Sufficient Release of Antibiotic by a Spacer 6 Weeks after Implantation in Two-stage Revision of Infected Hip Prostheses

Bernd Fink MD, Sebastian Vogt PhD,
Martin Reinsch PhD, Hubert Büchner PhD

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Abstract

Background Although antibiotic-loaded spacers are commonly used to treat periprosthetic infections, it is unclear whether spacers continue to release bactericidal levels of antibiotic 6 weeks after implantation.

Questions/purposes We asked whether an antibiotic can be detected in the tissue surrounding the spacer 6 weeks after implantation and whether the concentration is higher than the minimal inhibition concentration (MIC) previously determined for pathogens that are responsible for most periprosthetic infections.

Methods We removed 14 spacers used in two-stage septic revisions of infected hip prostheses 6 weeks after the primary implantations and determined the concentration of the antibiotics in the membrane formed between the spacer

and the neighboring bone on the acetabular and the femoral sides. In seven cases Copal cement with gentamicin and clindamycin were used, and in seven other cases vancomycin was added to the Copal cement. Concentrations of the spacer antibiotics in the neighboring tissue were determined by tandem mass spectroscopy.

Results All three antibiotics were detected in concentrations higher than their MIC. There were no differences between the groups regardless whether vancomycin was added to the cement, or whether the cement was applied with the acetabular cup spacer or with the stem spacer.

Conclusions We concluded that, using the spacer technique described in this study, 6 weeks after spacer implantation, the concentrations of antibiotic are sufficient to treat a periprosthetic infection.

Each author certifies that he or she has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

Each author certifies that his or her institution approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.

This work was performed at the Orthopaedic Clinic Markgröningen gGmbH, Markgröningen, Germany.

B. Fink (✉)

Clinic of Joint Replacement, General and Rheumatic
Orthopaedics, Orthopaedic Clinic Markgröningen gGmbH,
Kurt-Lindemann-Weg 10, 71706 Markgröningen, Germany
e-mail: b.fink@okm.de

S. Vogt, H. Büchner
Heraeus Medical GmbH, Wehrheim, Germany

M. Reinsch
Analytic Center Biopharm GmbH, Berlin, Germany

Introduction

Two-stage septic revision surgery is the most common method for treating infected hip endoprostheses [9, 14, 23]. A general advantage of the two-stage concept is that the surgical débridement is performed twice whereby the second operation allows for removal of residual organisms after the initial débridement. It has been possible to achieve a survival rate between 90% and 100% by using two-stage revision concepts for infected hip arthroplasties [13, 14, 16, 25].

In most two-stage revisions, an antibiotic-loaded spacer usually is placed in position for 6 to 12 weeks before the final prosthesis is implanted [7, 10, 19, 23]. The function of the spacer is to release the antibiotic into the infected bed of the prosthesis and to minimize soft tissue contractures, retain soft tissue tension, and so maintain reasonable functionality until a prosthesis can be reimplanted [10, 11].

It is important for the antibiotic effect of the spacer that the local antibiotic concentration is greater than the MIC for the pathogens that cause the periprosthetic infection and remains so for the entire spacer period. Otherwise there would be a danger of recurrence of the infection and appearance of resistant microorganisms. The question, whether a sufficient concentration of antibiotic in vivo is ensured for the entire spacer period, could not be answered satisfactorily in previous studies with spacers and the usual antibiotics. Previous in vivo studies of the release of antibiotics from bone cement have showed that only a minor portion (5%–18%) of gentamicin incorporated in bone cement is eluted [4, 33].

Several studies of local antibiotic concentrations in vivo have reported high concentrations in wound drainage fluid during the first few days after spacer implantation [4, 15, 36]. However, little data address the release of antibiotics from spacers in vivo after several weeks. Masri et al. [24] found tobramycin and vancomycin were eluted from bone cement in high concentrations after a mean of 118 days in 49 patients, and Hsieh et al. [21] reported similarly high elution from bone cement in 46 patients after a mean of 107 days for vancomycin and aztreonam. Bertazzoni Minelli et al. [3] reported a high level of release of gentamicin and vancomycin soon after implantation followed by lower but constant levels at 3 and 6 months after surgery.

Different antibiotics are released at different rates from the spacers and affect each other when in combination with other antibiotics [5]. Thus, one cannot make assumptions about one antibiotic based on evidence from another. One study recommends using Copal bone cement rather than Palacos for the spacers because of the former's ability to release higher amounts of antibiotic [6]. Other antibiotics sometimes are added to it according to the determined antibiotic sensitivity of the pathogen concerned, although this usually is vancomycin [6, 9]. By adhering to this concept, a 100% rate of infection control has been achieved [10, 20]. Thus, it can be assumed that a sufficiently high level of antibiotic had been maintained in these cases. However, the elution of antibiotic from the bone cement does not necessarily reflect the tissue concentrations, and the levels of antibiotic in vivo from these spacers at the end of the spacer implantation period have not been described.

We asked whether an antibiotic can be detected in the tissue surrounding the spacer 6 weeks after implantation and whether the concentration is higher than the MIC previously determined for pathogens that are responsible for most periprosthetic infections.

Patients and Methods

We studied 14 patients (eight women, six men) aged 69.9 ± 7.2 years who had undergone two-stage revision of

an infected hip prosthesis and whose spacers contained, appropriate for the sensitivity of the microorganisms concerned, Copal cement with gentamicin and clindamycin (Heraeus Medical, Darmstadt, Germany) in seven cases and additional vancomycin added to the Copal cement in seven other cases. The original diagnosis that led to the primary arthroplasty was osteoarthritis in all cases. We excluded patients with spacers containing other antibiotics from this study. All subjects gave informed consent to participate in the study and the protocol was approved by the research ethics boards of the institution.

The periprosthetic infection was diagnosed by aspiration of the hip, which is a standard procedure in our clinic before any revision of a hip prosthesis is performed, and bacteriologic cultivation of the aspirated fluid for 14 days according to Schäfer et al. [30]. Bacteriologic and histologic examinations according to the methods of Virolainen et al. [35], Atkins et al. [1], and Pandey et al. [28] of the membrane at the site of loosening, which was removed during the operation, were performed to confirm the original diagnosis (Table 1).

During revision after removal of the infected implant, the cup-shaped acetabular spacer was formed out of antibiotic-loaded cement (with a specific mixture of antibiotics recommended by the microbiologist). To avoid spacer fractures, the stem spacer component consisted of old prosthesis stem models, monoblock devices in most cases and no longer used for primary implantations, that were encased in antibiotic-supplemented cement and, just before implantation, coated in the patient's blood to facilitate easier removal as described previously [10, 11]. The two spacer components were connected by a metal headpiece.

We tested the pathogens causing the infection for their sensitivity to antibiotics. Based on the recommendations of the microbiologist, who specializes in periprosthetic infections, Copal bone cement was used for the spacer (seven cases), with gentamicin and clindamycin already mixed in during manufacturing (Heraeus Medical) if the microorganism was sensitive against gentamicin and clindamycin and because of the synergistic effect on the release of each antibiotic [6]. If the pathogens were resistant to one of these antibiotics, an extra 2 g vancomycin was added to 40 g Copal cement during the operation (seven cases). Copal cement was still used in these cases because antibiotic release from Copal of both antibiotics is prolonged and Copal is more effective in biofilm reduction compared with Palacos R-G or other cement [6, 27].

The cement was stirred in the absence of any vacuum because elution of antibiotic from hand-mixed cement is reportedly greater than that from cement mixed under vacuum because of the presence of air bubbles and their greater surface area [17].

Table 1. Demographic data and antibiotic levels

Patient number	Age (years)	Microorganism	Antibiotics in spacer	Systemic antibiotics for 2 weeks	Oral antibiotics for 4 weeks	Gentamicin ($\mu\text{g/g}$)		Clindamycin ($\mu\text{g/g}$)		MIC in mg/mL		Vancomycin ($\mu\text{g/g}$)		MIC in mg/mL
						Cup	Stem	Cup	Stem	Cup	Stem	Cup	Stem	
1	60	Staphylococcus epidermidis	Vancomycin in Copal	Flucloxacillin	Levofloxacin, rifampicin	21.85	12.88	63.85	50.60	≤ 4	≤ 0.5	177.24	38.18	≤ 4
2	75	Staphylococcus epidermidis	Vancomycin in Copal	Imipenem + cilastatin	Levofloxacin, rifampicin	2.55	19.02	53.25	24.90	≤ 4	≤ 0.5	15.00	17.00	≤ 4
3	77	Staphylococcus epidermidis	Vancomycin in Copal	Vancomycin, clindamycin	Linzolid	19.39	23.91	54.32	301.07	≤ 4	≤ 0.5	52.74	55.47	≤ 4
4	61	Staphylococcus aureus	Vancomycin in Copal	Vancomycin, rifampicin	Levofloxacin, rifampicin	9.82	3.01	120.49	127.00	≤ 4	≤ 0.5	64.72	57.78	≤ 2
5	69	Staphylococcus epidermidis	Vancomycin in Copal	Flucloxacillin	Levofloxacin, rifampicin	17.87	13.45	62.32	39.48	≤ 4	≤ 0.5	39.46	32.57	≤ 4
6	68	Staphylococcus aureus	Vancomycin in Copal	Vancomycin, rifampicin	Levofloxacin, rifampicin	22.23	17.45	49.93	51.69	≤ 4	≤ 0.5	22.69	36.78	≤ 2
7	67	Staphylococcus epidermidis	Vancomycin in Copal	Flucloxacillin	Loracarbef	5.47	4.13	57.67	67.89	≤ 4	≤ 0.5	40.22	17.14	≤ 4
8	66	Staphylococcus lugdunensis	Copal	Flucloxacillin	Ciprofloxacin, rifampicin	7.40	64.06	22.41	89.35	≤ 4	≤ 0.5	-	-	
9	86	Streptococcus group B	Copal	Benzyloxacillin	Amoxicillin	7.36	7.91	60.37	38.57	≤ 4	≤ 0.25	-	-	
10	79	Propionibacterium granulosum	Copal	Imipenem + cilastatin	Clindamycin	3.70	5.31	12.38	40.40	≤ 4	≤ 0.5	-	-	
11	61	Clostridium perfringens	Copal	Ciprofloxacin, benzyloxacillin	Ciprofloxacin, amoxicillin	50.93	45.76	322.29	224.56	≤ 4	≤ 0.5	-	-	
12	59	Staphylococcus capitis	Copal	Flucloxacillin	Levofloxacin, rifampicin	1.02	21.62	53.07	102.78	≤ 4	≤ 0.5	-	-	
13	73	Staphylococcus aureus	Copal	Flucloxacillin	Levofloxacin, rifampicin	1.80	22.34	50.07	89.67	≤ 4	≤ 0.5	-	-	
14	74	Streptococcus group B	Copal	Benzyloxacillin	Amoxicillin + clavulanic acid	1.78	5.25	133.70	329.73	≤ 4	≤ 0.5	-	-	

Table 2. Results of the validation*

Analyte	Precision [†]	Accuracy [†]	Linearity	Stability [‡]	Stability [§]
Gentamicin	9%–15%	–9%–1%	10–750 ng/g	48 hours	4 hours
Vancomycin	4%–18%	–12%– 4%	2.5–2500 ng/g	48 hours	4 hours
Clindamycin	7%–17%	–9% - 4%	2.5–2500 ng/g	120 hours	4 hours

* According to the FDA guidelines for industry; [†]interassay (n = 15 at five concentration levels); [‡]extracts in the automatic sampler at 4 °C; [§]extracts at room temperature.

The patients were discharged after 2 weeks of parenteral antibiotic therapy and mobilization with partial weight-bearing on the surgically treated leg (Table 1). The high bioavailability of the antibiotics rifampicin and ciprofloxacin allowed their oral administration from the second day after surgery. After an additional 4 weeks of oral antibiotic treatment, we removed the spacers and reimplanted a hip prosthesis. Reimplantation was performed after 6 weeks on a regular basis if the CRP level was low (< 20 mg/L), as described in a previous study [10].

When the spacers were removed, the layer of connective tissue that had formed between the cement spacer and the bone of the acetabular cup and the stem was easily loosened because of the poor quality of the cementing process, and could be removed completely. The individual membranes from the acetabular and the stem side then were treated separately and the tissue levels of gentamicin, clindamycin, and vancomycin assessed by high-pressure liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), as described subsequently.

The tissue samples were prepared and analyzed with three independent methods that were developed and validated according to the FDA Guidance for Industry: Bioanalytical Method Validation [34]. Each method validation included precision, accuracy, linearity, selectivity, and stability investigations (Table 2).

Preparation of tissue samples, calibration standards, and quality control samples was performed as follows: for gentamicin and vancomycin, internal standard (tobramycin for gentamicin, 4'-hydroxyacetophenone for vancomycin) and trichloroacetic acid were added to 1 g of a homogenized sample; for clindamycin, internal standard (lincomycin hydrochloride) and acetonitrile were added to 1 g of homogenized sample. Then, the samples were vortexed (for gentamicin and vancomycin), or mixed with an Ultra Turrax® (IKA®-Werke GmbH & Co. KG, Staufen, Germany) (for clindamycin), and sonicated. After sonicating, the samples were centrifuged and the supernatant used for LC-MS/MS analysis (for gentamicin and vancomycin). For clindamycin, triglycerides were removed from the supernatant with hexane. The hexane phase was discarded and the acetonitrile phase evaporated to dryness. Then, reconstitution solution was added and the extract was used for LC-MS/MS analysis.

Chromatographic separation was performed on a modular HPLC 1200 Series (Agilent Technologies, Waldbronn, Germany) using a Luna C18 (II) column, 150 × 2 mm (Phenomenex, Aschaffenburg, Germany) at 25°C (for gentamicin) and at 40°C (for clindamycin and vancomycin). Injection volume was 10 µL for gentamicin, 2 µL for clindamycin, and 20 µL for vancomycin. For gentamicin, the mobile phase A was 0.11 mol/L trifluoroacetic acid/methanol (50:50) and mobile phase B was acetonitrile. For clindamycin and vancomycin, mobile phase A was 1% formic acid solution and mobile phase B was methanol. An isocratic separation was achieved for gentamicin with an A:B ratio of 95:5 at a flow rate of 0.25 mL/minute and for clindamycin with an A:B ratio of 50:50 at a flow rate of 0.25 mL/minute. A gradient separation was achieved for vancomycin with a starting A:B ratio of 100:0 at a flow rate of 0.3 mL/minute. The described conditions for gentamicin were used by Heller et al. [18], where the coeluted gentamicin components C1, C2, C2a and C1a were detected simultaneously. The apparatus used for detection of all three antibiotics was an API 4000 QTrap (Applied Biosystems, Darmstadt, Germany). Ionization was performed with an electrospray interface (positive polarity) using the mass selective detector in the multiple reaction monitoring mode (MRM). The chromatograms were evaluated by the software Analyst 1.4.2 (Applied Biosystems, Darmstadt, Germany).

The Wilcoxon test was used to compare antibiotic levels of the cup and the stem, and the Mann-Whitney test was used to compare concentrations of antibiotics between the spacers with and without additional vancomycin. We conducted the statistical analyses using the computer program SPSS for Windows (SPSS Inc, Chicago, IL, USA).

Results

All of the tissue samples contained levels of antibiotics that were greater than the MICs previously determined for the pathogens that had caused the periprosthetic infections (Table 1). There were no differences in the spacer-related concentrations of gentamicin and clindamycin regardless whether vancomycin had been added to the cement

(gentamicin: $p = 0.128$ for the cup and $p = 0.383$ for the stem; clindamycin: $p = 0.62$ for the cup and $p = 0.456$ for the stem, Mann-Whitney test) (Table 1). There also were no differences between levels associated with the acetabular cup and those with the stem ($p = 0.502$ for gentamicin, $p = 0.296$ for clindamycin, and $p = 0.375$ for vancomycin, Wilcoxon test) (Table 1).

Discussion

Antibiotic-loaded spacers are commonly used for treating periprosthetic infections, but it is not clear whether the antibiotics released from spacers result in bactericidal levels of antibiotic in the tissue for a period of 6 weeks after implantation. Therefore we asked whether an antibiotic can be detected in the tissue surrounding the spacer 6 weeks after implantation and whether the concentration is greater than the MIC previously determined for the pathogens that are responsible for most periprosthetic infections.

We recognize limitations to our study. First, we had a small number of patients. However, the data consistently showed bactericidal levels of antibiotic in the tissues. Second, we studied only several antibiotics and only one type of cement. Copal cement was used because antibiotic release from Copal of both antibiotics is greater and more prolonged and Copal is more effective in biofilm reduction than Palacos R-G cement or other cement [6, 27]. The data we obtained with the antibiotics we studied are not necessarily relevant for other mixtures of antibiotics. Our study simply showed the antibiotics we studied were released locally by the spacer during a 6-week implantation period in tissue concentrations that were sufficient for antibacterial activity. Third, we studied only one duration of implantation of 6 weeks because of the good clinical results with this concept [12]. Sufficient release of antibiotics after that period does not indicate adequate release of antibiotics after longer periods of spacer implantation. Longer periods of spacer implantation are not uncommon so further studies analyzing the tissue level of antibiotics after longer periods are necessary. Although these limitations effectively make this a pilot study for analyzing antibiotic levels in tissues surrounding a spacer, they are consistent with the high rate of infection control after two-stage procedures for periprosthetic infections of the hip [9]. Fourth, the antibiotic concentration in the tissue was measured as $\mu\text{g/g}$ tissue and then expressed as $\mu\text{g/mL}$ for the MICs. It can be assumed that the water content of this tissue is approximately 70% [12, 26] and that the antibiotics can dissolve in it. If this amount of water is taken into account during calculation of the concentrations, the MICs for the pathogens causing the periprosthetic infections are

more than exceeded in every case. Moreover, the objective of the study was to determine whether the MIC had been exceeded, not to determine the exact concentrations of each antibiotic. The aim of the study was to measure the concentration of the antibiotics at the site of the infected prosthesis, in other words, in the tissue immediately surrounding the spacer where the new prosthesis was to be implanted. There, the antibiotic levels are relevant for protecting the new implant from reinfection and are not necessarily the same as in the fluid of the joint which was measured in all the other studies [21, 24]. Fifth, three of the seven patients who had implantation of spacers containing added vancomycin also were treated with vancomycin systemically and this treatment could have had an effect on the local concentrations of vancomycin in the tissue surrounding the spacers. Because the local concentrations of vancomycin found for these patients did not differ from those found for the patients not given systemic vancomycin, it appears the systemic administration of vancomycin was minimal.

Our data with 14 two-stage revisions of infected hip prostheses confirm a combination of gentamicin and clindamycin in Copal bone cement and for vancomycin that had been added to the Copal cement provided MIC dosages in tissues after 6 weeks. The difference from other *in vivo* studies of antibiotic release by spacers over weeks of implantation is that these early studies assessed the local antibiotic concentration either by examining aspirates from the joint [24] or by examining the release of antibiotic from the spacers *in vitro* after their removal [3]. We are unaware of other studies examining antibiotic concentrations in the tissue immediately surrounding the spacer and thus at the site of the implantation of the new prosthesis.

Not all antibiotics can be mixed into cement because they must be available in powder form, be water soluble, and be thermostable. The most commonly used are gentamicin, clindamycin, vancomycin, tobramycin, aztreonam, ampicillin, and ofloxacin [10, 14, 17, 21]. Some authors use vancomycin and tobramycin as local antibiotics on a regular basis because they have a broad spectrum of activity [8, 22]. However, not all bacteria can be treated successfully with these agents (eg, some Gram-negative organisms), so this is an argument for investigating the antibiotic resistance pattern of the isolated bacteria and selecting a specific antibiotic for the treatment. Masri et al. [25] reported a success rate of 89.7% in their retrospective study involving bacteria-specific antibiotic mixed into the cement of a PROSTALAC® spacer (DePuy Orthopaedics Inc, Warsaw, IN, USA). We saw no reinfection of 36 cases with a minimum followup of 2 years using this concept for handmade spacers [10].

Antibiotics also affect each other's elution from the cement. The use of two antibiotics results in a synergistic

effect and the release of the individual components is greater than that of the single antibiotics on their own [2, 6, 29, 31]. In our study, however, the addition of vancomycin did not result in an increase in the release of the antibiotics present in Copal bone cement, namely, gentamicin and clindamycin. However, this can be explained by the high variance among patients in local concentrations of different antibiotics in the tissue surrounding the spacer components. We have no obvious explanation for this variance. Possible reasons could be nonhomogeneous release of the antibiotics at different locations of the same spacer and/or between the spacers, and nonhomogeneous diffusion of the antibiotics into the surrounding tissue. Nonhomogeneous release of antibiotics could be attributable to different amounts of abrasion of the spacer surfaces at the different interfaces, which then could result in new spacer surfaces with antibiotic release. Abrasion of the spacers used in this study has been described in another study [11]. Because the samples were always taken from the fibrous tissue directly surrounding the spacers, and the methodology of analyzing the antibiotics is validated, technical reasons for this variance seem unlikely. Elution of an antibiotic from hand-mixed cement is greater than that from cement mixed under vacuum because of the presence of air bubbles and their greater surface area [17]. However, the mechanical characteristics of hand-mixed cement are not as good [17]. Because the mechanical properties of the spacer cement do not have to fulfill the criteria associated with primary implantation of endoprostheses, we suggest the addition of several antibiotics to the cement spacer in the manner described here is a promising concept for treatment of periprosthetic infections.

One of the main concerns for local use of an antimicrobial agent in general is the appearance of resistant microorganisms. This could occur if the antibiotic levels decrease to below the MIC; but this was not the case in our study. Moreover, after cultivation for 14 days, there were no cases of microorganisms being detected in any of at least five samples taken from the membrane around each spacer during reimplantation.

Our data showing a local tissue concentration of antibiotics in excess of the MIC, even after 6 weeks of implantation, are consistent with data from the few studies analyzing antibiotic levels around the spacer in vitro or aspirates after several weeks [4, 21, 32]. Moreover the low rate of recurrence of infection with a two-stage approach supports the conclusion that the spacer releases sufficient, high amounts of antibiotics after that interval to treat infection and is not just fulfilling a physical, joint-maintaining function [9, 10, 25]. Additional studies are necessary to see if other antibiotic mixtures, other cements, and longer spacer intervals fulfill the same criteria.

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