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Pharmacodynamics of vancomycin and ampicillin alone and in combination with gentamicin once daily or thrice daily against *Enterococcus faecalis* in an *in vitro* infection model

Heather H. Houlihan[a], Daniela P. Stokes[b] and Michael J. Rybak[a,b]*

[a]The Anti-Infective Research Laboratory, Department of Pharmacy Services, Detroit Receiving Hospital/University Health Center and College of Pharmacy and Allied Health Professions, 4201 St Antoine; [b]Department of Internal Medicine, Division of Infectious Diseases, School of Medicine, Wayne State University, Detroit, MI 48201, USA

We compared the pharmacodynamic activities of vancomycin and ampicillin with or without gentamicin once daily or thrice daily in an *in vitro* infection model with fibrin–platelet clots (FPCs) infected with *Enterococcus faecalis*. Antibiotics were administered as a bolus to simulate human pharmacokinetics with regimens consisting of vancomycin 1 g q12h, ampicillin 2 g q6h and gentamicin 1.3 mg/kg q8h and 5 mg/kg qd. Model experiments were performed in duplicate over 72 h. FPCs were removed from the models in triplicate at 0, 8, 24, 32, 48 and 72 h, weighed, homogenized, diluted and plated to determine colony counts. Additional FPCs were removed at over 72 h post-antibiotic dose to determine antibiotic concentrations. The inoculum density at 72 h was used to compare bactericidal activity between the regimens. Overall, all antibiotic regimens containing either ampicillin or vancomycin significantly ($P < 0.01$) decreased the bacterial load at 72 h compared with the growth control although monotherapy regimens with either vancomycin or gentamicin had little impact. Ampicillin was superior to vancomycin with or without the addition of gentamicin ($P < 0.01$). There were no significant differences in reduction of bacterial density at 72 h between the combination of ampicillin or vancomycin plus gentamicin q8h and ampicillin or vancomycin plus gentamicin once daily. This was despite achieving unmeasurable FPC gentamicin concentrations after the 8 h time point during the once-daily aminoglycoside regimen. Vancomycin plus gentamicin either every 8 h or once daily was significantly ($P < 0.01$) better than vancomycin alone. Ampicillin plus either of the two gentamicin regimens was also better than ampicillin alone but this did not reach statistical significance. Our data suggest that once-daily gentamicin in combination with ampicillin or vancomycin demonstrates equivalent bacterial reductions to combination therapy with thrice-daily gentamicin. Once-daily aminoglycoside combination therapy for the treatment of enterococcal endocarditis warrants further investigation.

Introduction

Enterococcus has become an important pathogen in the current era because of its unique antibiotic resistance profile. It creates therapeutic dilemmas even in cases where multiple acquired drug resistance is not a problem. One of these dilemmas involves the treatment of enterococcal endocarditis, which represents up to 20% of all cases of infective endocarditis (IE).[1–5]

A bactericidal effect is necessary to cure infected cardiac valve vegetations, and this is usually achieved by a synergic combination of a cell wall-active agent (β-lactam or glycopeptide) with an aminoglycoside.[6–10] The American Heart Association's consensus statement on the treatment of adults with IE advocates a 4–6 week course of this regimen (with gentamicin in traditional thrice-daily dosing) for the management of enterococcal endocarditis.[11] This therapeutic approach has been debated continually for two main

*Corresponding author. Tel: +1-313-745-4554; Fax: +1-313-993-2522; E-mail: mrybak@dmc.org

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reasons: the potential for nephrotoxicity associated with prolonged aminoglycoside therapy in individuals with already compromised clinical status, and the cost associated with prolonged hospitalization or extended intravenous catheter treatment. Our theoretical and clinical knowledge about once-daily administration of aminoglycosides has increased tremendously in the past few years.[12–15] Since this administration method minimizes nephrotoxicity while preserving efficacy in most Gram-negative and some Gram-positive infections, several experimental and clinical studies have been conducted to evaluate the role of this therapy in IE, where the microenvironment of infected vegetations affects the pharmacodynamics of antibiotics quite differently from those of other infections.[16–24]

It is well known that the addition of an aminoglycoside to cell wall-active agents does not improve the efficacy for treatment of *Staphylococcus aureus* endocarditis, with the exception of uncomplicated right-sided methicillin-sensitive *S. aureus* endocarditis in injecting drug users.[25,26] Although monotherapy with cell wall-active agents alone is usually bactericidal against streptococci and staphylococci, combination with aminoglycosides does shorten the duration of bacteraemia (which has implications for the development of IE complications).[25] Accordingly, current IE recommendations include aminoglycosides as a short-term optional additional treatment.[11]

Several animal models have been designed to evaluate the use of aminoglycosides once daily for treatment of enterococcal endocarditis. The results, however, have been conflicting, favouring both once-daily or multiple daily dosing of aminoglycosides in combination with a β-lactam or a glycopeptide.[19,22] It has been pointed out that these studies differ in experimental design making it difficult to support the conclusion of either of them. In addition, there is evidence from an investigation using an *in vitro* infection model with simulated human antibiotic phamacokinetics that once-daily aminoglycosides in combination with β-lactams or glycopeptides may be a viable option for the treatment of enterococcal infections.[24] In this model, however, antibiotics and bacteria interact without regard to penetration problems and/or special conditions such as high inoculum, stationary phase organisms, etc., which reduce the efficacy of antibiotics when used for the treatment of endocarditis. In an attempt to help resolve this controversy, we designed a study utilizing an *in vitro* infected fibrin–platelet clot (FPC) model to compare the combination of ampicillin or vancomycin with gentamicin administered thrice versus once daily against *Enterococcus faecalis*.

Materials and methods

Bacterial isolate

A clinical isolate of *E. faecalis* (OG1X) was used in all experiments.[17]

Antimicrobial agents

Vancomycin, ampicillin and gentamicin analytical powders were purchased from Sigma Chemicals (St Louis, MO, USA), vancomycin for injection from Eli Lilly & Co. (Indianapolis, IN, USA), ampicillin for injection from Apothecon (Princeton, NJ, USA) and gentamicin for injection from Fujisawa USA, Inc. (Deerfield, IL, USA).

Susceptibility and synergy determination

MICs and MBCs were determined by a microdilution technique with an inoculum of 5×10^5 cfu/mL following NCCLS guidelines.[27] The FIC index for each drug combination was determined following NCCLS guidelines where synergy was defined as an FIC index of <0.5, and antagonism was defined as an FIC index of $\geqslant 4.0$.

Medium

Mueller–Hinton broth (Difco, Detroit, MI, USA) supplemented with 12.5 mg/L of magnesium and 25 mg/L of calcium (SMHB) was used for all model experiments and susceptibility testing.

Preparation of infected clots

Briefly, bacterial inocula were prepared by inoculating two or three colonies of bacteria from a fresh overnight tryptic soy agar (Difco) plate into 10 mL of SMHB, then incubating at 37°C for 24 h on a rotator. After centrifugation at 3500**g** for 15 min, the supernatant was removed and the pellet was resuspended with a small quantity of SMHB to achieve an inoculum of approximately 10^{10} cfu/mL. Infected FPCs of approximately 1 g were prepared by mixing 0.9 mL of cryoprecipitate from donors (volunteers from the American Red Cross, Detroit, MI, USA), 0.1 mL of organism suspension, 0.05 mL of platelet suspension (platelets obtained from healthy volunteers and mixed with normal saline provides 250000–500000 platelets per clot) and 0.05 mL of aprotinin solution (Sigma Chemicals; 2000 kIU/mL) in a sterile, siliconized, 1.5 mL Eppendorf tube. A sterile monofilament line was placed into the cryoprecipitate–bacteria mixture. Bovine thrombin (GenTrac, Middleton, WI, USA; 5000 U) was reconstituted with 5.0 mL sterile calcium chloride (50 mmol), and 0.1 mL of the thrombin was added to the cryoprecipitate–bacteria mixture.[28] The gelatinous mixture was removed from the Eppendorf using a sterile 21-gauge needle. The final inoculum obtained in the clots was approximately 10^9 cfu/g.

In vitro model

A one-compartment *in vitro* infection model capable of simulating human pharmacokinetics in the presence of viable bacteria was utilized (Figure 1).[29,30] The one-

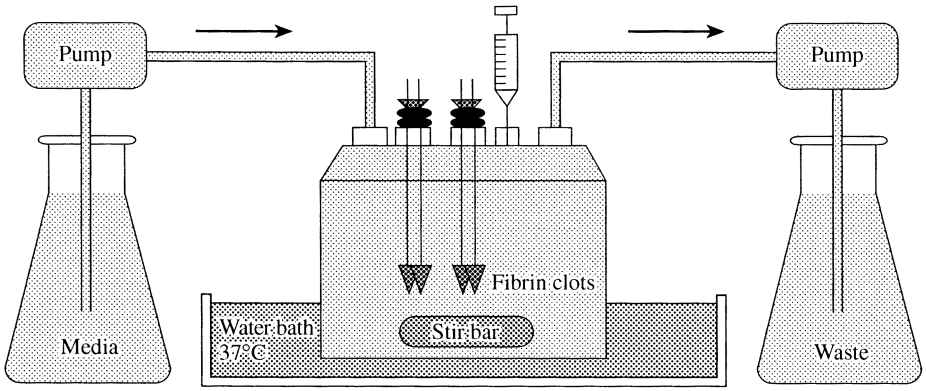

Figure 1. *In vitro* infection model with FPCs.

compartment glass models (500 mL) are fitted with sample ports from which 27 infected FPCs were suspended in each model and sealed with a rubber stopper. Fresh SMHB was supplied and removed from the models via a peristaltic pump set to achieve human simulated half-lives of 6 h for vancomycin, 1.5 h for ampicillin and 3 h for gentamicin. The models contained a magnetic stir bar for continual mixing of the antibiotics. Vancomycin was administered as a bolus into the model to simulate a regimen of 1 g every 12 h, ampicillin 2 g every 6 h and gentamicin 1.3 mg/kg every 8 h and 5 mg/kg every 24 h. The target antibiotic concentration for vancomycin q12h regimen was a peak of approximately 30 mg/L and trough of approximately 5–10 mg/L, and for ampicillin a peak of approximately 70 mg/L and trough of approximately 4 mg/L. The target antibiotic concentrations for the gentamicin q8h regimen were a peak of approximately 5 mg/L and a trough of approximately 1.0 mg/L, for the gentamicin q24h regimen were a peak of approximately 15 mg/L and a trough of $<$1.0 mg/L. For combination regimen experiments, the elimination rate was set for the drug with the shortest half-life, the drug with the longest half-life was supplemented.[31] Infection models were placed in a water bath and maintained at 37°C. Each infection model experiment was conducted over 72 h and performed in duplicate to ensure reproducibility.

Pharmacokinetic/pharmacodynamic analysis

Samples (0.5 mL) from the broth of all infection model central compartments were obtained at 0.25, 0.5, 1, 2, 4, 6, 8, 24, 32, 48 and 72 h in duplicate for determination of antibiotic concentrations and stored at –70°C until analysis. The antibiotic peak/trough and half-lives were calculated from plots of the concentration versus time plots.

Three FPCs were removed from each model (total of six at each time point) at 0, 0.25, 2, 4, 8, 24, 32, 48 and 72 h post-infusion for analysis of antibiotic concentrations. Determination of cfu/g was obtained from the samples that were removed at 0, 8, 24, 32, 48 and 72 h. The FPCs were weighed and homogenized using a mini-beadbeater (Biospec Products, Bartlesville, OK, USA) and trypsin solution (62.5 mg of trypsin (Lot 26H71305, Sigma) with 5.0 mL of normal saline). Cold normal saline was used for dilution of the samples and 20 μL was plated on tryptic soy agar (TSA) plates in triplicate, incubated for 24 h at 37°C and the colonies counted. Our limit of detection for this method has been determined previously to be 100 cfu/mL.[30] Potential antibiotic carryover samples (0.1 mL) were diluted in 0.9 mL of normal saline, and this mixture was filtered through a 45 μm pore-size filter (Gelman Sciences, Ann Arbor, MI, USA). The values for the six samples were averaged for each time point and plotted as $\log_{10}$ cfu/g versus time. Total reduction in the $\log_{10}$ cfu/g over 72 h was determined and the time to achieve a 99.9% reduction was calculated by linear regression.

Antibiotic assays

Vancomycin and gentamicin model concentrations were determined by fluorescence polarization immunoassay (Abbott Diagnostics TDx; Irving, TX, USA), which has a sensitivity of 2.0 mg/L for vancomycin and 0.27 mg/L for gentamicin. Standard curves were prepared in SMHB. Vancomycin and gentamicin fibrin clot concentrations could not be assayed using the fluorescence polarization immunoassay because of turbidity of the suspension. Vancomycin, ampicillin and gentamicin FPC concentrations and ampicillin broth concentrations were therefore determined by well-diffusion bioassay methodology using *Bacillus subtilis* spore suspension No. 6633 (Difco). The limits of detection for this assay are 5 mg/L for vancomycin, 0.5 mg/L for ampicillin and 0.5 mg/L for gentamicin, with intra-day coefficients of variation of $<$3.5%, $<$8.1% and $<$3.6%, respectively. The r^2 for all assays ranged from $\geqslant$0.982 to 0.999 over the concentration of 5–25 mg/L for vancomycin, 0.5–50 mg/L for ampicillin and 0.5–10 mg/L for gentamicin. Concentrations outside the standard curve were diluted in sterile water then multiplied by the appropriate dilution factor to determine the final concentration. All standards and unknowns were run in quadruplicate.

Statistical analysis

The changes in $\log_{10}$ cfu/g and time required to achieve 99.9% killing for each regimen over 72 h were compared using ANOVA with Tukey's test for multiple analysis. *P* values of <0.05 were considered significant. All statistical evaluations were performed using SPSS Statistical Software (Release 6.1.3, SPSS, Inc., Chicago, IL, USA).

Results

Susceptibility testing

The MIC/MBCs of this isolate to vancomycin, ampicillin and gentamicin are 2/8 mg/L, 2/64 mg/L and 16/16 mg/L, respectively. The FIC index for the combination of ampicillin and gentamicin was 0.6 (combination MICs of 1.0 and 1.0 mg/L for ampicillin and gentamicin, respectively) indicating an additive effect. The FIC index for the combination of vancomycin and gentamicin was 0.3 (combination MICs of 0.25 and 1.0 mg/L for vancomycin and gentamicin, respectively) indicating synergy.

Pharmacokinetics and pharmacodynamics

Antibiotic FPC, central compartment model concentrations, peak/trough and half-lives are listed in Table I. No differences were noted for antibiotic concentrations obtained from monotherapy or combination models and therefore, the results were combined. Overall, FPC concentrations of vancomycin appeared to accumulate slightly and remain quite similar to the drug concentration in the central compartment broth over the same time interval. Ampicillin FPC concentrations reached a peak at 2 h and the trough concentrations were nearly identical to that of the broth. FPC concentrations of the gentamicin qd regimen reached a peak of 2.4 mg/L at 2 h and remained undetectable at the end of each dosing interval, indicating a lack of drug accumulation. FPC concentrations of gentamicin q8h regimen reached a peak of 1.4 mg/L, just above half that reached in the qd regimen, and maintained detectable troughs (with the exception of 8 h) throughout the entire dosing interval (Table I). Vancomycin and ampicillin broth concentrations were above the MIC for the entire experiment for all regimens and gentamicin concentrations were below the MIC for both regimens throughout the dosing interval.

Overall, there was a minimal reduction of bacterial density in FPCs for monotherapy regimens of vancomycin or gentamicin (either regimen; Figures 2 and 3.). Ampicillin alone, in contrast, reduced the bacterial inocula by

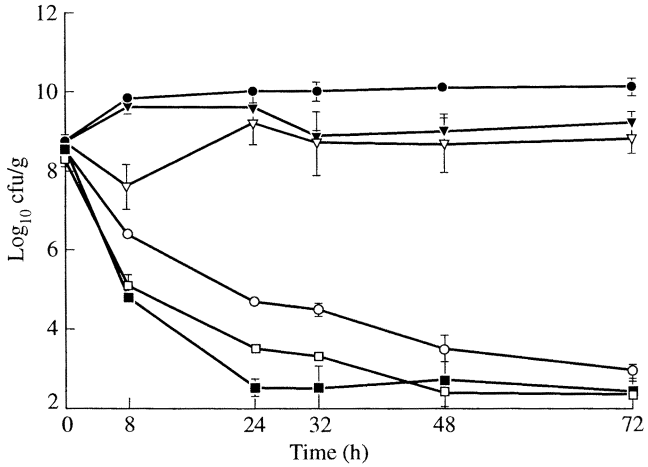

Figure 2. Bactericidal activity of ampicillin, gentamicin and the combination of ampicillin and gentamicin either q8h or q24h against *E. faecalis* OG1X. ●, Growth control; ▼, gentamicin q8h; ▽, gentamicin q24h; ○, ampicillin q6h; ■, ampicillin q6h + gentamicin q8h; □, ampicillin q6h + gentamicin q24h.

Table I. Antibiotic concentrations in FPCs and central compartment model[a]

	Ampicillin (mg/L)		Vancomycin (mg/L)		Gentamicin q8h (mg/L)		Gentamicin q24h (mg/L)	
Time (h)	clot	model	clot	model	clot	model	clot	model
0.25	16.9 ± 0.21	68.0 ± 1.7	5.1 ± 0.15	29.1 ± 1.2	0.83 ± 0.01	5.25 ± 0.24	1.56 ± 0.04	12.69 ± 0.32
2	26.5 ± 0.84	36.7 ± 0.64	13.4 ± 0.28	22.9 ± 0.61	1.40 ± 0.02	3.53 ± 0.07	2.40 ± 0.07	8.40 ± 0.19
4	12.3 ± 0.57	16.4 ± 0.70	13.7 ± 0.49	19.5 ± 0.57	0.95 ± 0.01	2.37 ± 0.06	2.20 ± 0.14	5.13 ± 0.04
8	16.1 ± 0.28	41.8 ± 0.92	14.9 ± 0.21	13.0 ± 0.13	< 0.50	0.95 ± 0.01	0.82 ± 0.01	2.53 ± 0.09
24	3.5 ± 0.05	3.5 ± 0.03	18.8 ± 0.35	14.9 ± 0.54	0.61 ± 0.02	0.99 ± 0.07	< 0.50	< 0.50
32	15.2 ± 0.28	32.5 ± 0.64	18.3 ± 0.25	19.3 ± 0.13	0.77 ± 0.01	1.11 ± 0.04	0.71 ± 0.01	3.40 ± 0.19
48	4.0 ± 0.07	3.3 ± 0.14	17.4 ± 0.56	13.9 ± 0.54	0.94 ± 0.02	1.17 ± 0.04	< 0.50	UD
72	3.0 ± 0.13	5.2 ± 0.18	5.0 ± 0.42	15.6 ± 0.07	1.13 ± 0.07	1.08 ± 0.03	< 0.50	UD

[a]Infection model central compartment antibiotic peak/trough and half-life: ampicillin peak/trough, half-life = 74.8 ± 1.1/6.2 ± 0.42 mg/L, 1.7 ± 0.02 h; vancomycin peak/trough, half-life = 29.8 ± 1.9/ 9.4 ± 1.2 mg/L,6.6 ± 0.39 h; gentamicin q8h peak/trough, half-life = 5.6 ± 0.11/0.96 ± 0.01 mg/L, 3.2 ± 0.13 h; gentamicin q24h peak/trough, half-life = 13.3 ± 0.28/0.06 ± 0.07 mg/L, 3.1 ± 0.10 h.
UD, undetectable.

>5.5 $\log_{10}$ cfu/g at 72 h ($P < 0.001$). The addition of gentamicin significantly ($P < 0.001$) improved bactericidal activity of vancomycin whether given as a single daily dose or by traditional dosing. Although there was a slight trend for increased killing when gentamicin (either regimen) was added to ampicillin, this was not statistically significant (Figures 2 and 3). For the combination regimens, there was no apparent difference between gentamicin administered once daily or thrice daily with regard to cfu/g reduction at 72 h. Ampicillin combined with gentamicin (qd or q8h) achieved the greatest bacterial reduction (>6 $\log_{10}$ cfu/g) at 72 h. Ampicillin in combination with gentamicin either qd or q8h was significantly better than vancomycin plus gentamicin either qd or q8h in reduction of the bacterial density at 72 h ($P < 0.05$). No regimens reached our limit of detection (1×10^2 cfu/g). Ampicillin was the only monotherapy regimen to achieve a 99.9% kill within the 72 h experiment. Similar to the overall reduction in cfu/g at 72 h, ampicillin plus gentamicin either qd or q8h was superior to vancomycin plus gentamicin either qd or q8h in achieving a 99.9% kill within the allotted 72 h period (Table II).

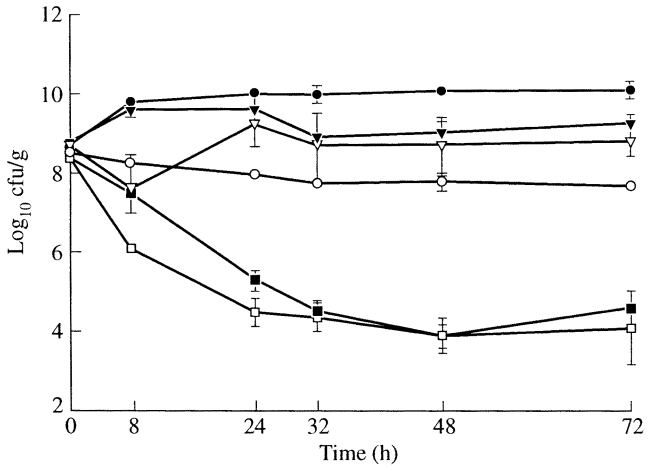

Figure 3. Bactericidal activity of vancomycin, gentamicin and the combination of vancomycin and gentamicin either q8h or q24h against *E. faecalis* OG1X. ●, Growth control; ▼, gentamicin q8h; ▽; gentamicin q24h; ○, vancomycin q12h; ■, vancomycin q12h + gentamicin q8h; □, vancomycin q12h + gentamicin q24h.

Discussion

It has long been established that reliable bactericidal activity against enterococci occurs only with the combination of a β-lactam or glycopeptide antibiotic with an aminoglycoside.[2,6–10] Since the advent of single-dose aminoglycoside therapy, investigators have hypothesized that synergy between the aminoglycoside and β-lactam or glycopeptide may be jeopardized by once-daily administration of the aminoglycoside. This judgement is based upon the premise that synergy between an aminoglycoside and the β-lactam can only occur via continuous interaction or contact between the two antibiotics and that the post-antibiotic effect demonstrated *in vitro* has not been shown *in vivo*.[19,32,33] Our data suggest that ampicillin or vancomycin administered in combination with gentamicin given thrice daily or once daily are equivalent in reducing the bacterial density load of *E. faecalis* within simulated vegetations. This is in the light of the fact that the gentamicin FPC and broth concentrations for the once-daily regimen and the q8h regimen never reached or exceeded the MIC during the dosing interval. It has been suggested that the concentration of an antibiotic inside the vegetation should be maintained above the MIC throughout the dosing interval to prevent loss of efficacy.[32] However, this is not the case for the aminoglycosides, since enterococcus is intrinsically

Table II. Residual organisms at 72 h and time to 99.9% reduction in bacterial density in the *in vitro* model

Antibiotic regimen	Time (h) to 99.9% reduction in cfu/g	cfu/g at 72 h
Growth control	NA	10.1 ± 0.21
Gentamicin q8h	NA	8.6 ± 0.12
Gentamicin qd	NA	8.8 ± 0.36
Ampicillin q6h	25.3 ± 0.37[d]	2.9 ± 0.17[a,c]
Ampicillin q6h + gentamicin q8h	17.2 ± 2.31[b,c]	2.4 ± 0.51[a,c]
Ampicillin q6h + gentamicin qd	20.6 ± 1.32[c]	2.3 ± 0.37[a,c]
Vancomycin 1 g q12h	NA	7.7 ± 0.05[a]
Vancomycin 1 g q12h + gentamicin q8h	33.6 ± 0.50[b]	4.6 ± 0.12[a,b]
Vancomycin 1 g q12h + gentamicin qd	28.2 ± 0.64[b]	4.1 ± 0.92[a,b]

[a]Regimens statistically ($P < 0.001$) different from growth control.
[b]Regimens statistically ($P < 0.001$) different from monotherapy.
[c]Regimens statistically ($P < 0.001$) different from vancomycin monotherapy or combination.
[d]Regimens statistically ($P < 0.01$) different from vancomycin + gentamicin q8h.
NA, not achieved.

resistant to low levels of aminoglycosides. It would seem that the concentration fraction of the aminoglycoside needed to produce synergy (FIC or possibly the fractional bactericidal concentration) would be the target concentration required during the dosing interval. Since this fraction was only obtained at the FPC site for approximately 4–8 h during the once-daily regimen and approximately 4 h for the q8h regimen, it may suggest that synergy may occur without continuous contact between the aminoglycoside and the β-lactam or glycopeptide during the dosing interval. Factors that may be important in this interaction include antibiotic penetration characteristics, concentration gradient effects, organism antibiotic uptake, concentration-dependent killing and a synergic post-antibiotic effect.[18] It has also been suggested that vancomycin activity against enterococci is inferior to that of ampicillin.[1] Our data support this premise since ampicillin alone or in combination with gentamicin was superior to vancomycin monotherapy or combination therapy with gentamicin. Differences in killing between ampicillin and vancomycin for monotherapy and in combination demonstrated in our experiments may be also be related to the difference in fibrin penetration. Although our methods did not allow us to determine the extent of antibiotic penetration throughout the fibrin clot, autoradiography studies performed in rabbits have demonstrated that glycopeptides such as teicoplanin at least initially remain concentrated at the periphery of the vegetation. In contrast, β-lactams such as penicillin and amoxycillin diffuse progressively across the vegetation although a concentration gradient exists between the periphery and the core for these drugs. Aminoglycosides such as tobramycin achieve a rapid and a homogeneous diffusion pattern throughout the vegetative mass.[18]

In recent years there have been several investigations attempting to address the issue of once-daily aminoglycosides for the treatment of IE. These studies evaluated the efficacy of either gentamicin or netilmicin administered once or thrice daily in combination with ampicillin, penicillin, vancomycin or ceftriaxone against various streptococcal species, including viridans streptococci, *S. aureus* and *E. faecalis*.[16,19–24,33,34]

The results of most of the studies to date, including one human study, which evaluated the use of once-daily aminoglycosides for streptococcal endocarditis, indicate that this method of administering aminoglycosides is equal to that of multiple dose aminoglycoside therapy.[16,19–23]

The data for enterococcal endocarditis, however, are not so consistent. Fantin and Carbon demonstrated in a rabbit endocarditis model that combination therapy with a traditional dosing schedule of aminoglycoside was clearly superior to the same total dose administered once daily.[19] Nevertheless, Gavaldà *et al.* recently demonstrated equal efficacy of aminoglycoside administered either once or thrice daily in the same total daily dose in a model of *E. faecalis* endocarditis.[22] These two studies differed in the time of initiation of the treatment after inoculation of the vegetations in the rabbits (48 h compared with 24 h) and in the pharmacokinetics of the β-lactam (the latter study simulated human pharmacokinetics). Like Gavalda *et al.*, we found once-daily administrations to be equal to multiple daily dosing of aminoglycosides. Further, our results are in agreement with the results demonstrated in the *in vitro* study performed by Schwank and Blaser.[24] They found no significant differences in the reduction in cfu between thrice-daily and once-daily administration of netilmicin in combination with ampicillin using the same isolate previously used by Fantin and Carbon in their rabbit endocarditis model, which favoured multiple dosing of the aminoglycosides.[19] In addition, both human and animal pharmacokinetics were simulated to evaluate this potential artefact in explaining the differences in the results of these two investigations. They also found, as we did, that although monotherapy with amoxycillin (and penicillin) was as effective as combination therapy with an aminoglycoside, the addition of the aminoglycoside (netilmicin) significantly improved the activity of vancomycin against *E. faecalis*. It is unclear why the addition of the aminoglycoside was more pronounced with vancomycin than with amoxycillin or in our case ampicillin. It may be related to differences in susceptibility of enterococci to the β-lactam or simply related to the fact that the definition for synergy was met with this combination as opposed to addition with ampicillin and gentamicin.

In conclusion, the bactericidal activity of once-daily versus thrice-daily aminoglycoside in combination with ampicillin or vancomycin was not different in our *in vitro* infection model. These results have important implications for the use of once-daily aminoglycoside therapy in the treatment of enterococcal endocarditis. Not only is the combination of the β-lactam or glycopeptide with an aminoglycoside necessary to establish bactericidal effect, but the use of once-daily aminoglycosides may be less toxic and more convenient than traditionally dosed aminoglycoside regimens. Further studies establishing the efficacy of once-daily aminoglycoside therapy for the treatment of enterococcal infections are warranted.

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