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Determining potential of PMMA as a depot for rifampin to treat recalcitrant orthopaedic infections

Stefanie M. Shiels[a,*], David J. Tennent[a,b], Kevin S. Akers[c,d], Joseph C. Wenke[a]

[a] Extremity Trauma and Regenerative Medicine Task Area, US Army Institute of Surgical Research, JBSA-Fort Sam Houston, TX, United States
[b] Department of Orthopaedics, San Antonio Military Medical Center (Brooke Army Medical Center), JBSA-Fort Sam Houston, TX, United States
[c] US Army Institute of Surgical Research, JBSA-Fort Sam Houston, TX, United States
[d] Infectious Disease Service, San Antonio Military Medical Center (Brooke Army Medical Center), JBSA-Fort Sam Houston, TX, United States

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ABSTRACT

Background: Open fractures are often complicated by infection. In cases of severe soft tissue and vascular injury, systemic antibiotics may be ineffective due to their inability to reach and provide direct antimicrobial activity to the zone of injury. High antibiotic concentrations within the wound can be achieved with reduced systemic toxicity by using local antibiotic delivery. As bacteria associated with musculoskeletal injuries frequently form biofilms, antibiotic selection is important. Herein, the use of rifampin, an antibiotic with activity against biofilms, delivered via polymethylmethacrylate (PMMA) beads is evaluated for use in a traumatic musculoskeletal wound model.

Methods: PMMA beads loaded with rifampin, or combinations of rifampin and vancomycin, were prepared and evaluated for time to curing, drug release kinetics *in vitro*, and infection prevention *in vivo* using a well-established rat model of musculoskeletal infection. A segmental bone defect was created and contaminated with methicillin susceptible *Staphylococcus aureus* (UAMS-1). Wounds were debrided, irrigated, and treated with PMMA beads, containing rifampin or combinations of rifampin plus vancomycin, following a 6-h (early) or 24-h (delayed) treatment. After 14 days, tissue, implants, and beads were removed for bacterial quantification and assessed for rifampin resistance.

Results: There was a direct association between loaded concentration and release kinetics of the rifampin and vancomycin from PMMA beads. Higher rifampin concentrations delayed PMMA curing times. The addition of vancomycin to PMMA resulted in more rapid release of rifampin from beads. However, the highest concentration of rifampin loaded PMMA beads (10% wt/wt) was the only treatment to significantly reduce bacterial counts. No rifampin resistance was observed.

Conclusion: Although higher concentrations of rifampin resulted in significant reductions of bacteria, these levels extended PMMA curing times and transformed PMMA material characteristics. While these characteristics make the material unsuitable for weight-bearing applications, such as total joint arthroplasty, the use of rifampin-loaded PMMA beads may be an effective intervention in a contaminated traumatic extremity wound due to its ability to eradicate biofilms.

Introduction

Infection rates following high-energy orthopaedic trauma continue to remain high despite advances in clinical care [1]. Bacteria colonize orthopaedic wounds and begin forming biofilms, thereby decreasing the effectiveness of common antimicrobials. A 100- to 1000-fold increase in antimicrobial concentrations is often required to eradicate the biofilm [2]. These local levels are not clinically achievable by systemic means without causing significant toxicity, however, but can be achieved by applying antibiotics directly to the fracture site.

Vancomycin is often used as an antibiotic for local application using polymethylmethacrylate (PMMA) beads to prevent and treat musculoskeletal infections based on its history of use, wide availability, and minimal host tissue toxicity [3]. Vancomycin is highly compatible with PMMA: it elutes steadily from PMMA over several days, is not deactivated by PMMA's exothermic curing reaction, and does not interfere with the polymerization process of PMMA [4]. While vancomycin exhibits effectiveness against gram-

* Corresponding author at: US Army Institute of Surgical Research, 3698 Chambers Pass, Bldg 3611, JBSA-Fort Sam Houston, TX 78234, 1-210-539-3654, United States.
E-mail addresses: stefanie.m.shiels.ctr@mail.mil, scrumlett@gmail.com (S.M. Shiels).

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positive bacteria, it performs poorly against established biofilms, a characteristic related to its mechanism of action [5]. Although vancomycin is widely used for traumatic orthopaedic infections, PMMA beads loaded with vancomycin (10% wt/wt) failed to eradicate a bacterial biofilm infection in a preclinical model [5]. Rifampin, a rifamycin antibiotic, inhibits bacterial DNA transcription, thus its mechanism of action is independent of cellular division making it effective against bacteria with reduced metabolism, such as those found in biofilms [6]. This property is believed to contribute to its overall performance against biofilms, including those of *Staphylococcus aureus*, a primary cause of musculoskeletal infections [7]. The medical treatment of prosthetic joint infections with rifampin-containing systemic antimicrobial regimens has improved implant retention rates [8]. In a traumatic wound, however, damaged and ischemic tissues may prevent efficient antibiotic tissue diffusion following systemic administration, thus limiting concentrations available in the wound.

Unlike other antibiotics more commonly incorporated into PMMA, rifampin interferes with the polymerization process by acting as a free radical scavenger and preventing hardening [9–11]. Consequently, this interaction results in low rifampin release, which can be overcome by increasing rifampin loading or incorporating a co-delivery agent. Although the antimicrobial elution from PMMA is less efficient than vancomycin, the lower minimum biofilm eradication concentration (MBEC) of rifampin for *staphylococcal* biofilms could result in effective biofilm eradication even when low concentrations of rifampin are released [6]. While the structural softening caused by incorporation of rifampin into PMMA makes this approach ineffective for arthroplasty [10,11], the use of rifampin-loaded PMMA has not been previously investigated as an antimicrobial delivery vehicle in a preclinical trauma model of musculoskeletal infection, where structural joint support is not required.

The purpose of this project was to evaluate the utility of rifampin-loaded PMMA beads for the local treatment of established *staphylococcal* orthopaedic infections using *in vitro* methods and a well-established *in vivo* model of musculoskeletal trauma.

Methods

Rifampin-loaded PMMA was systematically evaluated *in vitro* and *in vivo*. During bead preparation, PMMA texture and curing time were observed and recorded. Following curing, rifampin release kinetics were evaluated. Bead compositions were down-selected for *in vivo* evaluation based on curing time and release kinetics.

Materials

PMMA beads were loaded with rifampin (R) (Sigma Aldrich, St. Louis MO) or a combination of rifampin and vancomycin (V) (Sigma Aldrich, St. Louis, MO) (Table 1). The concentrations evaluated were chosen to reflect dosages currently available and convenient for incorporation with PMMA in the operating room. Briefly, the powder component of a 40 g kit of Palacos® R (Zimmer Biomet, Warsaw, IN) was premixed with antibiotic powder for 1 min, followed by addition of the liquid monomer, which was mixed to form a paste. The paste was either spread over a silicone mold to form 3 mm x 3 mm cylinders and allowed to cure, or rolled into balls for a touch test to determine curing behavior. For purposes of this study, curing was defined as the point when the material was no longer sticky to touch and maintained a cohesive structure. The antibiotic-loaded PMMA beads were stored at 4°C until use.

In vitro *release kinetics*

To measure antibiotic elution from PMMA beads, two 3 mm by 3 mm beads were added to 1 ml of sterile phosphate buffered saline (PBS) and placed into an incubator at 37 °C (n = 4). At the designated time points, the PBS eluate was completely removed and replenished with fresh PBS. The eluates were stored at 4°C until antibiotic concentrations were determined spectrophotometrically. Rifampin was measured by its absorbance at 335 nm and values correlated to a standard curve. Vancomycin was measured by high performance liquid chromatography (HPLC) at a detection wavelength of 210 nm, as previously reported [5].

In vivo *animal infection*

This study, approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical Research, was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals. As described previously, a critical-size defect was created in the right femur of 57 Lewis rats (Harlan Laboratories, Indianapolis, IN; mean weight of 337 ± 18 g) (Fig. 1) [12]. Briefly, each animal was given preemptive sustained-release buprenorphine, anesthetized with isoflurane, and the right hindlimb prepared for surgery in a sterile manner. A 3-cm incision was made over the lateral right thigh, and the femoral shaft was exposed. A 24-mm radiolucent polyacetyl plate was affixed to the femur with six 0.9 mm threaded stainless steel Kirschner-wires and a 6-mm segment of bone was removed with a reciprocating saw under copious saline irrigation. Each animal was inoculated with an average $3.59 \times 10^5 \pm 2.00 \times 10^4_{SEM}$ colony forming units (CFU) of UAMS-1, a clinical methicillin-susceptible *Staphylococcus aureus* osteomyelitis isolate, via an absorbable collagen matrix, and the wound was closed in layers. Animals were randomly selected for both time to treatment initiation and treatment type (Table 2). The high 10% rifampin loading (R10) was chosen based on its *in vitro* kinetics

Table 1
Composition of PMMA beads

Group Name	Rifampin (R)		Vancomycin (V)	
	% wt/wt bead	g rifampin powder (per PMMA kit)	% wt/wt bead	g vancomycin powder (per PMMA kit)
R10	10	6	–	–
R4	4	2.4	–	–
R4V1.7	4	2.4	1.7	1
R4V5	4	2.4	5	3
R2	2	1.2	–	–
R2V1.7	2	1.2	1.7	1
R2V5	2	1.2	5	3
R1	1	0.6	–	–
R1V1.7	1	0.6	1.7	1
R1V5	1	0.6	5	3

Beads are 3 mm diameter x 3 mm tall cylinders containing rifampin (R), with or without vancomycin (V), at the weight percentages indicated.