Injury, Int. J. Care Injured 46 (2015) 249–253

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

Activity of bone cement loaded with daptomycin alone or in combination with gentamicin or PEG600 against *Staphylococcus epidermidis* biofilms

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ARTICLE INFO

Article history:
Accepted 16 November 2014

Keywords:
Bone cement
Daptomycin
Vancomycin
Gentamicin
Biofilm
S. epidermidis
Microcalorimetry
Infection
Arthroplasty
Two-stage exchange

ABSTRACT

Daptomycin is a promising candidate for local treatment of bone infection due to its activity against multi-resistant staphylococci. We investigated the activity of antibiotic-loaded PMMA against *Staphylococcus epidermidis* biofilms using an ultra-sensitive method bacterial heat detection method (microcalorimetry).

PMMA cylinders loaded with daptomycin alone or in combination with gentamicin or PEG600, vancomycin and gentamicin were incubated with *S. epidermidis*-RP62A in tryptic soy broth (TSB) for 72 h. Cylinders were thereafter washed and transferred in microcalorimetry ampoules pre-filled with TSB. Bacterial heat production, proportional to the quantity of biofilm on the PMMA, was measured by isothermal microcalorimetry at 37 °C. Heat detection time was considered time to reach 20 μW. Experiments were performed in duplicate.

The heat detection time was 5.7–7.0 h for PMMA without antibiotics. When loaded with 5% of daptomycin, vancomycin or gentamicin, detection times were 5.6–16.4 h, 16.8–35.7 h and 4.7–6.2 h, respectively. No heat was detected when 5% gentamicin or 0.5% PEG600 was added to the daptomycin-loaded PMMA.

The study showed that vancomycin was superior to daptomycin and gentamicin in inhbiting staphylococcal adherence in vitro. However, PMMA loaded with daptomycin combined with gentamicin or PEG600 completely inhibited *S. epidermidis*-biofilm formation.

PMMA loaded with these combinations may represent effective strategies for local treatment in the presence of multi-resistant staphylococci.

Introduction

One- or two-stage revision represents the current standard method for managing infected total hip or knee arthroplasties [1]. In a two-stage exchange the procedure consists of debridement including removal of the device, local and intravenous antibiotic therapy, and re-implantation after 2 weeks (short interval) or after more than 6 weeks (long interval) [1]. Currently, polymethylmethacrylate (PMMA) bone cement spacers loaded with antibiotics, such as gentamicin, tobramycin and vancomycin, are used in two-stage revision [2]. The elution of commonly used antibiotics from the PMMA has been well investigated both in vitro and in vivo [3–5]. Several studies have shown that antibiotics added to cement do not reach significant concentrations in the bloodstream to cause systemic toxicity, and simultaneously, high concentrations of

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http://dx.doi.org/10.1016/j.injury.2014.11.014
0020–1383/

antibiotics can be achieved locally [3]. For an optimal local treatment, the antibiotic-loaded cement must release a sufficient concentration of antibiotics at the site of infection through a controlled delivery, in order to avoid possible side effects and prevent the emergence of resistance [5].

Staphylococcus epidermidis and *Staphylococcus aureus* are the major bacterial pathogens involved in infected arthroplasties [1,6]. An emergence of staphylococci resistant to antibiotics commonly used in cement, such as gentamicin, has been reported [7]. In addition, the increased prevalence of methicillin-resistant (MRSA) or vancomycin-resistant staphylococci (VRSA) creates a need of new antibiotics for local treatment [8,9]. Daptomycin is a lipopeptide antibiotic exhibiting a concentration-dependent killing activity against Gram-positive pathogens in vitro, including MRSA, VRSA, penicillin-resistant *Streptococcus pneumonia*, and ampicillin and vancomycin-resistant enterococci [10].

The mechanic properties and drug-release of daptomycin-loaded PMMA were recently studied [11]. A daptomycin-content of 2.5% or 5% was demonstrated to not affect the compressive strength of the cement [11]. The elution profile showed an initial burst between 6 and 12 h, a decreased release between 12 and 24 h, followed by a stable release up to 96 h. Interestingly, an addition of the aminoglycoside tobramycin increased the release of daptomycin, which could be explained by a greater porosity in the presence of both antibiotics [11]. Another strategy to improve the antibiotic release is to add a filler or a water-soluble component to the cement that will increase the porosity and water penetration, and thus the elution of the drug [12–15]. An improved daptomycin release from PMMA has been demonstrated in the presence of xylitol and glycine [13]. Polyethylene glycol (PEG) is another water-soluble excipient known to mix well into hydrophobic polymers, such as PMMA and polylacto caprolactone (PCL), and the addition of PEG was demonstrated to increase the release-rate of water-soluble drugs [16].

The aim of this in vitro study was to evaluate the efficiency of PMMA loaded with daptomycin alone or in combination with gentamicin or the additive PEG600, in the prevention of biofilm formation of *S. epidermidis*. For comparison, PMMA loaded with gentamicin or vancomycin was tested. For assessment of the biofilm quantity on the cement, bacterial heat production was measured by a sensitive and accurate bacterial heat detection method, isothermal microcalorimetry [17,18].

Material and methods

Study organism

A laboratory biofilm-forming strain of *S. epidermidis*, RP62A, was used in all experiments [17,18]. The strain is susceptible to gentamicin, vancomycin and daptomycin, with minimal inhibitory concentrations (MICs) of 2, 1 and 0.5 μg/mL, respectively.

Antibiotic-loaded cement

Palacos® PMMA cement was provided by Heraeus (Heraeus Medical GmbH, Wehrheim, Germany) as cylinders (Ø 6.5 mm × 10 mm). The cement was preloaded by the manufacturer with daptomycin, vancomycin or gentamicin at 2.5% or 5% wt/wt (i.e. 1 g or 2 g per 40 g cement). A combination-loading of daptomycin and gentamicin was tested at 2.5%, 3.75% and 5% wt/wt of both antibiotics. In addition, with the purpose to increase the daptomycin activity, the addition of the polyethylene glycol PEG600 to the daptomycin-loaded cement was evaluated. The amount of PEG600 added was 10 times lower than the daptomycin content, i.e. 0.25% of PEG600 for 2.5% of daptomycin etc.

Biofilm formation

Biofilm formation was performed according to a protocol from Clauss et al. [17,18]. Briefly, a cement cylinder was inserted in a 50 mL Falcon tube containing 2.7 mL Tryptic Soy Broth (TSB) supplemented with 50 mg/mL $CaCl_2$ (necessary for daptomycin activity) and soaked for 15 min. The growth media was then inoculated with 300 μL of *S. epidermidis* suspension with a Mc Farland density of 0.5 ($\sim 10^7$ CFU/mL) for a final concentration of ($\sim 10^6$ CFU/mL) and incubated for 72 h at 37 °C at static conditions. After the incubation the cylinder was transferred to a 50 mL Falcon tube prefilled with 5 mL PBS, and washed (shaken by hand) followed by removal of the PBS. The washing procedure was repeated 5 times. Experiments were performed in duplicate.

Detection of biofilm bacteria by microcalorimetry

The biofilm-imbedded cylinder was transferred with sterile forceps into a microcalorimetry ampoule filled with 1 mL TSB and mixed by inversion. After sealing the ampoules, they were lowered into a 48-channel batch microcalorimeter (Thermal Activity Monitor, model 3102 TAM III; TA Instruments, New Castle, DE). Heat flow (in μW) at 37 °C was measured for 72 h. The experimental detection limit was set at 20 μW to distinguish microbial heat production from the thermal background. The results were plotted as heat flow over time and the heat detection time recorded. Experiments were performed in duplicate.

Results

To determine the anti-adherence and anti-biofilm activity of different antibiotics, heat production of *S. epidermidis* present on the PMMA cylinders was measured by microcalorimetry. The start of heat production is proportional to the bacterial load in the sample, e.g. the more biofilm present on the cylinder, the faster a heat signal can be detected [19]. The time to heat detection was calculated for the different samples (Table 1).

The table summarises the detection time of bacterial heat production for the antibiotic loadings tested. When comparing the different antibiotics, vancomycin was the most efficient in preventing *S. epidermidis* biofilm formation during a 72-h bacterial exposure, in comparison to gentamicin and daptomycin at both 2.5% (Fig. 1A) and 5% wt/wt (Fig. 1B). The concentration-dependent activity of daptomycin was demonstrated by prolonged heat detection time for the 5%-loading compared to the 2.5%-loading, with heat detection times of 5.6–16.4 h and 3.3–5.1 h, respectively. No prolongation in time to heat detection was observed with a higher amount of gentamicin and vancomycin.

Fig. 2 illustrates the heat produced by *S. epidermidis* present on cement loaded with both daptomycin and gentamicin. A 2.5%-loading of the antibiotic combination did not prevent bacterial adherence and no delay in heat detection time was observed compared to the positive control (PMMA only). However, when

Table 1
Time to detection (in duplicate) of heat produced by *S. epidermidis* biofilm present on the PMMA bone cement with different antibiotic loadings.

Amount (wt/wt%)	Time to detection (h)				
	DAP	VAN	GEN	DAP + GEN	DAP + PEG600
2.5	3.3–5.1	29.8–48.7	6.8–13.3	<1	1.8–2.0
3.75	nd	nd	nd	>72	>72
5	5.6–16.4	16.8–35.7	4.7–6.2	>72	>72

Note: The time to heat detection of the antibiotic-free PMMA was 5.6–7.0 h. All PMMA cylinders were incubated in TSB inoculated with *S. epidermidis* for 72 h before the microcalorimetric test. DAP: daptomycin, VAN: vancomycin, GEN: gentamicin. Nd: not done.