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ORIGINAL PAPER

Characterisation of in vivo release of gentamicin from polymethyl methacrylate cement using a novel method

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Abstract
Purpose The purpose of this study was to investigate the in vivo elution kinetics of gentamicin from bone cement by assessing antibiotic levels in the urine.
Methods Urinary samples of 35 patients who had undergone primary total hip arthroplasty were collected post-operatively. Gentamicin concentrations were analysed using the fluorescence polarisation immunoassay technique.
Results The mean duration of urinary gentamicin release in all cases was 43 days (range 13–95). There was still detectable gentamicin at the final collection in 20 % (7/35) of cases, and in these cases, the mean gentamicin release was 71 days.
Conclusions From the assessment of urinary gentamicin, we were able to demonstrate the biphasic gentamicin elution from bone cement. In addition, there were detectable concentrations of the antibiotic from the urinary samples for prolonged periods of up to two to six months. Our study indicates that the assessment of urinary antibiotics can offer a non-invasive method of monitoring the in vivo release kinetics of antibiotics from bone cement.

Introduction

Prosthetic joint infection (PJI) remains one of the most feared complications of joint arthroplasty. The incidence of orthopaedic periprosthetic infection is estimated to be approximately 1.5–2.5 % in primary hip and knee replacement and 3.2–5.6 % in the revision cases [20].

Biofilm formation is central to the development of PJI and occurs during the first 24 hours after implantation [9]. Once established bacterial biofilms are very hard to treat and often require removal of the implants. The goal in prosthetic joint surgery is therefore to prevent biofilm formation in these early stages—the so-called race for the surface [14].

Numerous techniques have proven efficacy in reducing the PJI rate. These include the clothing and practice of theatre personnel, skin disinfection, wound lavage, theatre air decontamination and antibiotic prophylaxis. The latter is administered by both systemic and local routes.

Systemic antibiotic prophylaxis has been shown to reduce PJI in numerous studies and most successful if administered one hour before surgery. Carlsson et al. [6] reported upon the seven year data of a double-blind study originally started by Ericson et al. [12]. They showed an infection rate of 2 % in the antibiotic group and of 15.4 % in the control group. Long-term protection was afforded by perioperative antibiotics suggesting that implantation at the time of surgery is the most common cause of infection. Late haematogenous seeding accounted for only 0.8 % of cases.

Although serum and bone concentrations above the minimum inhibitory concentration (MIC) for planktonic susceptible bacteria are achieved with cephalosporins this may not be sufficient to eradicate the biofilm sessile bacteria that are the problem in PJI [22]. There is therefore a need for high-dose antibiotic delivery to the host-implant interface where the biofilm forms and this can be achieved with the use of antibiotic-loaded acrylic cement (ALAC).

In 1970 Buchholz and Engelbrecht at the Endoklinik incorporated gentamicin in polymethyl methacrylate (PMMA) for the treatment of PJI [5]. It remains standard practice to date to use ALAC as part of the antimicrobial prophylaxis in primary total joint arthroplasty and its

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efficacy as a prophylactic agent has been described. Antibiotic-loaded cement can also be used in revision arthroplasties involving implant retention treatment, as well as two-stage procedures [8]. Comparing plain against gentamicin-loaded Palacos cement in total hip arthroplasty (THA), Buchholz et al. [4] reported an infection rate of 4.85 % for the plain and only 1.63 % for the ALAC at three years. Similarly, Josefsson and Kolmert also reported six- and fourfold reductions respectively in infection rates with the use of prophylactic ALAC [16].

Antibiotic elution from acrylic bone cement has been described in three types of study—in vitro, animal and human studies, the majority being in vitro studies. The elution as described by these studies follows a biphasic pattern with early high release followed by long-term low-level release. In addition, the majority of the antibiotics remained as a reservoir within the cement.

The simple agar plate technique was the standard method used to assay antibiotic elution up to the end of the 1980s [3]. During the 1990s quantitative methods were added including dilutional bioassays. These represented an improvement with respect to the simple agar plate technique as they provided an approximate concentration of the eluted antibiotic [17]. Newer available techniques such as fluorescence polarisation immunoassay (FPIA) and high-performance liquid chromatography (HPLC) are far more sensitive and can accurately quantify the concentration of antibiotic released as the experiment progresses [18, 26].

Gentamicin was the antibiotic chosen by Buchholz and Engelbrecht in 1970 to add to Palacos cement [4]. It has proven to be a very successful combination [19]. Gentamicin is an ideal antibiotic for inclusion in PMMA as it possesses the following characteristics: broad antibacterial spectrum (Gram-positives and Gram-negatives), bactericidal action, high specific antibacterial potency, low rate of primary resistance, minimal resistance development, low protein binding, low sensitisation potential, high water solubility, chemical and thermal stability [24].

There is a paucity of human studies describing the release of gentamicin after primary THA compared to animal and laboratory studies. In addition the literature is predominately old (1980s) and uses outdated and insensitive assay techniques. A limitation of early studies is the requirement of invasive joint fluid aspiration, some of which have been reported to be up to 5.5 years [24].

More recently, Fink et al. [13] assayed antibiotic elution from 14 antibiotic-loaded cement spacers removed six weeks following their implantation in two-stage revision for prosthetic hip joint infections. Gentamicin, clindamycin and vancomycin concentrations were determined using HPLC coupled to tandem mass spectroscopy. Similarly, Anagnostakos et al. [1] studied the antibiotic elution from 17 hip spacer patients undergoing revision surgery for PJI. Daily elution samples were obtained from fluid in Redon drains which were inserted at the time of surgery and left in situ for seven and 13 days for the spacers and the beads respectively. The antibiotic concentrations were determined using FPIA.

A non-invasive technique that can monitor gentamicin release from the ALAC with modern assay techniques would be ideal. Gentamicin is highly water-soluble and shows both thermal and chemical stability. Although both nephro- and ototoxicity are recognised as risks of parenteral administration these have not been demonstrated when incorporated in bone cement [25]. This is because aminoglycosides are not metabolised systemically and serum concentrations remain very low after release from the cement [24]. Hence a urinary assay would be a useful direction for investigation. The aim of this study was to investigate the in vivo elution kinetics of gentamicin-loaded bone cement in THA patients.

Materials and methods

Patient recruitment

Patients were recruited from the pre-operative assessment clinic of the Avon Orthopaedic Centre. Ethical approval had been granted for this clinical study and informed written consent was given in each case. Patients undergoing primary THA using cemented implants were recruited and informed consent for prospective follow-up was taken. The cement used was Palacos® R-40 with Gentamicin Cement (Schering-Plough, UK) and this is the standard cement for this orthopaedic unit. In no cases was the surgeon's choice of implant or cement altered because of the study.

Exclusion criteria

Patients were excluded if they were concurrently receiving gentamicin or suffered from renal failure as based on a history or review of the hospital records. If a patient received a single intramuscular gentamicin injection post-operatively to cover the insertion or removal of a urinary catheter then the results from the urinary samples were disregarded for three days. This decision was based upon advice from the Microbiology Department and required the patient to have renal function within normal physiological limits during this period. If the renal function was abnormal the case was excluded from the trial.

Table 1 Urinary gentamicin concentrations after cemented primary hip arthroplasty

Case[a]	D	G	D	G	D	G	D	G	D	G	D	G	D	G	LD
P001	20	1.41	27	1.01	40	0.72	95	0.45	178	<0.06					95
P002	8	0.52	11	0.51	18	0.68	32	0.03	73	0.01	159	<0.06			18
P003	8	0.23	16	1.85	28	0.15	41	0.16	82	<0.06					41
P005	11	2.9	27	1	41	<0.06	82	<0.06	171	<0.06					27
P007	14	0.29	28	<0.06	42	0.09	90	<0.06	172	<0.06					42
P009	14	5.22	20	1.06	27	0.47	41	0.17	82	<0.06	165	<0.06			41
P010	14	0.93	17	0.55	20	0.46	27	0.34	41	0.16	82	0.08			82
P014	13	0.82	28	0.74	42	0.23	82	0.11							82
P015	13	0.13	27	<0.06	41	<0.06	82	<0.06							13
P017	14	5.38	21	0.78	28	0.89	41	0.65	83	<0.06	172	<0.06			41
P018	22	0.08	29	0.07	43	<0.06	84	<0.06	166	<0.06					29
P019	15	1.13	22	0.18	29	0.23	42	<0.06	97	<0.06	166	<0.06			29
P021	8	1.68	10	0.39	24	0.11	31	0.11	45	<0.06	86	<0.06			31
P022	8	7.05	13	9.48	20	1.39	27	0.12	40	<0.06	76	<0.06	165	<0.06	27
P026	8	9.48	15	8.37	22	1.64	28	0.27	41	0.15	77	<0.06	166	<0.06	41
P027	12	0.46	19	0.2	26	0.1	39	0.08	88	<0.06	170	<0.06			39
P029	12	0.17	19	0.07	26	0.07	39	0.04	88	<0.06	170	<0.06			26
P030	14	0.12	20	0.17	28	0.13	42	<0.06	83	<0.06	165	<0.06			28
P031	12	0.19	19	0.1	40	0.08	80	<0.06							40
P032	11	1.05	15	0.27	21	0.27	35	0.27	42	<0.06	83	<0.06	173	<0.06	35
P034	12	0.71	19	0.3	26	0.14	40	<0.06	80	<0.06	163	<0.06			26
P035	13	0.38	21	0.16	62	0.12	100	<0.06	172	<0.06					62
P037	13	0.21	26	0.15	40	0.11	81	<0.06	157	<0.06					40
P038	13	0.67	26	0.23	40	0.20	81	0.08	164	<0.06					81
P042	17	0.54	24	0.17	45	0.08	86	0.07							86
P043	15	0.1	26	0.25	33	<0.06	47	<0.06	88	<0.06					26
P044	9	1.36	20	0.18	30	0.09	37	<0.06	85	<0.06					30
P046	11	1.29	28	0.52	41	0.16	83	<0.06							41
P047	10	0.74	17	0.12	30	<0.06	37	<0.06	99	<0.06					17
P048	12	0.67	15	0.23	28	0.2	42	0.08	83	<0.06					42
P050	15	0.95	22	0.33	29	0.21	42	0.15	84	<0.06					42
P052	15	1.67	26	1.03	33	0.61	47	0.19	88	0.11					88
P054	14	0.18	21	0.15	28	<0.06	42	<0.06	90	<0.06					21
P055	14	0.63	21	0.23	35	0.12	86	0.1							86
P056	8	1.47	10	1.27	17	0.5	27	0.14	48	<0.06	83	<0.06			27

D interval between urinary sampling and operation in days, *G* urinary gentamicin concentration in mg/L, *LD* last urinary sampling with detectable gentamicin in days since operation

[a] Case number identifier

Urinary sampling

Gentamicin sulphate is rapidly and completely excreted by the renal system. It does not undergo metabolism within the body and hence the urinary levels are a true reflection of the release from the cement at any given time.

Post-operatively 2-ml urine samples were collected every two days until discharge from hospital. Domiciliary samples were then collected at two, three, four, six and 12 weeks. In selected early cases a final sample was collected at the six month stage.

All samples were frozen at −70 °C and then tested in batches. The gentamicin concentration was tested using an FPIA technique in the Abbott TDx system (Abbott Laboratories, Chicago, IL, USA). The sensitivity of this assay for gentamicin is 0.06 mg/L.

Data were analysed using SPSS v11 statistical software (SPSS Inc., Chicago, IL, USA). Data were tabulated and simple descriptive statistical analysis performed. An in vivo

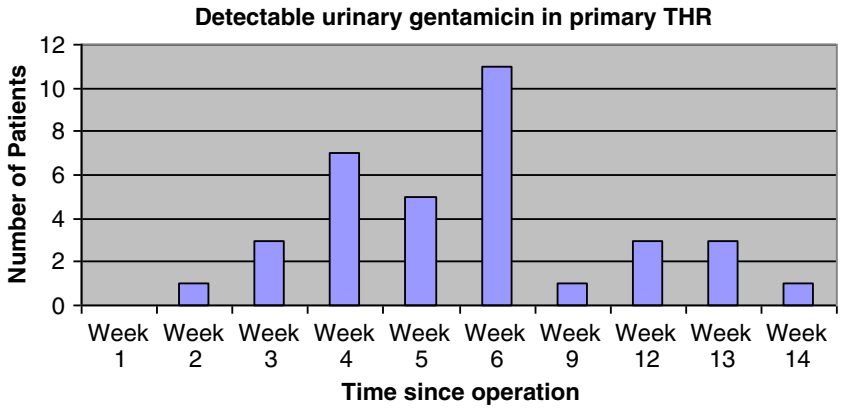

Fig. 1 Duration of in vivo gentamicin release

elution profile was produced for Palacos® R-40 with Gentamicin Cement (Schering-Plough, UK) used in THA.

Results

Patient recruitment and clinical details

There were 38 patients who gave post-operative urine samples to this study. Of these, 35 (92 %) patients were followed up for 12 or more weeks and their data are presented in Table 1.

Two brands of cement were used: Palacos® R-40 with Gentamicin Cement (Schering-Plough Europe, Brussels, Belgium) in 32 cases (91 %) and Refobacin®-Palacos® Cement (Biomet Merck, Merck Biomaterials GmbH, Darmstadt, Germany) in the remaining three (9 %). These cements employ the same PMMA base but the gentamicin (0.5 g) originates from different factories; the quantity of gentamicin was the same for both cements (0.5 g per 40 g pack).

The THAs were fully cemented (both the cup and stem components being implanted with cement) in 19 (54 %) of the cases. The remaining 16 cases (46 %) were hybrid arthroplasties, where only the stem was implanted with cement. Each hybrid case used two '40 g' mixes of cement compared to the three for each fully cemented arthroplasty.

The mean duration of detectable gentamicin release was 47 days for the fully cemented arthroplasties compared with 39 days for the hybrid cases. This difference was not significant (p=0.15 paired t test).

Intramuscular gentamicin

Sixteen (46 %) of the patients had an intramuscular injection of gentamicin in the immediate post-operative period to cover the removal of a urinary catheter. As the protocol devised by the microbiologists excluded results from the following three days it was decided to compare only the data values from one week post-operatively. This also conformed to the aim of this experiment to study the medium- to long-term release of gentamicin from cemented THAs. In addition, many studies have described the early in vivo release profiles [24].

In vivo gentamicin release profile

The concentration of gentamicin in the urine samples was measured until the sensitivity of the assay was exceeded. As the samples were collected at regular intervals the data were then grouped according to the time post surgery when urinary gentamicin was last detected.

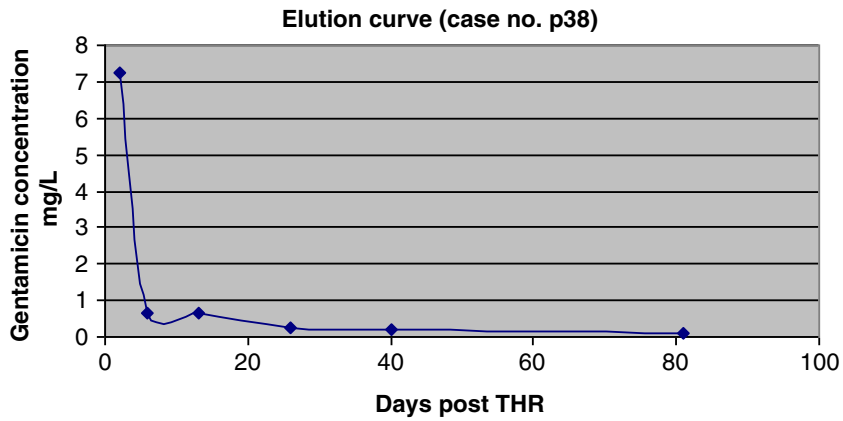

Fig. 2 A typical urinary gentamicin elution curve

The mean duration of urinary gentamicin release was 43 days with a range of 13–95 days. The duration of the in vivo elution is illustrated in Fig. 1.

A typical elution curve from the data is presented in Fig. 2 and it can be seen that the concentrations rapidly reach an asymptotic phase.

Of the 35 cases studied, seven (20 %) still had detectable gentamicin concentrations at their final collection. The mean duration of gentamicin release in these seven cases was 71 days with a range of 31–88 days.

Discussion

A urinary gentamicin assay is an effective and non-invasive means of monitoring the release of gentamicin from ALAC in primary THA. It reveals the recognised biphasic release profile.

There is a paucity of in vivo elution studies, but the consensus is that after primary hip arthroplasty gentamicin is detectable within the urine for approximately two weeks [7, 24]. This is due to two main reasons: firstly, it would be unfeasible to subject patients to multiple arthrocenteses for prolonged periods of time and secondly, older analytic techniques had relatively lower sensitivity.

The average (mode) release period in our group of 35 patients was six weeks and the longest was 14 weeks (95 days). The in vivo elution curve matches those described in other studies, with high initial release followed by a rapid drop and asymptotic phase [2, 21]. This study demonstrates that the use of a sensitive immunoassay will detect gentamicin release for significantly longer.

It is significant that gentamicin can be measured for an average of 71 days after primary THA. Indeed in 20 % of cases gentamicin was still recordable at the last sampling. This is therefore clear evidence of prolonged elution of gentamicin from the cement in primary THA.

Although the urinary gentamicin concentration does not represent the level present adjacent to the prostheses, it does give a useful guide to release rate. However, it should be noted that even intra-articular measurements do not reflect the gap concentration at the bone-cement interface [15]. The corollary of this is that the urinary detected gentamicin suggests that the significantly higher interface concentrations are being achieved in vivo.

This will ensure that the biomaterials are protected against the development of a bacterial biofilm for at least six weeks. This ensures that the race for the surface is not a sprint but instead a long-distance event. Importantly the implants will be protected for the period during which the principle soft tissue healing occurs

This supports the registry observations that ALAC is an independent factor that reduces the risk of revision [10, 23] and the clinical studies that have shown prolonged statistically significant protection against PJI [16].

One concern is the prolonged low-level release which has the theoretical potential to promote resistance in any bacteria present at the host-implant interface. Although ALAC is proven to reduce the rate of PJI, those infections that do occur are likely to demonstrate higher antimicrobial resistance [11].

The major weakness of this study is that the urinary gentamicin estimations were made on discrete urinary samples rather than on pooled daily outputs. Therefore although the data do represent absolute gentamicin concentrations, without urinary output volumes these are difficult to convert to true elution data (total amount of gentamicin released against time). The decision to use discrete urine samples was made pragmatically and reflected the difficulties of collecting 24 hour urine samples in this elderly population. We have therefore made an assumption that over the prolonged period of sampling there would be relatively constant daily urinary output, hence minimising day-to-day variations in gentamicin release. However, as the total weight of cement used in each patient was not recorded, it would have been difficult to relate total gentamicin urinary excretion to the amount implanted and one of the strengths of this study lies in proof of concept that urinary excretion can be used to evaluate gentamicin release from implanted cement.

In summary, the use of this novel analysis technique reveals more prolonged release of gentamicin from ALAC in patients undergoing primary THA. This may in part explain the favourable registry figures from the use of ALAC in THA.

Conflict of interest None.

References

1. Anagnostakos K, Wilmes P, Schmitt E et al (2009) Elution of gentamicin and vancomycin from polymethylmethacrylate beads and hip spacers in vivo. Acta Orthop 80:193–197
2. Armstrong M, Spencer RF, Lovering AM et al (2002) Antibiotic elution from bone cement: a study of common cement-antibiotic combinations. Hip Int 12:23–27
3. Beeching NJ, Thomas MG, Roberts S et al (1986) Comparative in-vitro activity of antibiotics incorporated in acrylic bone cement. J Antimicrob Chemother 17:173–184
4. Buchholz HW, Elson RA, Heinert K (1984) Antibiotic-loaded acrylic cement: current concepts. Clin Orthop Relat Res 190:96–108
5. Buchholz HW, Engelbrecht H (1970) Depot effects of various antibiotics mixed with Palacos resins. Chirurg 41:511–515
6. Carlsson AK, Lidgren L, Lindberg L (1977) Prophylactic antibiotics against early and late deep infections after total hip replacements. Acta Orthop Scand 48:405–410

7. Chohfi M, Langlais F, Fourastier J et al (1998) Pharmacokinetics, uses, and limitations of vancomycin-loaded bone cement. Int Orthop 22:171–177
8. Choi HR, von Knoch F, Kandil AO et al (2012) Retention treatment after periprosthetic total hip arthroplasty infection. Int Orthop 36:723–729
9. Costerton JW (2005) Biofilm theory can guide the treatment of device-related orthopaedic infections. Clin Orthop Relat Res 437:7–11
10. Dale H, Hallan G, Hallan G et al (2009) Increasing risk of revision due to deep infection after hip arthroplasty. Acta Orthop 80:639–645
11. Elson RA, Jephcott AE, McGechie DB et al (1977) Bacterial infection and acrylic cement in the rat. J Bone Joint Surg Br 59-B:452–457
12. Ericson C, Lidgren L, Lindberg L (1973) Cloxacillin in the prophylaxis of postoperative infections of the hip. J Bone Joint Surg Am 55(4):808–813, 843
13. Fink B, Vogt S, Reinsch M et al (2011) Sufficient release of antibiotic by a spacer 6 weeks after implantation in two-stage revision of infected hip prostheses. Clin Orthop Relat Res 469:3141–3147
14. Gristina AG, Costerton JW (1985) Bacterial adherence to biomaterials and tissue. The significance of its role in clinical sepsis. J Bone Joint Surg Am 67:264–273
15. Hendriks JG, Neut D, van Horn JR et al (2005) Bacterial survival in the interfacial gap in gentamicin-loaded acrylic bone cements. J Bone Joint Surg Br 87:272–276
16. Josefsson G, Kolmert L (1993) Prophylaxis with systematic antibiotics versus gentamicin bone cement in total hip arthroplasty. A ten-year survey of 1,688 hips. Clin Orthop Relat Res 292:210–214
17. Kuechle DK, Landon GC, Musher DM et al (1991) Elution of vancomycin, daptomycin, and amikacin from acrylic bone cement. Clin Orthop Relat Res 264:302–308
18. Lovering AM, White LO, MacGowan AP et al (1996) The elution and binding characteristics of rifampicin for three commercially available protein-sealed vascular grafts. J Antimicrob Chemother 38:599–604
19. Malchau H, Herberts P (1998) Prognosis of total hip replacement. Revision and re- revision rate in THR. A revision-risk study of 148,359 primary operations. Proceedings of AAOS, 65th Annual Meeting of the American Academy of Orthopaedic Surgeons, 19–23 March 1998, New Orleans
20. Montanaro L, Speziale P, Campoccia D et al (2011) Scenery of Staphylococcus implant infections in orthopedics. Future Microbiol 6:1329–1349
21. Sterling GJ, Crawford S, Potter JH et al (2003) The pharmacokinetics of Simplex-tobramycin bone cement. J Bone Joint Surg Br 85:646–649
22. Stewart PS, Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. Lancet 358:135–138
23. Swedish Hip Arthroplasty Register (2010) Annual report 2010. http://www.shpr.se
24. Wahlig H, Dingeldein E (1980) Antibiotics and bone cements. Experimental and clinical long-term observations. Acta Orthop Scand 51:49–56
25. Whelton A (1984) The aminoglycosides. Clin Orthop Relat Res 190:66–74
26. White LO, MacGowan AP, Lovering AM et al (1994) Assay of low trough serum gentamicin concentrations by fluorescence polarization immunoassay. J Antimicrob Chemother 33:1068–1070