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Does sodium fluoride in bone cement affect implant fixation

Part II: Evaluation of the effect of sodium fluoride additions to acrylic bone cement and the fixation of titanium implants in ovariectomized rabbits

MIKAEL SUNDFELDT[1,2*], JAN PERSSON[3], JANOS SWANPALMER[4], ANN WENNERBERG[1,5], JOHAN KÄRRHOLM[2], CARINA B. JOHANSSON[1], LARS V. CARLSSON[1,2]
[1]*Department of Biomaterials/Handicap Research*
[2]*Department of Orthopedics, Sahlgrenska University Hospital, Institute for Surgical Sciences, University of Gothenburg*
[3]*Department of Surgery of Borås Hospital, Sweden*
[4]*Department of Radiation Physics, Sahlgrenska University Hospital, University of Gothenburg*
[5]*Department of Prosthetic Dentistry/Dental Material Science, University of Gothenburg*

Bone integration of threaded implants made of cured polymethylmethacrylate containing sodium fluoride or commercially pure (c.p.) titanium were studied in normal and estrogen deficient New Zealand white rabbits. Nine had been ovariectomized through laparoscopy and nine served as controls. Four weeks after the ovariectomy two threaded implants made of cured bone cement with or without sodium fluoride addition were inserted in each tibia. One threaded commercially pure titanium implant was inserted in each patello–femoral joint flush to the cartilage. Six weeks after implant insertion measurement of the peak removal torque necessary to loosen the implants and light microscopical histomorphometrical investigations of tissue integration were performed. In the ovariectomized rabbits addition of sodium fluoride to the cement resulted in increased area of bone in the threads ($p = 0.04$), but no corresponding effect could be noted in the controls. The removal torque was lower in the ovariectomized rabbits compared to the non-ovariectomized when comparing implant with sodium fluoride addition ($p = 0.02$). The bone tissue response and the removal torque of the titanium implants were not influenced by ovariectomy in these rabbits.

Introduction

Many women in need of joint replacement surgery are menopausal with reduced endogenous estrogen production and increased risk of osteoporosis. Loss of trabecular bone around the prosthesis may increase the risk of loosening, osteolysis and fracture of the subchondral bone. Addition of sodium fluoride to bone cement may hypothetically speed up the early formation of bone at the interface and thereby improve fixation [1]. The interface might become more stable due to substitution of calcium with fluoride in the hydroxyapatite crystals. This stabilized interface would decrease the risk of early migration and osteolysis. In our previous study of such cement we did not, however, find any experimental support for this hypothesis in a rabbit model [2]. In the present study we hypothesized that addition of sodium fluoride to bone cement might be more advantageous in a situation where the bone is deprived of estrogen. Estrogen deficiency in the adult skeleton will increase the remodeling and resorption rate of bone [3] and may endanger implant fixation. In this study we used ovariectomized rabbits to evaluate whether sodium fluoride added to bone cement could improve short-term fixation and bone formation around implants in a

*Author to whom all correspondence should be addressed: Box 412, SE 405 30 Göteborg, Sweden.

bone bed with altered turn-over. In addition the fixation of commercially pure titanium implants was also studied in the femur of the same rabbit model.

Methods

Bone cement

Polymethylmethacrylate (PMMA) implants were made from commercially available bone cement (Cemex®, Tecres S.p.A., Italy) with a powder to liquid ratio of 3 : 1, and 13 wt % of the powder being barium sulfate as a radiopacifier. The Cemex® bone cement was mixed at room temperature and cast as round rods. In the fluoride cement a proportion of the barium sulfate was substituted with 6% sodium fluoride by weight.

Tibial implants

Rods of cured bone cement (Cemex®) with and without sodium fluoride were made of vacuum mixed cement (Optivac®, Scandi Med Implant AB, Sjöbo, Sweden). Screw shaped implants of an outer diameter of 3.7 mm and a total length of 8 mm were manually turned from these rods. Details of the manufacturing, sterilization and quality control have been described previously [2].

Femoral implants

Screw shaped implants turned from rods of c.p. titanium (c.p. Ti, grade 1) were used. Their outer diameter and length were 3.7 and 4.1 mm. Slotted heads enabled later biomechanical tests (removal torque). The implants were degreased in trichlorethylene, rinsed in absolute ethanol in an ultrasonic bath and finally sterilized in an autoclave prior to insertion in the knee joint. The implants inserted on the right side were submitted to removal torque testing (RTQ) and from the RTQ values a mean interfacial shear strength was calculated as previously described [2]. Implants on the left were processed for histological evaluation as undecalcified cut and ground sections.

Animals, anesthesia and surgical technique

Eighteen mature female New Zealand white rabbits were included in the study, under a protocol approved by the Local Animal Ethic Committee at the University of Göteborg. For surgery the animals were anesthetized according to our previously presented protocol [2]. Nine animals underwent a laparoscopic ovariectomy four weeks prior to implant insertion (test group). The remaining animals (controls) did not undergo sham operation since the surgical trauma due to laparoscopic surgery was regarded to be minimal. Prior to ovariectomy, baseline measurements of the bone mineral levels were performed in the test group using triple-energy X-ray absorptiometry (TXA) [4, 5]. At the time of killing additional bone mineral measurements were conducted in the test group. The ovariectomy was done using a 1.7 mm laparoscope (Stortz®, Karl Stortz, Tuttlingen, Germany). Prior to surgery the abdomen was shaved and carefully washed with a mixture of iodine and 70% ethanol. A canulla was inserted through the abdominal layers penetrating the peritoneum, the abdomen was insufflated with carbon dioxide (CO_2) and the laparoscope was inserted through an introducer placed for the purpose. Two further instrument paths were placed; one small path for scissors and small intruments and one 5 mm Ethicon Endopath® (Ethicon Endo-Surgery, Johnson and Johnson, Cincinnati, USA). The ovaries were identified and the circulation was terminated by applying an Ethicon Ligaclip® 20 m/l (Ethicon Endo-Surgery, Johnson and Johnson, Cincinnati, USA) around the stalk of the ovary. The ovary was then removed by laparoscopic scissors and withdrawn through the Ethicon Endopath®. The procedure was then repeated on the contralateral side. The small wounds created by the instrument paths were closed by non resorbable sutures. Post-operatively, the animals were allowed unrestricted movement.

Four weeks later the implants were inserted. The shaved skin of the hindlegs was carefully washed with a mixture of iodine and 70% ethanol. Local anesthesia with 1.0 ml of 5% lidocain (Xylocaine®, Astra, Södertälje, Sweden) was injected, in the region of the tibial tuberosity and at the medial part of the patello–femoral joint, where the incisions were planned. The skin and fascial layers were opened and closed separately. During all surgical sequences we followed our previously presented protocol [2]. All implants were inserted by the same surgeon.

Tibial implant insertion

The periosteum was gently elevated from a small area on the proximal-medial tibia. A hole was drilled through the medial cortex by a small burr followed by drills of increasing diameter and was finally threaded to fit the two cured bone cement implants. Right and left sides were operated in the same manner. Implants with addition of sodium fluoride were used on the right side and the left side served as control.

Femoral implant insertion

An incision in each knee joint area was made through a medial approach. The patella was dislocated during the preparation of femur. The center of the patello–femoral articulation was identified and a small hole was drilled, enlarged with successively larger drills and was threaded to fit the c.p. titanium implant. The screws were inserted flush to the cartilage.

The animals were kept in separate cages after the last operation; allowed unrestricted weight bearing immediately post-operatively. They received analgesics; 0.3 ml buprenorphin subcutaneously (Temgesic® 0.3 mg/ml, Schering-Plough, Stockholm, Sweden) post-operatively and twice a day the following three days. Six weeks after implant insertion the animals were killed by intravenous injection of pentobarbital and ethanol (Pentobarbital® 100 mg/ml, Apoteksbolaget, Uppsala, Sweden).

Mechanical testing and histomorphometrical analysis

Immediately after death removal torques of the right side femoral implants and the distal tibial implants were

TABLE I Tibial implants made of polymethylmethacrylate with (test) and whithout (control) sodium fluoride addition in non ovariectomized and ovariectomized rabbits. Median and range of removal torque, bone to implant contact, area of bone in threads and area of bone in mirror images

Tibial implant	Test (NaF)	Control	p-value[1]
Non ovariectomized rabbits			
Fixation			
Removal torque (Ncm)	10 (5–16)	7 (1–17)	0.19
Bone formation			
Bone to implant contact (%)	14 (7–19)	5 (0–27)	0.11
Area of bone in threads (%)	66 (43–74)	66 (48–89)	0.89
Area of bone in mirror images (%)	80 (63–89)	77 (55–93)	0.51
Ovariectomized rabbits			
Fixation			
Removal torque (Ncm)	6 (4–9)	5 (2–11)	0.57
Bone formation			
Bone to implant contact (%)	8 (4–49)	3 (0–13)	0.091
Area of bone in threads (%)	72 (64–80)	64 (41–73)	0.036
Area of bone in mirror images (%)	74 (53–90)	60 (11–80)	0.093

[1]Wilcoxon signed rank test.

measured with the RTQ device [6]. Thereafter, all implants and their surrounding tissue were removed en bloc and immersed in 4% neutral buffered formaldehyde (pH 7.0). The histological preparation was identical to that presented in our previous study [2]. Histomorphometrical investigations of the ground sections involved quantification of the entire bone to implant contact, the bone area in all threads around each implant and the ''mirror image area'' of the tibial implants threads, i.e. the inner thread area is ''mirrored'' out to the surrounding bone tissue immediately outside the inner thread. The latter investigation aimed to observe whether bone cement containing sodium fluoride affected the surrounding bone. Shear strengths of bone against the femoral implants were calculated in the same manner as previously reported [2].

Bone mineral measurements

The amount of bone mineral in the rabbit tibiae was determined using TXA [4, 5]. The lower part of the tibial tuberosity, well out of the implant region of the ovariectomized rabbits, was chosen as the measurement site. The radiation beam was directed antero-posteriorly through the bone. Bone mineral determination was performed before removal of the ovaries and post mortem. The values obtained being expressed in g/cm^2. Ash weight of an 8 mm long sagittal midshaft section of the left radius and ulna was also determined; the specimen being ashed in air in a platinum crucible at 1000 °C for 24 h.

Statistics

Wilcoxon signed rank tests were used for comparisons between test and control implants in the same animal (Table I). The Mann Whitney U-test was used for comparisons between ovariectomized and non ovariectomized animals (Tables II and III).

Results

One animal was excluded from the test group since one ovary was found *in situ* at post mortem exploration.

Quantitative investigation

Tibial implants

Analysis of screws with a Link ISIS energy dispersive X-ray system verified sodium fluoride at the test implant surface but not in the control as previously reported [2].

The torque required to loosen the screws made of cement with sodium fluoride was lower in the ovariectomized group compared to the non-ovariectomized group (Table III). In the ovariectomized group a larger bone area around cement implants containing fluoride was observed ($p = 0.04$). In the control group there was no difference between the two sides (Table I).

Femoral implants

The removal torque and shear strength of the femoral screws were not significantly influenced by estrogen deprivation (Table II). The relative amount of bone tissue surrounding the femoral implants (bone area) and the

TABLE II C.p. titanium implants inserted in femur in ovariectomized (O) and non-ovariectomized (NO) rabbits. Median and range of removal torque, shear strength, bone to implant contact and area of bone in threads

Femoral implant	O	NO	p-value[1]
Removal torque (Ncm)	13 (8–19)	13 (6–24)	0.89
Shear strength data (N/mm^2)	3.1 (1.0–7.2)	2.3 (1.1–7.1)	0.39
Bone to implant contact (%)	20 (9–35)	22 (11–51)	0.38
Area of bone in threads (%)	57 (43–72)	49 (26–71)	0.41

[1]Mann Whitney U-test.

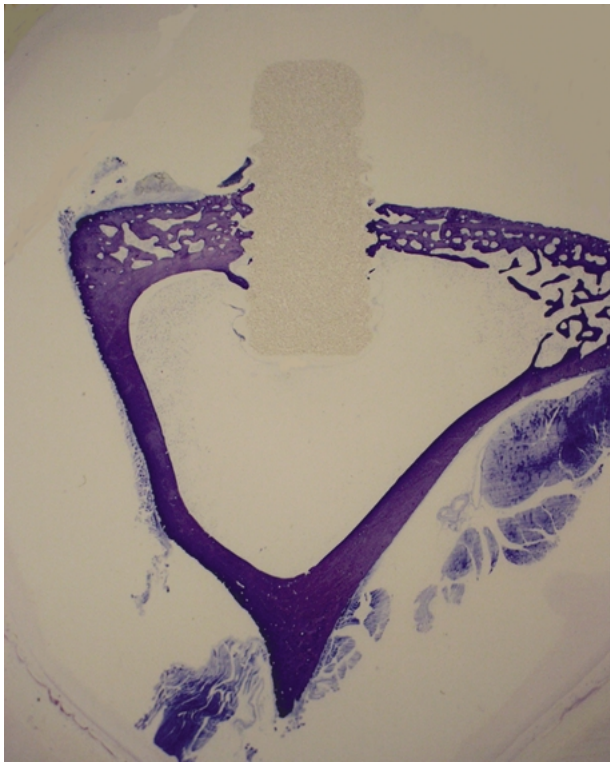

Figure 1 Undecalcified ground section (about 10 μm) of a screw shaped implant with an outer diameter of 3.7 mm made of Cemex® cement in a rabbit tibia. Toluidin blue staining.

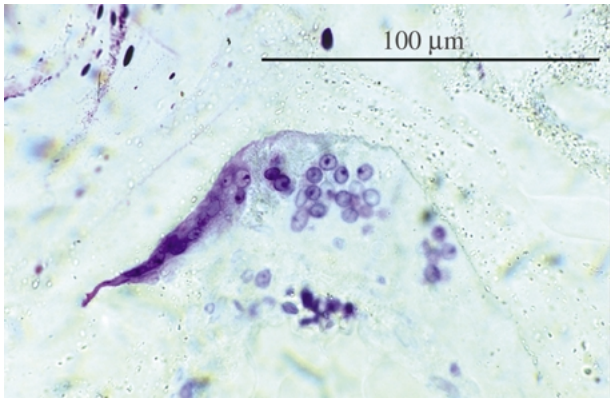

Figure 2 Undecalcified ground section (about 10 μm) demonstrating a multinucleated giant cell observed in a thread of a screw shaped implant made of Cemex® cement in a rabbit tibia. Toluidin blue staining. Bar 100 μm.

bone to metal contact was similar in the ovariectomized and control group (Table II).

The bone mineral density in the tibia ($p = 0.26$) and the content of bone mineral in the midshaft sections of the ulna and radius ($p = 0.74$) were not influenced by ovariectomy.

Qualitative investigation

Tibial implants (Fig. 1)

No major qualitative differences could be observed between ovariectomized and non-ovariectomized animals. The following description is therefore valid for both groups. The sodium fluoride (test) implants had more newly formed periosteal bone immediately adjacent to the implant surface than the controls, but

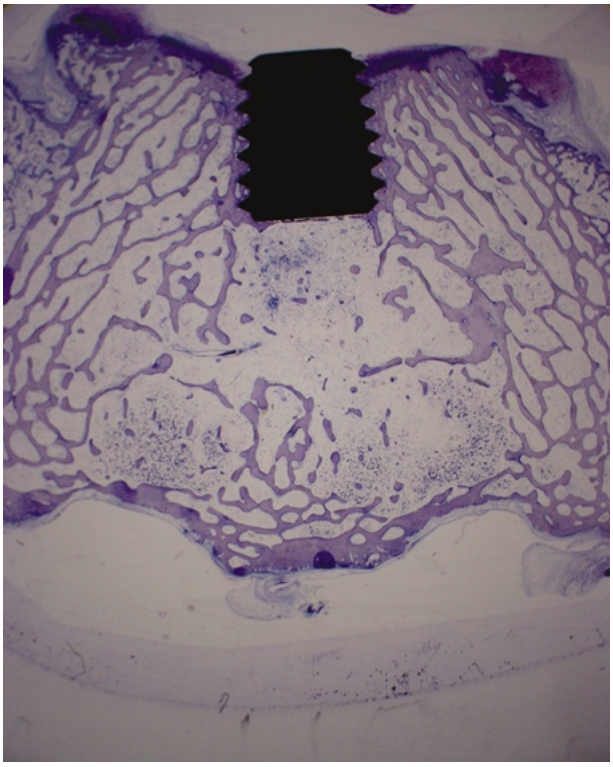

Figure 3 Undecalcified ground section (about 10 μm) of a screw shaped implant with an outer diameter of 3.7 mm made of commercially pure titanium inserted in a rabbit knee joint. Toluidin blue staining.

endosteal bone formation was sparse around both test and control implants. In general there was a thick, cellular rich connective tissue capsule covering almost all of the implant surface area in the bone marrow region. This connective tissue was denser beside the test implants and looser around the controls. Mostly macrophages and lymphocytes were observed in the capsule and multinucleated giant cells in the interface. The cellular composition did not differ between test and control. Fewer signs of remodeling were observed around the test implants than in the controls. There was more resorption on the bone surface in close proximity to the implants than on the bone at some distance away from the implants. Areas of less mineralized bone structure could be observed in the interface region. There was more foreign body reaction than an inflammatory reaction. Irrespective of test or control implants large numbers of multinucleated giant cells were observed in the threads with no bony contact (Fig. 2).

Femoral implants

There was an area of resorption observed close to the cartilage in both ovariectomized and nonovariectomized animals. The implants were surrounded by bone in both groups, and there were no obvious differences (Fig. 3).

Discussion

Estrogen affects both osteoclasts and osteoblasts. Thereby it has an important role in the metabolism of bone not least by its role in post menopausal osteoporosis [3, 7]. Mori *et al.* [8] showed that in estrogen deficient animals the time to achieve fixation of the implant is

TABLE III Ovariectomy vs. non-ovariectomy tibial implants. Median and range of removal torque, bone to implant contact, area of bone in threads and area of bone in mirror images of the polymethylmethacrylate tibial implants with sodium fluoride (test) or without sodium fluoride (control), ovariectomized (O) compared to non ovariectomized (NO) rabbits

Tibial implant	O	NO	*p*-value[1]
Fixation			
Removal torque (Ncm) control	5 (2–11)	7 (1–17)	0.66
Bone formation			
Bone to implant contact (%) control	3 (0–13)	5 (0–27)	0.38
Area of bone in threads (%) control	64 (41–73)	66 (48–89)	0.60
Area of bone in mirror images (%) control	60 (11–80)	77 (55–93)	0.083
Fixation			
Removal torque (Ncm) test	6 (4–9)	10 (5–16)	0.016
Bone formation			
Bone to implant contact (%) test	8 (4–49)	14 (7–19)	0.33
Area of bone in threads (%) test	72 (64–80)	66 (43–74)	0.092
Area of bone in mirror images (%) test	74 (53–90)	80 (63–89)	0.36

[1]Mann Whitney U-test.

prolonged. Ovariectomy and low-calcium diet in rabbits reduced the bone mineral content, and the time for new bone formation to occur around titanium implants in the tibia was increased. Another group found reduced bone-implant contact for c.p. titanium implants in the tibiae of ovariectomized rats [9]. Sodium fluoride is a well documented osteoblast recruiter but the bone becomes more brittle. Oral treatment with sodium fluoride has not demonstrated any advantages over placebo concerning vertebral fracture rate [10–12]. However, the use of sodium fluoride as a ''kick starter'' of bone formation to speed up implant stabilization after joint replacement may enhance implant incorporation. The formation of fluoro-apatite in the vicinity of the implant may also reduce osteoclastic activity during the ''healing-in'' period of the implant. Thus, it interferes in the early post operative bone remodeling phase, which may be critical for implant stability. Reduction of osteoclastic activity might be an important factor in this process as indicated by Hilding *et al*. [13]. They found that peroral treatment with clodronate could improve early fixation of the tibial component of total knee arthroplasties. In our study the addition of fluoride had no demonstrable effect on fixation, but increased the area of bone around implants in animals deprived of estrogen. If this finding is an effect of increased osteoblastic activity or reduced osteoclastic activity or both remains unknown. In an earlier study [2] we showed that the cured and machined bone cement implants with addition of sodium fluoride (test) had a smoother surface compared to control. This might have decreased the test RTQ data recorded when screws made of fluoride containing cement were studied. Surface texture has been shown to be as important as the biomaterial for bone apposition [14, 15]. The results from our previous study [2] with sodium fluoride indicated that the negative effect of a smooth surface may be compensated for by addition of sodium fluoride. However, bone cement in the clinical situation is administrated non-cured into trabecular bone in a marrow cavity and the surface texture is dependent on the cast of the cement into the trabecular system. Thus, our results do not exclude the possibility that sodium fluoride addition to bone cement may improve fixation in the clinical situation and especially in post-menopausal women.

The laparoscopic approach is probably less harmful to the animals than the open procedure and may therefore introduce less confounding factors in the model. No significant differences were found between the femoral implants. The TXA and ash weights also demonstrated no significant differences between test and control, indicating that the animals were estrogen deprived but not osteoporotic. The 10 week period between ovariectomy and killing may have been too short to develop osteoporosis. Mori *et al*. [8] used a rabbit model in which ovariectomy was combined with low calcium diet and titanium implants inserted in the tibia. Despite prolonged healing time in osteoporotic bone, intimate contact between bone tissue and the implant did develop. Martin *et al*. [16] inserted two porous titanium alloy press-fit implants in the humeri of ovariectomized Beagle dogs. They showed that the push-out strength was reduced in ovariectomized animals. The amount of fibrous tissue ingrowth was increased, but the mean bone volume was not changed in the ovariectomized group.

Johansson *et al*. [17] investigated fluoride coated c.p. titanium implants in rabbit tibia. After three months they found higher removal torques and improved bone to implant contact when this coating had been used. Jiang *et al*. [18] compared oral administration of sodium fluoride or intramuscular administration of estrogen to ovariectomized rats. Bone strength and density were examined. They found that fluoride increased bone mass but had decreased bone strength compared to estrogen. According to our study local release of sodium fluoride from bone cement in an unloaded implant has a positive effect on bone formation during estrogen deprived circumstances in a rabbit model. Local release of sodium fluoride from bone cement affects only the bone bed close to the implant; avoiding the negative effects attributed to oral treatment [10, 12].

Conclusions

The release of sodium fluoride from bone cement is non linear, with the highest concentration achieved in the first 24 h and thereafter rapidly reduced [1]. This local release may improve the important early fixation of a joint arthroplasty and reduce the risk of revisions due to

aseptic loosening. The benefits may be even more obvious in estrogen deficient bone. However, additional clinical studies in conjunction with joint replacement components are needed to evaluate whether sodium fluoride has a positive effect on implant stability in estrogen deficient women.

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