Efficacy of EBL-1003 (apramycin) against Acinetobacter baumannii lung infections in mice

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Abstract

Objectives: Novel therapeutics are urgently required for the treatment of carbapenem-resistant Acinetobacter baumannii (CRAB) causing critical infections with high mortality. Here we assessed the therapeutic potential of the clinical-stage drug candidate EBL-1003 (crystalline free base of apramycin) in the treatment of CRAB lung infections.

Methods: The genotypic and phenotypic susceptibility of CRAB clinical isolates to aminoglycosides and colistin was assessed by database mining and broth microdilution. The therapeutic potential was assessed by target attainment simulations on the basis of time-kill kinetics, a murine lung infection model, comparative pharmacokinetic analysis in plasma, epithelial lining fluid (ELF) and lung tissue, and pharmacokinetic/pharmacodynamic (PKPD) modelling.

Results: Resistance gene annotations of 5451 CRAB genomes deposited in the National Database of Antibiotic Resistant Organisms (NDARO) suggested >99.9% of genotypic susceptibility to apramycin. Low susceptibility to standard-of-care aminoglycosides and colistin was assessed by database mining and broth microdilution. The therapeutic potential was assessed by target attainment simulations on the basis of time–kill kinetics, a murine lung infection model, comparative pharmacokinetic analysis in plasma, epithelial lining fluid (ELF) and lung tissue, and pharmacokinetic/pharmacodynamic (PKPD) modelling.

Conclusions: This study provides proof of concept for the efficacy of EBL-1003 in the treatment of CRAB lung infections. Broad in vitro coverage, rapid killing, potent in vivo efficacy, and a high probability of target attainment render EBL-1003 a strong therapeutic candidate for a priority pathogen for which treatment options are very limited.

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Introduction

Carbapenem-resistant Acinetobacter baumannii (CRAB) strains are characterized by nosocomial transmission, high-level drug resistance, and high mortality. CRAB has consistently been ranked at the top of both the World Health Organization’s (WHO’s) list of
crucial priority pathogens and the US Centers for Disease Control and Prevention's (CDC's) most urgent health threats [1,2]. CRAB strains are primarily of concern in ventilator-associated bacterial pneumonia (VABP) and bloodstream infections (BSIs) in critical ill patients, and treatment options often rely on combination therapy that includes a last-resort drug, often colistin [3,4]. Drug resistance is prevalent among clinical A. baumannii isolates, and the proportion of CRAB strains has reached a concerning level of up to 90% in some parts of the world [5].

Aminoglycosides used to play an important role in treatment of CRAB, representing a common constituent in drug combination therapy. However, the use of standard-of-care aminoglycosides such as amikacin is frequently compromised by increasing levels of drug resistance [6]. Various mechanisms confer resistance to aminoglycoside in A. baumannii, including 16S ribosomal RNA methyltransferases (RMTases) or aminoglycoside-modifying enzymes (AMEs) such as N-acetyltransferases (AACs), O-phospho-transferases (APHs), and O-nucleotidyltransferases (ANTs) [7,8]. RMTase-mediated pan-aminoglycoside resistance has been observed with particularly high prevalence in CRAB and other carbapenem-resistant Gram-negative isolates, stressing the serious threat of antimicrobial resistance and the pressing need for new treatment options [9,10].

EBL-1003, a crystalline free base of apramycin, is a candidate drug currently in clinical development with support from the Innovative Medicines Initiative's European Gram-Negative Antibacterial Engine (IMI ENABLE) [11]. Based on its unique structure, comprising an unusual bicyclic octose moiety, apramycin evades almost all clinically relevant AMEs and is also unaffected by RMTase-mediated pan-aminoglycoside resistance [12]. Modification by the N-acetyltransferase AAC(3)-IV remains the sole clinically relevant resistance mechanism for apramycin [12,13]. Several research groups have independently reported outstanding in vitro activity of apramycin against highly drug-resistant clinical isolates of A. baumannii [12,14,15].

Based on these encouraging reports on apramycin activity, we sought proof of concept for the therapeutic efficacy of EBL-1003 in pulmonary infections, and insights into lung penetration and therefore the probability of target attainment (PTA) in respiratory CRAB infection.

Methods

We analysed the resistance gene annotations of CRAB isolates deposited in the National Database of Anti- biotic Resistant Organisms (NDARO) and subsequently assessed the activity of EBL-1003 against a panel of clinical A. baumannii isolates. The efficacy of EBL-1003 against CRAB was further investigated in a murine lung infection model. We used matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to visualize drug distribution in the lung. Lastly, we studied the pharmacokinetics and pharmacodynamics (PKPD) to assess the PTA in epithelial lining fluid (ELF). Full details of all methods are provided in the web-only Supplementary Material.

Results

Genotypic analysis of CRAB clinical isolates

First, we wanted to understand the prevalence of aminoglyco-side resistance in a large panel of CRAB isolates. Towards this end we analysed the resistance gene annotations of 5707 clinical A. baumannii isolates deposited in the National Database of Anti- biotic Resistant Organisms (NDARO) and classified 5451 isolates (95.5%) as carbapenem-resistant (CRAB) based on carbapenemase annotations in those genomes; 4305 (78.0%) of these CRAB genomes contained at least one aminoglycoside resistance gene. The most prevalent aminoglycoside resistance genes were aph(3’)-I, annotating to 2275 (41.7%) CRAB genomes, followed closely by the RMTase armA (2164; 39.7%) and the AMEs aac(6’)-I (1507; 27.7%), aph(3’)-IV (1366; 25.1%), and aac(3)-I (1002; 18.4%) (Fig. 1A); aac(3)-IV was the least prevalent aminoglycoside resistance gene and annotated to only one (0.02%) of the 5451 CRAB genomes. The only colistin resistance gene in the CRAB isolates was mcr-4 found in two genome annotations (0.04%).

From the annotation of aminoglycoside resistance genes, we inferred an aminoglycoside resistance for each isolate that we translated into genotypic resistance to individual aminoglycosides, and the lack of any resistance gene into genotypic susceptibility (Fig. 1B, Supplementary Material Table S1). This allowed us to compare the genotypic susceptibility to apramycin in deposited NDARO isolates with that to other aminoglycosides and colistin. Genotypic susceptibility of CRAB isolates to apramycin and to colistin was >99.9%. The genotypic susceptibility to standard-of-care aminoglycosides was considerably lower than for apramycin or colistin.

Antimicrobial susceptibility of CRAB clinical isolates

Next, we determined the in vitro activity of EBL-1003 in comparison to standard-of-care drugs in a panel of 100 geographically and phenotypically diverse A. baumannii isolates, of which 86 were identified as CRAB (meropenem MIC $\geq 4$ μg/mL). In the overall panel, 99 A. baumannii isolates were susceptible to EBL-1003 (applying an apramycin epidemiological cut-off value (ECOFF) of 16 μg/mL [12]), and the MIC90 for EBL-1003 was 8 μg/mL. The MIC0, amikacin, tobramycin, gentamicin and plazomicin was $\geq 128$ μg/mL. Of the 86 CRAB isolates, 85 were apramycin susceptible and 79 showed susceptibility to colistin (MIC <2 μg/mL) while all other tested drugs failed to provide sufficient coverage of the CRAB isolates in the panel (Fig. 2). All of the 68 A. baumannii isolates with a pan-aminoglycoside-resistant phenotype had an apramycin MIC of $\leq 16$ μg/mL. Notably, 63 A. baumannii isolates and 57 CRAB isolates (66.3%) were confirmed respiratory origin.

The in vitro activity of EBL-1003 against CRAB led us to further explore the bactericidal activity in time-kill experiments. For subsequent analyses we selected the XDR strain A. baumannii AR Bank #0282, which is characterized by OXA-23- and OXA-66-mediated carbapenem resistance [16]. The MIC of EBL-1003 was determined as 4 μg/mL for this strain (Supplementary Material Table S2). Concentrations as low as 3 μg/mL (0.75-fold MIC) resulted in CFU reductions, and concentrations of $\geq 16$ μg/mL (≥4-fold MIC; $\frac{1}{4}$ of Cmax observed in mice after a single administration of 20 mg/kg) irreversibly suppressed regrowth of persister phenotypes (Supplementary Material Fig. S1).

Lung efficacy dose fractionation studies, lung PK, and MALDI-MS imaging

We then tested the efficacy of EBL-1003 in a murine A. baumannii lung infection model with the strain AR Bank #0282. All mice survived the predefined study period of 26 h post infection. A subcutaneous dose of 5 mg/kg EBL-1003 at q6h (total daily dose of 20 mg/kg) resulted in a $>2$-$\log_{10}$ (>99%) reduction in CFU counts in lung tissue at 24 h when compared with the start of treatment. Doses of $>5$ mg/kg at q6h resulted in a $>4$-$\log_{10}$ (>99.9%) reduction in bacterial burden (Fig. 3A, Supplementary Material Fig. S2).

The pharmacokinetics and lung penetration of EBL-1003 were assessed in the same lung infection model as the efficacy. For a single dose of 20 mg/kg, the Cmax in plasma was 67 μg/mL at
$T_{\text{max}} = 0.167$ h. Overall, the $C_{\text{max}}$ of EBL-1003 in the ELF was lower than in plasma, but the AUC in ELF resembled the AUC in plasma (Fig. 3B,C). Hence, EBL-1003 penetrated into the lung efficiently and reached an AUC$_{0-24,\text{ELF}}$/AUC$_{0-24,\text{plasma}}$ ratio of 0.88 at a dose of 125 mg/kg. The AUC$_{\text{plasma}}$ of 339 h × μg/mL for this dose in mice roughly corresponds to the expected therapeutic AUC in humans (Fig. 3B). The human dose predicted to achieve a similar AUC$_{\text{plasma}}$ exposure level is approximately 30 mg/kg [17].

Tissue distribution of EBL-1003 in the lungs was further studied by MALDI-MSI after subcutaneous administration in healthy mice. We were thus able to visualize the penetration of EBL-1003 into the lung tissue. The MS images indicated that EBL-1003 was distributed evenly across the entire lung, and confirmed peak tissue concentration at about one hour post administration. Drug clearance over time in lung tissue was in agreement with decreasing drug concentration in ELF (Fig. 3D).
PKPD and probability of target attainment (PTA) analysis

Next, we integrated plasma and ELF data with the results of the efficacy study (pharmacodynamic, PD) to study which PK/PD index reveals the best correlation. PK/PD indices based on plasma and ELF concentrations had similar correlations. The two indices $\frac{\text{AUC}_{\text{ELF}}}{\text{MIC}}$ and $\frac{\text{C}_{\text{max,ELF}}}{\text{MIC}}$ showed good correlation with $R^2$ values of 0.82 and 0.87, respectively, whereas $\frac{T}{\text{MIC}}$ showed lower $R^2$ values of 0.59–0.66. The AUC and the $C_{\text{max}}$ were therefore much better predictors of CFU reduction in lung than the time over MIC. $\frac{\text{AUC}_{\text{ELF}}}{\text{MIC}}$ target values for stasis, $1\text{-log}_{10}$ and $2\text{-log}_{10}$ CFU reduction in lung tissue after a single subcutaneous dose of 400 mg/kg in healthy mice. Tissue sections were analysed by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) quantifying EBL-1003 relative to kanamycin as internal standard. Ion distribution images of $m/z$ 578.24 (potassiated EBL-1003) at (I) 1 h, (II) 2 h, (III) 4 h post administration, and (IV) in control tissue. Ion intensities are shown using a rainbow scale with maximum intensity scaled to 3%. Scale bar 1 mm; lateral resolution 100 µm.

Discussion

The findings in this study provide the first proof of concept for lung penetration and the efficacy of apramycin in lung infections in mice. Results were assessed in a PKPD evaluation that supports target attainment in ELF for the predicted therapeutic dose of EBL-1003 in humans. The study further confirms and extends on previous reports of the superior in vitro activity of apramycin against Acinetobacter baumannii clinical isolates by analysing the relevant resistance gene annotations in an unprecedented high number of 5451 CRAB genomes.

In CRAB genomes deposited in NDARO, we screened for gene annotations and inferred a resistance phenotype based on this genotypic information which allowed us to assess a large number of isolates. However, in this screening, unspecified resistance mediated by drug efflux and potential gradual effects on susceptibility (intermediate versus resistant) could not be considered, and thus genotypic assessment might not in all cases fully reflect the phenotype. Based on AMEs and RMTases as the major aminoglycoside resistance mechanisms in CRAB, we inferred a very high genotypic susceptibility from the low prevalence of apramycin and colistin resistance genes in CRAB. Colistin is a last-resort drug often used in therapeutic regimens for CRAB infection, but is only used in patients with limited treatment options because of the very high nephrotoxicity of this drug [3,18,19]. Our database analysis suggested high susceptibility to apramycin, rendering EBL-1003 an alternative candidate drug with a coverage of CRAB isolates that compares well with that of colistin. Our data also provide a
rationale for limited clinical utility of other aminoglycoside antibiotics in CRAB infections, providing further support for the specific differentiation of EBL-1003 within its own drug class.

Both EBL-1003 and colistin also showed very good in vitro coverage of 100 clinical A. baumannii isolates, the majority of which were CRAB and/or pan-aminoglycoside-resistant. Time-kill experiments confirmed rapid multi-log bactericidal activity of EBL-1003 against the XDR A. baumannii isolate AR Bank #0282. Phenotypic assessments in the present and in previous studies are in agreement with the genotypic resistance profiling of the CRAB genomes deposited in the NDARO, indicating that EBL-1003 and colistin are the only two effective antibiotics in the panel tested against CRAB. Although a relatively small number of isolates was assessed in vitro in comparison to the genotypic analysis, the results are generally in agreement with previous studies on the susceptibility of A. baumannii and other Gram-negatives to apramycin [12,15,20,21]. Genetic information was unavailable for the isolates tested in this study, but it is reasonable to assume that the single clinical isolate resistant to EBL-1003 carries the aac(3)-IV resistance gene, which has been described as the only apramycin resistance mechanism of clinical relevance [12,13]. Notably, a significant proportion of the clinical isolates included in our in vitro assays were of respiratory origin, and respiratory CRAB infections have been reported as one of the largest hospital burdens, with colistin often left as the only effective treatment option currently available on the market [18,22].

The in vivo efficacy of apramycin has previously been demonstrated by James Kirby and colleagues in a murine thigh infection model [23]. To our best knowledge, this was the first and thus far the only proof of concept for the remarkable efficacy of apramycin
against *A. baumannii*. The outstanding *in vitro* activity of EBL-1003 in our murine *A. baumannii* lung infection model supports the notion of apramycin as a potent alternative to colistin in the treatment of respiratory CRAB infections, and provides proof of concept for the efficacy in lung infections as well. As was expected, based on the *in vitro* time–kill analysis, apramycin led to highly potent killing of *A. baumannii* AR Bank #0282 with low fAUC/MIC targets in both ELF and plasma.

The excellent efficacy of EBL-1003 in the lungs of infected mice indicated sufficient penetration into lung tissue and ELF. Indeed, our pharmacokinetic studies revealed a high AUC in ELF resembling the AUC in plasma, suggesting a lung penetration of 88% at a dose predicted to result in human therapeutic plasma exposure. MS imaging suggested even tissue distribution of the candidate drug. The human AUC lung penetration ratio of aminoglycosides has been reported to range from a low 13% for plazomicin to a high 77% for gentamicin [24–26]. High lung penetration and efficacy of EBL-1003 in mice warrants further clinical assessment of EBL-1003 to demonstrate sufficient drug penetration into human ELF after parenteral administration in humans.

In our model, the EBL-1003 exposure in plasma and ELF (fAUCplasma and fAUCELF, respectively) achieved by doses corresponding to the predicted therapeutic dose for systemic *Escherichia coli* infections in humans (30 mg/kg) were considerably higher than the predicted target values for *A. baumannii* [17]. PK/PD predictions suggested >99.9% PTA for MIC < 8 μg/mL in the lungs of healthy individuals and even higher PTA in patients with a reduced (80 mL/min) creatinine clearance, representative of typical patients in the target patient population [27,28]. In the absence of a model for the human PK of EBL-1003, human concentration time profiles were simulated from a model of human PK of gentamicin. While being an assumption, this hypothesis is backed up by the chemical similarity of the two molecules and the similarity of their PK in various animal species [17].

The present study emphasizes the great potential of EBL-1003 in the treatment of *A. baumannii* infections based on its unprecedented coverage of multidrug-, carbapenem- and aminoglycoside-resistant isolates, its efficient lung penetration, and its promising PTA. Intrinsically lower toxicity of EBL-1003 has been proposed to provide for an improved safety margin when compared to colistin or other aminoglycosides antibiotics [29,30].

The WHO has not only prioritized CRAB infections as the most critical medical need in antibacterial therapy, as indicated above. It has also monitored the clinical development activities in the field to find a critical gap between the prioritized pathogens and the current clinical pipeline [11]. The current pipeline is dominated by β-lactam/β-lactamase inhibitor combinations, which have in part been successful in addressing critical infections with carbapenem-resistant *Enterobacteriales* (CRE), but have demonstrated little benefit in treating *P. aeruginosa* and *A. baumannii*. Of the 52 antibiotics listed in the last WHO clinical pipeline report in 2019, EBL-1003 is one of only five candidate drugs that may hold promise for efficacy against CRAB infections. Further evaluation in patients will be required to underscore the clinical utility and lasting success of new antibiotics in the treatment of CRAB infections.

**Author contributions**

KB and SNH analysed the NDARO data base. KB, KH, SC, DH and SNH coordinated the in vitro microbiology work. SC performed time–kill experiments. KB and SNH coordinated the in vivo microbiology including plasma and ELF pharmacokinetics. AN, RS, BP and PEA contributed to the MS imaging of lung tissue. VAC and LEF performed PK analysis. PKPD evaluation, and PTA simulations. LEF and SNH conceptualized the study. KB, VAC, ECB, LEF and SNH wrote the manuscript. All authors reviewed and approved the manuscript.

**Transparency declaration**

ECB and SNH are cofounders and shareholders of Juvabis AG (Zurich, Switzerland). All other authors declare no competing interests for this work. The University of Zurich has utilized the non-clinical and preclinical services program offered by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health. Some of the research leading to these results was conducted as part of the ND4BB European Gram-Negative Antibacterial Engine (ENABLE) Consortium (www.nd4bb-enable.eu) and has received funding from the Innovative Medicines Initiative Joint Undertaking (www.imi.europa.eu) under grant agreement no 115583, resources of which are composed of financial contributions from the European Union’s Seventh Framework Programme (FP7/2007-2013) and The European Federation of Pharmaceutical Industries and Associations (EFPIA) companies in-kind contribution. The ENABLE project is also financially supported by contributions from academic and small and medium-sized enterprise (SME) partners. This work was further supported by the Swedish Research Council (grant number 2018-05501), the Swedish Foundation for Strategic Research (grant number RIF14-0078), and the Science for Life Laboratory to PEA. This work was further supported by the University of Zurich, Institute of Medical Microbiology.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.12.004.

**References**


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