Consistent rates of kill of *Staphylococcus aureus* by gentamicin over a 6-fold clinical concentration range in an *in vitro* pharmacodynamic model (IVPDM)

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**Objectives:** To compare the effect of a 6-fold range in gentamicin concentration on the bacterial killing of *Staphylococcus aureus*.

**Methods:** Six 24 h duplicate experiments were performed using an *in vitro* pharmacodynamic model (IVPDM) which was inoculated with $10^6$ cfu/mL *S. aureus* (ATCC 29213) and subjected to desired initial gentamicin concentrations of 0, 5, 10, 15 and 20 mg/L. A 2 h half-life was emulated for gentamicin. Samples were drawn at 0.5, 1, 1.5, 2, 3, 4, 6, 9 and 24 h to quantify cfu/mL and gentamicin concentration. These samples were subjected to serial saline dilution to prevent antibiotic carryover and to produce a countable number of colonies. Pre- and post-gentamicin MIC values were performed for *S. aureus*. Duplicate 24 h kill curves were generated for each experiment and assessed for statistical difference (two-way ANOVA) between the slopes of the kill curves and time to 3 log kill.

**Results:** Kill curve slopes were analysed out to the 2 h time point and no statistical difference was found between the different concentrations ($P > 0.05$). Time to 3 log kill was not significantly different between the concentrations. Post-exposure gentamicin MIC values were within one tube dilution of the pre-exposure MIC value (0.25 mg/L).

**Conclusions:** These data demonstrate that clinical gentamicin concentrations kill *S. aureus* with equivalent effectiveness and that the use of higher doses of aminoglycosides would probably not improve bacterial kill rates.

Keywords: single daily dose, adjunctive aminoglycosides, *S. aureus*, pharmacodynamics

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**Introduction**

*Staphylococcus aureus* is one of the most prevalent pathogens in the United States and is often associated with skin infections, infective endocarditis and bacteraemia. A common strategy for treating *S. aureus* bacteraemias and endocarditis is to use an antibiotic combination of a semi-synthetic penicillin or a glycopeptide combined with an aminoglycoside, most often gentamicin, as adjunct therapy. This antibiotic combination has been found to decrease the duration of bacteraemia and fever but does not affect mortality. Despite the common usage of gentamicin in clinical practice, the bacterial kill relationship of gentamicin concentration to *S. aureus* is unknown. This raises the question, would there be any benefit to dosing the gentamicin in a single daily dose (SDD) approach (7 mg/kg every 24 h) or instead continuing to use the multi daily dose (MDD) strategy (80 mg every 8 h or 1.5 mg/kg every 8 h)?

Aminoglycosides are known concentration-dependent killers of Gram-negative organisms. However, the relationship between concentration and rate of bacterial kill of gentamicin with Gram-positive organisms such as *S. aureus* is unknown. A study addressing this same question against enterococci found little value using more aggressive aminoglycoside dosing strategies.

A typical gentamicin peak serum concentration achieved with conventional dosing is 5–8 mg/L. When SDD is used, a much higher gentamicin peak of 20–25 mg/L is desired.

The purpose of this work was to determine whether higher clinical concentrations of gentamicin are more effective in killing *S. aureus*.

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Activity of clinical concentrations of gentamicin against *S. aureus*

**Materials and methods**

**Model description**

Experiments were performed in an *in vitro* pharmacodynamic model (IVPDM) described previously. The pharmacodynamic model consisted of a sealed glass chemostat filled with cation-adjusted Mueller–Hinton broth (CAMHB; Difco, Detroit, MI, USA). Experiments were run in duplicate. Each chemostat had a volume of ~275 mL. An initial desired inoculum of 10⁶ cfu/mL of *S. aureus* was used for each experiment. Chemostats were placed in a water bath maintained at 37°C and agitated using a magnetic stir bar. The desired starting peak concentration of antibiotic was achieved using a single bolus dose of antibiotic at the start of each experiment. In order to simulate desired rates of drug elimination, the drug-free medium was pumped using a SciQ 400 pump (Watson Marlow, Norway) into the chemostats, thus displacing an equal volume of the drug-containing medium into the waste beaker. The pump rate (Ko mL/h) was set to simulate a gentamicin half-life (t₁/₂) of 2 h (Ko = 0.693/t₁/₂ × volume of chemostat).

**Antibiotic**

Gentamicin was purchased from the Sigma Chemical Co. (Victoria, Australia). Gentamicin stock solutions, with a concentration of 1 mg/mL, were frozen at –80°C.

**Bacteria**

Methicillin-susceptible *S. aureus* (ATCC 29213) was used for all experiments.

**Susceptibility testing**

Pre- and post-exposure gentamicin MICs were determined using 96-well microtiter trays and broth microdilution techniques. All wells were inoculated with 10⁵–10⁶ cfu/mL. MICs were determined in accordance with NCCLS guidelines. The study organism, *S. aureus* (ATCC 29213), was also used as the control strain. We did not expect to see an inoculum effect with gentamicin.

**Time–kill experiments**

Six 24 h experiments including a growth control and five duplicate experiments exhibiting four different desired peak concentrations (C_max) of gentamicin (5, 10, 15 and 20 mg/L) (Table 1) were used to examine the effect of different clinical concentrations on killing of *S. aureus*.

Samples were taken from the experiment at time 0, which was prior to the introduction of gentamicin, and every half hour for the first 2 h. Additional post-antibiotic levels were obtained at 3, 4, 6, 9 and 24 h. Gentamicin carryover was prevented by serial saline dilution. Following incubation for 24 h at 37°C, bacterial colonies were counted and recorded on data sheets. The accuracy range for colony counting in our lab was 300–3000 colonies per plate. The lower limit of accuracy in our lab was 300 cfu/mL. To achieve countable colonies we used serial saline dilutions. Colony counts < 300 cfu/mL were not included in our data analysis.

**Analysis**

Time–kill curves were constructed and compared for each gentamicin experiment. Bacterial kill curve slopes were calculated by linear regression using Graph Pad Prism 4 software (Graph Pad Software Inc., San Diego, CA, USA) at 0–2 h of gentamicin exposure to examine the possibility of a concentration-dependent effect. The slopes then compared for statistical significance using a two-way ANOVA (Prism 4). Gentamicin concentrations were determined using an Axsym System (Abbott Laboratories, Abbot Park, IL, USA) commercial fluorescence polarization immunoassay. The range of detectable concentrations for this test was 0.3–10 mg/L (r = 0.994). Samples in which the anticipated concentration would exceed this range were diluted. Two control values (3 and 8 mg/L) were used in this assay (coefficient of variation values for these controls were 3.7% and 3.6%, respectively).

Time to 3 log kill (T3K) was assessed by using slopes obtained from linear regression out to the 2 h time point. Time was calculated using the equation γ = mx + b. T3K values for each experiment were then compared by using a two-way ANOVA (Prism 4). Statistical significance was defined as P < 0.05.

**Results**

**Susceptibility testing**

The pre-exposure MIC for *S. aureus* 29213 was 0.25 mg/L. Post-exposure MICs were either identical to pre-exposure MICs or increased by one tube dilution.

**Pharmacokinetics**

The target peak concentrations for the experiment were 5, 10, 15 and 20 mg/L. The desired half-life was 2 h. The actual peaks were 3.2, 6.6, 11 and 14 mg/L with measured half-lives of 2.2, 2.0, 2.1 and 2.2 h, respectively. Because we failed to obtain a measured peak of ~20 mg/L, an additional experiment was performed. The actual peak concentration for that experiment was 19.3 mg/L with a half-life of 2.5 h.

**Pharmacodynamics**

Figure 1 displays the kill curve data and bacterial regrowth over the 24 h period of the experiment. Although our peak concentrations fell short of the predicted values, we still achieved a 6-fold range that would allow us to discern the concentration relationship that best predicted eradication of *S. aureus* by gentamicin.

Kill curve slopes were used to analyse the rate of bacterial kill by different concentrations of gentamicin in this experiment. A steeper slope indicates a more rapid rate of kill as shown in the decline of bacterial cfu/mL. From the time of initial gentamicin...
Discussion

Aminoglycosides remain useful antibiotics but are known to cause nephrotoxicity and ototoxicity. With the advent of single daily dosing, many supporters believed that this new regimen could lead to not only better clinical outcomes, but also reduced toxicity. While this theory is yet to be proven definitively in a large, well-designed human trial, many smaller studies have been inconclusive in their assessment of this new dosing strategy for aminoglycosides.

The kill curve data in our experiment showed an insignificant difference in slopes when analysed out to the 2 h time point. Since our experiment covered over a 6-fold range in concentrations with no statistical difference in rate of kill, this would lead us to believe that increased clinical concentrations of gentamicin are not more effective in killing S. aureus than lower concentrations. In addition, the T3K, which occurred within the first 2 h of the experiments, was also not statistically different between the experiments.

A limitation of the present study is whether or not this data can be extrapolated to mimic a more clinically relevant scenario. Gentamicin is not recommended as monotherapy for any infection caused by S. aureus. Rather gentamicin is used as an adjunct to agents with good efficacy against Gram-positive infections such as vancomycin or nafcillin. Clinically the combination caused by S. aureus is not recommended as monotherapy for any infection. Rather, gentamicin is used as an adjunct to agents with good efficacy against Gram-positive infections such as vancomycin or nafcillin. Clinically the combination could lead to not only better clinical outcomes, but also reduced toxicity.

A 3 log kill (99.9% reduction in cfu/mL) was achieved within the range of 1.2–1.61 h for all experiments. This difference was not statistically different (P > 0.05) between either the models or the different gentamicin concentrations.

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References

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