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Pharmacodynamics of plazomicin and a comparator aminoglycoside, amikacin, studied in an in vitro pharmacokinetic model of infection

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ABSTRACT

The new aminoglycoside plazomicin shows in vitro potency against multidrug-resistant Enterobacteriales. The exposure–response relationship of plazomicin and the comparator aminoglycoside amikacin was determined for *Escherichia coli*, while for *Klebsiella pneumoniae* only plazomicin was tested. An in vitro pharmacokinetic model was used. Five *E. coli* strains (two meropenem-resistant) and five *K. pneumoniae* strains (two meropenem-resistant) with plazomicin MICs of 0.5–4 mg/L were used. Antibacterial effect was assessed by changes in bacterial load and bacterial population profile. The correlation between change in initial inoculum after 24 h of drug exposure and the AUC/MIC ratio was good (plazomicin $R^2 \geq 0.8302$; amikacin $R^2 \geq 0.9520$). *Escherichia coli* plazomicin AUC/MIC ratios for 24-h static, –1, –2 and –3 log drop were 36.1 ± 18.4, 39.3 ± 20.9, 41.2 ± 21.9 and 44.8 ± 24.3, respectively, and for amikacin were 49.5 ± 12.7, 55.7 ± 14.8, 64.1 ± 19.2 and 73.3 ± 25.3. *Klebsiella pneumoniae* plazomicin AUC/MIC ratios for 24-h static, –1, –2 and –3 log drop were 34.0 ± 15.2, 46.8 ± 27.8, 67.4 ± 46.5 and 144.3 ±129.8. Plazomicin AUC/MIC ratios >66 and amikacin AUC/MIC ratios >57.7 were associated with suppression of *E. coli* growth on 4 × or 8 × MIC recovery plates. The equivalent plazomicin AUC/MIC to suppress resistance emergence with *K. pneumoniae* was >132. The plazomicin AUC/MIC for 24-h static effect and –1 log reduction in *E. coli* and *K. pneumoniae* bacterial load was in the range 30–60. Plazomicin AUC/MIC targets aligned with those of amikacin for *E. coli*.

1. Introduction

Plazomicin (plazomicin sulfate, ACHN-490) is a new aminoglycoside that was developed to overcome aminoglycoside-modifying enzymes, the most common aminoglycoside resistance mechanism in Enterobacteriales, with in vitro potency against extended-spectrum β-lactamase (ESBL)-producing, carbapenemase-producing and aminoglycoside-resistant strains. Plazomicin has been studied in two phase 3 clinical trials. One evaluated the efficacy and safety of plazomicin versus colistin as part of definitive combination therapy for serious infections due to carbapenem-resistant Enterobacteriaceae. A variety of infections, including bloodstream infection, hospital-acquired or ventilator-associated pneumonia, complicated urinary tract infection (cUTI) and acute pyelonephritis, were included. When used as part of a combination regimen, plazomicin treatment was associated with reduced all-cause mortality at 28 days overall and in the subgroup of bloodstream infection patients compared with colistin combination treatment [1]. In a second study, the efficacy and safety of plazomicin monotherapy was assessed versus meropenem for the treatment of cUTI including acute pyelonephritis. Plazomicin met the primary objective of non-inferiority and demonstrated a significantly higher composite cure rate than meropenem [2]. Plazomicin was approved in June 2018 by the US Food and Drug Administration (FDA) for the treatment of cUTI caused by susceptible *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Enterobacter cloacae*.

The in vitro potency of plazomicin has been assessed as part of the ALERT (Antimicrobial Longitudinal Evaluation and Resistance Trends) global surveillance programme. When tested against isolates collected from 26 European and adjacent countries during 2014–2015, plazomicin showed MIC_{50}/MIC_{90} values (minimum inhibitory concentrations for 50% and 90% of the isolates, respectively) of 0.5/1 mg/L (range 0.12–16 mg/L) against *E. coli* and 0.25/0.5 mg/L (range ≤0.06 to >128 mg/L) against *K. pneumoniae* [3]. Furthermore, against all Enterobacteriales species, the plazomicin MIC_{50}/MIC_{90} values were 0.5/2 mg/L, whilst against carbapenem-resistant Enterobacteriales they were 0.25/128 mg/L

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[3]. Plazomicin has also been shown in vitro to have some antibacterial synergy with piperacillin/tazobactam and ceftazidime against multidrug-resistant Enterobacteriaceae, including aminoglycoside-resistant strains and those encoding β-lactamases (e.g. CTX-M-15, TEM) and carbapenemases (e.g. KPC, OXA-48) [4], however studies on the emergence of resistance are scarce.

Plazomicin pharmacokinetics for a 15 mg/kg dose has been studied in healthy volunteers. The peak serum concentration (C_{max}) was 76.0 ± 19.6 mg/L, the 24-h area under the concentration–time curve (AUC_{0-24}) was 263 ± 65.9 mg/L•h, the half-life ($t_{1/2}$) was 3.5 ± 0.5 h and the volume of distribution was 18.5 ± 4.7 L [5].

The aim of the current study was to define the exposure–effect relationship for plazomicin and a comparator aminoglycoside, amikacin, against a range of Enterobacteriales in terms of changes in bacterial load and risk of emergence of resistance as measured by changes in population profile and pathogen MIC. The results of such a body of work provide translational pharmacokinetic/pharmacodynamic (PK/PD) targets to help support and explain clinical trial findings in terms of microbiological outcome and emergence of resistance. In addition, the results of pre-clinical studies such as described here in conjunction with clinical data provide a rationale for the dosing regimen for plazomicin and its clinical breakpoints.

2. Materials and methods

2.1. Pharmacokinetic model

A FerMac 310 fermentation system (Electrolab, Tewkesbury, UK) in vitro pharmacokinetic model, as previously described [6], was used to simulate free drug concentrations of plazomicin or amikacin. Plazomicin and amikacin were administered once daily (every 24 h) or twice daily (every 12 h). The apparatus consists of a polycarbonate culture chamber with an additional chamber for infusion of plazomicin or amikacin ensuring the correct half-life, connected via aluminium and silicone tubing to a reservoir containing broth. The culture chamber was also connected to a waste via silicone tubing. The contents of the culture chamber were diluted with broth from the reservoir using an Ismatec pump (Cole Palmer, Hanwell, UK) to simulate the individual drug half-lives. The initial inoculum was 10^6 CFU/mL and the temperature was maintained at 37 °C with constant agitation.

2.2. Media, antimicrobial agents and bacterial counting

Mueller–Hinton broth (100%) (Thermo Fisher, Basingstoke, UK) was used for all experiments, and nutrient agar plates (Thermo Fisher) were used to recover bacterial strains from the model. Aliquots (1 mL) were taken from the central compartment at 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 36 and 48 h, the antibiotic was neutralised using ion exchange paper (P81) and the samples were then plated onto nutrient agar plates using a spiral plater (Don Whitley Scientific Ltd., Bingley, UK) for determination of total bacterial counts. A second untreated aliquot was plated onto nutrient agar plates containing 1 ×, 2 ×, 4 × and 8 × MIC of plazomicin or amikacin for the strain tested to detect changes in population profile. Any isolates growing on these plates were stored at −70 °C and subsequently had their plazomicin or amikacin MICs determined by agar dilution according to Clinical and Laboratory Standards Institute (CLSI) guideline [7]. MICs were assessed by concurrent agar dilution MIC testing of single survivors at 48 h and of parental (naïve) strains. The risk of emergence of resistance was stratified by AUC/MIC.

2.3. Drugs

Plazomicin was supplied by Achaogen (South San Francisco, CA, USA) and amikacin was supplied by Sigma (Poole, UK). Solutions were prepared according to the manufacturer's instructions.

2.4. Bacteria

Five strains of *E. coli*, three of which were supplied by JMI Laboratories (North Liberty, IA, USA), were employed. Of the three strains from JMI, strain AECO 1174 contained an ESBL and OXA-48 enzyme, with a meropenem MIC of 16 mg/L; strain AECO 1175 contained an ESBL and NDM like-enzyme, with a meropenem MIC of 4 mg/L; and strain AECO 1177 contained an ESBL, with a meropenem MIC of 0.03 mg/L. The other two *E. coli* were wild-type isolates from Southmead Diagnostic Laboratory (SMH 64979 and SMH 64982). Five strains of *K. pneumoniae* were also used, three of which were provided by JMI Laboratories. Of the three strains from JMI, AKPN 1169 contained an ESBL and KPC enzyme, with a meropenem MIC ≥ 32 mg/L; AKPN 1170 contained an ESBL, KPC and AAC(6′)-Ib enzyme, with a meropenem MIC of 8 mg/L, a gentamicin MIC of 4 mg/L and an amikacin MIC of 32 mg/L; and AKPN 1171 contained an ESBL enzyme, with a meropenem MIC of 0.03 mg/L. Two additional strains from the Southmead Diagnostic Laboratory collection (SMH 41965 and SMH 41966) were also used (Supplementary Table S1). MICs for amikacin and plazomicin were confirmed using the CLSI broth macrodilution method in triplicate for each strain [7].

2.5. Antibiotic assays

Plazomicin concentrations were determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS) by Alturas Analytics (Moscow, Russia). Amikacin was measured by QMS™ (quantitative microsphere system) immunoassay on an Indiko Plus Analyser (Thermo Fisher).

2.6. Pharmacokinetics

Dose-ranging experiments were performed based on the plasma concentration of a plazomicin once-daily 15 mg/kg dose, that is C_{max} = 75 mg/L after a 0.5-h infusion, total drug AUC = 246 mg/L•h and $t_{1/2}$ = 3.4 h. Amikacin dose ranging was also based on a 0.5-h infusion, with $t_{1/2}$ = 2.5 h and C_{max} = 50.0 mg/L. AUC/MICs ranged from 1.65–1056 for *E. coli* strains and from 8.25–880 for *Klebsiella* strains, with 7 to 12 drug exposures for each strain. As protein binding of both agents is low (<10%), it was not taken into account.

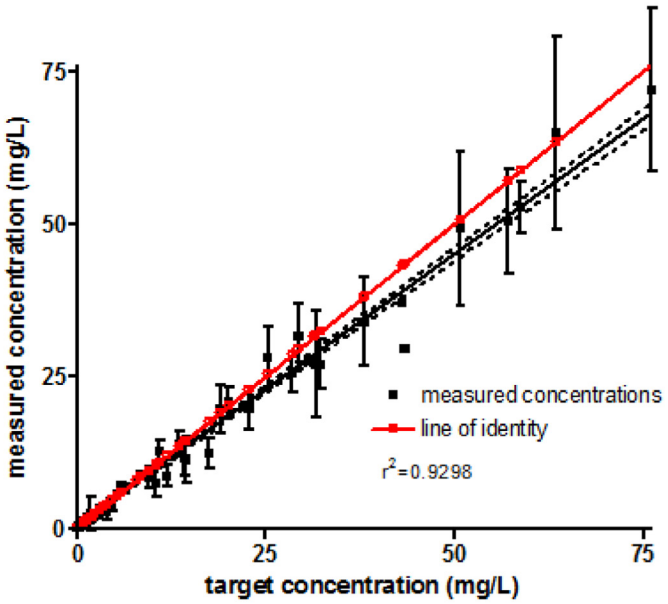
2.7. Data analysis

The relationship between AUC/MIC and antibacterial effect for each strain described by log reduction at 12, 24 and 48 h was described using a Boltzmann sigmoid E_{max} equation: Y = bottom+(top–bottom)/{1+exp[(V50–x)/slope]} using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA; https://www.graphpad.com). For the two species tested (*E. coli* and *K. pneumoniae*), the AUC/MIC values related to static and cidal effects were measured for each individual strain, and the mean ± standard deviation (S.D.) was calculated for each species.

2.8. Emergence of resistance

Changes in population analysis profiles for each strain were assessed by growth on plates containing 2 ×, 4 × and 8 × aminoglycoside MICs. Colonies growing on the 4 × or 8 × MIC plates at 48 h were subsequently assessed for MIC change.

a) Plazomicin



b) Amikacin

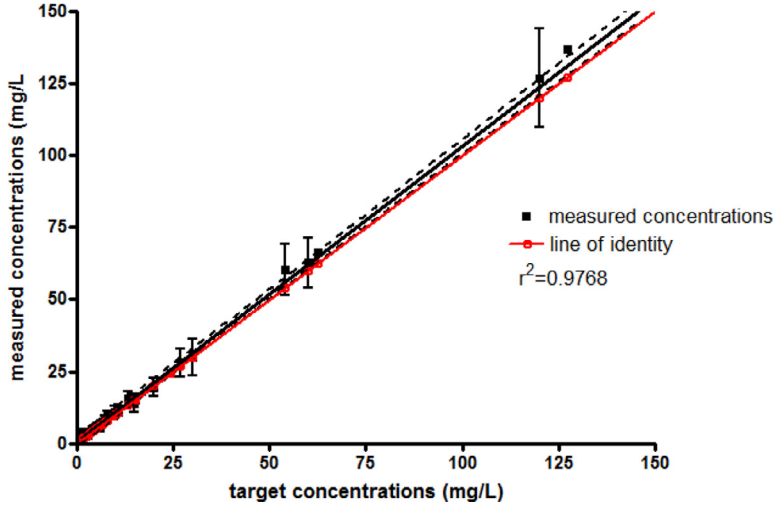


Fig. 1. Target and measured (mean ± standard deviation) concentrations for (a) plazomicin and (b) amikacin.

3. Results

3.1. Bacterial strains and drug pharmacokinetics

Five *E. coli* strains and five *K. pneumoniae* strains were employed in the various plazomicin and amikacin simulations. The median MICs of the triplicate tests for the *E. coli* strains were as follows: strain AECO 1174, amikacin MIC = 2 mg/L, plazomicin MIC = 0.5 mg/L; strain AECO 1175, amikacin MIC = 2 mg/L, plazomicin MIC = 1 mg/L; strain AECO 1177, amikacin MIC = 2 mg/L, plazomicin MIC = 2 mg/L; strain SMH 64979, plazomicin MIC = 2 mg/L; and strain SMH 64982, plazomicin MIC = 4 mg/L. The median plazomicin MICs for the *K. pneumoniae* strains were as follows: strain AKPN 1169, MIC = 1 mg/L; AKPN 1170 MIC = 1 mg/L; AKPN 1171, MIC = 2 mg/L; SMH 41965, MIC = 0.5 mg/L; and SMH 41966, MIC = 0.5 mg/L (Supplementary Table S1).

Target and measured concentrations were in good agreement, with $R^2 = 0.9298$ for plazomicin concentrations, and $R^2 = 0.9768$ for amikacin concentrations (Fig. 1).

3.2. Impact of dosing regimen on AUC/MIC targets

Three strains of *E. coli* were used (AECO 1174, AECO 1175 and AECO 1177) and once-daily (every 24 h) dosing of amikacin or plazomicin was compared with twice-daily (every 12 h) dosing to produce the same AUC_{0-24} (mg/L•h). AUC/MIC targets for static and –1, –2, –3 log drop in initial *E. coli* inoculum were calculated at 12, 24 and 48 h. For both plazomicin and amikacin, the AUC/MICs for

Table 1
Comparison of AUC/MIC ratios for plazomicin and amikacin against *Escherichia coli* ($n = 3$ strains) with 24-h and 12-h dosing

Antibacterial effect	Plazomicin AUC/MIC (mean ± S.D.)		Amikacin AUC/MIC (mean ± S.D.)	
	24-h dosing	12-h dosing	24-h dosing	12-h dosing
At 12 h				
Static	7.6 ± 2.5	9.4 ± 5.6	17.5 ± 18.3	16.1 ± 10.0
–1 log drop	14.5 ± 3.5	14.0 ± 6.4	26.4 ± 27.6	22.8 ± 12.5
–2 log drop	30.7 ± 9.3	23.8 ± 11.0	41.2 ± 42.0	32.4 ± 12.4
–3 log drop	81.4 ± 34.5	52.5 ± 38.9	68.8 ± 64.6	59.3 ± 11.9
At 24 h				
Static	141.3 ± 77.4	39.8 ± 24.9	96.3 ± 20.8	49.7 ± 12.7
–1 log drop	152.2 ± 88.4	44.3 ± 27.8	104.4 ± 22.8	55.7 ± 14.8
–2 log drop	164.9 ± 105.8	47.3 ± 28.5	112.0 ± 23.2	64.1 ± 19.2
–3 log drop	242.5 ($n = 2$)	52.3 ± 31.1	123.1 ± 31.8	73.3 ± 25.3
At 48 h				
Static	189.1 ± 78.6	71.6 ± 50.5	133.6 ± 52.3	78.6 ± 35.9
–1 log drop	221.7 ± 73.9	75.6 ± 48.9	143.4 ± 59.3	81.0 ± 36.6
–2 log drop	253.1 ± 67.0	77.5 ± 47.4	150.2 ± 68.0	81.8 ± 37.8
–3 log drop	370.9 ($n = 2$)	83.6 ± 44.9	170.9 ± 95.4	84.2 ± 38.9

AUC, area under the concentration–time curve; MIC, minimum inhibitory concentration; S.D., standard deviation.

Table 2
Plazomicin AUC/MIC values for static and log drop effects against *Escherichia coli*

Antibacterial effect	AUC/MIC					Mean ± S.D. (CV%)
	Strain AECO 1174 (MIC = 0.5 mg/L)	Strain AECO 1175 (MIC = 0.5 mg/L)	Strain AECO 1177 (MIC = 2 mg/L)	Strain SMH 64979 (MIC = 4 mg/L)	Strain SMH 64982 (MIC = 4 mg/L)	
At 24 h						
Static	67.3	18.6	33.5	33.1	28.2	36.1 ± 18.4 (51.0%)
–1 log drop	75.0	20.9	37.2	28.8	27.8	39.3 ± 20.9 (53.2%)
–2 log drop	79.1	23.9	38.9	35.3	28.8	41.2 ± 21.9 (53.3%)
–3 log drop	87.1	27.2	42.7	36.3	30.6	44.8 ± 24.3 (54.5%)
At 48 h						
Static	129.7	46.2	38.8	56.2	22.9	58.8 ± 41.5 (70.6%)
–1 log drop	131.8	52.5	42.6	57.5	25.6	62.0 ± 40.9 (65.9%)
–2 log drop	131.8	55.3	45.2	60.3	26.6	63.8 ± 40.1 (62.9%)
–3 log drop	134.9	64.6	51.3	61.4	31.6	68.8 ± 39.2 (56.9%)

AUC, area under the concentration–time curve; MIC, minimum inhibitory concentration; S.D., standard deviation; %CV, coefficient of variation.

static and –1 log drop in bacterial load were similar for once-daily and twice-daily dosing regimens at 12 h, but at 24 h and 48 h once-daily dosing simulations required markedly larger AUC/MIC values for the equivalent antibacterial effect, e.g. static effect (Table 1; Supplementary Figs S1 and S2). Once-daily dosing was also associated with changes in *E. coli* population profiles up to higher AUC/MIC values than twice-daily dosing (Supplementary Table S2). 48-h AUC/MIC targets were greater than those at 24 h both for plazomicin and amikacin (Table 1).

We also tried to more accurately simulate the impact of the plazomicin gamma phase on AUC/MIC targets, however validating such low plazomicin concentrations was not possible so this approach was not used further (Supplementary Table S3).

3.3. Plazomicin AUC/MIC targets for Escherichia coli and Klebsiella pneumoniae: bacterial load and changes in population profiles

AUC/MIC was related to log change in viable counts at 24 h and 48 h for each strain; the R^2 for curve fit varied from 0.8302–0.9997 depending on the strain. The individual strain AUC/MIC ratios for static effect and log drop in *E. coli* are shown in Table 2. The 24-h static, –1 log, –2 log and –3 log AUC/MIC plazomicin targets were 36.1 ± 18.4, 39.3 ± 20.9, 41.2 ± 21.9 and 44.8 ± 24.3, respectively. For the *K. pneumoniae* strains tested, the 24-h static, –1 log, –2 log and –3 log AUC/MIC targets for plazomicin were similar at 34.0 ± 15.2, 46.8 ± 27.8, 67.4 ± 46.5 and 144.3 ± 129.8, respectively (Table 3). Table 4 shows the population profiles for *E. coli* and *K. pneumoniae* summarising the growth on 4 × and 8 × MIC culture plates both in terms of the number of experimental simulations with each AUC/MIC that showed growth as well as the bacterial load on those plates. For the *E. coli* strains, no changes in population profile were noted up to 48 h for plazomicin AUC/MIC ratios of >66, while the equivalent value, with one exception, for *K. pneumoniae* strains was >132. Plazomicin MICs performed on colonies recovered from 4 × or 8 × MIC plates at 48 h had plazomicin MICs in the range 0.5–32 mg/L.

3.4. Amikacin AUC/MIC targets for Escherichia coli: bacterial load and changes in population profiles

AUC/MIC was related to log change in viable counts at 24 h and 48 h for each strain; the R^2 for curve fit varied from 0.7655–0.9974 for each strain. The individual strain AUC/MIC ratios for 24-h static, –1 log, –2 log and –3 log drop for 12-h dosing were 49.7 ± 12.7, 55.7 ± 14.8, 64.1 ± 19.2 and 73.3 ± 25.3, respectively (Table 1). Table 5 shows the population profiles for *E. coli* summarising the growth on 4 × and 8 × MIC plates at 24 h and 48 h. No changes in population profiles were seen over 48 h with an amikacin AUC/MIC of >57.7. Amikacin MICs performed on colonies recovered from 4 × or 8 × MIC plates at 48 h had amikacin MICs in the range 8–128 mg/L.

4. Discussion

Human studies of the aminoglycoside exposure–response relationship precede the development of the modern PK/PD drug assessment paradigm. Total drug gentamicin C_{max} values were related to clinical outcome in patients with serious Gram-negative

Table 3
Plazomicin AUC/MIC values for static and log drop effects against *Klebsiella pneumoniae*

Antibacterial effect	AUC/MIC					Mean ± S.D. (CV%)
	Strain AKPN 1169 (MIC = 1 mg/L)	Strain AKPN 1170 (MIC = 1 mg/L)	Strain AKPN 1171 (MIC = 2 mg/L)	Strain SMH 41965 (MIC = 0.5 mg/L)	Strain SMH 41966 (MIC = 0.5 mg/L)	
At 24 h						
Static	18.5	38.0	27.7	58.2	27.5	34.0 ± 15.2 (44.6%)
–1 log drop	33.7	42.7	34.9	95.5	27.2	46.8 ± 27.8 (59.3%)
–2 log drop	63.5	49.0	47.1	147.9	29.7	67.4 ± 46.5 (69.0%)
–3 log drop	226.5	57.5	65.0	333.4	39.2	144.3 ± 129.8 (89.9%)
At 48 h						
Static	11.5	49.6	65.8	103.5	68.4	59.7 ± 33.4 (55.9%)
–1 log drop	25.7	53.1	74.1	167.1	113.5	86.7 ± 55.2 (63.7%)
–2 log drop	59.3	59.3	84.7	251.2	184.1	127 ± 86.1 (67.4%)
–3 log drop	134.3	67.3	333.4	278.6		183.9 ± 115.9 (63.15%)

AUC, area under the concentration–time curve; MIC, minimum inhibitory concentration; S.D., standard deviation; %CV, coefficient of variation.

Table 4
Changes in population profiles of plazomicin in *Escherichia coli* and *Klebsiella pneumoniae*

AUC/MIC	Growth on plates containing:							
	E. coli				*K. pneumoniae*			
	At 24 h		At 48 h		At 24 h		At 48 h	
	4 × MIC	8 × MIC	4 × MIC	8 × MIC	4 × MIC	8 × MIC	4 × MIC	8 × MIC
0	2/5; 3.7[a]	0/5; –	2/5; 3.9	1/5; 3.9	3/4; 2.8 ± 0.2	0/4; –	2/5; 5.1	2/5; 4.8
33	1/5; 8.1	1/5; 5.3	2/5; 7.8	1/5; 8.0	3/5; 4.4 ± 1.2	3/5; 3.9 ± 1.6	4/5; 6.2 ± 0.2	1/5; 3.6
66	1/5; 6.0	0/5; –	1/5; 5.7	1/5; 5.7	2/5; 4.8	2/5; 4.8	3/5; 6.0 ± 1.1	2/5; 6.2
132	0/5; –	0/5; –	0/5; –	0/5; –	2/5; 3.6	1/5; 4.4	3/5; 4.2 ± 1.4	2/5; 5.2
198	0/2; –	0/2; –	0/2; –	0/2; –	0/5; –	0/5; –	0.4/–	0/5; –
264	0/4; –	0/4; –	0/4; –	0/4; –	0/5; –	0/5; –	0/5; –	1/5; 2.72

AUC, area under the concentration–time curve; MIC, minimum inhibitory concentration.
[a] Number of experiments with growth on MIC-containing recovery plates/total number of experiments performed; mean ± standard deviation bacterial count (log_{10} CFU/mL) on plates with growth.

Table 5
Changes in population profiles to amikacin in *Escherichia coli*

AUC/MIC	Growth on plates			
	At 24 h		At 48 h	
	4 × MIC	8 × MIC	4 × MIC	8 × MIC
0	3/3; 4.9 ± 3.3[a]	3/3; 3.5 ± 0.4	3/3; 4.1 ± 1.5	1/3; 3.8
15.4	3/3; 7.5 ± 1.2	2/3; 6.9	2/3; 8.2	3/3; 50. ±2.7
19.2	3/3; 7.4 ± 1.5	2/13; 7.1	2/3; 8.4	3/3; 5.1 ± 2.6
28.9	2/3; 8.3	2/3; 7.9	3/3; 8.0 ± 0.5	2/3; 8.2
38.5	2/3; 6.4	2/3; 6.4	3/3; 7.2 ± 2.0	2/3; 8.1
57.7	2/3; 5.7	2/3; 5.8	2/3; 8.1	2/3; 8.2
115.7	0/3; –	0/3; –	0/3; –	0/3; –
230.8	0/3; –	0/3; –	0/3; –	0/3; –

AUC, area under the concentration–time curve; MIC, minimum inhibitory concentration.
[a] Number of experiments with growth on MIC-containing recovery plates/total number of experiments performed; mean ± standard deviation bacterial count (log CFU/mL) on plates with growth.

infection from the urinary tract, wounds, lungs and bloodstream [8,9]. One of the first clinical descriptions linking a pharmacodynamic index to clinical response employed data on aminoglycosides; the C_{max}/MIC ratio showed a graded exposure–response after adjustment for underlying severity of illness [10].

Although total drug C_{max}/MIC ratio and C_{max} was related to outcome in these studies, this was mainly because aminoglycosides were monitored by trough and peak concentrations, but other pharmacodynamic indices could also be related to clinical outcome, e.g. AUC/MIC [11]. Subsequently, the relationship between the aminoglycoside C_{max}/MIC ratio and clinical outcome has been confirmed in hospital-acquired Gram-negative pneumonia, Gram-negative bloodstream infection in patients receiving haemodialysis, patients with severe sepsis and patients with Gram-negative ventilator-associated pneumonia [12–15]. Various C_{max}/MIC targets have also been suggested, e.g. C_{max}/MIC ratio of ≥10 [12], C_{max}/MIC ratio ≥6 [13] and C_{max}/MIC ratio ≥8 [14] for clinical response or microbiological cure. Clinical response has also been linked to AUC/MIC as well as C_{max}/MIC both for Gram-negative and Gram-positive infections treated with an aminoglycoside [12,16]. It is likely that AUC/MIC is the dominant driver for aminoglycoside efficacy in humans, as systematic reviews and meta-analyses of trials of single daily doses versus multiple daily doses of aminoglycosides have failed to show a consistent advantage in efficacy for single-dose therapy [17,18]. Despite this relative wealth of aminoglycoside PK/PD data in humans, there is little pre-clinical in vitro or in vivo data to help define pharmacodynamic index targets both for reduction in pathogen load and risk of resistance emergence. We recently summarised these data [19], which indicated that for gentamicin, amikacin and tobramycin, AUC/MIC targets for 24-h bacteriostatic effect were in the range 30–70 and those for –1 log kill were in the range 80–100. Our previous data derived from the same model system as used here indicated that for aerobic Gram-negative rods the mean ± S.D. for amikacin free-drug AUC/MIC associated with a 24-h static effect, –1 log drop and –2 log drop was 51.0 ± 26.7, 71.6 ± 27.6 and 92.2 ± 29.8, respectively [19].

Plazomicin pharmacodynamics have been studied in vitro and in vivo. As expected, plazomicin is bactericidal against Gram-negative rods, including those resistant to other aminoglycosides [20,21], and has a post-antibiotic effect against *E. coli* and *Klebsiella* spp. [22]. Based on data derived from a neutropenic murine thigh infection model of *Klebsiella* spp. and carbapenem-resistant Enterobacteriales, the median (range) AUC/MIC for net bacterial stasis effect was 23.6 (5.7–49.4), whilst that for a –1 log drop was 85 (8.1–518.3) [23,24]. Based on a murine lung infection model, the

median (range) plasma AUC/MIC target associated with a –2 log drop was 38 (16–137). In this study, AUC/MIC was identified as the dominant pharmacodynamic driver [25]. Data from the current study using an in vitro pharmacokinetic model indicate similar results to those previously obtained in murine thigh and lung infection model, with an mean ± S.D. (range) AUC/MIC ratio for 24-h bacteriostatic effect for *E. coli* or *Klebsiella* spp. of 36.1 ± 18.4 (18.6–67.3) and 34.0 ± 15.2 (18.5–58.2), respectively. Equivalent values for a –2 log drop were 41.2 ± 21.9 (23.9–79.1) for *E. coli* and 67.4 ± 46.5 (29.7–147.9) for *K. pneumoniae*. The AUC/MIC ratios associated with –1 log drops are less in the in vitro model compared with in animals; however, bactericidal activity is easier to demonstrate in vitro than in vivo. Plazomicin AUC/MIC targets in this study were similar to those of amikacin for *E. coli*, confirming that plazomicin pharmacodynamics are aligned within the aminoglycoside class.

In addition to defining AUC/MIC targets for bacteriostatic and cidal effects, we were also able to study the impact of plazomicin exposures on changes in bacterial population profiles and MICs for *E. coli*. Plazomicin AUC/MIC ratios of >66 were able to suppress the emergence of resistance with *E. coli*, whilst for *K. pneumoniae* the equivalent ratio was >132. Amikacin AUC/MIC ratios of >57.7 abolished changes in *E. coli* population profiles, which is similar to the previous value we reported with amikacin (AUC/MIC > 60) [19]. The observation that the AUC/MIC ratio required to prevent changes in population profiles is similar for plazomicin and amikacin and also similar to the AUC/MIC value for 24-h bacteriostatic effect is also in keeping with much other data [26].

Of interest is the effect of frequency of dosing of plazomicin and amikacin on the AUC/MIC targets for reduction in bacterial load and emergence of resistance. Administration twice-daily and once-daily produced equivalent AUC/MIC values at 12 h post-dosing, however with 24-h dosing AUC/MIC values were notably higher at 24 h and 48 h and were not in alignment with previously described in vitro data [19]. For this reason, we elected to use twice-daily aminoglycoside dosing to define AUC/MIC targets in the in vitro model. Similarly, in this pre-clinical model, twice-daily dosing was also more effective at suppressing changes in population profiles.

In conclusion, these data show that plazomicin pharmacodynamics is similar to amikacin as a comparator agent and that a plazomicin AUC/MIC target of 30–60 for reduction in bacterial load is suitable for translation into human PK/PD modelling. An AUC/MIC ratio of >130 is associated with suppression of resistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.07.001.

Declarations

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Competing interests: AK is a stockholder in Achaogen; KMK is an employee and stockholder in Achaogen; APM has received commercial grants from Merck, Paratek, VenatoRx, Bayer, Achaogen, AiCuris, Polyphor, Pfizer and Roche; IMI Industrial Partners – AiCuris, Melinta, MedImmune (AZ); Public Sector – NIHR England, MRC (UK), Innovate UK, Newton Fund (FCO). All other authors declare no competing interests.

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