Release of tobramycin from tobramycin-containing bone cement in bone and serum of rabbits

M. W. NIJHOF*, W. J. A. DHERT, P. B. J. TILMAN, A. J. VERBOUT
Department of Orthopaedics, University Cluster of Orthopaedics, University Hospital Utrecht, G05.228, P.O. Box 85500, NL 3508 GA Utrecht, The Netherlands

A. FLEER
Department of Medical Microbiology, University Children's Hospital and Eijkman-Winkler Institute, University Hospital Utrecht, Utrecht, The Netherlands

Tobramycin release from tobramycin-containing bone cement was studied *in vivo* in a rabbit model. After insertion of cement into the right femur, tobramycin concentration as a function of time for up to 28 days was measured in serum and bone of rabbits. Tobramycin release in the femoral cortex adjacent to the cement was found to exceed the minimal inhibitory concentration for *Staphylococcus aureus* Wood 46. Serum tobramycin concentrations were below the systemic toxicity threshold.

1. Introduction

Since 1969, various combinations of antibiotics and bone cement have been used in the prevention and treatment of arthroplasty infections [1–3]. Tobramycin, like other aminoglycosides, is heat-stable, which makes it suitable for incorporation in polymethylmethacrylate (PMMA). In addition, tobramycin is potentially active against the most frequently found causative infective organisms in arthroplasty surgery (*Staphylococcus* species and aerobic Gram-negative bacilli).

In order to be effective in preventing or treating arthroplasty infections, antibiotic-containing bone cement must fulfil two requirements. Firstly, the antibiotic should elute in a concentration which exceeds the susceptibility level for the infecting bacteria. This is the minimal inhibitory concentration (MIC), defined as the lowest concentration of antibiotic that prevents visible growth after an 18–24 h incubation *in vitro*. Secondly, this concentration should be reached at the site of contamination or infection. Thus in arthroplasty surgery, the antibiotic concentration should be sufficiently high (i.e. well above the MIC) at the site of the implant, especially at the bone–cement interface and in the surrounding bone. In addition, the antibiotic concentration in serum should not exceed toxic levels.

Release studies of tobramycin-containing bone cement are limited. Seyral *et al.* [4], Miclau *et al.* [5] and Lawson *et al.* [6] demonstrated *in vitro* elution of tobramycin from PMMA-bone cement. The presence of tobramycin in wound drainage, serum and urine of patients who underwent total hip arthroplasty with tobramycin-containing cement has been shown [7, 8]. Also tobramycin release from cement beads and spacers has been studied *in vivo* [9–11].

In the present study, we determined the release of tobramycin from tobramycin-containing bone cement in an animal model. Tobramycin release in serum and bone as a function of time was measured in rabbits, after insertion of tobramycin-containing cement into the femur. The longest follow-up period was 28 days.

2. Materials and methods

2.1. Animals

A total of 39 healthy adult female New Zealand White rabbits (Ico : NZW, Broekman Instituut BV, Someren, The Netherlands), ranging in weight from 2.8–3.5 kg, were obtained 1 week prior to surgery to acclimatize to the housing in the Central Animal Laboratory Institute, Utrecht University. The animals were caged individually, fed with 80–100 g antibiotics-free rabbit diet (Hope Farms Standard laboratory Diet LKK-20, Hope Farms BV, Woerden, The Netherlands) and water *ad libitum*.

2.2. Cement

Surgical Simplex® bone cement, containing 1.0 g tobramycin as a sulfate in 60.0 g methyl methacrylate (batch N6043), was supplied by Howmedica Inc., Rutherford, NJ, USA.

* Author to whom all correspondence should be addressed.
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This radiopaque bone cement is a mixture of a liquid component (monomer) and a powder component (polymer), both sterilized. The composition of the powder component is: polymethyl methacrylate (6.0 g), methyl methacrylate-styrene copolymer (30.0 g), barium sulfate (4.0 g), tobramycin (as a sulfate, 1.0 g). The liquid component (20 ml) is composed of methyl methacrylate (97.4% vol/vol), *N*,*N*-dimethyl-*p*-toluidine (2.6% vol/vol) and hydroquinone (75 ± 15 p.p.m.). The bone cement (4 °C) was vacuum-mixed (0.9 bar) for 1 min on the surgical table (Simplex® cement vacuum-mixer, Howmedica Inc., Rutherford, NJ, USA).

2.3. Experimental design

Tobramycin-containing bone cement was introduced into the medullary canal of the right femur of the rabbits. At 1, 3, 7, 14, 21 and 28 days, groups of six rabbits were killed and the tobramycin levels in bone adjacent to the cement were assayed by fluorescence polarization immunoassay. Bone from the left femur without cement served as control. Tobramycin serum levels were also measured.

The experimental design was approved by the institutional review board (Animal Care and Use Committee, Faculty of Medicine, Utrecht University).

2.4. Anaesthesia

Surgery was performed under general inhalation anaesthesia. The anaesthesia was prepared by an intramuscular injection of 0.2 mg methadone (methadone HCl 10 mg ml^{-1}), 0.2 mg acepromazinemaleate (Vetranquil®, Sanofi Sant BV, Maassluis, The Netherlands) and 0.5 mg atropine (Atropine-sulfate, Kobivet, Etten-Leur, The Netherlands). A pressure line was introduced into the auricular artery for measuring blood pressure. Subsequently, anaesthesia was induced by an intravenous injection of 8–12 mg etomidate (Hypnomidate®, Janssen pharmaceutica BV, Tilburg, The Netherlands). An endotracheal tube (No. 3) was introduced through which the anaesthesia was maintained with a 1 : 1 mixture of nitrous oxide, oxygen and halothane 1% (Albic BV, Maassluis, The Netherlands). If the blood pressure fell after insertion of the cement, dopamineNaCl (0.25 mg ml^{-1}, ICN Pharmaceuticals Holland BV, Zoetermeer, The Netherlands) was injected intravenously.

2.5. Operative technique

Surgery was performed under strict aseptic conditions. The skin of the outer right thigh was clipped and the rabbit was placed with its left side on the table. The operative area was disinfected with a 2% tincture of iodine and isolated by sterile drapes. Subsequently, a skin incision (approximately 3 cm) was made over the trochanter tertius of the right femur, parallel to the femur shaft. The trochanter tertius was exposed by splitting the fascia, retracting the femoral biceps and coccygeofemoral muscles posterio-medially and scraping the periost. Using an air-pressured AO minidrill, the cortex was penetrated by a small drill (diameter 1.2 mm). Subsequently, this hole was widened and the femoral canal was reamed with drills and fraises up to 4.0 mm in width, until a silicon tube (monitor line, outer diameter 3.0 mm) could be inserted. After vacuum-mixing of the PMMA–tobramycin bone cement, a small 6 ml syringe, with a 2 cm long silicon tube (outer diameter 3.0 mm, inner diameter 1.5 mm) attached to it, was filled with cement. The syringe was weighed on an electronic balance (PT150, Sartorius-Instrumenten BV, Nieuwegein, The Netherlands). Before insertion of cement, the medullary canal was washed with sterile physiologic saline and suctioned. The syringe was placed in an adapted device on an applicator gun and approximately 1.2 ml cement was injected gently into the femoral canal, while the syringe was slowly being retracted. Subsequently, the syringe was weighed again in order to know the exact amount of cement injected. After polymerization of the cement, and wound drainage with sterile saline solution, the fascia, subcutis and cutis were closed with Vicryl® 3-0 (Ethicon® GmbH & Co KG, Norderstedt, Germany). Pain relief was provided by 0.3 ml nalbufine (Nubain®, Lamepro BV, Raamsdonkveer, The Netherlands) i.m. immediately postoperative, and subsequently 0.3 ml buprenorfine (Temgesic®, Rechitt and Colman Products, Kingston-upon-Hull, UK) i.m. (if necessary, buprenorfine injection was repeated postoperatively).

2.6. Follow-up

Postoperatively, localization of the cement was evaluated by routine AP and lateral X-rays of the right femur. The rabbits recovered in a temperature-controlled recovery cage.

The rabbits were monitored by a daily clinical examination, with special attention to wound healing, the presence of a fracture, eating, activity level and body temperature.

At 1, 3, 7, 14, 21 and 28 days, groups of six rabbits were killed with an overdose of pentobarbital N_2 (Euthesate®, Apharmo BV, Arnhem, The Netherlands) intravenously.

2.7. Tobramycin assay technique

Tobramycin concentrations in bone and serum were measured by fluorescence polarization immunoassay (TDX, Abbott Laboratories, Chicago, IL, USA). The minimal detectable tobramycin concentration was 1.0 μg g^{-1} in bone and 0.10 μg ml^{-1} in serum.

2.7.1. Bone

The left (control) and right femur from all animals were excised and cleaned of tissue debris. First a bone fragment was taken from the left femur from a region corresponding to the right femur. Secondly, using a high-speed dental drill with a circular metal saw, the external surface of the right femur was notched circumferentially at each end of the shaft and longitudinally on two sides, posterior and anterior. An osteotome was used to free the lateral half of the bone from the medial half, with the cement core *in situ*

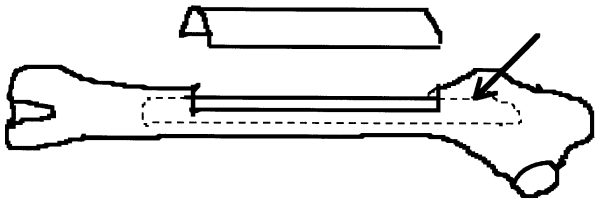

Figure 1 Femur of rabbit with lateral half of cortex excised. Cement (dotted line) was injected proximally (arrow).

(Fig. 1). Care was taken not to damage the cement. Only full-thickness cortex adjacent to the cement was used. The bone fragments of the lateral half of each femur were ground in a metal mortar. Samples of 1.00 g bone were homogenized in 10.0 ml phosphate buffered saline (PBS, pH 7.4) using a Polytron PT 3100 tissue grinder (Kinetica Benelux BV, Best, The Netherlands), 3 min at 2500 r.p.m. and 5 min at 6000 r.p.m. The homogenate was stored at 4 °C overnight. Following centrifugation (3000 r.p.m., 7 min, 4 °C) the supernatant was removed for tobramycin TDX assay.

2.7.2. Serum

Blood samples were taken from the auricular vein of all rabbits on the day of killing. The 28 day rabbits also had tobramycin serum levels measured preoperatively, then at 12 h, 1, 3, 7, 14 and 21 days postoperatively. These samples were centrifuged at 3000 r.p.m. for 9 min at 4 °C, after which the serum was collected for tobramycin TDX assay.

2.8. E-test

The minimal inhibitory concentration (MIC) of tobramycin against *Staphylococcus aureus* Wood 46 was determined using the E-test (E test®, AB Biodisk, Solna, Sweden) [12].

2.9. Statistical analysis

Dixon's Q-test was used to determine outliers in extreme values of tobramycin concentration [13].

3. Results

The mean weight ($\pm$ standard error of the mean, S.E.) of cement inserted was 1.24 ± 0.03 g.

Two rabbits died during surgery. Histology showed fat and bone marrow embolisms in lungs and heart. All other animals recovered well. Post-operative X-rays showed no fractures or cement outside the femur. A fracture was seen at necropsy in one rabbit at day 21 (excluded from study).

Fig. 2 shows the tobramycin concentration (mean $\pm$ S.E.) for a period of up to 4 weeks in serum and bone of both the operated right femur and the left femur (control). Extreme values in each follow up group of rabbits were not outliers (Dixon's Q test, $p < 0.05$). Peak tobramycin concentrations in the right and left

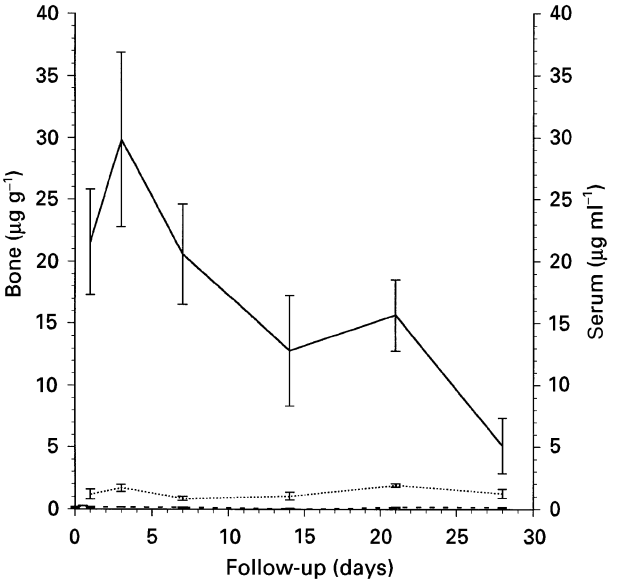

Figure 2 Tobramycin release (mean $\pm$ S.E.) in bone of (—) right femur (insertion of cement) and (⋯) left femur (control), and (– – –) in serum.

femur were reached on day 3 (29.9 ± 7.0 versus 1.7 ± 0.3 μg g^{-1} bone, respectively, mean $\pm$ S.E.).

The tobramycin concentration in the right femur decreased to 5.10 ± 2.2 μg g^{-1} (mean $\pm$ S.E.) over 28 days.

The tobramycin concentrations in serum remained constant throughout the study, just above the detection limit of 0.1 μg ml^{-1} (highest concentration at 12 h 0.25 ± 0.0 μg ml^{-1}, mean $\pm$ S.E.). The minimal inhibitory concentration of tobramycin for *Staphylococcus aureus* Wood 46 was determined as 0.125 μg ml^{-1}.

4. Discussion

The exact mechanism of antibiotic release from PMMA-bone cement is not yet clear. Amongst others, elution characteristics of antibiotic containing bone cement depend on the concentration of antibiotic, and surface area-to-volume ratio of cement and presence of voids and cracks in cement [4, 5, 11, 14–19]. Therefore, it is difficult to predict exactly the release pattern of a certain preparation of antibiotic-containing bone cement from earlier studies, especially from those using different types of antibiotic and/or bone cement. An efficacy study on such a preparation used in arthroplasty surgery, should be preceded by a release study *in vivo*.

Antibiotic concentrations in bone adjacent to the implant exceeding MIC levels of bacteria, which most frequently cause arthroplasty infection, can be indicative for efficacy of the antibiotic-containing bone cement. In this study, tobramycin concentrations in the cortex adjacent to the inserted bone cement in the femur of rabbits exceeded the MIC for *Staphylococcus aureus* Wood 46 for up to 28 days: on day 3 (peak level) mean tobramycin concentration of 239 times MIC was measured, and on day 28 still 40.8 times MIC. In addition, the measured tobramycin concentrations in bone seemed to be low enough to prevent bone toxicity; in recent studies, local bone

concentrations of tobramycin below 200–500 $\mu g\,ml^{-1}$ are recommended [20, 21].

Two serious side effects of aminoglycosides are nephrotoxicity and ototoxicity. The threshold for nephrotoxicity is reported as 6.0 $\mu g\,ml^{-1}$ [10]. Only serum levels of tobramycin well below this threshold could be detected in our study.

Because of the relatively small size of the femur of the rabbit compared to human, the risk of creating a rise in intramedullary pressure by entrapment of air during insertion of cement is present. This rise in intramedullary pressure is a major pathogenic factor for the development of fat embolism syndrome [22]. Not surprisingly, histology showed embolisms to be the cause of death of two rabbits who died just after insertion of the cement.

In conclusion, after insertion of tobramycin-containing bone cement in the femur of a rabbit, the tobramycin release in bone up to 28 days, results in high concentrations next to the cement. Serum tobramycin concentrations are well below systemic toxicity threshold. Studies on prevention of actual implant infection are currently being performed at our institute.

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