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Daptomycin combinations as alternative therapies in experimental foreign-body infection caused by meticillin-susceptible *Staphylococcus aureus*

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ABSTRACT

Whilst levofloxacin (LVX) in combination with rifampicin (RIF) is considered the optimal treatment for prosthetic joint infection (PJI) caused by meticillin-susceptible *Staphylococcus aureus* (MSSA), no therapeutic alternatives have been accurately evaluated. Based on the high effectiveness of the combination of daptomycin (DAP) plus RIF against meticillin-resistant *S. aureus* (MRSA) in this setting, in this study the efficacy of DAP+RIF and DAP+LVX combinations was tested as alternative therapies for foreign-body infections (FBIs) caused by MSSA. A tissue-cage infection model was performed using an MSSA strain. Male Wistar rats were treated for 7 days with LVX, DAP, RIF or the combinations LVX+RIF, DAP+RIF and DAP+LVX. Antibiotic efficacy was evaluated by bacterial counts from tissue cage fluid (TCF) and the cure rate was determined from adhered bacteria. Resistance was screened. Monotherapies were less effective than combinations ($P<0.05$), and resistance to DAP and RIF emerged. DAP+RIF (decrease in bacterial counts in TCF, −4.9 log CFU/mL; cure rate, 92%) was the most effective therapy ($P<0.05$). There were no differences between LVX+RIF (−3.4 log CFU/mL; 11%) and DAP+LVX (−3.3 log CFU/mL; 47%). No resistant strains appeared with combined therapies. In conclusion, the combinations DAP+RIF and DAP+LVX showed good efficacy and prevented resistance. DAP+RIF provided higher efficacy than LVX+RIF. These DAP combinations were efficacious alternatives therapies for MSSA FBI. Further studies should confirm whether DAP+RIF may be useful as a first-line therapy in the setting of PJI caused by MSSA.

1. Introduction

The combination of levofloxacin (LVX) plus rifampicin (RIF) is considered the standard reference for oral treatment of acute prosthetic joint infection (PJI) caused by meticillin-susceptible *Staphylococcus aureus* (MSSA) and managed with debridement and implant retention or with one-stage exchange [1,2]. The benefits of early RIF administration in this setting are well documented, and the fluoroquinolones have high synergistic efficacy and protect against the development of resistant strains [3–6]. However, despite optimal therapeutic management, some clinical and microbiological failures occur, especially when implant retention is attempted [2,4]. Moreover, occasions arise when these drugs often cannot be administered owing to intolerance or allergy, and no adequately evaluated alternatives exist [7].

Daptomycin (DAP) has bactericidal activity against growing and non-growing *S. aureus* and may be suitable against bacteria embedded within a biofilm [8,9]. In difficult-to-treat infections in humans, current recommendations encourage the use of DAP at high doses (8–10 mg/kg/day) in combination with other antibiotics [10]. However, the efficacy of such combined therapies has mainly been studied against meticillin-resistant *S. aureus* (MRSA) strains but has not fully explored in MSSA infection. This is the case for DAP+RIF combination, which has been proposed as one of the most effective

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anti-MRSA treatments for PJI based on results obtained in experimental models [11–13]. To date, DAP as anti-MSSA therapy can only be expected to have similar efficacy to that for MRSA, but this has not been confirmed or evaluated in comparison with other anti-MSSA alternative therapies. With this in mind, our group recently evaluated the efficacy of a DAP plus cloxacillin (CLOX) combination using a rat tissue-cage infection model and found that it was as effective as CLOX+RIF, which is the most common intravenous treatment used in the first weeks of PJI [14] caused by MSSA.

The rat tissue-cage infection model is a validated method that can provide relevant information about the pathogenesis and treatment of foreign-body infections (FBIs) [15,16]. For the present study, this model was used with MSSA infection to test the efficacy of DAP+RIF and DAP+LVX combinations as alternative therapeutic options when the reference LVX+RIF cannot be used.

2. Materials and methods

2.1. Micro-organism

MSSA strain ATCC 29213 was used for all in vitro and in vivo studies.

2.2. Antimicrobial agents

For in vitro experiments, purified antibiotic powder was re-suspended according to laboratory recommendations. Antibiotics were purchased from the following laboratories: DAP (Novartis, Barcelona, Spain); RIF (Sanofi-Aventis, Madrid, Spain); and LVX (Sigma-Aldrich, Madrid, Spain). For in vivo experiments, the commercial product was diluted to achieve a final volume suitable for administration to rats.

2.3. In vitro studies

The medium broth was supplemented with 50 mg/L calcium chloride dihydrate (Merck KGaA, Darmstadt, Germany) for all in vitro experiments with DAP.

2.3.1. Preparation of inocula

Bacteria from overnight cultures on 5% blood agar plates (Becton Dickinson, Madrid, Spain) were grown for 3 h in Muller–Hinton broth (MHB) (Becton Dickinson). They were then centrifuged and re-suspended to use in the MHB macrodilution method with a final inoculum of 10^5 CFU/mL under exponential growth conditions.

For experiments in stationary phase, bacteria recovered from an overnight culture in MHB were centrifuged and re-suspended in a nutrient-restricted medium [phosphate-buffered saline (PBS), 1% glucose (VWR Chemicals, Leuven, Belgium) and 4% MHB], thus ensuring that bacteria remained stable for up to 24 h under these conditions. A macrodilution method with a high inoculum of 10^8 CFU/mL was used [15].

2.3.2. Minimum inhibitory and minimal bactericidal concentrations

For bacteria in log phase, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using standard recommendations [17]. MBCs for bacteria in stationary phase were determined using methodology described elsewhere [15,18]. In log phase, the respective MICs and MBCs were 0.5 mg/L and 1 mg/L for DAP, 0.5 mg/L and 1 mg/L for LVX, and 0.015 mg/L and 0.12 mg/L for RIF. The corresponding MBCs of these antibiotics in stationary phase were 32, 4 and >8 mg/L.

2.3.3. 24-h kill curves

For the novel DAP+RIF and DAP+LVX combinations, kill curves in log phase (using standard methodology) [19] and in stationary phase (following previously reported methodology) [15] were performed. The concentrations of antibiotics selected for kill curves in log phase were pre-fixed at subinhibitory and clinically achievable levels that were above the MIC. Owing to bacterial tolerance to antibiotics expressed in stationary phase, drug concentrations tested were higher than in log phase.

For all experiments, bactericidal activity was defined as a $\geq 3 \log_{10}$ decrease in CFU/mL of the initial inoculum by 24 h. The results of combination therapy were compared with the most active single drug; synergy, indifference and antagonism were then defined as a ≥ 2 log increase in killing, a <2 log change (increase or decrease) in killing, and a ≥ 2 log decrease in killing, respectively. To avoid carry-over antimicrobial agent interference, the sample was placed on the plate in a single streak down the centre and was allowed to be absorbed into the agar until the plate surface appeared dry; the inoculum was then spread over the plate. As described in detail elsewhere [20], this methodology was checked by comparing the results obtained with the centrifugation and re-suspension of the fluid from tubes of killing curves.

2.4. Animal studies

2.4.1. Animal model

The experimental protocol complied with European (Directive 2010/63/EU) and Spanish (RD 53/2013) legislation on animal experimentation, and the Ethics Committee for Animal Experiments of the University of Barcelona (Barcelona, Spain) approved the animal model that had previously been standardised by our group [14,15]. Male Wistar rats weighing 220–250 g at the beginning of the experiments were used. Rats were given food and water ad libitum. Two tissue cages with two polymethylmethacrylate coverslips (CVs) (Mecanizados del Besós, Badalona, Spain) were subcutaneously implanted in rats that were previously anaesthetised with an intraperitoneal injection of ketamine (Centauro Servicios Veterinarios, Barcelona, Spain) plus xylazine (Laboratorios Calier S.A., Barcelona, Spain). After 3 weeks of surgery, tissue cage fluid (TCF) was checked for sterility and was then percutaneously infected with 0.1 mL of an MSSA preparation ($0.2–2 \times 10^6$ CFU/mL). One week after inoculation, TCF was obtained by percutaneous puncture to quantify bacterial counts (Day 1). Therapy was then administered intraperitoneally for 7 days. At 24 h and 4 days after the end of treatment (Days 8 and 11, respectively), TCF was again recovered to perform bacterial counts. To obtain samples during the experiment, rats were anaesthetised with isoflurane (Abbvie Farmaceutica S.L.U., Madrid, Spain). All animal subjects were sacrificed on Day 11 by cardiac puncture of thiopental. CVs were then removed and processed to quantify adherent bacteria and the infection cure rate [15].

In fact, the tissue-cage infection model mimics a biofilm infection and allows analysis of the bacterial population attached to the CV and those released from the biofilm, which are located in the TCF and are also tolerant to antibiotics [21].

The criterion for efficacy was defined as a decreased bacterial count in TCF from the beginning to the end of the treatment; the criterion was evaluated twice, on Days 8 and 11. Antibiotic efficacy against adherent bacteria from the CV removed on Day 11 was also evaluated by determining bacterial counts. Finally, the infection cure rate was calculated from the TCF and CV on Day 11 and was defined as the percentage of samples with bacterial counts under the limit of detection with respect to the total samples. For all cases, the lower limit of detection of bacterial counts was 10 CFU/mL.

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