DAPTOMYCIN VERSUS VANCOMYCIN IN A METHICILLIN-RESISTANT
STAPHYLOCOCCUS AUREUS ENDOPHTHALMITIS RABBIT MODEL:
BACTERICIDAL EFFECT, SAFETY AND OCULAR PHARMACOKINETICS

Running title: Intravitreal daptomycin and endophthalmitis

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ABSTRACT

Staphylococcus aureus is a frequent cause of acute endophthalmitis and this virulent bacteria is often associated with a poor visual outcome. We investigated, in this study, the bactericidal efficacy and the safety of intravitreal daptomycin, a lipopeptide antibiotic with a Gram-positive broad-spectrum, compared with intravitreal vancomycin, in a methicillin-resistant S. aureus endophthalmitis rabbit model. The pharmacokinetics and pharmacodynamics of daptomycin were also studied in the infected eyes. Rabbits were randomly divided into three treatment groups (n=8) and one untreated group (n=4), to compare the effect of single intravitreal injections of 0.2 mg and 1 mg of daptomycin (DAP 0.2 and DAP 1 groups respectively) with 1 mg of intravitreal vancomycin (VAN 1 group). Vitreal aspirations and grading of ocular inflammation were regularly realised until euthanasia on day 7. In the DAP 0.2 group, 62.5% of the eyes were sterilised and the mean bacterial count presented a reduction of 1-log-unit. In the DAP 1 and VAN 1 groups, the infection was eradicated (100% and 87.5% of eyes sterilised respectively) with a 4-log-units reduction of the mean bacterial count. DAP 1 group ensured non-inferiority bactericidal efficacy with VAN 1 group and was superior to other regimens in limiting the ocular inflammation and preserving the architecture of the ocular structures (p<0.05). The elimination half-life ($T_{1/2\text{el}}$) of daptomycin was independent of the administered dose ($38.9 \pm 16.5$ hours and $40.9 \pm 6.7$ hours respectively for the DAP 0.2 and DAP 1 groups) and was significantly longer than $T_{1/2\text{el}}$ of vancomycin ($20.5 \pm 2.0$ hours for the VAN 1 group) (p<0.05). This antibiotic could therefore be considered for the treatment of Gram-positive-bacteria intra-ocular infections.

Keywords: daptomycin, Staphylococcus aureus, endophthalmitis, vancomycin, pharmacokinetics, pharmacodynamics, rabbit.
INTRODUCTION

Acute bacterial endophthalmitis is a rare but vision-threatening eye disease. This infection and inflammation of the deep structures of the eye is mainly caused by colonisation with exogenous bacteria following penetrating trauma (post-traumatic) or intraocular surgery (post-operative) and can result in blindness if not rapidly and properly managed. The leading causative organisms of post-traumatic and post-operative endophthalmitis are Gram-positive bacteria, preponderant on the normal ocular surface, with a significant percentage of Staphylococcus aureus, an especially virulent pathogen with a poor visual outcome (9, 12, 28). Current treatment regimens include a Gram-positive broad-spectrum coverage with a 1 mg vancomycin direct intravitreal injection (3, 16, 27). According to recent reports, even if the proportion of methicillin resistance in S. aureus isolates responsible for endophthalmitis is increasing (10), no vancomycin resistance has yet been declared. However, there is a need for a therapeutic alternative because of the decreasing trend for susceptibility to vancomycin in non-ocular S. aureus related infections (7, 25).

Daptomycin is a lipopeptide antibiotic approved for skin, soft-tissue infections and bacteraemias, including those associated with right-sided endocarditis. Its spectrum of activity is similar to that of vancomycin but daptomycin is also active against vancomycin-intermediate and vancomycin-resistant S. aureus and vancomycin-resistant enterococci (13). The use of daptomycin in an experimental endophthalmitis model has only been reported once: a single dose of 0.2 mg of intravitreal daptomycin is safe and effective in a rabbit coagulase-negative Staphylococcus epidermidis endophthalmitis (8).

The aim of this study was to compare the bactericidal activity and safety of different dosages of intravitreal daptomycin with 1 mg of intravitreal vancomycin, the reference treatment, in experimental endophthalmitis caused by S. aureus. In addition, we studied the pharmacokinetics and pharmacodynamics of daptomycin in the infected rabbit eyes.
MATERIALS AND METHODS

Bacterial strain and growth conditions
The *S. aureus* strain used in this study was isolated in the bacteriology laboratory of the Strasbourg University Hospital (France), from a human corneal ulcer. Classification and identification used a MALDI-BioTyper™ system for mass spectrometry (Microflex®, Bruker Daltonics, Bremen, Germany). Isolate was tested for susceptibility to oxacillin, vancomycin and daptomycin using the VITEK 2 system (VITEK® 2, bioMérieux, Marcy l’Etoile, France). Susceptibility breakpoints and interpretive criteria were used from the 2011 recommendations of the Antibiogram Committee of the French Microbiology Society guidelines (1). Etest® were carried out to determine the vancomycin and daptomycin MICs (Etest®, bioMérieux, Marcy l’Etoile, France). The presence of the Panton-Valentine leukocidin, A and B exfoliative toxins, and A, B, C, D enterotoxins were studied by immunoprecipitation using Ouchterlony method and reversed passive latex agglutination using a toxin detection kit (Oxoid®, Dardilly, France), to determine the toxin profiling of the *S. aureus* strain. *S. aureus* were cultivated on Columbia agar plates with 5% sheep blood (bioMérieux, Marcy l’Etoile, France) for 18-24 h in an aerobic atmosphere at 37°C.

Animals care
Twenty-eight female New Zealand albino rabbits weighing between 3 and 4 kg were used in this study. They were obtained and cared for in the animal research facility of the Bacteriology Institute of the University of Strasbourg in accordance with the recommendations of the European Community guidelines for the use of experimental animals (European directive 2010/63/EU). Animals had *ad libitum* access to rabbit pellets and water throughout the study, and were housed in individual cages with controlled temperature (21°C) and light cycle (12/24 h). All *in vivo* testing conformed with the Association for Research in
Vision and Ophthalmology (ARVO) statement on animal use and the study protocol was approved by the regional veterinary services direction.

**Anaesthesia and euthanasia drug protocols**

The anaesthesia protocol, used prior to each eye puncture, consists in an intramuscular injection of a mixture of 30 mg/kg body weight of ketamine (Ketamine Virbac®, Carros, France) and 4 mg/kg body weight of xylazine (Rompun® 2%, Bayer Pharma, Puteaux, France). Additionally, a drop of oxybuprocaine (Oxybuprocaine Faure® 1.6 mg/0.4 mL, Novartis Pharma SAS, Rueil Malmaison, France) was instilled. At the end of the study, the animals were euthanatised with 5 mL of sodium pentobarbital (Dolethal®, Vetoquinol SA, Lure, France) administrated intravenously.

**Experimental endophthalmitis model.**

A 27-gauge needle assembled on a 1 mL tuberculin syringe was inserted 3 mm from the limbus and directed toward the center of the eyeball, avoiding the crystalline lens. The right eye of all animals received approximately 500 colony forming units of *S. aureus* in a volume of 0.1 mL. This bacterial suspension was prepared from *S. aureus* colonies harvested from an 18 h to 24 h culture, and diluted in sterile 0.9% of NaCl (wt/vol). Purity and count were verified by plating aliquots of the serially diluted samples on 5% sheep blood agar plates, incubated overnight at 37°C. Left eyes served as non infected control eyes.

**Preparation of the antibiotic intravitreal injection.**

Daptomycin (Cubicin®, Novartis France, Paris, France) and vancomycin (Vancomycine Sandoz®, Sandoz SAS, Levallois-Perret, France) were obtained from commercial sources. Intravitreal solutions were freshly prepared from injectable powders, diluted in sterile ophthalmic balanced salt solution (BSS) to the desired concentrations.
Study design

After the clinical onset of endophthalmitis, all the rabbits received 0.1 mL antibiotic or BSS intravitreal injection in the infected right eye. Rabbits were randomly divided into four groups: three treatment groups and one untreated group. One group of eight animals received 0.2 mg of daptomycin (DAP 0.2 group), the second and the third, each composed of eight animals, received 1.0 mg of daptomycin (DAP 1 group) and 1 mg of vancomycin (VAN 1 group). In the untreated group, 0.1 mL of BSS was injected to four animals (BSS group). Left control eyes received no injection. Day 0 corresponded to the day of this antibiotic intravitreal injection. All the eyes were clinically examined on day 0 and 2, 4 and 7 days after. Serial 50 to 100 µL vitreous samples were withdrawn from the right eyes under sterile conditions and through 27 gauge needles on day 0, 1, 2, 4 and 7 for bacterial counts and/or dosage of antibiotic residual concentrations. Vitreous samples obtained from the left eyes (control group) served for bacterial cultures on day 7. All animals were eventually euthanatised on day 7 and four animals from each group were enucleated for histological assessment.

Bacterial count

Fifty microliters of the vitreous samples was serially 10-fold diluted in 450 µL of sterile 0.9% (wt.vol) NaCl. A 100 µL volume of each dilution was spread on a 5% sheep blood agar plate and incubated overnight at 37°C to be counted after 24h. The limit of detection was 1 log_{10} CFU/mL.

Clinical examination of the eyes

The clinical investigation was conducted by a single ophthalmologist (M.S.) and according to the Nussenblatt criteria (17). Briefly, five increasing levels of severity of damage were scored for the anterior segment and annexes, and five other levels were scored for the posterior...
segment observed by direct ophthalmoscopy (Heine®). The sum of these two scores constituted the ocular inflammation score (on a scale of 0, indicating normal findings, to increasing values corresponding to increasing severity of abnormal findings with 8 as a maximum score). Endophthalmitis corresponded to a Nussenblatt score higher than 2.

**Histopathologic examination of the eyes**

Immediately after enucleation, the eyes were fixed in 4% buffered formaldehyde (v/v). After being embedded in paraffin, they were sectioned in 5 µm thick transverse sections and stained with haematoxylin and eosin. The slides were analysed histopathologically by an ophthalmic pathologist (L.M.) and graded according to the scheme detailed in Table 1 (18, 20).

**Drug assay: daptomycin and vancomycin in rabbit vitreous**

Concentrations of daptomycin were determined using a high-performance liquid chromatography (HPLC) assay, developed and validated for the quantification of the drug in vitreous samples at the bacteriology laboratory in our hospital (S.L.). Vitreous samples collected from rabbits were stored at 80°C until analysis. Frozen vitreous samples were thawed at room temperature and subjected to protein precipitation as follows. Vitreous aliquots of 50 µL were prepared by addition of an equal volume of a methanol:acetonitrile 1:2 (v/v) mixture and vortexed for 5 s. After a slow rotation for 10 min at 30 rpm this mixture was centrifuged for 5 min at 10,000 g. Finally, 20 µL of the supernatant were injected into the prominence HPLC® system (Shimadzu USA Manufacturing Inc., Canby, OR, USA) comprising a LP-20AT pump and a SPD-20A UV-visible detector. A Rheodyne® model 7725i manual sample injector (Rheodyne, Rohnert Park, CA, USA) was used with a 20 µL loop. Chromatographic separation was achieved on a reversed-phase C18, 150×4.6 mm, 3 µm Uptisphère Interchim® column (Interchim, Montluçon, France) with a mobile phase consisting of an isocratic mixture of 20 mM phosphate buffer and 40% acetonitrile, and the pH adjusted...
to 3.5 with phosphoric acid. A 1.0 mL/min flow rate was used and the detection wavelength was set at 224 nm. Under these conditions, the retention time for daptomycin was 7.5 min. The LC solution Shimadzu® software (Shimadzu France, Champs sur Marne, France) was used for acquiring and processing the data. The quantification limit of the daptomycin assay was 0.5 µg/mL in vitreous. The method was linear over the concentration range of 0.5 to 500 µg/mL, with a mean correlation coefficient of 0.9988. Quality control standards were prepared at final concentrations of 1.0, 50.0 and 250 µg/mL. Interday and intraday accuracy of the method respectively ranged from 92.0 to 103% and 93.8 to 101%. The precision values (coefficients of variation) ranged from 0.98 to 3.58% for intraday precision and 2.66 to 3.03% for the interday precision. Vitreous levels of vancomycin were determined by Enzyme Multiplied Immunoassay (Viva-E® Drug Testing System, Siemens, USA). Haemorrhagic vitreous samples were excluded from the study.

Pharmacokinetic/pharmacodynamic (PK/PD) analysis

To address the problem of intersubject variation, we calculated individual PK parameters for each rabbit of the three different treatment groups (DAP 0.2, DAP 1 and VAN 1 groups). The measured vitreous concentration-time data were graphically displayed with a semilogarithmic scale. On the basis of correlation coefficient R² and least-squares regression analysis, vitreous antibiotic concentration-time data best fit monoexponential equation, for each animal providing three or four concentration values at different time points. Exponential least-squares regression analysis was realised for all the animals of the three groups. Maximum vitreous concentrations (Cmax), corresponding to initial concentrations immediately after the antibiotic intravitreal injection, were estimated by extrapolation of the regression curves to time zero. The respective equations of the regression curves, were used to calculate the following pharmacokinetics parameters for each animal: terminal elimination constant (Kel) was the slope of the regression line, terminal elimination half-time (T1/2el) was calculated as ln2/Kel.
and areas under the concentration-time curve (AUC) were measured using the log trapezoidal rule as follows: \[ \text{AUC}_{1-2} = \frac{(C_1-C_2)}{K_{el}} \]. We calculated AUC between 1 day and 7 days (AUC_{1-7d}) and AUC between 0 and 24 hours (AUC_{0-24h}). Finally, the Cmax/MIC and AUC_{0-24h}/MIC ratios were calculated for the PD analysis.

Statistical analysis

All data were expressed as mean ± standard deviation. Differences among the groups were tested for significance using a non parametric Kruskal-Wallis test (Prism®, version 5.04, Graph Pad software Inc., San Diego, CA, USA). Then, if a significant difference occurred, Dunn’s multiple comparison post-test was used to compare each pair of groups. Dose proportionality assessment of daptomycin was realised using the approach proposed by Smith et al. (26). Log-transformed, dose-normalised AUC_{1-7d} values for the two groups of daptomycin (DAP 0.2 and DAP 1) were evaluated by one-way ANOVA and ratios of geometric means and their corresponding confidence interval 90% were reported and compared with the accepted interval defined by FDA guidance (26). We used the R software (R Development Core Team, 2011. Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL) to realise this statistical analysis.
RESULTS

The mass spectrum of the *S. aureus* strain was similar to the *S. aureus* ATCC 29737 reference strain, with a log score of 2.332. This strain was methicillin-resistant and susceptible to vancomycin and daptomycin (Table 2) and study of the toxin profiling (Table 3) revealed the presence of type A enterotoxin and the absence of Panton-Valentine leukocidin.

Bacterial count

The sizes of the injected bacterial inoculums did not differ significantly among the different treatment groups (p>0.05, Kruskal-Wallis test). Just before the antibiotic intravitreal injection (day 0), all infected right eyes displayed bacterial growth with comparable bacterial counts (p>0.05, Kruskal-Wallis test). On day 4 and day 7, 62.5% of the eyes treated with a 0.2 mg intravitreal injection of daptomycin (DAP 0.2 group), 87.5% of the eyes treated with a 1 mg intravitreal injection of vancomycin (VAN 1 group) and 100% of the eyes that received a 1mg intravitreal injection of daptomycin (DAP 1 group) were sterilised (Fig.1). Eyes from the BSS group (100%) showed an increasing bacterial growth from day 0 to day 7 while vitreous cultures from the control left eyes did not demonstrate any bacterial growth. Bacterial counts are presented in Table 4. Mass spectrometry confirmed the sole presence of *S. aureus* in all the colonies which were isolated from positive vitreous samples.

Clinical examination

The baseline clinical examination revealed normal ocular structures and both eyes of all rabbits scored zero on the ocular inflammation grading scale, using Nussenblatt criteria. Throughout the study no inflammation was observed in the left eyes. The clinical scores of the inoculated eyes are presented at the different time points in Fig. 2. On Day 0, endophthalmitis was clinically diagnosed for all inoculated eyes (scores higher than 2) and the ocular inflammatory scores were not significantly different between the different groups.
For all time points, the clinical scores were similar among the different antibiotic-treated groups (VAN 1, DAP 0.2 and DAP 1 groups) \((p>0.05, \text{Kruskal-Wallis test})\). On days 2 and 4, while clinical scores in the DAP groups (DAP 0.2 and DAP 1) were significantly lower than those of the BSS group, this difference was not found with the VAN 1 group. On day 7, only the DAP 1 group differed from the untreated BSS group \((p<0.05, \text{Dunn’s multiple comparaison test})\).

**Histopathologic analysis**

The left control rabbit eyes displayed normal and well-distinguished layers. The histopathologic scores of the inoculated rabbit eyes are presented in Fig. 3 and when compare with the untreated group (BSS) (score 9.5 ± 0.58), a significant difference appeared only with the DAP 1 group (score 1 ± 0.82) \((p<0.01, \text{Dunn’s post-test})\). Eyes from the DAP 0.2 and DAP 1 groups presented a normal or partially inflamed vitreous with always a normal retinal architecture (Fig. 4.A). The anterior chamber was free of inflammatory cells but was sometimes partially filled with fibrin. These eyes were less inflamed than those in the VAN 1 group. Indeed, the retina in the VAN 1 group eyes were partially infiltrated and necrotic even if some well-organised retinal layers were seen. Inflammatory cells were observed migrating from the vitreous into the retina (Fig. 4.B). The eyes from the BSS group exhibited the most severe inflammation in all ocular structures. Inflammatory cells migrated in greater numbers into the corneal periphery, forming corneal ring abscesses. Inflammatory cells, fibrin and sometimes erythrocytes filled the anterior segment and the vitreous. The retinal structures were indistinguishable because of the necrosis (Fig. 4C).

**Antibiotics assay**

The time course of the measured antibiotic vitreous concentrations and corresponding statistical data (mean ± standard deviation) are presented in Fig. 5. Twenty-four hours (day 1)
after the intravitreal injection of 0.2 mg (DAP 0.2 group) and 1 mg (DAP 1 group) of
daptomycin, antibiotic vitreous concentrations were respectively 87 ± 25.0 and 287 ± 118.0 µg/mL, while the residual concentrations reached respectively 3 ± 1.6 and 25 ± 20.0 µg/mL at
day 7. Vancomycin vitreous concentrations were 266 ± 29.0 µg/mL on day 1 and 3 ± 1.4 µg/mL on day 7 in the VAN 1 group.

**PK/PD analysis**

In the DAP 0.2, DAP 1 and VAN 1 groups, the plot of the log of measured vitreous antibiotic
concentrations versus time gave nearly straight lines with correlation coefficients close to 1
($R^2_{DAP 0.2} = 0.974 ± 0.035$, $R^2_{DAP 1} = 0.979 ± 0.018$ and $R^2_{VAN 1} = 0.943 ± 0.085$). $T_{1/2el}$ were
38.9 ± 16.5 hours, 40.9 ± 6.7 hours, and 20.5 ± 2.0 hours respectively for the DAP 0.2, DAP 1
and VAN 1 groups. The $T_{1/2el}$ of the DAP 0.2 and DAP 1 groups were similar, while a
significant difference were observed between the VAN 1 group and the both groups of
daptomycin ($p<0.05$, Dunn’s post-test). The $AUC_{1-7d}$ were 3,629 ± 2,705 mg/L.h, 15,417 ±
9,734 mg/L.h and 11,052 ± 4,935 mg/L.h respectively for the DAP 0.2, DAP 1 and VAN 1
groups. The $AUC_{1-7d} (DAP 1) / AUC_{1-7d} (DAP 0.2)$ estimated ratio was equal to 4.62 with a
confidence interval IC$_{90\%} = [1.91; 11.2]$. Concerning the pharmacodynamic parameters of the
different treatment groups, $C_{max}/MIC$ ratios were ranged from 1,817 to 7,157 mg/L, while
$AUC_{0-24h}/MIC$ ratios from 29,359 to 138,934 mg/L.h. The results are presented in Table 5.
The rabbit model is considered suitable for evaluation of the activity, toxicity and pharmacokinetics of antimicrobials in experimental endophthalmitis (globe size, aqueous humor turnover rate and blood-ocular barriers are comparable to those of human eyes) (2, 4).

In this study, we used a methicillin resistant *S. aureus* strain, a very virulent pathogen often responsible for poor visual outcome. Antibacterial activity of daptomycin was here assessed *in vivo* because of its known rapid and concentration-dependent bactericidal *in vitro* efficacy against *S. aureus* in comparison with the slow and time-dependent bactericidal *in vitro* efficacy of vancomycin (29).

**Bactericidal efficacy**

In this study, increasing doses of daptomycin improved the bactericidal efficacy in the eye. A single 0.2 mg intravitreal injection of daptomycin was not effective in sterilising all infected eyes. It resulted in an approximately 1-log-unit reduction in the mean bacterial count 7 days after the antibiotic intravitreal injection. This bacterial count reduction failed to reach a level of statistical significance with the untreated group (BSS group). In contrast, single 1.0 mg intravitreal injections of daptomycin or vancomycin reduced bacterial counts in the eyes by approximately 4-log-units, and eradicated the ocular infection. However, failure of the 0.2 mg daptomycin intravitreal treatment is surprising since daptomycin vitreous concentration in the DAP 0.2 group was approximately 2000 times higher than the daptomycin MIC$_{90}$ of the inoculated *S. aureus* on day 1 and 47 times higher on day 7. In pharmacodynamic studies of concentration-dependant antibiotics, when antibiotic concentrations are eight times higher than the MIC, bactericidal activity was predominantly and strongly associated with a clinical response (6, 11).

**PK/PD analysis**
After a 0.2 or 1.0 mg intravitreal injection (DAP 0.2 and DAP 1 groups), daptomycin vitreous concentration-time curves showed a single exponential decline in the period from 1 to 7 days after administration. With a first sampling time at 24 hours, a distribution phase could have been missed to predict the model followed by the ocular daptomycin distribution. In this study, we didn’t take a sample of the vitreous humor immediately after intravitreal injection to limit the number of the eye punctures and to favour the collection of samples during an extended period (seven days). We decided with this attitude to analyse the ocular elimination process of daptomycin. The results showed that the terminal elimination half-time of daptomycin was constant and independent of the administered dose (38.9 hours and 40.9 hours respectively for the DAP 0.2 and DAP 1 groups) and T_{1/2el} of both groups of daptomycin was significantly longer than T_{1/2el} of vancomycin (20.5 hours in the VAN 1 group). The IC_{90%} \{1.91; 11.2\} of the AUC_{1-7d}(DAP 1) / AUC_{1-7d}(DAP 0.2) ratio was not contained completely in the acceptance interval [4;6.25]. Consequently, it is not statistically possible to conclude that the AUC is proportional to the both doses studied. Further studies are needed to conclude to the distribution model and the dose proportionality of daptomycin after administration by intravitreal injection in this endophthalmitis model.

According to the literature, daptomycin displays concentration-dependent killing against S. aureus, and efficacy is well-correlated with the Cmax/MIC and AUC_{0-24h}/MIC ratios, using data based on serum concentrations (14, 22). However, the ocular pharmacokinetic/pharmacodynamic relationship determining the efficacy of daptomycin against S. aureus into the eye, especially after a direct intravitreal injection, are not known. In this experimental model of endophthalmitis, very high Cmax/MIC and AUC_{0-24h}/MIC ratios did not appear to be predictive of antibacterial efficacy. Indeed, in the DAP 0.2 group Cmax/MIC = 2,499 and AUC_{0-24h}/MIC = 45,257 mg/L.h were not sufficient to provide a satisfactory bactericidal activity, but 1 mg of daptomycin was a bactericidal treatment presenting no failure in curing the endophthalmitis with Cmax/MIC = 7,157 and AUC_{0-24h}/MIC = 7,157 and AUC_{0-24h}/MIC = 45,257 mg/L.h were not sufficient to provide a satisfactory bactericidal activity, but 1 mg of daptomycin was a bactericidal treatment presenting no failure in curing the endophthalmitis with Cmax/MIC = 7,157 and AUC_{0-24h}/MIC = 45,257 mg/L.h.
$\frac{\text{MIC}}{\text{MIC}} = 138,934 \text{ mg/L.h.}$ The pharmacodynamic profile should be accurately described in future studies. The composition of vitreous humor with severe inflammation in infected eyes may impact the bactericidal activity of daptomycin. Markedly binding to vitreal proteins and/or conformational modifications could affect the percentage of free, stable and active drug. The importance of high protein binding (90-93% of plasma protein binding, according to Benvenuto et al. (5)) has already been underlined by Safdar et al (22). In a neutropenic murine thigh model infected by S. aureus, bactericidal activity and in vivo efficacy of daptomycin was analysed by using free drug concentrations. In our study, daptomycin vitreous concentrations were very high and protein binding could have been saturated in the DAP 1 group while not in the DAP 0.2 group. This could be one explanation for the failure of the low dosage of daptomycin in the present study in spite of the very high pharmacodynamics ratios.

Clinical examination and histopathologic analysis

The direct intravitreal injection is the best route to treat bacterial endophthalmitis because it shunts the blood-ocular barrier which limits the ocular penetration of antibiotics from the bloodstream, providing intravitreal antibiotic concentrations higher than those obtained after a systemic administration. However, ocular toxicity is a real problem correlated with high antibiotic concentrations in the eye. Indeed aminoglycosides may induce retinal toxicity and macular ischaemia with permanent damage after a single intravitreal injection (21, 23). In this study, the 1 mg daptomycin dosage directly injected into the eye was superior to other regimens in limiting the ocular inflammation and preserving the architecture of the ocular structures. It also appeared that the retina, including the photoreceptor layers, was preserved histologically. However, toxicity of such doses of intravitreal daptomycin on retinal function must be assessed in further studies.
One of the limitations of this study is that repeated punctures of the infected eyeball of each animal may reduce the antibiotic concentration after dilution in the newly secreted aqueous humor. There is also a risk of breaching the blood-ocular barrier and of damage to the ocular tissues. However, the same procedure was performed in the vancomycin group and it is likely that modifications occurred in the same proportion in all groups. Additionally, haemorrhagic samples were excluded from the analysis because of the possible impact on the antibiotic elimination and the clinical and histopathologic examinations. The findings in this study show differences with a previously published study. Comer et al. (8) show safety and efficacy of a 0.2 mg intravitreal daptomycin in an adult pigmented rabbit eye model of *S. epidermidis* endophthalmitis. No growth was observed in any infected eyes (seven eyes) 48 h after the antibiotic injection. No changes on the electroretinogram pattern were found. The photoreceptor layer was preserved. Therefore, the 0.2 mg dose appeared to be safe for the retinal functions and retinal toxicity was only observed for higher doses (i.e. moderate and severe scotopic and photopic waveforms and the photoreceptor layer was missing). This type of photoreceptor destruction in DAP 1 samples was not found. The difference in the rabbits’ strain (Dutch belted vs New-Zealand) and weight (respectively 2.2 - 2.5 kg vs 3.0 - 4.0 kg) could partially explain the difference observed, because the vitreous volume of the tested rabbits could vary significantly. Ocular pigmentation could also play a role since affinity of daptomycin to melatonin could affect the intra-ocular pharmacokinetics of this antibiotic, as it has already been implicated in some studies comparing intraocular penetration of systemic antibiotics in albino and pigmented rabbits (15, 19). The difference in the tested bacterial species (*S. aureus vs S. epidermidis*) could modify the bacterial toxin profiling and could explain differences in strain virulence and histopathologic analysis. Finally, the absence of vitreous daptomycin assay in the Comer study makes it impossible to compare these studies. To our knowledge, there is only one study dealing with ocular penetration of daptomycin in human (24). In this study, daptomycin was administered intravenously at a 10 mg/kg single
dose to a patient presenting an MRSA endogenous endophthalmitis. The vitreous
concentration 42 hours after administration of the dose was 12.43 mg/L and therapeutic
efficacy was not assessed in the absence of intraocular cultures. There are no data available
after direct intravitreal administration in human. Even if numerous ocular PK/PD studies are
conducted on rabbits because of the anatomic characteristics of the rabbit eye (i.e., globe size
and aqueous humor turnover rate are comparable to those of human eyes), caution must be
taken in extrapolating these results to humans.
In conclusion, this study demonstrates evidence of the effectiveness of daptomycin for the treatment of experimental staphylococcal endophthalmitis. A 1 mg dose of daptomycin ensures non-inferiority bactericidal efficacy with a 1 mg dose of vancomycin. This is the first report to study the ocular pharmacokinetics and pharmacodynamics of daptomycin in the eye. Daptomycin presented a long terminal elimination half-time significantly higher than vancomycin to the studied doses (p<0.05). Clinical and histopathological examinations showed limited ocular inflammation and preservation of the architecture of the ocular structures after sterilisation of the treated eye. Daptomycin should therefore be considered for the treatment of Gram-positive-bacteria intraocular infections. Further studies are needed to determine the toxicity of daptomycin on visual function and the PK/PD profile.

ACKNOWLEDGEMENTS

We would like to thank Dr Julien Godet for conducting the statistical analysis of the dose proportionality of daptomycin.

Transparency section: nothing to declare.

None of the authors has financial interest in this work.
REFERENCES


FIGURE LEGENDS

FIG 1. Percentage of eyes with viable bacteria at different time points after a single intravitreal injection of 1 mg of vancomycin (VAN 1) and 0.2 mg (DAP 0.2) or 1 mg (DAP 1) of daptomycin in rabbit eyes infected by methicillin-resistant S. aureus. BSS corresponds to the infected but untreated eyes group.

FIG 2. Effects of a single intravitreal injection of 1 mg of vancomycin (VAN 1) and 0.2 mg (DAP 0.2) or 1 mg (DAP 1) of daptomycin on the ocular inflammation score (expressed in cumulative average points) in infected rabbit eyes. Error bars represent the standard deviation. BSS corresponds to the infected but untreated eyes group. Asterisks represent a statistically significant difference in comparison with the BSS group (Dunn’s multiple comparison post-test) with the following scale of symbols: *, **, *** and **** mean, respectively, p<0.05, p<0.01, p<0.001 and p<0.0001.

FIG 3. Effects of a single intravitreal injection of 1 mg of vancomycin (VAN 1) and 0.2 mg (DAP 0.2) or 1 mg (DAP 1) of daptomycin on the histopathologic score (expressed in cumulative average points) in infected rabbit eyes. Error bars represent the standard deviation. BSS corresponds to the infected but untreated eyes group. Asterisks represent a statistically significant difference in comparison with the BSS group (Dunn’s multiple comparison post-test) with the following scale of symbols: *, **, *** and **** mean, respectively, p<0.05, p<0.01, p<0.001 and p<0.0001.

FIG 4. Histopathologic sections from enucleation specimen stained with hematoxylin-eosin, showing vitreous (asterisk) and retina (arrow) in the different rabbit groups. A = right eye after a 1 mg intravitreal injection of vancomycin (VAN 1 group), showing an infiltrated...
vitreous and a partially infiltrated and necrotic retina with some normal retina. (original magnification x 200). B: right eye after a 1 mg intravitreal injection of daptomycin (DAP 1 group), showing a non infiltrated vitreous and a normal retina. (original magnification x 400).

C: right eye after an intravitreal injection of a balanced salt solution (BSS group) showing a completely filled vitreous with infiltrate and a totally necrotic and desorganised retina with no retina layer intact (original magnification x 200). D: Left control eye, with no inflammation of vitreous and a normal retina (original magnification x 400).

FIG 5. Daptomycin and vancomycin vitreous concentrations versus time after administration of a single intravitreal injection of 0.2 mg (A: DAP 0.2 group) or 1 mg (B: DAP 1 group) of daptomycin and 1 mg of vancomycin (C: VAN 1 group).
A. DAP 0.2 group

Time (days after the antibiotic intravitreal injection)

B. DAP 1 group

Time (days after the antibiotic intravitreal injection)

C. VAN 1 group

Time (days after the antibiotic intravitreal injection)
**TABLE 1. Grading of endophthalmitis severity**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Cornea</th>
<th>Anterior chamber</th>
<th>Vitreous</th>
<th>Retina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No infiltration of inflammatory cells</td>
<td>Normal</td>
<td>No inflammation</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Partial-thickness infiltration of inflammatory cells</td>
<td>Partially filled with fibrin, no inflammatory cells</td>
<td>Infiltratory cells</td>
<td>Partially infiltrated and partially necrotic, some normal retina seen</td>
</tr>
<tr>
<td>2</td>
<td>Segmental full-thickness infiltration of inflammatory cells</td>
<td>Partially filled with fibrin, inflammatory cells</td>
<td>Partially filled with abscesses of infiltrate</td>
<td>Totally infiltrated and partially necrotic, no normal retina, some retinal layers intact</td>
</tr>
<tr>
<td>3</td>
<td>Total full-thickness infiltration of inflammatory cells</td>
<td>Completely filled with fibrin, inflammatory cells</td>
<td>Completely filled with infiltrate</td>
<td>Totally necrotic, no retina layer intact</td>
</tr>
</tbody>
</table>
TABLE 2. Oxacillin, vancomycin and daptomycin MICs for the *S. aureus* strain

<table>
<thead>
<tr>
<th>Strain</th>
<th>MICs (mg/L)</th>
<th>Susceptibility a</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> (ATCC 29737)</td>
<td>Oxacillin &gt; 2</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>Vancomycin = 0.5</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Daptomycin = 0.064</td>
<td>S</td>
</tr>
</tbody>
</table>

Abbreviations: a R : resistant; S : susceptible

TABLE 3. Toxin profiling of the *S. aureus* strain

<table>
<thead>
<tr>
<th>Strain</th>
<th>Toxin a</th>
<th>Technique</th>
<th>Presence (+) / Absence (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> (ATCC 29737)</td>
<td>LukFPV, LukSPV, EtA, EtB, SEA, SEB, SEC, SED</td>
<td>Immunoprecipitation using Ouchterlony method</td>
<td>− / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reversed passive latex agglutination using a toxin detection kit (Oxoid®, Dardilly France)</td>
<td>+ / −</td>
</tr>
</tbody>
</table>

a LukFPV and LukSPV = proteins F and P of the Panton-Valentine leukocidin; EtA and EtB = A and B exfoliative toxins; SEA, SEB, SEC and SED, = A, B, C, D enterotoxins
TABLE 4. Bacterial counts of the vitreous samples at different time points after a single intravitreal injection of 1 mg of vancomycin (VAN 1) and 0.2 mg (DAP 0.2) or 1 mg (DAP 1) of daptomycin in rabbit eyes infected by methicillin-resistant *S. aureus*.

The bacterial counts are expressed as mean ± standard deviation. BSS corresponds to the infected but untreated eyes group. The limit of detection is 1 log_{10} CFU/mL. Asterisks represent a statistically significant difference in comparison with the BSS group (Dunn’s multiple comparison post-test) with the following scale of symbols: *, ** and *** mean, respectively, p<0.05, p<0.01 and p<0.001.

<table>
<thead>
<tr>
<th>Rabbit groups</th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSS (n=4)</td>
<td>4.95 ± 0.94</td>
<td>6.82 ± 1.06</td>
<td>6.18 ± 2.42</td>
</tr>
<tr>
<td>VAN 1 (n=8)</td>
<td>5.65 ± 1.53</td>
<td>1.59 ± 1.66 **</td>
<td>1.54 ± 1.52 **</td>
</tr>
<tr>
<td>DAP 0.2 (n=8)</td>
<td>4.14 ± 1.48</td>
<td>2.16 ± 1.74 *</td>
<td>2.86 ± 2.58</td>
</tr>
<tr>
<td>DAP 1 (n=8)</td>
<td>4.65 ± 1.92</td>
<td>1.0 ± 0.00 ***</td>
<td>1.0 ± 0.00 **</td>
</tr>
<tr>
<td><em>p</em> (Kruskal-Wallis test)</td>
<td>&gt; 0.05</td>
<td>0.0006</td>
<td>0.003</td>
</tr>
</tbody>
</table>
TABLE 5. Ocular pharmacokinetic/pharmacodynamic parameters

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>DAP 0.2</th>
<th>DAP 1</th>
<th>VAN 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{el}$</td>
<td>0.48 ± 0.16</td>
<td>0.42 ± 0.08</td>
<td>0.82 ± 0.09</td>
</tr>
<tr>
<td>$T_{1/2el}$ (h)</td>
<td>38.8 ± 16.5</td>
<td>40.9 ± 6.7</td>
<td>20.5 ± 2.0</td>
</tr>
<tr>
<td>AUC1-7d (mg/L.h)</td>
<td>3,629 ± 2,705</td>
<td>15,417 ± 9,734</td>
<td>11,052 ± 4,935</td>
</tr>
<tr>
<td>MIC (mg/L)</td>
<td>0.064</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>$C_{max}$ (mg/L)</td>
<td>160 ± 159</td>
<td>458 ± 296</td>
<td>908 ± 533</td>
</tr>
<tr>
<td>AUC0-24h</td>
<td>2,896 ± 2,755</td>
<td>8,892 ± 5,589</td>
<td>14,679 ± 8,100</td>
</tr>
<tr>
<td>$C_{max}$/MIC (mg/L)</td>
<td>2,499 ± 2,487</td>
<td>7,157 ± 4,627</td>
<td>1,817 ± 1,066</td>
</tr>
<tr>
<td>AUC0-24h/MIC (mg/L.h)</td>
<td>45,257 ± 43,043</td>
<td>138,934 ± 87,332</td>
<td>29,359 ± 16,199</td>
</tr>
</tbody>
</table>

AUC1-7d and AUC0-24h = area under the curve between 1 and 7 days and between 0 and 24h after the intravitreal antibiotic injection; $K_{el}$ = terminal elimination constant; $T_{1/2el}$ = terminal elimination half-life; MIC = minimum inhibitory concentration; $C_{max}$ = antibiotic maximum concentration in vitreous humor. All parameters are expressed as mean ± standard deviation.