Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-012-1594-x

ARTICLE

The optimal aminoglycoside and its dosage for the treatment of severe *Enterococcus faecalis* infection. An experimental study in the rabbit endocarditis model

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Received: 27 December 2011 / Accepted: 14 February 2012

Abstract Aminoglycosides are recommended for the treatment of *Enterococcus faecalis* infections, especially in severe and bacteremic infection. However, the optimal aminoglycoside or the optimal dosage remains uncertain. This study aimed to compare the activity of four aminoglycosides against *E. faecalis* (gentamicin, netilmicin, tobramycin, and amikacin) and two dosages of gentamicin. One clinical strain of *E. faecalis* was used to induce aortic endocarditis in the study rabbits. Each aminoglycoside was infused daily over 3 days with a computer-regulated flow simulating human pharmacokinetics of 15 mg/kg/day for amikacin, 6 mg/kg/day for netilmicin, and 3 mg/kg/day for gentamicin and tobramycin. Additionally, two dosages of gentamicin (simulating 3 or 6 mg/kg/day) were compared over 1 or 3 days of treatment. The in vivo efficacy was assessed according to the bacterial count in vegetations, in comparison with a control group. Of the four aminoglycosides tested, only gentamicin and netilmicin showed significant antibacterial efficacy after 3 days of treatment. After only 1 day of treatment, the high dosage of gentamicin (6 mg/kg/day) was more effective than the standard dosage (3 mg/kg/day). Among the tested aminoglycosides, gentamicin showed the best efficacy, with the best results after 24 h of treatment for the highest dosage.

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Published online: 08 March 2012

Introduction

The treatment of patients with severe infections requires an immediately effective antibiotic. The place of aminoglycosides in combination with beta-lactams or glycopeptides is recognized for the treatment of enterococcal infections; one example of recommendation is the first-line treatment of bacterial endocarditis [1]. The bactericidal effect of aminoglycosides has been demonstrated in vivo in experimental studies [2, 3]. This effect was particularly observed when aminoglycosides were administered once daily, simulating human pharmacokinetics in animals [4]. The prescription of gentamicin for the treatment of severe bacteremic infections due to *Enterococcus faecalis*, in combination with glycopeptides or beta-lactams, is usually recommended. However, there is no certainty on the optimal aminoglycoside or the optimal dosage to guide physicians' prescriptions [5]. The early and consistent efficacy of aminoglycosides in such infections needs to be determined.

The aim of the study was to define the aminoglycoside drug and dosage that would be the most effective against *E. faecalis* in an experimental endocarditis model. Two experiments were performed. The first compared the efficacy of various aminoglycosides and the second experiment assessed the most effective dosage of gentamicin, which is the most commonly recommended aminoglycoside for these infections.

Materials and methods

A clinical strain was investigated (*E. faecalis* HM 1061) and the minimum inhibitory concentrations (MICs) of amikacin, gentamicin, netilmicin, and tobramycin were determined by the microdilution method in Mueller–Hinton broth [6]. In vivo studies were carried out on New Zealand white female rabbits (CEGAV S.S.C., France). The animals were kept in individual

cages and allowed free access to food and water throughout the experiment. The experimental protocol was approved by the Committee of Animal Ethics of the University of Nantes. Animals were treated in accordance with French national regulations. Aortic valve endocarditis was induced [7] by retrograde transaortic valvular catheterization under anesthesia, followed 24 h later by an intravenous injection of 10^8 colony forming units (CFU), in the marginal ear vein. Twenty-four hours after inoculation, infected animals were randomized into control and treated groups (five or more animals per group). The control animals were euthanized at treatment onset. Only one control group was necessary to assess the antibiotic in vivo effect at both stages of experimentation. The main judgment criterion was the comparison of the bacterial count between the control group and each of the treated groups.

First experiment: The treated animals were randomized into four groups (five or more animals per group). Aminoglycosides were administered to rabbits in a single daily dose, simulating the kinetics of a human dosage of amikacin (15 mg/kg/day), gentamicin (3 mg/kg/day), netilmicin (6 mg/kg/day), and tobramycin (3 mg/kg/day), as previously published [4, 8]. The aminoglycosides were given over 3 days using a computerized system with automatic syringes managed by software to adapt the infusion rate.

Second experiment: The treated animals were randomized into two gentamicin groups (five or more animals per group). Gentamicin was administered to rabbits in a single daily dose, simulating the kinetics of a human dosage of 3 or 6 mg/kg/day [4]. Gentamicin was given over 24 h or 3 days using the computerized system described for the first experiment.

At the end of the treatments in both experiments (corresponding to 24 h after the last daily infusion), animals were euthanized with an intravenous 100-mg thiopental bolus. After thoracotomy and cardiectomy, endocarditis vegetations were removed and immediately stored on ice. The samples were then weighed, homogenized in 0.5 mL of saline buffer, and plated on Trypticase soy agar plates using a spiral system. Dilutions at 10−1, 10−2, and 10−4 were performed to eliminate potential carry-over effects. Viable counts after 24 h of incubation at 37°C were expressed as the mean ± standard deviation (S.D.) log10 CFU per gram of vegetation. For all these aminoglycosides, and both gentamicin dosages, pharmacokinetics data have been previously published, demonstrating peaks at 22.4±6.8 mg/l for gentamicin 3 mg/kg/d, 48.7±4.0 mg/l for gentamicin 6 mg/kg/d, 45.5±4.3 mg/l for netilmicin, 24.2±2.0 mg/l for tobramycin, and 49.5±1.6 mg/l for amikacin [4, 8, 9]. These high plasmatic concentrations could be considered as efficacious, by referring to the targeted concentrations in humans' therapeutics. The mean CFU per gram of vegetation for the experimental and control groups were compared by analysis of variance (ANOVA) plus a Bonferroni test for intergroup comparisons (StatView, Abacus Concepts Inc.). Statistical significance was defined as a p-value<0.05.

Table 1 In vivo results after 3 days of treatment

Experimental groups	log CFU/g±95% CI (n)
Controls	9.1±0.5 (8)
Amikacin once daily 15 mg/kg/d	8.1±1.0 (5)
Gentamicin once daily 3 mg/kg/d	3.7±0.7 (5)*
Netilmicin once daily 6 mg/kg/d	4.2±0.8 (5)*
Tobramycin once daily 3 mg/kg/d	7.7±0.8 (5)

*p<0.0001 versus controls, amikacin, and tobramycin (Bonferroni's test after ANOVA)

Results

The MICs of amikacin, gentamicin, netilmicin, and tobramycin were 256, 16, 16, and 16 mg/L, respectively.

After 3 days of experimental treatment, the mean bacterial counts in each treated group were compared to the control group (pre-treatment measure). No differences were observed between the amikacin and tobramycin groups and the control animals. A significant decease of bacterial counts in endocarditis vegetations was observed only for two therpautic regimens: gentamicin (3 mg/kg/day) and netilmicin (6 mg/kg/day) (Table 1). A significant difference was observed between these effective treatments and amikacin and tobramycin.

In the second experiment comparing two doses of gentamicin (3 and 6 mg/kg/day; standard and high dosage, respectively), the bacterial counts were significantly lower after treatment with the high dosage for 1 day (Table 2), but no significant antibacterial effect was observed with the standard dosage. After 3 days of treatment, there was no difference in the bacterial counts between both gentamicin dosages.

Table 2 In vivo results after treatment by gentamicin at two different dosages

Experimental groups	log CFU/g±95% CI (n)	
Controls	9.2±0.4 (8)	
	Treatment duration	
	24 h	3 days
Gentamicin once daily 3 mg/kg/d	8.2±0.5 (7)	3.7±0.7 (6)**
Gentamicin once daily 6 mg/kg/d	6.8±1.2* (7)	3.5±0.2 (6)**

*p<0.05 versus controls and gentamicin 3 mg/kg/d (Bonferroni's test after ANOVA)

**p<0.05 versus controls (Bonferroni's test after ANOVA)

Discussion

The results of these experimental studies show an antibacterial advantage for the CHOICE of gentamicin (or netilmicin) among the tested aminoglycosides and, specifically, for the administration of a high dose (6 mg/kg/day) of gentamicin at the first treatment day of severe *E. faecalis* infection.

The in vivo activity of aminoglycosides against enterococcal strains is recognized, particularly in combination with beta-lactams or glycopeptides [2, 3, 10–12]. The usually recommended dosage (3 mg/kg/day) is lower than the most effective dosage at the first treatment day in this study (6 mg/kg/day) [1].

In critically ill patients, severe infection can result in septic shock, which can cause an aberrant pharmacokinetic profile of aminoglycosides [13, 14]. Particularly, the distribution volume is increased, resulting in a peak level decrease and a half-life increase of the drugs [14]. The prognosis of severe infection depends on the efficacy of antibacterial therapy during the early stage of treatment. Considering that peak concentration is predictive of efficacy, and the pharmacokinetic and pharmacodynamic profiles of aminoglycosides previously described [12–14], a high dosage of aminoglycoside at the early stage of treatment in such infections could be more clinically efficacious. The human pharmacokinetic simulation and the severity of this endocarditis model advocate for the consideration of these results in clinical therapeutics. The results of this experimental study could form the basis of clinical recommendations for the treatment of severe enterococcal infections.

Acknowledgments The authors thank Dr. Laney Weber for the English linguistic support and pre-editing (BioScience Writers).

Funding No external sources.

Conflicts of interest Nothing to declare.

Ethical approval In vivo studies were approved by the animal study committee of the University of Nantes, France.

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