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In vitro activity of gentamicin as an adjunct to penicillin against biofilm group B *Streptococcus*

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Objectives: Group B *Streptococcus* (GBS) increasingly causes invasive disease in non-pregnant adults, particularly in elderly persons and those with underlying diseases. Combination therapy with penicillin plus gentamicin has been suggested for periprosthetic joint infection. The postulated synergism of this combination is based on experiments with planktonic bacteria. We aimed to assess the efficacy of this combination against sessile bacteria.

Methods: Four different GBS strains were used. We compared results of MICs with those of minimal biofilm eradication concentrations (MBECs), applied chequerboard assays to the MBEC device and calculated the fractional inhibitory concentration index. Synergism was evaluated with time–kill assays against bacteria adherent to cement beads, using penicillin (0.048, 0.2 and 3 mg/L), gentamicin (4 and 12.5 mg/L) and a combination thereof. Results were evaluated via colony counting after sonication of beads and scanning electron microscopy.

Results: MBEC/MIC ratios were 2000–4000 for penicillin and 1–4 for gentamicin. In chequerboard assays, synergism was observed in all four isolates. In time–kill assays, penicillin and 12.5 mg/L gentamicin showed synergism in two isolates. In the other two isolates 12.5 mg/L gentamicin alone was as efficient as the combination therapy.

Conclusions: These *in vitro* investigations show activity of 12.5 mg/L gentamicin, alone or as an adjunct to penicillin, against four strains of biofilm GBS. This concentration cannot be achieved in bone with systemic administration, but can be reached if administered locally. The combination of systemic penicillin plus local gentamicin indicates a potential application in orthopaedic-device-associated GBS infections. Studies with a larger number of strains are required to confirm our results.

Introduction

Group B *Streptococcus* (GBS) increasingly causes invasive GBS disease in non-pregnant adults, particularly in elderly persons and those with underlying diseases. Several reports have highlighted the role of GBS in periprosthetic joint infections (PJIs),[1] GBS being a frequently found species among all streptococcal PJIs. Treatment concepts are not established, but combination therapy with penicillin plus gentamicin has been suggested during the first 2 weeks of treatment.[2] Similar recommendations have been made for infective endocarditis (IE).[3] These suggestions were derived from *in vitro* studies showing synergism against planktonic bacteria with these antibiotics.[4] Here, we investigated this postulated synergism against four different strains of GBS in sessile form.

Methods

For this study, four strains were used. We used two clinical isolates obtained from patients with PJI (BE07-1b) and IE (BE05-1). NEM316 (a frequently used strain for laboratory experiments[5]) and Col2 (BE12-2, a strain obtained from a colonized woman that showed high biofilm production on crystal violet staining) were used as controls. The MIC and minimal biofilm eradication concentration (MBEC) were measured via the microbroth dilution method. Penicillin (benzylpenicillin sodium, Grünenthal Pharma AG, Mitlödi, Switzerland) and gentamicin (Salutas Pharma, Holzkirchen, Germany) were supplied from the clinical pharmacy of the University Hospital (Bern, Switzerland). Chequerboard assays were applied to the MBEC device (Innovotech Inc., Edmonton, Alberta, Canada) and the fractional inhibitory concentration index (FICI) was calculated. A synergistic effect was defined as FICI $\leq$0.5. In addition, synergism was evaluated with time–kill assays against bacteria adherent to a foreign body, as described previously.[6]

Table 1. Antimicrobial susceptibilities (mg/L) and FICIs

Isolate	MIC determined by microbroth dilution		MBEC		
	PEN	GEN	PEN	GEN	FICI
PJI	0.03 (0.016–0.04)	23.4 (15.6–31.25)	75 (75–150)	31.25 (15.6–31.2)	0.23 (0.06–0.38)
IE	0.04 (0.016–0.04)	15.6 (7.8–15.6)	75 (75–300)	62.5 (31.2–62.5)	0.39 (0.18–0.75)
NEM316	0.04 (0.032–0.04)	15.6 (7.8–15.6)	18.7 (9.4–18.7)	15.6 (7.8–15.6)	0.11 (0.11–0.11)
Col2	0.02 (0.016–0.04)	7.8 (7.8–15.6)	37.5 (4.7–37.5)	7.8 (7.8–7.8)	0.38 (0.38–0.5)

PEN, penicillin; GEN, gentamicin.
All measurements were repeated three or more times. Results are presented as median and range.

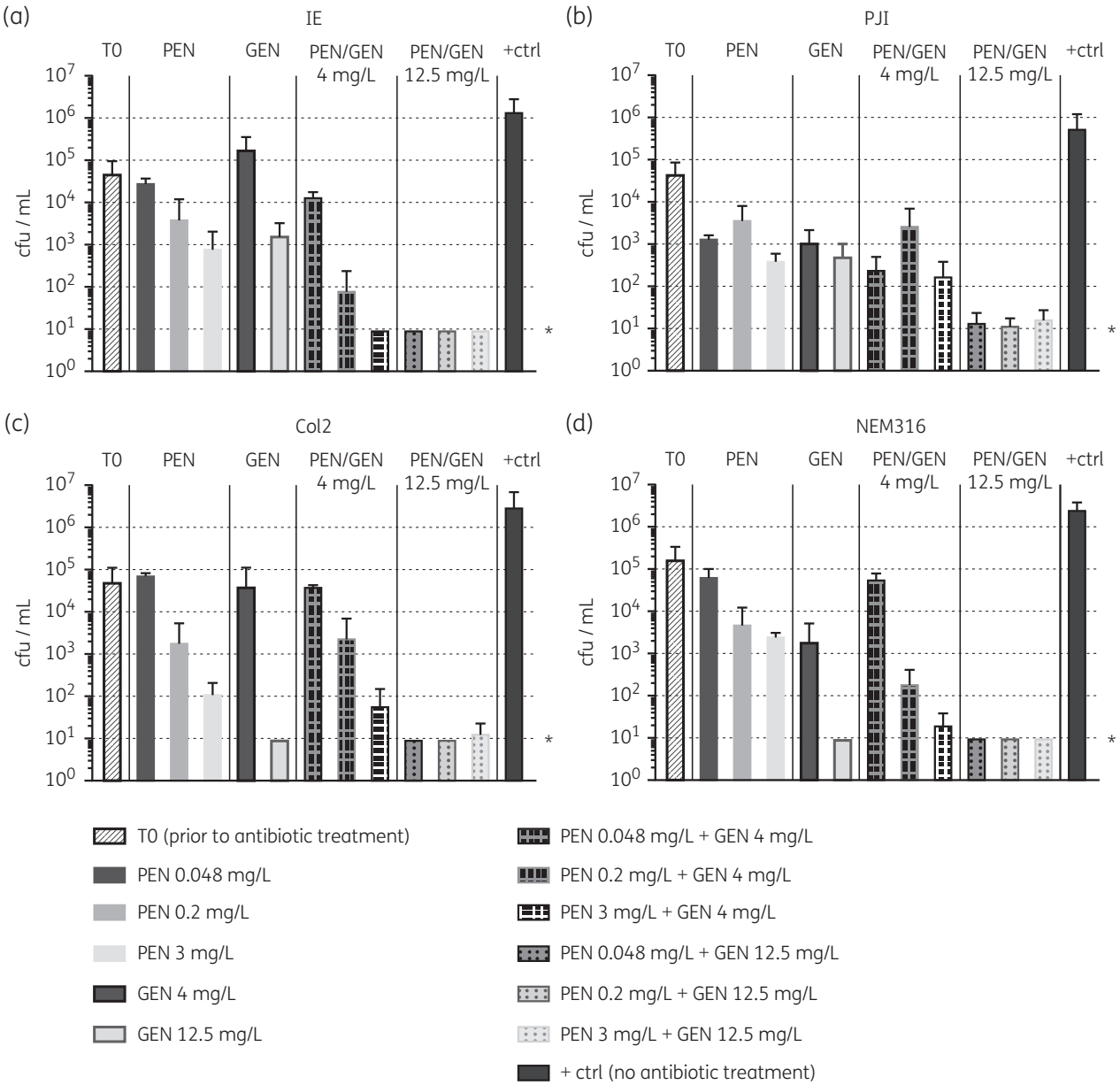

Figure 1. Time–kill assays of sessile GBS. After antibiotic exposure for 12 h, bacteria were dislodged from cement beads via sonication and cfu counted. The following strains were used: (a) IE, a GBS strain obtained from a patient with IE; (b) PJI, a GBS strain obtained from a patient with PJI; (c) Col2, a GBS strain obtained from a vaginally colonized woman (control strain); and (d) NEM316, control strain.[5] Bacteria adhered to a cement bead for biofilm formation for 24 h. An asterisk indicates the limit of detection. The data shown represent means with standard deviations. T0, timepoint 0; PEN, penicillin; GEN, gentamicin.

In brief, cement beads (Biomet, Dordrecht, the Netherlands) were incubated with 10^5–10^6 cfu/mL GBS for 24 h to form a biofilm and then washed to remove non-sessile bacteria. Then the biofilm was challenged with antibiotics for 12 h. Antimicrobial concentrations were as follows: penicillin, 0.048, 0.2 and 3 mg/L; gentamicin, 4 and 12.5 mg/L; and combinations thereof. The rationale for selecting these penicillin concentrations was based on the following arguments: (i) 0.048 mg/L is a concentration that was considered as an approximation of 1 × MIC for all four strains (Table 1); (ii) 3 mg/L is a penicillin serum concentration in adults 4 h after completion of intravenous administration of 5 million IU of penicillin;[7] and (iii) 0.2 mg/L is an extrapolation from serum trough levels (3 mg/L)[7] to 15% bone penetration of penicillin (i.e. bone–serum ratio in humans 0.1–0.2).[8] Gentamicin concentrations were selected on the basis of peak serum levels in adults after intravenous administration of 1 mg/kg (4 mg/L in serum) or 3 mg/kg (12.5 mg/L in serum) body weight (bw).[9] After antibiotic exposure, biofilm was dislodged via sonication (3210, Branson Ultrasonics Corporation, Geneva, Switzerland) at 40 kHz for 5 min and dilutions were plated for assessing the number of cfu.

Synergy was defined as a 100-fold (≥2 log) increase in killing at 24 h with the combination therapy in comparison with the most active single drug. Colony count results were paralleled by imaging with a scanning electron microscope. All assays were repeated at least three times.

Results

MIC, MBEC and FICI results are presented in Table 1. Penicillin MBEC/MIC and gentamicin MBEC/MIC ratios were 2000–4000 and 1–4, respectively. In chequerboard assays, synergism of penicillin plus gentamicin was observed in all isolates. Time–kill assay results are presented in Figure 1(a–d). With penicillin monotherapy, the colony count reduction ranged from <0.5 to 2.5 logs. There was an insignificant tendency that the higher the penicillin concentration, the more effective the killing of sessile GBS. In the two control strains (Col2 and NEM316), a gentamicin concentration of 12.5 mg/L alone reduced the number of cfu by >3 logs (Figure 1c and d). This was not observed in the two clinical strains (IE and PJI) or when 4 mg/L gentamicin alone was used. In penicillin/gentamicin combination assays using 4 mg/L gentamicin, >3 log cfu reduction was only seen in one strain (IE; Figure 1a) and when 3 mg/L penicillin was used. The most effective combination was seen with penicillin (all three concentrations) plus 12.5 mg/L gentamicin. The mathematical criteria for synergism were fulfilled in two clinical isolates (PJI and IE). In the control isolates, Col2 and NEM316, this was not the case, because the effect of 12.5 mg/L gentamicin alone was similar to that of penicillin plus 12.5 mg/L gentamicin. In accordance with the time–kill assay results, scanning electron microscopy showed eradication of biofilm from the cement beads after IE and PJI had been treated with 0.2 mg/L penicillin plus 12.5 mg/L gentamicin (Figure 2).

Discussion

Our investigations confirm the results of a previous study on sessile bacteria, showing a high penicillin MBEC/MIC ratio and a low aminoglycoside MBEC/MIC ratio.[10] Reduction of β-lactam MBEC after adding gentamicin has been observed in some enterococcal isolates.[11] The results suggest a variability of biofilm susceptibility to antibiotics between each strain that is difficult to assess with the measurement of MBECs only. Nonetheless, these data indicate that gentamicin (alone or in combination therapy) at a sufficiently high concentration has activity against biofilm GBS, provided that there is no high-level gentamicin resistance.[12]

In clinical practice, gentamicin can be administered at 3 mg/kg bw in a single dose or divided into two to three doses per day (i.e. 2 × 1.5 or 3 × 1 mg/kg bw). Our investigations showed marked killing rates of adherent bacteria when we used 12.5 mg/L gentamicin, corresponding to the peak serum concentration after single-dose administration in adults (i.e. 1 × 3 mg/kg bw). The translation of these results into clinical practice requires caution. It is unknown

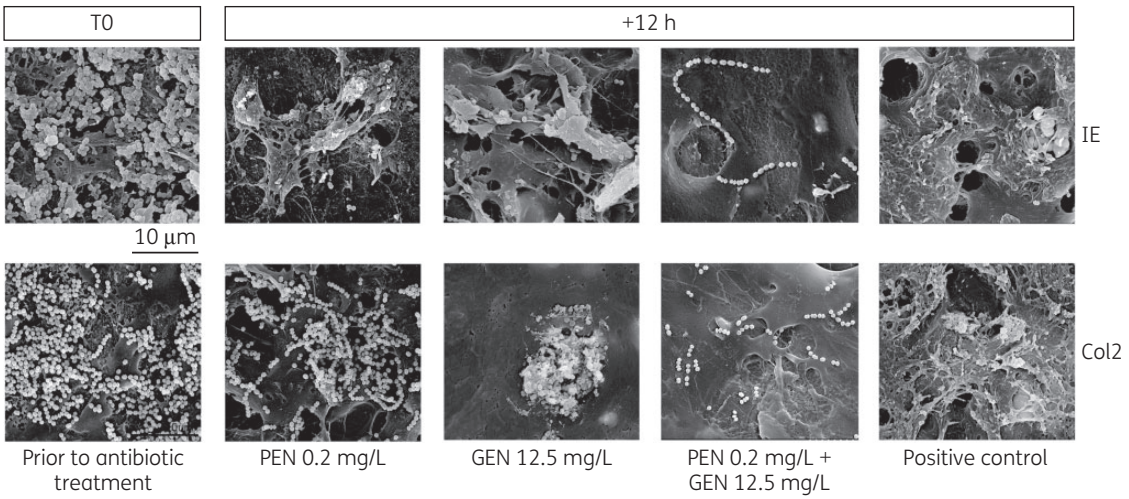

Figure 2. Scanning electron microscopy of cement beads inoculated with GBS biofilm. GBS IE (upper row) represents clinical isolates and Col2 (lower row) is a control isolate. Twenty-four hours after inoculation with GBS, and prior to antibiotic exposure, biofilm is visible on cement beads (T0, timepoint 0). Penicillin (0.2 mg/L) alone shows little effect in biofilm killing after 12 h of antibiotic exposure (second column from left). Gentamicin (12.5 mg/L) alone markedly reduced the biofilm of GBS Col2, but not of GBS IE (third column from left). The anti-biofilm effect in clinical and control isolates with 0.2 mg/L penicillin plus 12.5 mg/L gentamicin is shown in the second column from right. The positive controls (GBS strains without antibiotic exposure) show continuous growth in biofilm (right column).

how the bacterial state cultured on cement beads can be compared with endocardial vegetation on a heart valve or a biofilm in PJI. The killing rates of bacteria presented here are based on exposure to a single dose and antibiotics are not—as they are *in vivo*—metabolized. In other words, the effect of concentration fluctuation in a patient could not be reflected in this *in vitro* study, since fixed antibiotic concentrations were used for 12 h. However, we used penicillin concentrations that are considered as trough levels in humans (i.e. 3 mg/L in serum and 0.2 mg/L in bone). No recommendation about the duration of aminoglycoside treatment can be made. A retrospective study on GBS IE indicated that aminoglycoside treatment beyond 7 days had a higher heart failure rate and is likely not beneficial.[13] The expanding population affected by invasive GBS disease, namely, elderly persons and patients with comorbidities, is more prone to the nephrotoxicity of aminoglycosides. Of note, the combination of penicillin (0.2 or 3 mg/L) plus gentamicin (12.5 mg/L) is unlikely to occur in patients treated for GBS PJI because—considering the recommended dosing—penicillin concentrations rarely fall below 3 mg/L in serum or 0.2 mg/L in bone,[7] and a gentamicin concentration of 12.5 mg/L is not achieved in bone when administered intravenously. Moreover, in infections affecting tissues or biological fluids the pH is acidic. Low pH increases MICs of aminoglycosides for Gram-positive cocci.[14] Hence, the efficacy of systemically administered aminoglycosides against Gram-positive cocci in bone is uncertain.

Penicillin (both concentrations, 0.2 and 3 mg/L) plus $\geq$12.5 mg/L gentamicin is possible in bone when penicillin is given systemically and gentamicin locally. Degradable drug delivery systems in bone have shown high levels of gentamicin release into the bone (e.g. >600 mg/L during the initial 48 h).[15] At these concentrations, antibacterial activity is likely, even in a low-pH milieu.

Our *in vitro* investigations show activity of gentamicin—alone for two control strains or as an adjunct to penicillin for two clinical strains—against biofilm GBS when a concentration of 12.5 mg/L, but not 4 mg/L, is used. This concentration cannot be achieved in bone with systemic administration, but can be reached if administered locally. The translation of these findings into clinical practice requires further studies with a higher number of strains and the use of *in vivo* models. The combination of systemic penicillin plus local gentamicin indicates a potential application in orthopaedic-device-associated infections.

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Transparency declarations

None to declare.

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