In-vivo Efficacy of Apramycin in Murine Infection Models

Martin Meyer¹, Pietro Freihofer¹, Michael Scherman², Joanne Teague³, Anne Lenaerts², Erik C. Böttger¹*

¹ Institut für Medizinische Mikrobiologie, Nationales Zentrum für Mykobakterien, Universität Zürich, Zürich, Switzerland
² Mycobacterial Research Laboratories, Department of Microbiology, Colorado State University, Fort Collins, Colorado, USA
³ Euprotec Limited, Manchester, United Kingdom

* corresponding author

Institut für Medizinische Mikrobiologie
Universität Zürich
Gloriastrasse 30/32
8006 Zürich
Switzerland

phone: +41 44 634 26 60
fax: +41 44 634 49 06
email: boettger@imm.uzh.ch

Running title: Apramycin in-vivo efficacy

Keywords: Aminoglycosides, apramycin, in-vivo efficacy, tuberculosis, MRSA, septicaemia, ototoxicity
Abstract

Apramycin is a unique aminoglycoside with a dissociation of antibacterial activity and ototoxicity. We assessed the antibacterial efficacy of apramycin in two murine models of infection: *Mycobacterium tuberculosis* aerosol infection and *Staphylococcus aureus* septicaemia. In both infection models the efficacy of apramycin was comparable to that of amikacin. These results suggest that apramycin has the potential to become a clinically useful agent against drug resistant pathogens and support further development of this promising unique aminoglycoside.
Emerging resistance globally threatens the efficacy of antibiotics (1), arguably one of the most significant achievements in human medicine. The prospects for novel classes of antibiotics are scarce (2) and there is an urgent need to improve available antibiotic compounds (3).

The first aminoglycoside, streptomycin, was introduced almost 70 years ago and since then numerous aminoglycosides have been isolated, and new derivatives have been synthesized (4). Aminoglycosides have broad-spectrum activity and are used for treatment of serious life-threatening infections, including tuberculosis (5). As with all antibiotics, resistance to aminoglycosides has evolved in the past decades and significantly impairs the clinical utility of these compounds (6). Resistance to aminoglycosides is conferred primarily by aminoglycoside-modifying enzymes, which inactivate these compounds by various modifications of their NH₂ or OH substituents (7). In addition, methyltransferases that modify bacterial rRNA, the molecular target of aminoglycosides, confer high-level resistance to all clinically used aminoglycosides (8, for review 9), including the most recently developed plazomicin, a semi-synthetic aminoglycoside that has recently completed phase II clinical trials for complicated urinary tract infections (10, 11).

Apramycin is a structurally unique aminoglycoside, characterized by a bicyclic sugar moiety and a 4-monosubstituted 2-deoxystreptamine ring (12). In-vitro apramycin shows broad-range antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria including Pseudomonas aeruginosa (13, 14). As a result of its unique structure, apramycin is not inactivated by most of the known aminoglycoside-modifying enzymes (14), including N-acetytransferases (AAC), O-nucleotidyltransferases (ANT) and O-phosphotransferases (APH). In addition, apramycin has been found to be the only aminoglycoside active against producers of rRNA methylases (11). Apramycin also shows high antimycobacterial activity in-vitro (14), including multidrug-resistant strains of Mycobacterium tuberculosis and rapidly growing nontuberculous mycobacteria, such as M. abscessus, M. massiliense and M. bolletii, which are
difficult to treat, obnoxious lung pathogens in patients with cystic fibrosis or bronchiectasis (15).

Of note, the antimycobacterial activity of apramycin \textit{in-vitro} matches that of amikacin, an established second-line antituberculous agent and reference aminoglycoside used for treatment of MDR tuberculosis and for treatment of lung infections with \textit{M. abscessus} (15, 16).

The clinical use of aminoglycosides is limited by their toxicity, in particular ototoxicity. The ability of these compounds to cause irreversible hearing damage affects ~20% of patients following brief courses of treatment and more than 90% of patients after long-term regimens (17, 18). Aminoglycoside ototoxicity is linked to the destruction of the sensory cells of the inner ear and consistently has been associated with both natural and semi-synthetic aminoglycosides (17).

However, we have recently made the surprising observation that apramycin shows a unique dissociation of antibacterial activity and ototoxicity, conferring it a low ototoxic potential (14). The low ototoxicity of apramycin together with its promising \textit{in-vitro} antibacterial activity prompted us to investigate the \textit{in-vivo} efficacy of this unique aminoglycoside for treatment of infectious diseases.

The \textit{in-vivo} efficacy of apramycin as antituberculous agent was tested in a low-dose aerosol infection model in female IFN-gamma knock-out mice (Jackson Laboratories, Bar Harbour, ME) as described (19, 20). Evaluation of the antituberculous activity of apramycin \textit{in-vivo} was performed in an acute \textit{M. tuberculosis} infection model to provide an assessment of drug efficacy against replicating bacteria. The animal efficacy studies were performed according to the guidelines of the Colorado State University Institutional Animal Care and Use Committee, and approved under Protocol Number: 13-4263A. Mice (5-6/group) were challenged with an aerosol of \textit{M. tuberculosis} Erdman (TMC 107, ATCC 35801) via a GlasCol aerosol chamber in a certified ABSL-3 laboratory according to guidelines of the Institutional Animal Care and Use Committee. Mice were infected in a single aerosol run (target dose 100 CFU per mouse). Control mice were sacrificed the day after aerosol challenge to enumerate the bacteria instilled in the
lungs after infection, which was approximately 140 CFU. The MIC of *M. tuberculosis* Erdman to apramycin and to the comparator amikacin is 1.5 mg/L for both compounds. Starting 13 days after challenge, mice received drug (200 mg/kg subcutaneous) once daily for 9 consecutive days. For endpoint analysis, mice were euthanized and lungs collected at day 22 following challenge. The left lung lobe was homogenized for enumeration of CFUs by plating dilutions of the organ homogenates on 7H11 agar plates. The CFUs were converted to logarithms, which were then evaluated by a one-way ANOVA analysis of variance followed by a multiple comparison analysis of variance using the Tukey test (SAS Software program, Research Triangle Park, NC). Differences were considered significant at the 95% level of confidence. Figure 1 shows the CFUs in the lungs of mice following experimental infection. Apramycin demonstrated significant *in-vivo* efficacy in reducing bacterial burden in lungs when compared to saline controls (p <0.001), showing a 2.4 log_{10} CFU reduction versus the control after 9 consecutive days of treatment. Apramycin’s antituberculous activity compared well to that of amikacin (1.8 log_{10} reduction, p >0.05), an aminoglycoside and well established second-line agent for treatment of multidrug-resistant tuberculosis.

The *in-vivo* efficacy of apramycin as a broad-range antibacterial agent was tested in a neutropenic model of *Staphylococcus aureus* sepsicaemia (21). The animal experiments were carried out at Euprotec Limited, Manchester, United Kingdom, under UK Home Office Licenses with ethical approval from the University of Manchester Ethics committee. Outbred male mice (strain Hsd:ICR CD-1) were supplied by Charles River UK. Mice were rendered temporarily neutropenic by immunosuppression with cyclophosphamide at 200mg/kg 4 days before infection and 150mg/kg 1 day before infection by intraperitoneal injection. The immunosuppression regimes lead to neutropenia starting 24 hours post administration which continues throughout the study. For *in-vivo* infection a methicillin resistant strain of *Staphylococcus aureus*, clinical isolate MRSA AG041, was used – for the MIC profile of MRSA strain AG041 see Table 1. 24
hours post the second round of immunosuppression mice were infected with *S. aureus* MRSA AG041 by intravenous injection into the lateral tail vein using ~1 x10^7 CFU/mouse. Apramycin was administered at 16, 32 and 80 mg/kg / dose (equivalent to 2 times, 4 times and 10 times the MIC value in mg/kg). Linezolid (20 mg/kg / dose) was used as positive control. Antibacterial treatment was initiated 1 hour post infection and delivered subcutaneously at 10 mL/kg (linezolid was given by intravenous bolus injection). All drugs were administered at 1, 9 and 17h post infection. At 1h (pre-treatment group) or 24h post infection blood samples were collected, and at 24h post infection kidneys were removed for CFU determination. The data for apramycin were generated in two different and independent experiments, both of which are given in Fig. 2. Figure 2 shows the CFUs in mice with experimental septicaemia. In animals infected with methicillin-resistant *S. aureus*, treatment with apramycin reduced the bacterial burden both in blood and in kidney. Compared to the vehicle treated mice, drug treatment reduced bacterial burden in the kidneys between 2 to 5 log_{10} and in blood between 2 to 3 log_{10} in a dose-dependent manner.

Our data show that the excellent antibacterial *in-vitro* activity of apramycin reported previously for a wide range of infectious pathogens (14), including strains resistant to multiple antibiotics, extends to *in-vivo* activity in two different standard models of infection: experimental *S. aureus* septicaemia and *M. tuberculosis* pneumonia. While apramycin’s unique structure precludes its inactivation by most of the known aminoglycoside-modifying enzymes, mutational alterations of 16S rRNA which confer resistance to amikacin and kanamycin in *M. tuberculosis* and *M. abscessus* (22-25) also confer resistance to apramycin (14). The *in-vivo* efficacy of apramycin was comparable to that of the comparator aminoglycoside amikacin. As with all animal models, the results presented here do not ensure efficacy of apramycin in treatment of human infectious diseases. However, the demonstration of efficacy in mouse infection models illustrates the potential clinical use of this unique aminoglycoside against serious infections. The mouse
aerosol infection and septicaemia model used here are established models to measure pre-clinically the efficacy of antibiotics (19-21).

The need for new antibiotics active against antibacterial-resistant microbes is undisputed. Emerging and high-levels of antibiotic resistance including various pathogens are observed in all areas of the world. The emergence and spread of carbapenem-resistant *Enterobacteriaceae* endowed with rRNA methylases conferring resistance to all clinically used aminoglycosides (11, 26), has added further urgency to the situation, given that carbapenem and aminoglycoside antibiotics have been the last resort for treating infections by multidrug-resistant *Enterobacteriaceae* (27). Our results on the *in-vivo* efficacy of apramycin in established murine models of infection testify to the potent antibacterial activity of this compound and together with its promising biocompatibility (14) support the further evaluation of the safety and efficacy of this unique aminoglycoside for further development.

Acknowledgements

We thank Swapna Vaddi and Pia Thommes for help with the *S. aureus* infection experiments, Lisa Woolhiser for help with the *M. tuberculosis* infection experiments, and Susanna Salas for typing the manuscript. The study was supported by the University of Zurich and by NIAID IDIQ Contract Task Order MHSN2722010000091/01.

The University of Zurich has filed a patent for the use of apramycin in treatment of human infectious diseases.
References


Figure 1. *In-vivo* antituberculous activity of apramycin and comparator in a murine model of acute, low dose aerosol infection. Log_{10} CFU in the lungs of *M. tuberculosis* infected mice are given. At day 13 following infection, mice were drug treated for 9 days and lungs were collected for CFU determinations at day 22.

Figure 2. *In-vivo* activity of apramycin and comparators in a neutropenic murine model of *S. aureus septicaemia*. A) Bacterial burden in kidney, CFU/g tissue. B) Bacterial burden in blood, CFU/ml. Given are the geometric mean and the data points for single animals. Log reductions are indicated. The pre-treatment bar indicates the bacterial load at the initiation of treatment. LOD: limit of detection. Amikacin data were taken from reference 14 and are included for comparison (amikacin at 16 and 40 mg/kg is equivalent to 4 and 10 times the MIC value in mg/kg).
Table 1  
In-vitro activities of apramycin and comparator antibiotics against the methicillin-resistant *Staphylococcus aureus* strain used in the in-vivo efficacy study

<table>
<thead>
<tr>
<th>Organism</th>
<th>Phenotype</th>
<th>Strain</th>
<th>MIC (μg/L)</th>
<th>Apramycin</th>
<th>Amikacin</th>
<th>Kanamycin</th>
<th>Paromomycin</th>
<th>Ciprofloxacin</th>
<th>Oxacillin</th>
<th>Cefoxitin</th>
<th>Linezolid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>methicillin-resistant</td>
<td>A6941</td>
<td>4.0-8.0</td>
<td>4.0</td>
<td>2.0-4.0</td>
<td>2.0-4.0</td>
<td>0.5</td>
<td>8.0</td>
<td>12.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>