High local concentrations without systemic adverse effects after impaction of netilmicin-impregnated bone

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Background **When cancellous bone is impregnated with antibiotics the subsequent release of antibiotics from the bone shows a high early release. Hence, impaction of large amounts of netilmicin-impregnated bone may cause toxic netilmicin values in serum.**

Patients and methods **We studied kidney and otovestibular function after impacting 50 g of netilmicin-impregnated cancellous bone during revision hip or knee arthroplasty in 20 patients. The bone was impacted in the acetabulum (n = 8), proximal femur (n = 9) and distal femur/proximal tibia (n = 3). Serum creatinine concentration was measured and audiometry was performed before and after the operation. Netilmicin concentrations in serum, joint fluid, and in urine were recorded postoperatively at regular intervals. We analyzed pharmacokinetics in two study groups receiving bone impregnated with netilmicin (50 mL), at either 50 mg netilmicin/mL (group I) or 100 mg netilmicin/mL (group II).**

Results **Neither netilmicin-induced renal toxicity, nor otovestibular toxicity was registered. Peak serum netilmicin values in group I and group II were 0.9 (0.5–1.3) mg/L and 1.8 (0.6–4.0) mg/L, respectively (p = 0.04). Peak netilmicin concentrations in wound drainage fluid in group I and group II were 237 (9–647) mg/L and 561 (196–1132) mg/L, respectively (p = 0.01). In both groups, netilmicin was recovered in urine samples for approximately 4 weeks.**

Interpretation **50 grams of cancellous bone impregnated with 100 mg/mL netilmicin solution was impacted in the hip or knee joint with no adverse effects. Extremely high local concentrations of netilmicin in joint fluid were recorded postoperatively. The use of antibioitic-impregnated cancellous could be an option when performing revision of hip and knee prostheses.**

Due to the antibacterial and physiochemical properties of antibiotic-impregnated bone cement, aminoglycosides have been preferred when incorporating antibiotics in bone cement (van de Belt et al. 2001). However, it has been speculated that the sustained release of antibiotics from bone cement may have adverse effects, and authors have argued for the use of biodegradable vehicles for antibiotic delivery (Hope et al. 1989, van de Belt et al. 2001). When there is loss of bone stock during revision of a loose hip or knee prosthesis, morselized cancellous allograft is impacted before implantation of the new prosthesis (Sloof et al. 1984, Stockley et al. 1992, Gie et al. 1993, Benjamin et al. 2001). There have been reports that cancellous bone can act as a carrier of antibiotics (Witsø et al. 1999, 2000, 2002, Winkler et al. 2000). Large quantities of aminoglycosides are released from antibiotic-impregnated cancellous bone, both in vitro and in vivo. All aminoglycosides share the potential for renal and otovestibular toxicity (Ristuccia 1984). Hence, clinical use of cancellous bone impregnated with aminoglycosides could have toxic side effects.

We evaluated possible adverse effects on kidney and otovestibular function when impacting netilm-

icin-impregnated cancellous bone during revision hip and knee arthroplasty. In addition, we analyzed the pharmacokinetics in two study groups receiving bone impregnated with two different netilmicin concentrations.

Patients and methods

20 patients with aseptic loosening of either a hip or knee prosthesis who also had a loss of acetabular, femur or tibia bone stock were included consecutively in the study. Criteria for exclusion from the study were: a) patients with a history of renal pathology or presently impaired renal function, defined as serum creatinine > 120 μmol/L (males) or > 100 μmol/L (females), b) patients with any previous history of serious otovestibular pathology (except presbyacusis), c) dehydrated patients, d) patients who were pregnant or breast-feeding, e) patients receiving concurrent administration of vancomycin or cephalosporin, and f) patients in whom pre- or peroperative evaluation indicated an infected prosthesis.

At inclusion in the study, auditory evaluation (audiometry) was performed. Baseline serum creatinine concentration was defined as the mean value of three consecutive values measured within 72 h before the operation. All operations took place in an operating theatre with laminar airflow. Cancellous bone was prepared and impregnated with antibiotics as described previously (Witsø et al. 1999). Briefly, a cancellous allograft (femoral head) was morselized in an orthopedic bone mill (Aesculap, HARRIS, GB 044). 50 g of bone was impregnated using 50 mL netilmicin. Patients received cancellous bone impregnated with either 50 mg netilmicin/mL (group I) or 100 mg netilmicin/mL (group II). After 10 minutes, the antibiotic solution was strained from the bone through gauze. Then the fluid was pressed out of the bone by twisting the gauze to leave almost completely dry bone. The study period commenced with the impaction of the antibiotic-impregnated bone in the acetabulum, femur or tibia. If more than 50 g bone was needed for impaction, the extra amount of bone was not impregnated with antibiotics. If bone cement was used in addition to bone impaction, antibiotics were not added to the cement. After skin closure, wound fluid from the hip or knee joint was drained using a standardized closed system set ("bellovac FG 14/4.7 mm", Astra Tech AB, Sweden). As part of the routine used at our department, patients received systemic antibiotic prophylaxis with dicloxacillin (2 g + 1 g + 1 g; 30 minutes preoperatively and 8 and 16 hours postoperatively). If the patient was allergic to penicillin, clindamycin (600 mg + 300 mg + 300 mg) was used instead.

Postoperatively, the patients were monitored continuously for potential side effects. Serum creatinine concentrations were recorded daily for 1 week. Serum concentrations of netilmicin were measured every hour for 6 hours after impaction and thereafter every sixth hour until serum concentrations were below 1.0 mg/L, and then weekly until the serum concentration of netilmicin was below 0.1 mg/L. Joint fluid was collected in the closed wound drainage system bags. Whenever possible, the bags were changed at regular intervals, i.e. after approximately 6, 12, 24 and 36 hours postoperatively. The amount of netilmicin in urine was recorded for as long as the patient had postoperative epidural sedation and a bladder catheter. After removal of the bladder catheter, netilmicin concentration in urine was measured daily until the concentration was below 1.0 mg/L, and weekly until it was below 0.1 mg/L. When calculating the amount of netilmicin excreted in urine after removal of the bladder catheter, we used an estimated diuresis of 1500 mL/day. The study period ended when netilmicin concentrations were below 0.1 mg/L in both urine and serum. At the end of the study period, serum creatinine was measured and otovestibular function was evaluated by audiometry.

Netilmicin concentration was determined using fluorescence polarization immunoassay (FPIA) technology (TDx/TDxFLx Netilmicin Assay System, Abbott Laboratories, Diagnostics Division, Abbott Park, IL 60064, USA). In this assay, 0.09 mg/L of netilmicin is the lowest detectable concentration.

Ethical approval

The study was approved by the Regional Ethics Committee and the Norwegian Medicines Control Authority. Each patient signed a confirmation of consent.

Definitions

Nefrotoxicity: Increase from the baseline serum creatinine concentration by 50% on two consecutive occasions (Rybak et al. 1999).

Ototoxicity: An impairment of auditory threshold of at least 15 dB at two adjacent frequencies in one or both ears, registered at two consecutive registrations (Rybak et al. 1999).

Statistics

If not otherwise stated, results are presented as arithmetic mean (dispersion as total range). Statistical analysis was performed using Student's t-test. Statistical significance was defined as $p < 0.05$.

Results

27 patients were considered for participation in the study. 7 were excluded, due either to elevated serum creatinine concentration (n = 6), or a history of otovestibular pathology (n = 1). The remaining 20 patients included in the study consisted of 12 women and 8 men, age 71 (43–86) years. There were no dropouts. 17 hip and 3 knee exchange arthroplasties were performed. The following revision prostheses were used: 13 cemented acetabular components, 7 cemented femoral components, 5 uncemented femoral components (including 3 long stem reconstruction prostheses), and 3 cemented knee prosthesis. At revision, cancellous bone impregnated with netilmicin was impacted in the acetabulum (n = 8), the proximal femur (n = 9) or the distal femur/proximal tibia (n = 3). Each patient received 50 g of cancellous bone impregnated with netilmicin. The total amount of cancellous bone used per revision was 90 (50–200) g. In all patients except one, cancellous bone impregnated with netilmicin was impacted adjacent to the joint. In the other patient, the impregnated bone was impacted distally in the greater trochanter and an additional amount of 30 g of nonimpregnated cancellous bone was impacted in the proximal part of the trochanter. Operation time averaged 200 (120–390) min. Time from completed cancellous bone impaction to skin closure was 60 (30–105) min. Estimated blood loss during the operation was 1.3 (0.3–3) L.

1 patient had a postoperative increase in serum creatinine concentration of more than 50% of baseline values at two consecutive occasions due to hypovolemia. The serum creatinine level normalized after 48 hours. This patient had a peak serum netilmicin concentration of 1.2 mg/L, and 12 hours postoperative serum netilmicin was below 1.0 mg/L. In all patients the second audiogram was performed more than 2 weeks after the operation. In 1 patient, the preoperative audiogram was unintentionally deleted before the postoperative audiogram was recorded. The audiogram taken at the end of the study period showed changes typical of presbyacusis. The postoperative peak netilmicin serum concentration of this patient was low (1.2 mg/L), with no risk of ototoxicity. We observed a decreased auditory threshold postoperatively in 1 patient. This patient had a peak serum netilmicin value of 1.0 mg/L, and the case was diagnosed as otosclerosis. Netilmicin-induced nephrotoxicity or otovestibular toxicity was thus not registered. Furthermore, we did not observe any hypersensensitivity reactions.

In the two study groups the peak concentrations and total amount of netilmicin recovered in serum, joint fluid and urine were as follows. Peak values of netilmicin in serum: group I: 0.9 (0.4–1.3) mg/L; group II: 1.8 (0.6–4.0) mg/L ($p = 0.04$). Peak netilmicin values in joint fluid: group I: 237 (9–647) mg/L; group II: 561 (311–1132) mg/L ($p = 0.01$). The total amount of netilmicin in joint fluid: group I: 175 (2–573) mg; group II: 483 (176–901) mg ($p = 0.01$). Peak values in urine: group I: 17 (5–35) mg/L; group II: 22 (6–51) mg/L ($p = 0.4$). Total amount in urine: group I: 67 (23–120) mg; group II: 95 (21–194) mg ($p = 0.1$). Duration of excretion in urine: group I: 25 (14–36) days; group II: 31 (12–94) days ($p = 0.5$). The total amount of netilmicin recovered in joint fluid and urine for each patient was 242 (113–659) mg in group I and 626 (221–1095) mg in group II ($p < 0.01$). All details with regard to individual patients are shown in Table 1.

Netilmicin concentrations in serum above 2.0 mg/L were measured for more than one consecutive recording in only two patients. Netilmicin concentrations in serum were below 1.6 mg/L after 6 h, and below 1.0 mg/L 12 h after completed impaction of the bone in all patients. Consecutive recordings of serum netilmicin concentrations during the first 12 h for patients in group I and group II are

Table 1. Peak values and the total amount of netilmicin recorded in serum, wound drainage fluid and urine from patients in groups I and II

A	B	C	D	E	F	G	H	I
Group I								
1	M/82	A	0.5	103	89	5	52	29
2	F/70	T	0.8	336	256	15	48	15
3	F/86	K	0.8	647	573	35	86	36
4	F/43	A	1.0	176	12	59	25	
5	F/70	T	1.1	165	10	26	7	14
6 [a]	M/79	T	1.2	9	2	15	111	29
7	F/51	K	1.3	324	104	24	53	28
8	M/59	A	0.4	239	174	7	23	22
9	F/72	T	1.0	196	198	19	120	33
10	F/56	A	0.5	174	64	8	50	23
Group II								
1	M/68	A	4.0	792	901	45	194	29
2	F/72	T	0.9	349	298	12	62	33
3	M/70	K	1.6	958	841	12	101	94
4	F/72	T	0.7	466	317	9	128	38
5	M/74	A	0.9	196	178	6	43	13
6	M/75	T	0.6	1132	873	9	21	12
7	M/83	T	2.2	538	489	30	126	23
8	F/74	A	1.2	325	176	34	100	23
9	F/80	A	3.4	311	335	51	126	27
10	F/79	T	2.3	547	426	10	51	21

[a] indicates the only patient in which antibiotic-impregnated bone was impacted deep in the trochanter region with nonimpregnated bone impacted on top, adjacent to the joint.

A Patient no.
B Sex/age
C Site of impaction
  A Acetabulum
  T Trochanter
  K Knee
D Peak values in serum, mg/L
E Peak values in wound fluid, mg/L
F Total amount in wound fluid, mg
G Peak values in urine, mg/L
H Total amount in urine, mg
I Excretion in urine, days

shown in Figure 1. 1 week postoperatively, the netilmicin concentrations in serum were below measurable concentrations. The total amount of joint fluid averaged 1.20 (0.26–2.5) L; drained for 30 (18–41) h. The consecutive recorded concentrations of netilmicin in joint fluid for each patient are listed in Table 2. The netilmicin concentration in urine over the first 10 days postoperatively is shown in Figure 2.

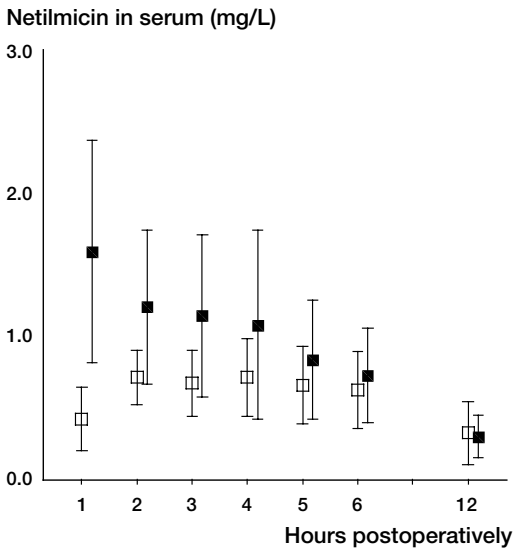

Figure 1. Illustration of the relationship between netilmicin concentration in the impregnation fluid and serum netilmicin concentration during the first 12 hours after impaction of cancellous bone impregnated with netilmicin 50 mg/mL (□ = group I) or 100 mg/mL (■ = group II). Error bars are 95% confidence interval of mean.

Discussion

The toxicity of aminoglycosides has been associated with persistent exposure to elevated serum levels of the drug, i.e., high and prolonged trough serum values. Netilmicin has been administered as a once-daily dose with high peak serum levels (18–26 mg/L) and with no recordable toxic effects (Hollender et al. 1989, Gilbert 1991). In our study the highest netilmicin concentration recorded in serum was 4.0 mg/L, which is far below toxic levels. In all patients, serum netilmicin concentrations were above 1.0 mg/L for only a few hours. There was a direct proportional relationship between antibiotic concentration in the impregnating fluid and peak antibiotic levels in serum. The results suggests that patients with normal renal function can probably receive far more than 50 g of morselized cancellous bone impregnated with 100 mg/mL netilmicin, with no risk of toxic side effects. It may be argued that our study sample was small. However, the preliminary results of several pilot studies formed the basis for the estimation of the study sample.

Table 2. Netilmicin concentrations (mg/L) in the wound drainage fluid collected after 2h, 5–9h, 14–19h, 23–30h and 35–41h postoperatively

Patient no.	Site of impaction	Hours postoperatively 2	5–9	14–19	23–30	35–41
Group I						
1	Acet		103	47	36	
2	Troch		336	33	8	
3	Knee	647	143	33	13	
4	Acet			176	62	
5	Troch		165	70	14	
6	Troch		9	2	1	
7	Knee	324	73	13		
8	Acet		239	63	31	
9	Troch		196	58		30
10	Acet		174	88		25
Group II						
1	Acet			792	258	
2	Troch		349	163	109	
3	Knee		958	180	26	
4	Troch		466	145	88	
5	Acet		196	40	8	
6	Troch		1132	97	9	4
7	Troch		538	157	90	
8	Acet		325	62	6	
9	Acet		311	97		70
10	Troch		547	168		47

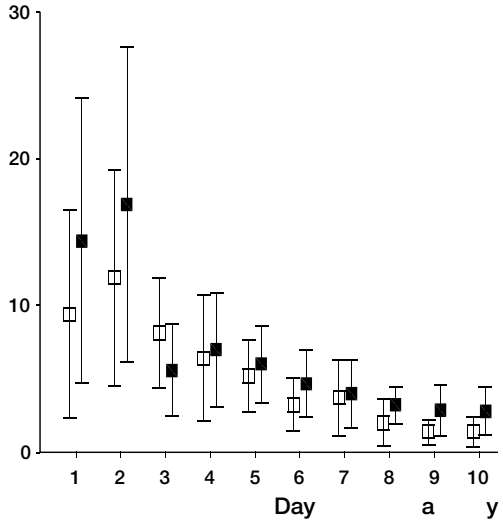

Figure 2. The excretion profile of netilmicin in urine was similar in the two study groups (□ = group I, ■ = group II). Error bars are 95% confidence interval of mean.

Previous peak values (mean) in drainage fluid from the hip joint have ranged from 25 to 118 mg/L of gentamicin (Wahlig and Dingeldein 1980, Wahlig et al. 1984, Salvati et al. 1986, Bunetel et al. 1989, 1990, Lindberg et al. 1991) and 9–43 mg/L of tobramycin (Soto-Hall et al. 1983, Brien et al. 1993, Masri et al. 1998, Della Valle et al. 2001). To our knowledge, the netilmicin peak values in drainage fluid recorded in our study have been the highest recorded to date.

In general, minimum inhibitory concentration (MIC) of netilmicin against staphylococci is low (Wiedemann et al. 1996): MIC90 0.25 mg/L *(S. aureus)* and 0.125 mg/L (coagulase-negative staphylococci). Hence, when impacting cancellous bone impregnated with netilmicin at 100 mg/L, the netilmicin concentration in the joint fluid during the first few hours postoperatively is considerably higher than the MIC of netilmicin against most strains of *S. aureus* and *S. epidermidis*. High local concentration of antibiotics can be advantageous and necessary for the eradication of biomaterial-centered bacterial contamination and high peak values result in enhanced antibacterial efficacy (Gerber 1988). The postantibiotic effect (defined as the period of suppression of bacterial growth after cessation of exposure of the bacteria to an active antibiotic) is longer after a high aminoglycoside concentration (Kapsunik et al. 1988, Gilbert 1991). Finally, in nature, bacteria adhere to surfaces (Costeron et al. 1978, Geesey et al. 1978). MIC testing against bacteria adherent to human tissue surfaces or biomaterials has demonstrated increased bacterial resistance compared to MIC testing in the microbiology laboratory with bacteria in the planktonic phase (Gristina et al. 1989, 1991, Naylor et al. 1990, Webb et al. 1994). This is partly explained by the creation of a biofilm that is impermeable to antibiotics.

50 grams of cancellous bone impregnated with netilmicin (50 mL) at 100 mg/mL has the potential to release approximately 1000 mg of netilmicin in vitro (Witsø et al. 2002). In-vitro studies have reported an almost complete release of netilmicin from cancellous bone impregnated with netilmicin (Witsø et al. 2002). During a revision arthroplasty, approximately 2000–3000 mL of saline is used for washing. In addition, a considerable amount of

bleeding was observed in the interval from completed impaction to skin closure in some patients. Since much of the saline and blood in the operation field was removed using absorbent gauze, we were unable to determine exactly the degree of possible loss of netilmicin before skin closure. However, despite the abovementioned bias, the total amount of netilmicin recovered in urine and wound drainage fluid in group II was 579 (221–1095) mg, i.e., 58 (22–100)% of the theoretical maximum amount of the drug delivered. Furthermore, approximately 75% of the total amount of netilmicin released postoperatively was recovered in wound drainage fluid. We estimated the diuresis to be 1500 mL when calculating the amount of netilmicin recovered in the urine after removal of the bladder catheter. This could expose the study to a bias. However, the amount of netilmicin recovered in urine after removal of the bladder catheter constituted only 10 (1–20)% of the total amount of netilmicin recovered in urine and wound drainage fluid.

We have not studied whether antibiotic impregnation of the graft could have a detrimental effect on osteogenesis. In-vitro studies on local application of aminoglycosides have shown that local levels of tobramycin of < 200 μg/mL have little or no effect on osteoblast replication, while a concentration of 400 μg/mL significantly impairs cell replication and a concentration of 10 000 μg/mL causes cell death (Miclau et al. 1995). In a new study, Buttaro (Buttaro et al. 2003) showed that the healing process occurring when vancomycin-supplemented bone allografts were used for the treatment of tibia defects in pigs did not differ radiographically, histologically or immunohistochemically from that occurring when nonsupplemented allografts were used. In-vivo and clinical studies are needed to evaluate whether aminoglycosides inhibit restoration of the skeleton when using aminoglycoside-impregnated cancellous allografts.

In animal models of infected implants, the prolonged release of antibiotics from bone cement did not protect against late hematogenous infection (Elson et al. 1977, Blomgren and Lindgren 1981). One clinical study has associated the prolonged release of small amounts of antibiotics from bone cement with the emergence of resistant strains of S. epidermidis (Hope et al. 1989). This is in accordance with current knowledge of the emergence of resistant small colony variants of S. aureus after being exposed to aminoglycosides, both in vitro and in vivo (Musher et al. 1977, von Eiff et al. 1997). Consequently, some investigators have warned against the routine use of antibiotic-containing bone cement when performing primary hip arthroplasties (Duncan and Masri 1994, van de Belt 2001). Despite the lack of randomized clinical trials, however, the use of antibiotic-containing bone cement is commonly recommended when infected hip prostheses are revised (Duncan and Masri 1994, Garvin and Hansen 1995, Cordero 1999), and authors have warned against the use of uncemented prosthesis and the use of allograft when reimplanting a prosthesis after infection (Cordero 1999, Walenkamp 2000).

In conclusion, the use of antibiotic-impregnated cancellous bone might be an alternative or a supplement to the use of antibiotic-containing bone cement when performing revision of aseptic and septic loosened hip and knee prostheses.

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