Persistence of bacterial growth on antibiotic-loaded beads

Is it actually a problem?

Konstantinos Anagnostakos, Philip Hitzler, Dietrich Pape, Dieter Kohn, and Jens Kelm

Klinik für Orthopädie und Orthopädische Chirurgie, Universitätsklinikum des Saarlandes, Homburg/Saar, Germany
Correspondence AK: konstantinos.anagnostakos@uniklinik-saarland.de
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Background and purpose **Implantation of antibiotic-loaded beads is used for orthopedic infections. However, recent in vitro reports have emphasized that bacteria can persist on—or even colonize—antibiotic-impregnated bone cement. We therefore assessed whether bacterial adherence and growth could be determined on gentamicin- and gentamicin-vancomycin-loaded beads that had been removed after eradication of infection.**

Material and methods **We bacteriologically examined 18 chains of antibiotic-loaded beads (11 gentamicin-loaded, 7 gentamicin-vancomycin-loaded) that had been implanted because of orthopedic infections. Among the causative agents, *Staphylococcus epidermidis, Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) were the most frequent organisms identified.**

Results **In 4 cases (3 with *S. epidermidis* and one with MRSA), we found that there was persistence of bacterial growth on the beads. *S. epidermidis* strains persisted only on gentamicin-loaded beads, while MRSA could grow on gentamicin-vancomycin-impregnated cement. In one case, the emergence of a gentamicin-resistant *S. epidermidis* strain was observed despite the fact that preoperative samples of *S. epidermidis* from this patient had been susceptible to the antibiotic.**

Interpretation **Persistence of bacterial growth on bone cement remains a hazardous problem in orthopedic surgery. Adherence of bacteria to cement can lead to emergence of bacterial resistance to antibiotics and might result in clinical recurrence of infection.**

Since their introduction by Klemm (1979), antibiotic-loaded beads have been widely and successfully used in local treatment of orthopedic-related infections. Commercially available beads (e.g. Septopal) are usually impregnated with gentamicin only. However, various antimicrobial agents have also been tested in vitro and in vivo and have shown sufficient release from beads, with vancomycin being the most frequently used agent (Ozaki et al. 1998, Kelm et al. 2004). The selection of antibiotics is mostly based on identification of the pathogenic organism and the release characteristics of each antimicrobial agent from bone cement. Thus, in cases of multibacterial infection or when no causative bacterium has been identified, some authors recommend impregnation of bone cement with an aminoglycoside and a glycopeptide as a result of the wider antimicrobial spectrum and the enhanced antibiotic elution that can be gained from a synergistic effect between these antibiotic groups (Gonzalez Della Valle et al. 2001, Anagnostakos et al. 2005). This synergistic effect seems, however, to depend on the relative amounts of these antibiotic groups (Anagnostakos et al. 2006).

Recent reports have emphasized the emergence of bacterial strains with altered resistance profiles after adherence to antibiotic-loaded cement (Arciola et al. 2002). Using an animal model, Thornes et al. (2002) demonstrated the development of gentamicin resistance of primarily gentamicin-susceptible *Staphylococcus epidermidis* strains after exposure to gentamicin-loaded PMMA. Neut

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et al. (2001) showed growth of various organisms on 18 out of 20 explanted gentamicin-impregnated chains, with coagulase-negative staphylococci being the most commonly identified bacteria. *S. epidermidis* strains are capable of adhering to and accumulating on both gentamicin-loaded and gentamicin-clindamycin-loaded cement (König et al. 2001).

Persistence of bacterial growth on PMMA is apparently an increasing problem in orthopedic surgery. Almost all the relevant studies have, however, dealt with gentamicin-loaded bone cement. We prospectively examined bacterial growth persistence on gentamicin-loaded and gentamicin-vancomycin-loaded PMMA beads retrieved from patients after the clinical symptoms of infection had been eradicated.

Material and methods

From 2003 through 2005, we implanted 18 antibiotic-loaded chains for treatment of infections in 15 patients (Tables 1 A and B; see Supplementary article data).

Either Septopal chains (4.5 mg gentamicin/bead) (Merck, Darmstadt, Germany) or gentamicin-vancomycin-impreganted chains (1 g gentamicin, 2 g vancomycin, and 40 g Palacos) (Kelm et al. 2004) were used. The latter chains were produced by means of a mold. In each case, the selection of beads was done according to the following criteria: (1) size, length, and location of the infected area, (2) pathogenic organism, if isolated preoperatively, and its susceptibility to gentamicin and/or vancomycin, (3) known allergic reactions to vancomycin, and (4) the possibility of prolonged implantation or even non-removal of the beads due, for example, to the general condition of the patient or his/her willingness to undergo additional surgery (in these cases, gentamicin-vancomycin-loaded beads were implanted due to the known synergistic effect with prolonged antibiotic elution) (Kelm et al. 2004).

Postoperatively, systemic antibiotics were administered according to the antibiogram. If possible, neither gentamicin nor vancomycin was used. As soon as local signs of infection had subsided and the results of blood tests such as ESR and CRP had returned to normal, the chains were removed. In some cases, immediate chain explantation could not be performed, either because of the general medical condition of the patient or because the patient was not willing to undergo a second procedure in such a short time. In these cases the intravenous antibiotic therapy was given for 4 weeks, with oral antibiotics for 2 weeks after that. The overall mean implantation period was 89 days (86 days for Septopal chains and 94 days for gentamicin-vancomycin-loaded chains).

At explantation of beads, the beads and tissue samples were sent within 2 h for microbiological examination. The tissue samples were incubated on blood agar plates for 2 days. The beads were also incubated for 2 days on blood agar plates, and also in tryptic soya broth (Becton-Dickinson, Heidelberg, Germany). The bacteria identified on the PMMA were compared with the organisms isolated preoperatively, if known, and also compared for clinical relevance with the results of the tissue samples—if persistence of infection or reinfection occurred. Antibiotic susceptibility of all bacterial strains isolated was determined in a standardized way by means of the MicroScan WalkAway system (Dade MicroScan, Inc., Sacramento, CA) and then compared to the preoperative resistance profile. Pathogenic organisms isolated from the beads and tissues are listed in Table 2.

Results

With regard to the beads, all results from the agar plates and the tryptic soya broth were identical. In all cases where organisms were isolated, the number of colony forming units (CFU) was > 10^5/mL. Persistence of bacterial growth on the PMMA was observed in 22% of the cases (4/18) (nos. 1, 7, 8, and 9) (Table 2). *S. epidermidis* strains could be isolated on 3 gentamicin-loaded beads (cases 1, 8, and 9), In case 1, gentamicin resistance emerged after implantation of beads despite an apparent susceptibility of the *S. epidermidis* strain preoperatively. In one case (no. 7), an MRSA strain was able to survive on gentamicin-vancomycin-loaded cement. In the tissue samples, positive cultures were found in 3 cases (nos. 6, 7, and 8), which corresponded to one *S. aureus* strain, one *S. epidermidis* strain, and one MRSA strain, respectively.

Table 2. Pathogenic organisms and their antibiotic profiles prior to implantation of beads and after removal

Case	Pathogenic organism	Antibiotic susceptibility prior to beads implantation		after beads explantation		Organism identified beads	tissue
		gentamicin	vancomycin	gentamicin	vancomycin		
1	*S. epidermidis*	s	s	r	s	*S. epidermidis*	none
2	*S. epidermidis*	r	s	–	–	none	none
3	*S. aureus*	s	s	–	–	none	none
4	*P. mirabilis*	s					
	S. aureus	s	s	–	–	none	none
	P. aeruginosa	s					
5	*S. aureus*	s	s	–	–	none	none
6	*S. epidermidis*	r	s	–	–	none	S. aureus
						none	none
7	MRSA	s	s	s	s	none	MRSA
				s	s	MRSA	none
8	*S. epidermidis*	r	s	r	s	*S. epidermidis*	*S.epidermidis*
9	*S. epidermidis*	r	s	r	s	*S. epidermidis*	none
10	*S. aureus*	s	s	–	–	none	none
	S. aureus	s	s	–	–	none	none
11	*S. aureus*	s	s	–	–	none	none
12	*Corynebacterium*	s	s	–	–	none	none
13	n.b.i.	–	–	–	–	none	none
14	*S. aureus*	s	s	–	–	none	none
15	*S. aureus*	s	s	–	–	none	none
	Streptococcus	s	s	–	–		

s: susceptible; r: resistant; n.b.i: no bacterium isolated; –: not tested

With a total mean follow-up time of 16 months, we observed complications in 2 cases. Patient no. 6 presented again—4 months after removal of beads—with signs of infection; after explantation of the prosthesis and implantation of a spacer, the infection was eradicated. At a further follow-up of 2 years after re-implantation of a prosthesis, no re-infection had occurred. In the other case (no. 14), re-infection was observed 13 months after removal of the beads. Infection management consisted of surgical debridement and removal of hardware. At a new follow-up of 18 months, no further complications had occurred. In the remaining cases, no persistence of infection or re-infection was observed at a mean follow-up of 16 (2–38) months.

Discussion

We have found no other published in vivo studies examining whether bacteria can persist on both gentamicin-loaded and gentamicin-vancomycin-loaded beads. Various studies have shown adherence and growth of bacteria on antibiotic-loaded bone cement. Once adherent, some bacteria produce an extracellular matrix, called glycocalyx, or slime. Adherence and slime production are postulated to produce an environment that promotes bacterial colony growth and inhibits host antimicrobial responses and antibiotic action (Kendall et al. 1996). *S. epidermidis* can adhere to and grow on tobramycin-impregnated cement discs due to the protection provided by the biofilm mode of growth (Oga et al. 1992). Van de Belt et al. (2000) reported that biofilm formation by *S. aureus* was reduced on different gentamicin-loaded bone cements as compared to unloaded cements only during a short period, which depended on the initial high antibiotic release from the cement. Hence, worries have been expressed about the contribution of the use of antibiotic-loaded bone cement to the development of antibiotic-resistant microorganisms. In a retrieval study, coagulase-negative staphylococci were found to be responsible for 88% of infections in patients with the primary arthroplasty fixed with gentamicin-loaded cement, while at least one of the infecting strains of staphylococci was resistant to gentamicin (Hope et al. 1989).

In accordance with data in the literature, we found that *S. epidermidis* strains are also capable

of in vivo adherence to gentamicin-loaded bone cement. Interestingly, this occurred in 3 of 4 cases of *S. epidermidis* infection in our patients, indicating the potential of *S. epidermidis* for colonization of PMMA. Hanssen (2004) has recently reported that although much of the orthopedic concern is focussed on methicillin-resistant staphylococci and vancomycin-resistant enterococci, there is increasing evidence that gentamicin-resistant staphylococci are becoming increasingly prevalent in association with the use of gentamicin-loaded bone cement used for prophylaxis of arthroplasty. Thornes et al. (2002) pointed out that the emergence of antibiotic resistance among strains of *S. epidermidis* emphasizes the potential for the development of resistance in low-grade pathogenic bacteria that have an affinity for implanted materials.

In our MRSA infection, the strain could persist on both beads and tissue samples. However, since clinical findings and laboratory data were normal no further treatment procedures were performed. The persistence of the MRSA in the wound and on the beads indicates the potency of this bacterium. Perhaps the search for other antimicrobial agents for incorporation into PMMA may solve this problem.

Although the combination in an aminoglycoside-glycopeptide-loaded acrylic bone cement makes sense, very few studies have investigated the pharmacokinetic properties of such antibiotic-loaded cement media. Klekamp et al. (1999) studied the release of tobramycin and vancomycin from Simplex and Palacaos cement in vitro. They found that the release of tobramycin was compromised by the presence of vancomycin, whereas the release of vancomycin was not compromised by the presence of tobramycin. Another in vitro study by Penner et al. (1996) demonstrated that the combination of both antibiotics in PMMA enhances the release of tobramycin by 68% and of vancomycin by 103% in comparison to controls containing tobramycin or vancomycin alone. In vivo studies of tobramycin-vancomycin-impregnated hip and knee spacers have shown superior elution for tobramycin than for vancomycin; however, the elution of vancomycin was enhanced by the presence of tobramycin (Masri et al. 1998).

One previous study has shown that the antibiotic release from the same gentamicin-vancomycin-loaded beads as used in the present study can give sufficient gentamicin for 11 days and sufficient vancomycin for 4 months at an impregnation level of 1 g gentamicin and 2 g vancomycin per 40 g PMMA (Kelm et al. 2004). This indicates that prolonged implantation of beads is more likely to encourage the emergence of gentamicin-resistant bacterial strains rather than vancomycin ones, due to the inferior release characteristics at such a ratio of antibiotics. We made similar observations, which explain the higher rate of adherence and bacteria growth in the Septopal group compared to the group with gentamicin-vancomycin-loaded beads. However, it should be noticed that antibiotic-loaded beads in other antibiotic proportions ([aminoglycoside] > [glycopeptide]) may show results other than the ones we found.

In 2 of our cases, we noticed complications regarding re-infection. Kendall et al. (1996) have demonstrated that adherent bacteria can be found on biomaterial removed from asymptomatic patients. The possible cause may be that routine cultures often indicate no infection whereas extensive culture demonstrates growth persistence on PMMA (Neut et al. 2001). Perhaps prolonged incubation and the use of enrichment broth might increase the detection rates, in order to prevent such events (Neut et al. 2001).

Some reservations should be made with regard to the interpretation of our results. The mean duration of implantation of the beads was relatively long (86 days for the Septopal group and 94 days for the gentamicin-vancomycin group). If the beads had been removed after a shorter implantation period, perhaps the rates of bacterial persistence would have been reduced. Secondly, there are no exact data available about the precise mechanism of bacterial killing when two antibiotics are combined in bone cement, compared to what happens with monoantibiotic-loaded cement. Since the synergistic effect with regard to antibiotic release of both agents and, hence, their antimicrobial properties are dose-dependent, and may vary widely with different amounts of gentamicin and vancomycin incorporated into PMMA, our results should be interpreted with caution. Moreover, each type of cement has a “window of effectiveness” with regard to reduction of biofilm formation (Hanssen 2004); thus, our results are valid only for the use of

Palacos. Furthermore, we were only able to detect gram-positive organisms on the PMMA, and in all our cases there were monobacterial infections. Neut et al. (2005) stated that re-implantation after removal of an infected joint replacement has a high failure rate, especially when gram-negative organisms are involved. They also found that gentamicin-loaded cement stimulates slime production in *P. aeruginosa* infections. Antibiotic-loaded bone cement also stimulates the formation of small variant colonies (SVCs) with reduced sensitivity to gentamicin (Von Eiff et al. 2000). The combination of SCVs and persistent or relapsing infections has become more and more probable over the past decade, especially in patients with chronic osteomyelitis (Von Eiff et al. 2000). Until a study provides firm data regarding these issues, one should exercise caution in making generalizations from conclusions.

Our observations also have clinical relevance for the general use of antibiotic-loaded bone cement. If gentamicin-impregnated cement has been used in primary surgery, bacteria involved in these infections may already have survived a high concentration of gentamicin inside a prosthesis-related gap, and are probably gentamicin-resistant. Subsequent use of gentamicin-loaded cement is therefore less efficacious, while still carrying the risk of further selection of resistance.

PMMA without any antibiotic additives and also antibiotic-loaded PMMA can be colonized by bacteria. Unfortunately, there is no reliable method that would completely reduce the adherence of bacteria to acrylic bone cement to 0%. Most antimicrobial agents inhibit bacterial growth only over a limited time period. However, some encouraging results from in vitro studies have been reported concerning impregnation of acrylic bone cement with silver nanoparticles, with regard to bacterial killing and prevention of bacterial adherence (Alt et al. 2004). Perhaps this might be a valuable alternative in cases of multibacterial and multiresistant infections.

Based on our results, with infections caused by *Staphylococcus epidermidis* orthopedic surgeons should consider using gentamicin-vancomycin-loaded beads rather than Septopal beads. In the case of MRSA infections, gentamicin-vancomycin-loaded beads should be inserted for infection management; however, prolonged implantation may be associated with bacterial survival and, hence, persistence of infection. In the case of multibacterial infections, the use of gentamicin-vancomycin-impregnated beads should be also recommended due to the wider antimicrobial spectrum and the enhanced pharmacokinetic characteristics of both agents.

In summary, antibiotic-impregnated beads have proven efficacy in local treatment of infections. However, possible persistence of infection may be caused by persistence of bacterial growth on the PMMA. Adherence to cement can lead to the emergence of resistant bacteria irrespective of the fact that there may have been antibiotic susceptibility of the same organism preoperatively—and might, under certain circumstances, result in a clinical re-infection. With regard to eradication of infection, complete killing of the bacterial strain seems to be questionable: only suspension of an infection can be achieved.

Contributions of authors

PD, KD: study design. AK, HP, KJ: study design and writing.

No competing interests declared.

Supplementary data

Tables 1 A and B are available on the Acta Orthopaedica website (www.actaorthop.org), identification number 0803.

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