Antibiotic Bone Cement for the Treatment of Pseudomonas Aeruginosa in Joint Arthroplasty: Comparison of Tobramycin and Gentamicin-Loaded Cements

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Abstract: One hundred clinical isolates of Pseudomonas aeruginosa were collected from 22 medical centers throughout Europe and were challenged with two aminoglycoside-loaded bone cements, employing a modified in vitro Kirby–Bauer susceptibility model. The results of this study show that Simplex® P with tobramycin exhibits antibacterial activity against 98% of the strains tested, compared to 93% for Palacos with gentamicin. Additionally, for strains that were susceptible to the antibiotic bone cement formulations, the average zone of inhibition produced around the tobramycin-loaded cement disks was approximately 25% greater than that seen around the gentamicin-loaded cement disks. This difference was statistically significant ($p \ll 0.01$). Tobramycin-loaded bone cement is therefore the preferred formulation when addressing Pseudomonas aeruginosa in septic joint arthroplasty. © 2002 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 64B: 94–98, 2003

Keywords: bone cement; tobramycin; gentamicin; Pseudomonas aeruginosa; resistance

INTRODUCTION

One of the most devastating complications of total joint arthroplasty is infection of the prosthetic implant. The spectrum of causative bacteria in joint sepsis is broad, including aerobic gram-positive and gram-negative bacteria, as well as anaerobes and mycobacterium.[1–5] Pseudomonas aeruginosa is a virulent pathogen, which is arguably the most difficult to eradicate, once established within the joint.[6,7] Implants infected with this organism may require multiple revisions if eradication is not achieved.

The use of antibiotic bone cement for the fixation of joint prostheses provides surgeons several advantages, including delivery which is localized specifically to the implant site, and sustained at therapeutic levels for a period after the surgical site is closed. Selection of an antibiotic for incorporation into bone cement is limited by several criteria, including thermal stability to the exothermic polymerization of the cement. Additionally, it must be proven to elute from the cement in therapeutic concentrations, and have an appropriate spectrum of antibacterial coverage in order to be effective against the diverse range of causative pathogens in deep sepsis. The aminoglycosides are one class of antibiotics that have been shown to meet these requirements. The two aminoglycosides most commonly incorporated into acrylic bone cements are tobramycin and gentamicin. Gentamicin has been used predominantly in Europe, where it is formulated into several commercial antibiotic bone cement preparations. Another commercial formulation of antibiotic bone cement, which contains tobramycin, has recently been introduced in Europe. Tobramycin has historically found extensive use in North America, where surgeons routinely hand-mix it into individual doses of bone cement at the time of surgery.[8–10] This formulation of tobramycin in bone cement has also been used for fabrication of beads[11–13] and spacers[14,15] to address deep sepsis during revision procedures.

Of these two aminoglycosides, it has been reported that tobramycin has superior activity against Pseudomonas aeruginosa when compared to gentamicin[16–21] as well as reduced incidence of nephrotoxicity and ototoxicity.[22–24] Prior research comparing the two antibiotics has focused on typical systemic administration, which may or may not be correlated to the localized delivery from an acrylic bone cement. It is the intent of this study to compare the effectiveness of two commercially available aminoglycoside-containing bone cements against multiple isolates of Pseudomonas aeruginosa, employing a modified in vitro Kirby Bauer susceptibility model, which places antibiotic bone cement disks in direct contact with the Pseudomonas isolates.

MATERIALS

The two antibiotic bone cement formulations tested in this study were Surgical Simplex® P with Tobramycin (1.0 g as a

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TABLE I. Inhibition Around Test Disks (Zone size in mm)

ID	Location of Origin	Source of Isolate	Zone Tobra (mm)	Zone Genta (mm)	ID	Location of Origin	Source of Isolate	Zone Tobra (mm)	Zone Genta (mm)
1	Utrecht, NLD	Sputum	8.50	6.00	51	Linz, AUT	Blood	NZ/NI	NZ/NI
2	Utrecht, NLD	Urine	10.75	9.50	52	Linz, AUT	Blood	6.25	1.75
3	Utrecht, NLD	Sputum	8.25	7.25	53	Linz, AUT	Wound	9.00	8.00
4	Utrecht, NLD	Ear	8.25	5.75	54	Brussels, BEL	Blood	1.50	NZ/I
5	Utrecht, NLD	Sputum	6.00	1.75	55	Paris, FRA	Blood	9.25	7.50
6	Utrecht, NLD	Urine	11.50	11.00	56	Paris, FRA	Blood	1.50	NZ/NI
7	Utrecht, NLD	Wound	7.75	5.75	57	Paris, FRA	Blood	9.50	8.00
8	Utrecht, NLD	Feces	8.75	8.00	58	Paris, FRA	Wound	8.75	7.00
9	Utrecht, NLD	Sputum	8.00	6.00	59	Lyon, FRA	Blood	6.75	5.25
10	Utrecht, NLD	Urine	7.50	5.25	60	Lyon, FRA	Wound	2.25	1.75
11	Utrecht, NLD	Pus	7.75	6.00	61	Lille, FRA	Blood	9.50	6.00
12	Utrecht, NLD	Wound	8.25	6.50	62	Lille, FRA	Wound	1.75	NZ/I
13	Utrecht, NLD	Pus	7.75	6.25	63	Freiburg, DEU	Blood	8.75	5.50
14	Utrecht, NLD	Blood	6.50	5.25	64	Freiburg, DEU	Wound	8.50	6.50
15	Utrecht, NLD	Urine	10.75	7.50	65	Dusseldorf, DEU	Blood	9.75	8.25
16	Utrecht, NLD	Sputum	8.75	5.00	66	Dusseldorf, DEU	Wound	NZ/I	NZ/I
17	Utrecht, NLD	Sputum	7.75	6.00	67	Athens, GRC	Blood	9.00	6.25
18	Utrecht, NLD	Sputum	6.00	3.50	68	Athens, GRC	Wound	7.75	5.50
19	Utrecht, NLD	Ear	7.50	6.75	69	Athens, GRC	Wound	9.75	7.75
20	Utrecht, NLD	Sputum	7.25	5.00	70	Genoa, ITA	Blood	[a]	[a]
21	Utrecht, NLD	Pus	7.75	7.50	71	Genoa, ITA	Wound	7.50	6.50
22	Utrecht, NLD	Urine	7.25	7.50	72	Genoa, ITA	Wound	1.50	NZ/NI
23	Utrecht, NLD	Sputum	9.75	8.25	73	Rome, ITA	Blood	NZ/I	NZ/I
24	Utrecht, NLD	Knee puncture	10.00	8.00	74	Rome, ITA	Wound	10.50	8.00
25	Utrecht, NLD	Sputum	8.00	5.75	75	Warsaw, POL	Blood	9.00	7.50
26	Utrecht, NLD	Pus	8.00	6.25	76	Warsaw, POL	Wound	NZ/I	NZ/I
27	Utrecht, NLD	Pus	7.75	6.00	77	Cracow, POL	Blood	1.00	NZ/I
28	Utrecht, NLD	Wound	8.50	6.25	78	Cracow, POL	Wound	8.50	7.25
29	Utrecht, NLD	Urine	8.50	7.25	79	Coimbra, PRT	Blood	8.00	6.50
30	Utrecht, NLD	Ear	9.00	7.25	80	Coimbra, PRT	Wound	9.50	8.00
31	Utrecht, NLD	Sputum	8.25	5.25	81	Sevilla, ESP	Blood	9.75	6.75
32	Utrecht, NLD	Sputum	9.00	6.00	82	Sevilla, ESP	Wound	8.50	6.50
33	Utrecht, NLD	Sputum	10.00	7.75	83	Sevilla, ESP	Wound	10.25	5.75
34	Utrecht, NLD	Sputum	8.00	5.00	84	Madrid, ESP	Blood	10.50	8.00
35	Utrecht, NLD	Ear	9.00	6.50	85	Madrid, ESP	Wound	8.25	5.25
36	Utrecht, NLD	Urine	10.25	8.50	86	Barcelona, ESP	Blood	−11.25	8.75
37	Utrecht, NLD	Wound	8.50	6.00	87	Barcelona, ESP	Wound	10.00	7.00
38	Utrecht, NLD	Sputum	1.25	NZ/NI	88	Barcelona, ESP	Wound	9.00	5.50
39	Utrecht, NLD	Wound	8.25	7.25	89	Lausanne, CHE	Blood	8.75	6.25
40	Utrecht, NLD	Sputum	9.50	5.75	90	Lausanne, CHE	Wound	10.00	7.25
41	Utrecht, NLD	Sputum	8.50	6.50	91	London, UK	Blood	10.25	7.25
42	Utrecht, NLD	Sputum	10.50	7.25	92	London, UK	Wound	11.75	8.25
43	Utrecht, NLD	Sputum	7.50	6.00	93	Tirana, ALB	Wound	1.75	3.00
44	Utrecht, NLD	Sputum	6.75	5.50	94	Tirana, ALB	Wound	NZ/I	1.75
45	Utrecht, NLD	Sputum	7.25	5.75	95	Tirana, ALB	Wound	0.25	1.25
46	Utrecht, NLD	Wound	8.75	7.25	96	Ankara, TUR	Blood	8.75	6.75
47	Utrecht, NLD	Sputum	7.50	5.50	97	Ankara, TUR	Wound	9.75	7.25
48	Utrecht, NLD	Pus	8.75	7.50	98	Ankara, TUR	Wound	0.50	NZ/NI
49	Utrecht, NLD	Sputum	10.00	8.00	99	Ankara, TUR	Blood	8.50	6.25
50	Utrecht, NLD	Sputum	9.25	7.50	100	Tel Hashomer, ISR	Blood	9.00	7.50

[a] Results from Sample 70 were inconclusive, due to culturing of two organisms following incubation, with one being susceptible to the antibiotic bone cement disks, and the other exhibiting growth.

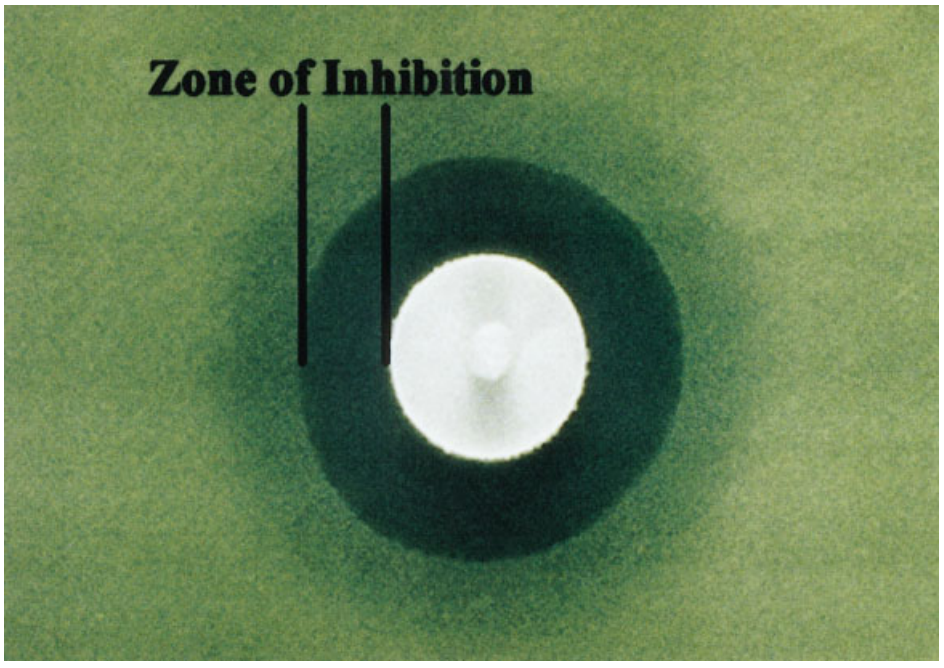

Figure 1. Zone of inhibition with ghost zone. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sulfate) per 40 g bone cement powder, and Palacos with gentamicin (0.5 g as a sulfate).

A total of 100 clinical isolates of Pseudomonas aeruginosa were collected from 22 medical centers in 15 countries throughout Europe. (Isolate descriptions are provided in Table I.)

METHODS

Sample Preparation

Single doses of each antibiotic bone cement were vacuum mixed and delivered into aluminum molds with cavities to produce uniform disk-shaped specimens with a diameter of 14 mm, and a thickness of 2 mm. One hour after the bone cement disks were molded, they were placed on a Mueller–Hinton agar plate that had been seeded with one of the Pseudomonas aeruginosa strains.

Test organisms were propagated and handled in accordance with ATCC recommendations[25] for broth media, agar, and incubation specifications. Organisms were prepared by inoculating TSB and incubating at 37 °C for 24 h. Microbial suspensions were adjusted to an absorbency of 0.325 with the use of a spectrophotometer (wavelength = 475 nm) and swabbed onto Mueller–Hinton agar plates. Each seeded plate was challenged with both a tobramycin-containing bone cement disk and a gentamicin-containing bone cement disk. Samples were tested in duplicate (i.e., two plates per organism). Following incubation for 24 h at 37 °C, the plates were examined for zones of inhibition around the disks.

Interpretation of Zones of Inhibition

The zone of inhibition is defined as the distance between the test disk and the edge of bacterial growth (Figure 1) It is common for a hazy, or ghost zone to exist between the areas of complete inhibition and full bacterial growth. This ghost zone is the result of partial inhibition and is not included in the measurement of the zone of inhibition.[26]

Samples that did not exhibit a zone of inhibition and had bacterial growth at the cement surface was reported as NZ/NI. In the case where no zone of inhibition was observed, but bacterial growth was inhibited at the cement surface, results were reported as NZ/I.

RESULTS

The zones of inhibition (mm) recorded around the antibiotic-containing bone cement disks are presented in Table I.

The data show a bimodal distribution of the zones of inhibition due to the presence of moderately susceptible strains (see Figure 2).

A well-defined discontinuity in the data provides a clear distinction between strains that are susceptible to the antibiotic activity of the disks and those that are moderately susceptible. Susceptible strains are thus defined as those exhibiting a zone of inhibition greater than 3 mm; whereas moderately susceptible strains exhibit zones $\leq$ 3 mm. Additionally, resistant strains are defined as those that exhibit no zone of inhibition, and no inhibition at the disk surface (NZ/NI).

Under these definitions, the antibacterial behavior of the two antibiotic bone cement formulations against the 100 strains of Pseudomonas aeruginosa tested is summarized in Table II.

By separating the data into resistant, moderately susceptible, and susceptible strains, a more normal distribution is seen, and thus a valid statistical comparison of the zones of inhibition can be made. The mean zone size of susceptible pseudomonas strains versus the two antibiotic bone cement formulations were as follows: Simplex with tobramycin, 8.71 ± 1.21 mm; Palacos with gentamicin, 6.78 ± 1.13 mm.

The approximate 25% difference in zone size between the two antibiotic bone cements was found to be statistically significant ($p << 0.001$) via student's t test, and by a paired-sample analysis.

TABLE II. Susceptibility Patterns of Pseudomonas Aeruginosa

	Resistant (NZ/NI)	Moderately Susceptible (zone ≤3 mm)	Susceptible (zone > 3 mm)
Simplex with tobramycin	2	14	84
Palacos with gentamicin	7	12	81

DISCUSSION

Drawing conclusive correlations between in vitro results for antibiotic bone cement studies and clinical performance is not straightforward, as the diffusion of antibiotics from an in situ polymerizing carrier into a vascular-compromised bone site is not easily modeled. When assessing the results of this study, however, the effectiveness of an antibiotic bone cement in inhibiting bacterial growth at the disk surface can be interpreted directly, because the application of the disks onto a seeded agar surface eliminates the issues related to diffusion. The significance of inhibiting this virulent pathogen at the surface of the antibiotic bone cement disks is recognized, and is supported by the work of Gristina and Casterton *et al*[27] who determined that surface colonization of bacteria is the key to the establishment of implant infection.

The results of this study show that Simplex with tobramycin exhibits superior activity in vitro against Pseudomonas Aeruginosa compared to Palacos with gentamicin. Of the 100 isolates tested, only two strains were resistant to Simplex with tobramycin, whereas seven strains were resistant to Palacos with gentamicin. These results are consistent with published reports regarding aminoglycoside resistance patterns in Pseudomonas aeruginosa, which cite a level of activity two to eight times greater for tobramycin than gentamicin, as demonstrated in both minimum inhibitory and minimum bactericidal concentration studies.[16–21]

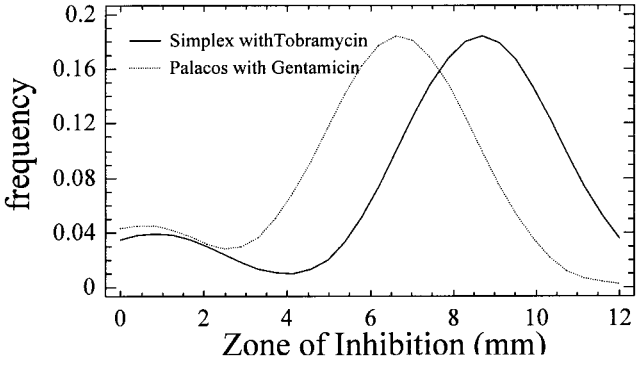

Figure 2. Frequency distribution—zone of inhibition.

The increased zone of inhibition around the Simplex with tobramycin disks may be attributed to a combination of increased susceptibility to that antibiotic, as well as the fact that the Simplex formulation contains twice as much antibiotic as Palacos with gentamicin. Aminoglycosides act through a concentration-dependent mechanism, whereby increasing doses will provide greater antibacterial effectiveness.[28] Numerous researchers have demonstrated that both Simplex with Tobramycin and Palacos with Gentamicin share typical antibiotic release traits, with a high initial burst release, followed by an exponential decay.[29–32] It has also been shown that the release of antibiotic is positively correlated to the amount of drug formulated into the cement.[33]

The results of this study provide compelling evidence that Simplex® P with tobramycin is an effective formulation when addressing Pseudomonas aeruginosa infections following joint arthroplasty. Another in vitro susceptibility study has shown this combination of antibiotic and bone cement to be effective against a wide range of bacteria common to joint arthroplasty including both aerobic gram-positive and gram-negative bacteria as well as anaerobes and mycobacterium.[34] Nijhof et al. utilized an in vivo rabbit model to demonstrate that Simplex with tobramycin was effective in preventing Staph aureus and Staph epidermidis infections, and that tobramycin was released into the surrounding bone at therapeutic levels,[35,36] while serum levels remained well below the limits of systemic toxicity.[36] The results of these studies, along with previously published in vitro,[31,32,37] in vivo,[35–37] and clinical[8–10,12,13] studies support the use of tobramycin-containing Simplex P during joint arthroplasty for the reduction of deep sepsis.

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