts (ii) in studies with *E. coli* NCTC 10418 exposed to regimens mirroring those in patient profiles associated with postdose 30-min peak concentrations of 4 mg/liter (a) and 8 mg/liter (b) ($n = 5$). Negative standard error bars are not shown to scale.

dependence of effect on $C_{max}$ leads logically to bolus administration regimens with long time intervals between doses. In contrast, dependence on the AUC indicates that the size of the dose rather than the details of administration is important (16, 22).

For these in vitro observations to offer an explanation of the efficacy of once-daily dosing regimens (12, 16), there is a requirement that plasma profiles are reflected in tissue concentration-time profiles. Available data for humans are confined to biliary and bronchial secretions; however, it is clear that concentration in plasma-time oscillations are reflected in damped oscillations in concentrations in tissue (15,

16, 18). Given the multiplicity of transfer sites and mechanisms in the liver and lungs, it appears that rapid, first-order transfer mechanisms apply to aminoglycosides at these sites. If this is a generally applicable principle of tissue transfer, then high peak concentrations of aminoglycosides in plasma would be desirable for treatment of most infections.

Concerns regarding enhancement of toxicity by a high $C_{max}$ logically follow; however, the general consensus is that there is no toxicity associated with transient elevation of concentration in plasma (5, 12, 16, 17, 21).

Principles of dosing design follow from these considerations. Increased in vitro kill and clinical efficacy and lack of

TABLE 1. Summary of results[a]

| Peak concn of drug[b] | $C_{max}$ (mg/liter) | $C_{max}$/MIC ratio | AUC (mg · hr/liter) | Bacterial count ($\log_{10}$) at: | | | |
|---|---|---|---|---|---|---|---|
| | | | | 2.5 h | 10.5 h | 24.5 h | 48.5 h |
| None (control) | | | | | | | |
| Bolus | 0 | | 0 | 8.17 ± 0.33 | 8.98 ± 0.04 | 9.01 ± 0.05 | 8.95 ± 0.02 |
| Infusion | 0 | | 0 | 8.52 ± 0.20 | 9.14 ± 0.19 | 9.19 ± 0.31 | 9.16 ± 0.27 |
| 4 mg/liter | | | | | | | |
| Bolus | 14.2 ± 0.9 | 28.4 ± 0.45 | 19.69 ± 2.31 | NGD | NGD | 4.28 ± 0.83 | 8.66 ± 0.15 |
| Infusion | 6.5 ± 0.4 | 13.0 ± 0.8 | 18.65 ± 2.84 | 2.36 ± 0.98 | 3.29 ± 0.19 | 8.80 ± 0.31 | 8.90 ± 0.26 |
| 6 mg/liter | | | | | | | |
| Bolus | 20.5 ± 1.4 | 41 ± 2.8 | 26.88 ± 2.11 | NGD | NGD | 3.57 ± 1.12 | 8.34 ± 0.85 |
| Infusion | 9.6 ± 0.5 | 19.2 ± 1.0 | 25.95 ± 1.13 | NGD | NGD | 7.87 ± 0.29 | 9.06 ± 0.36 |
| 8 mg/liter | | | | | | | |
| Bolus | 29.1 ± 1.8 | 58.2 ± 3.6 | 36.05 ± 1.97 | NGD | NGD | NGD | NGD |
| Infusion | 12.5 ± 0.5 | 25.0 ± 1.0 | 37.98 ± 5.08 | NGD | NGD | 3.50 ± 1.35 | 8.97 ± 0.43 |

[a] For all groups, $n = 5$. NGD, no growth detected.
[b] The peak concentrations of drug are for 30 min postdistribution.

clinical toxicity associated with high bolus peaks suggest that gentamicin could be given by intravenous bolus if "at-risk" patients are avoided (e.g., those with myasthenia gravis or relaxant ·anaesthesia). By extension, large-dose bolus regimens with long intervals between doses (e.g., 12 to 24 h) could be used at any level of aggregate clinical dose.

We advocate the bolus dosing approach with continuing careful review of clinical efficacy and toxicity.

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