ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2003, p. 3365–3367 Vol. 47, No. 10
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.10.3365–3367.2003

Teicoplanin-Containing Cement Spacers for Treatment of Experimental *Staphylococcus aureus* Joint Prosthesis Infection

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Received 30 May 2002/Returned for modification 9 February 2003/Accepted 15 July 2003

Using a rabbit model of methicillin-resistant *Staphylococcus aureus* knee-prosthesis infection, we studied the efficacy of teicoplanin cement alone or in combination with systemic intramuscular (i.m.) injections of teicoplanin. Seven days after infection, surgical debridement and removal of the infected prostheses were performed, and five rabbits were randomly assigned to one of five different treatment groups: untreated controls, prosthesis replacement by drug-free cement spacer, prosthesis replacement by teicoplanin-loaded cement spacer (1.2 g of teicoplanin/40 g of cement), i.m. injections of teicoplanin (20 mg/kg of body weight, twice a day for 7 days), or systemic antibiotic treatment combined with teicoplanin-loaded spacers. The most effective regimen combined systemic teicoplanin and antibiotic spacers.

Orthopedic infections by methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming more frequent after device implantation (6, 7), and these organisms are often resistant to many commonly used antibiotics.

Treatment of infection without removal of the prosthesis is associated with a high probability of therapy failure, especially for infections due to *Staphylococcus aureus* (2, 15). Before implanting a new prosthesis, in addition to systemic antibiotics, some surgeons use antibiotic-impregnated cement spacers for local delivery of antibiotics to facilitate the revision surgery (5).

Vancomycin or teicoplanin is usually used as first-line therapy for prosthesis infections because methicillin-resistant staphylococci remain sensitive to it. Because vancomycin can be stably incorporated into polymethylmethacrylate and elute well (10, 17), it is often loaded into cement spacers. Teicoplanin has also been studied as a local therapy (4).

Although antibiotic-impregnated cement spacers are being inserted more and more frequently, their use remains controversial because their efficacy has not been definitively proven (17) and because concerns persist regarding the potential toxicity of a local antibiotic or the emergence of resistant strains (9). Furthermore, the possibly deleterious effect of cement, acting as a foreign body, on the progression of the infection is unknown.

To date, no experimental study has evaluated the efficacy of antibiotic-loaded spacers in the treatment of joint arthroplasty infection (17). In the present study, we compared the efficacy of a teicoplanin-impregnated cement spacer alone with that of a teicoplanin-impregnated cement spacer combined with systemic teicoplanin, using a rabbit model of MRSA knee prosthesis infection that closely mimics human infection (1).

This model has been previously described in detail (1). Briefly, an orthopedic surgeon performed a partial knee replacement with a tibial component on the right knee of the rabbit. Immediately after surgery, the animals were inoculated with 10^8 CFU of MRSA in 0.5 ml injected into the knee close to the prosthesis. Seven days after infection, a surgical debridement was performed and the infected prosthesis was removed and either replaced with a cement spacer or not replaced. The rabbits were randomized to one of five different treatment groups: (i) untreated controls, (ii) prosthesis replacement by drug-free cement spacer, (iii) prosthesis replacement by teicoplanin-loaded cement spacer, (iv) i.m. injections of teicoplanin (20 mg/kg of body weight, twice a day for 7 days), or (v) combined systemic antibiotic treatment and antibiotic-loaded spacer.

The antibiotic-loaded spacer was obtained by mixing 1.2 g of teicoplanin powder with 40 g of powdered cement polymer before the addition of methylmethacrylate, as done in clinical practice and according to the manufacturer's instructions (CMW radiopaque bone cement; DePuy CMW, Blackpool, England). It was then molded to reproduce a facsimile of the silicone prosthesis in a sterile mold.

Four weeks after inoculation (on day 28), treated rabbits and the untreated controls were killed by intravenous injection of pentobarbital. Quantitative bacterial counts were performed as previously described (3). Results are expressed as means ± standard deviations (SD) of $\log_{10}$ CFU/g of bone.

For rabbits treated with teicoplanin-cement spacers, portions (0.1 ml) of each undiluted bone homogenate were also plated on brain heart infusion agar (Difco, Detroit, Mich.) containing teicoplanin at two times the MIC and four times the MIC, in order to detect mutant bacteria showing antibiotic resistance after 48 h of incubation.

In vitro and in vivo evaluation of teicoplanin diffusion from the impregnated spacers was measured by high-performance liquid chromatography with UV detection (λ = 224 nm) (8). The lower limit of detection was 2 μg of teicoplanin/ml. In

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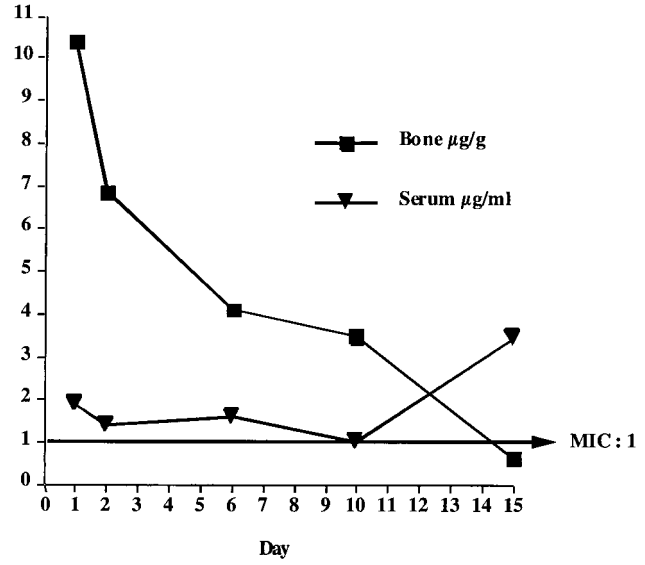

FIG. 1. In vivo concentrations of teicoplanin measured in serum (μg/ml) and bones (μg/g of bone) adjacent to the spacer. The horizontal line at 1 μg/ml represents the MIC for MRSA.

vitro results are expressed as percentages of teicoplanin released from the cement spacers to an immersion medium (amount released each day/total amount). In vivo teicoplanin concentrations were measured in serum and bones adjacent to the spacers in three infected rabbits.

Bacterial densities in bone of the experimental groups were compared by analysis of variance followed by Scheffe's test for multiple comparisons. Results are expressed as means ± SD. A P value of ≤0.05 was considered significant.

The teicoplanin MIC (1 μg/ml) and MBC (2 μg/ml) have been determined in a previous study (14).

At day 15 in vitro, only 1.5% of the teicoplanin had leached out of the cement, most of it during the first day (1.2% ± 0.3%). Thus, almost all of the antibiotic was retained within the cement. In vivo, the mean bone concentration was about 10 μg of antibiotic/g 1 day after implantation of the teicoplanin-loaded spacer (Fig. 1). On days 4 and 10, it was still four times the MIC but fell below this level on day 15. Mean concentrations in serum were low (<2 μg of teicoplanin/ml) but increased to 4 μg of teicoplanin/ml on day 15.

All animals that received drug-free cement spacers were infected (Table 1), with a mean bacterial count of 5.5 ± 0.7 $\log_{10}$ CFU/g of bone. This value was not significantly different from that found in the untreated control group, indicating that setting the bone with a cement spacer had no deleterious effect on the progression of infection.

Compared to results for the untreated controls, only those for the combination of i.m. teicoplanin and antibiotic-impregnated cement spacers were significant, with 67% of the animals having sterile bone and lower bacterial counts ($P < 0.05$).

The emergence of resistance was not detected with teicoplanin-loaded spacers in vitro. Also, no teicoplanin-resistant strain emerged in the bones of treated animals or in contact with the spacers recovered from animals treated with antibiotic-impregnated spacers alone.

TABLE 1. Efficacy of teicoplanin alone or in combination with teicoplain-loaded cement spacer for the treatment of experimental MRSA joint prosthesis infection in rabbits

Treatment	No. of rabbits	No. of rabbits with sterile bone	$\log_{10}$ CFU/g of bone (mean ± SD)
None	11	1	4.2 ± 1.3
Drug-free cement spacer	10	0	5.5 ± 0.7
Teicoplanin-loaded cement	12	4	3.9 ± 2.2
Teicoplanin by i.m. injection	11	1	3.7 ± 1.6
Teicoplanin-loaded cement plus teicoplanin by i.m. injection	12	8	2.2 ± 0.9[a]

[a] $P \leq 0.05$ versus results for rabbits receiving no treatment, and $P < 0.001$ versus results for rabbits receiving drug-free cement spacers.

Our data suggest that the use of teicoplanin-loaded cement spacers enhances the efficacy of systemic teicoplanin in eradicating infection after resection arthroplasty.

To date, there have been no adequately designed, randomized, controlled clinical trials with adequate numbers of patients and sufficiently long-term follow-up to guide therapy. Most available clinical data on the effectiveness of antibiotic and spacers were obtained in noncomparative open studies (5, 18). One randomized study evaluated the efficacy of gentamicin-impregnated polymethylmethacrylate beads in infected total hip and knee arthroplasties in 28 patients (12). To the best of our knowledge, no study has compared the efficacy of systemic antibiotics plus antibiotic-loaded spacers with that of either therapy alone. Therefore, therapeutic choices are essentially guided by information from in vitro and experimental studies. However, it has been noted that the in vitro MIC for an antibacterial agent is not a good predictor of clinical outcome (16). The experimental model used in this study reproduces a prosthetic knee infection similar to that observed in humans and is suitable for the comparative evaluation of antibiotic therapies (1, 3, 13, 14).

It must be noted that, in vitro, only a very small amount of the antibiotic was released from cement, and this release was completed within the first few days. However, it was sufficient to obtain in vivo teicoplanin concentrations in bone adjacent to the spacers that remained above the MIC for the MRSA test strain for 10 days. This observation could explain why local therapy enhanced the efficacy of systemic teicoplanin. We have previously shown that systemic [^{14}C]teicoplanin diffuses into the bone marrow and trabecular bones. Like [^{14}C]sparfloxacin (3) and other antibiotics, teicoplanin diffuses weakly into compact bones. Local teicoplanin might have a better or more sustained diffusion into infected tissues than i.m. teicoplanin. However, we found no difference between the efficacies of either treatment alone. The limited elution capacity of vancomycin (another glycopeptide antibiotic) from cement has also been described (10).

Fifteen days after inoculation, the mean concentration of teicoplanin in serum increased in animals that had received teicoplanin-impregnated cement spacers. This finding is consistent with what has been reported for a patient who had received vancomycin- and tobramycin-impregnated cement and had a high concentration of antibiotics in serum (J. W.

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Further experiments evaluating an antibiotic-loaded composite better able to release the antibiotic than bone cement should be conducted, taking care to avoid toxic blood levels and emergence of resistant bacteria. Indeed, glycopeptide-intermediate staphylococci are an emerging problem, and in this context, sustained subinhibitory concentrations of antibiotics could have detrimental effects (11).

These data showing a beneficial effect of teicoplanin-impregnated bone cements cannot be extrapolated to other types of local therapy. Other experiments using different antibiotics effective against bacteria responsible for infected arthroplasty infections are needed to better orient therapy of these difficult-to-treat complications.

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