High active local levels of vancomycin without nephrotoxicity released from impacted bone allografts in 20 revision hip arthroplasties

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Background Cancellous bone can act as a delivery vehicle for vancomycin without impairment of graft incorporation. However, local and systemic antibiotic levels, biological activity of vancomycin, interaction with antibiotic-loaded cement, and also nephrotoxicity of these composites have not yet been studied clinically.

Material and methods Blood, drainage and urine samples of 20 consecutive patients undergoing revision total hip arthroplasties with impaction grafting technique utilizing 1 g of vancomycin per femoral head were studied. Plain PMMA cement was used in 10 cases, while PMMA with gentamycin was used in 5 cases and tobramycin was used in the remaining 5 cases. Biological activity of vancomycin was studied using kinetic killing curves in three ATCC organisms (methicillin-sensitive Staphylococcus aureus, methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa). Quantification was done with fluorescent polarized immunoassay. Renal function was evaluated with preoperative and postoperative urea and creatinine.

Results Local active bactericidal levels of vancomycin reached 1 400 µg/mL (average 5-point level = 367 µg/mL) without nephrotoxicity. Vancomycin was present in urine until the fifteenth day. Both aminoglycosides in the cement had activity against Pseudomonas aeruginosa.

Interpretation Local levels of vancomycin were 35 times greater than the highest levels reported with vancomycin-loaded PMMA. A synergistic effect was observed between vancomycin released from impacted allografts and aminoglycoside-loaded PMMA.

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of the patients received vancomycin, tobramycin or gentamycin intravenously during or after surgery. Allografts were obtained from fresh frozen femoral heads from our own bank and were not treated with irradiation. 56 femoral heads were used (3 heads on average for each revision). Morsellized bone graft was fragmented manually in 0.4–0.6 cm bits and mixed for 15 min with 1 g of powdered vancomycin (Lilly Indianapolis, IN) per femoral head. Impaction grafting was performed by a routine technique as described by Slooff et al. (1984) for the acetabulum and by Gie et al. (1993) for the femur. Graft packing was made with two specific instrumentation sets (Primary Impaction Grafting Instruments, De Puy Int., Leeds, UK, and X-Change Revision Instruments System, Howmedica, Rutherford, NJ).

The components were fixed with Simplex-P radiopaque bone cement (Howmedica, Rutherford, NJ) in 5 patients, CMW1 (De Puy, Warsaw, IN) in 5 patients, Simplex with tobramycin cement (Howmedica) in 5 patients and CMW1 with gentamycin (De Puy) in 5 patients.

After implantation, 10 mL samples of the wound drainage were collected under sterile conditions at 1, 6, 12, 24 and 48 h, and immediately centrifuged and frozen at -20°C. Serum samples were obtained at 1, 6, 12, 24, 48 and 72 h and urine samples on days 1, 2, 3, 4 and 5. Nephrotoxicity was defined as an increase in serum creatinine of 0.5 mg/dL above pretreatment levels (Zaske 1992).

Statistics
Correlation analysis was performed by ANOVA, comparing preoperative and postoperative urea and creatinine. Pearson coefficient was used for comparison between vancomycin doses and local and urinary levels of vancomycin each day.

Results
The average vancomycin drainage level obtained by FPIA was 408 μg/mL during the first hour and 265 μg/mL after 48 h (Table, Figure 1). Average urine vancomycin concentration was 70 μg/mg of urine creatinine on the first day, and was undetectable at 45 days (Figure 2).

Methicillin-sensitive Staphylococcus aureus (MSSA-ATCC 29213) killing curves achieved bactericidal effect after 8 h of incubation when plain cement was used, whereas the same fall was achieved after 4 hours with antibiotic-loaded cement. Methicillin-resistant Staphylococcus aureus (MRSA-ATCC 43300) and Pseudomonas aeruginosas (PA-ATCC 27853). Tests were performed in tubes containing equal volumes of the drain sample and the microorganisms to be tested in Müller Hinton broth incubated at 35°C. Starting inocula were 4 × 10^5 to 6 × 10^5 colony-forming units (CFU)/mL. Aliquots were removed after 0, 4, 8 and 24 h and then plated quantitatively onto CLDE agar. Colonies were counted after overnight incubation. Incubation was prolonged 48 h if the colonies were small. A graph of log_{10}CFU against time was made. Bactericidal effect was defined as a 3-point drop in log_{10}CFU/mL compared to the initial inoculum after 6–8 h of incubation.

Quantification of vancomycin in every sample was performed by means of fluorescent polarized immunoassay (FPIA) (AxSYM System, Abbott Laboratories, Abbott Park, IL) (Jolley 1981, Jolley et al. 1981). The assay characteristics are: total standard deviation and coefficient of variation (%), 0.32 and 4.3, respectively, at 7 μg/mL; 1.04 and 2.9 at 35 μg/mL, and 2.97 and 4.2 at 75 μg/mL. Finally, the lower limit of detection was 2 μg/mL.

Renal function was evaluated with tests for blood urea and creatinine preoperatively and on days 1, 2, 3, 4 and 5. Nephrotoxicity was defined as an increase in serum creatinine of 0.5 mg/dL above pretreatment levels (Zaske 1992).
Local vancomycin levels (µg/mL) in the 20 cases determined by FPIA at 1 h, 6 h, 12 h, 24 h and 48 h postoperatively. Number of femoral heads used in each patient (A) and grams of vancomycin (B).

| Case | Local vancomycin levels (µg/mL) | A | B |
|------|---------------------------------|---|---|
| 1    | 116 56 63 65 41 2 2             | 2 | 2 |
| 2    | 456 383 382 418 382 4 4         | 4 | 4 |
| 3    | 199 255 327 360 323 2 2         | 2 | 2 |
| 4    | 533 466 439 379 239 4 4         | 4 | 4 |
| 5    | 109 96 83 87 71 3 3             | 3 | 3 |
| 6    | 649 494 359 259 281 2 2         | 2 | 2 |
| 7    | 409 517 462 330 228 2 2         | 2 | 2 |
| 8    | 555 1378 1400 1142 558 3 3      | 3 | 3 |
| 9    | 191 165 75 64 55 2 2            | 2 | 2 |
| 10   | 612 648 607 279 217 4 4         | 4 | 4 |
| 11   | 681 432 452 420 219 4 4         | 4 | 4 |
| 12   | 426 470 461 452 429 4 4         | 4 | 4 |
| 13   | 368 348 384 395 348 3 3         | 3 | 3 |
| 14   | 325 320 255 209 165 3 3         | 3 | 3 |
| 15   | 684 628 301 354 341 2 2         | 2 | 2 |
| 16   | 475 459 376 384 212 4 4         | 4 | 4 |
| 17   | 389 357 331 286 235 3 3         | 3 | 3 |
| 18   | 462 471 553 466 421 3 3         | 3 | 3 |
| 19   | 267 254 293 309 351 2 2         | 2 | 2 |
| 20   | 243 199 218 221 190 2 2         | 2 | 2 |

Figure 1. Average local vancomycin concentration (µg/mL) at 5 different time points. Bars represent SD.

Figure 2. Average urinary vancomycin level (µg/mg creatinine) at 1, 2, 3, 4, 5, 15 and 45 days after implantation. Bars represent SD.

Discussion

The capacity of cancellous bone allografts to act as a vehicle of vancomycin has been confirmed both in vitro and in vivo by means of bioassay (Witsø et al. 1999, 2000, Winkler et al. 2000). This makes it possible to reach local concentrations of this antibiotic that are higher than the 90% MIC for S. aureus. 1 gram of this antibiotic per femoral head causes a 400 times higher concentration than that on the second day, 0.85 on the third day, 0.86 on the fourth day, and 0.85 on the fifth day (overall range 0.3–1.6).

Nephrotoxicity, defined according the criteria described above, was not observed in any of these patients. No statistically significant differences were noted concerning serum laboratory nephrotoxicity parameters measured preoperatively and on days 1, 2, 3, 4 and 5 after implantation (p > 0.05). No statistical correlation was found between the number of femoral heads per g vancomycin used and local levels of vancomycin. We found a statistically significant correlation between grams of local vancomycin and urine levels on days 2, 3, 4, 5 and 15 (Pearson coefficient).
necessary to achieve antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Vancomycin is also effective against methicillin-resistant *Staphylococcus aureus* (MRSA).

In this clinical study, levels of vancomycin sufficient to inhibit multiplication of *S. aureus* were achieved without impairing renal function. Bactericidal effect occurred in both gram-positive organisms, and this was achieved in half the time when confronted to methicillin-sensitive *Staphylococcus aureus* (MSSA). Vancomycin proved to be more effective when combined with aminoglycosides in the cement.

We observed high local levels of vancomycin during the first 48 h after implantation, and in the local bone antibiotic levels were most likely toxic to even the most resistant organisms. Some microorganisms are sensitive to vancomycin at minimum serum levels of 4 μg/mL (Brien et al. 1993). Later, these levels should fall but could provide a toxic bactericidal environment for the remaining sensitive organisms. This fact could be explained by the finding of vancomycin urine levels 15 days after surgery. The urinary levels were related to the original amounts of antibiotic administered. Other authors have observed this same phenomenon using other local antibiotic delivery systems (Yu et al. 1992, Laurencin et al. 1993).

Local antibiotic levels of vancomycin reached peak values as high as 1 400 μg/mL in our patients (average 5-point level: 359 μg/mL). Different authors have found local vancomycin levels eluted from methylmethacrylate ranging from 0.76 to 11 μg/mL (Brien et al. 1993, Klekamp et al. 1999, Gonzalez Della Valle et al. 2001). In a recent study, the highest reported peak value of vancomycin eluted from cement (measured by FPIA) was 19 μg/mL (Gonzalez Della Valle et al. 2001). Brien et al. (1993) found undetectable local vancomycin levels in 30% of the cases when impregnated cement was used. The lowest vancomycin level observed in our study was 42 μg/mL at 48 h. According to these observations, adding vancomycin to the bone allograft instead of the cement would make it possible to achieve an average of 35 (2–70) times higher levels of this antibiotic. On the other hand, to reach such levels, it would be necessary to mix 35 g of vancomycin into every 40 mg of PMMA, causing deleterious mechanical effects.

Vancomycin and an aminoglycoside are often combined in the PMMA to achieve a potentially synergistic effect in the treatment of severe infections caused by methicillin-resistant *Staphylococcus aureus* (Masri et al. 1998, Klekamp et al. 1999). Although controversies on its use exist, vancomycin constitutes 18% of all antibiotics added to cement by hip reconstructive surgeons in the United States (Heck et al. 1995).

Nephrotoxicity has been defined as an increase in serum creatinine of 0.5 mg/dL above pretreatment levels (Zaske 1992). We found no patient with renal toxicity in this series. This can be explained because our sample size was not large enough to address the true effect of vancomycin on the kidneys. Lack of statistical significance in the differences between creatinine measurements may also be explained by small study power. However, the risk of vancomycin toxicity in combination with allografts is very low, since undetectable blood levels were observed in all our patients. Levels in the order of 60 μg/L are commonly achieved during intravenous therapy (Chohfi et al. 1998).

Gradual development of vancomycin resistance as a result of wide use, prolonged release in subtherapeutic doses, or the employment of large amounts is one of the current concerns. We consider that vancomycin-supplemented bone allografts should be used only for revisions of infected arthroplasties, or if there is a potential risk of infection.

In summary, vancomycin-supplemented bone allografts reached local concentrations that were 20–300 times higher than the 90% MIC for *S. aureus* at the 5 different time points measured, and without impairing renal function. These levels were highly superior to those reported for vancomycin-impregnated methylmethacrylate. The bactericidal effect occurred in both gram-positive organisms. Tobramycin and gentamycin samples proved to be effective against *Pseudomonas aeruginosa*. When confronted to MSSA there were synergistic effects between the biological activity of vancomycin and aminoglycosides in the cement. Bactericidal effects could be seen after 4 h instead of 8 h.
No competing interests declared.

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