Incorporation of raloxifene-impregnated allograft around orthopedic titanium implants impairs early fixation but improves new bone formation

A 4-week study in 12 dogs

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Background — The anti-osteoporotic drug raloxifene reduces the risk of vertebral fractures by increasing bone mass density. We investigated whether raloxifene offers any benefits in augmenting early fixation of orthopedic implants in the setting of impaction bone grafting.

Methods — 24 non-weight-bearing grafted gap implants were inserted bilaterally into the tibia of 12 dogs. The 2.5-mm peri-implant gap was filled with either raloxifene-impregnated or untreated bone allograft. Implants were harvested after 28 days. Implant fixation was assessed by mechanical testing and histomorphometric evaluation.

Results — Raloxifene-treated allograft reduced early implant fixation compared to untreated allograft, as measured by inferior maximum shear strength (p < 0.001) and apparent shear stiffness (p = 0.001). We found that the raloxifene group had more newly formed bone in the gap around the implant (p = 0.02), but also less allograft (p = 0.03).

Interpretation — The accelerated allograft resorption in the raloxifene group explained the impaired early fixation, despite its stimulation of new bone formation. Our results with local and possible high-dose treatment are not consistent with current theory regarding the mechanism of how systemic raloxifene administration counteracts the decrease in BMD in postmenopausal women. Instead of being solely anti-resorptive as generally held, our results indicate a possible anabolic side of raloxifene.

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Under physiological conditions, there is a close regulation and coupling of bone formation and resorption. The receptor activator of nuclear factor kappa-β ligand (RANKL)-to-osteoprotegerin (OPG) ratio is a critical parameter in this regulation (Crotti et al. 2004, Teitelbaum 2007), and the ratio is essential for the differentiation, recruitment, activation, and survival of the osteoclasts (Bashir et al. 2005, Leibbrandt and Penninger 2009).

Raloxifene is a second-generation selective estrogen receptor modulator (SERM) that is currently registered as a treatment against osteoporosis. SERMs are non-steroidal molecules capable of binding to estrogen receptors (ERs) and they act as estrogen agonists and/or antagonists depending on the tissue in question. Raloxifene has an analogous effect to that of estrogen on bone, and has been shown to increase bone mass density (BMD) and maintain bone strength (Evans et al. 1994, Turner et al. 1994, Yan et al 2010). Although the mechanism of action of raloxifene is not fully understood, recent in vitro and in vivo studies have shown an effect on osteoblasts via ERs, followed by lower levels of RANKL and higher expression of OPG. This interaction inhibits normal osteoclastic functioning.

The effect of raloxifene on the RANKL/RANK/OPG pathway has raised the question of whether treatment with raloxifene can enhance the fixation of orthopedic implants in the setting of impaction bone grafting. Hip revision treatment strategies currently favor non-cemented prostheses (Overgaard 2010), allowing impaction bone grafting to restore the patient's own bone stock to a condition closer to what was present during the primary arthroplasty (Toms et al. 2004).
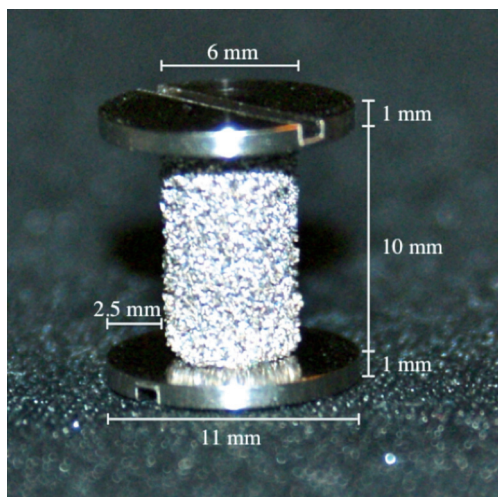
Figure 1. Titanium implant with measurements.

In the present study, we investigated whether raloxifene can alter the balance of bone formation and resorption in favor of less resorption, thereby preserving bone graft as a mechanical stabilizer of the implant and keeping it available as a scaffold for new bone. Our hypothesis was that raloxifene-impregnated morselized cancellous allograft bone would retain more bone and increase early implant fixation compared to normal, untreated morselized cancellous allograft, as evaluated by mechanical and histomorphometric analysis.

Materials and methods

Study design

The investigation was conducted as a paired study in 12 male dogs of the breed American hound. The dogs were bred for scientific purposes and the local Institutional Animal Care and Use Committee (IACUC), Minneapolis Medical Research Foundation (MMRF), Minneapolis, MN, approved the protocol. At the time of surgery, the animals had a mean age of 12 (11–15) months and weighed 32 (30–36) kg.

The implants were inserted in the proximal metaphyseal ends of each tibia. We used unloaded, cylindrical titanium-alloy implants (Ti-6A1-4V) coated with a porous surface (Gription Porous Coating; DePuy Inc), with a nominal diameter of 6.00 mm and a length of 10 mm (Figure 1) (Soballe 1993). We studied 2 groups: (1) titanium implant + untreated allograft; and (2) titanium implant + raloxifene-impregnated allograft.

Each animal received both a control implant and an intervention implant. To avoid contamination with raloxifene, the implant from the control group was always inserted as the first implant. For that reason, the surgeries were not performed blind but all preparations following euthanasia and mechanical and histological evaluation were carried out blind. 2 additional studies were carried out on the same animals, using the humerus and femur bones for investigation of other local treatment strategies and interventions with the aim of enhancing implant fixation. The humerus study examined the outcome of local endotoxins present on the implant surface, while nano-hydroxyapatite was added to bone allograft in the femur study. No systemic treatments were tested on the animals, so the 3 separate studies were not expected to interfere with each other.

Preparation of allograft

The raloxifene was purchased as raloxifene hydrochloride (R1402-1G; Sigma-Aldrich), a solid off-white powder that is almost insoluble in water. The raloxifene powder was weighed individually in doses of 10.0 mg and kept in small PCR tubes. Before application, the raloxifene was heat-sterilised at 121°C, and we confirmed its stability by nuclear magnetic resonance (NMR).

The allograft was prepared from the metaphyseal part of humerus and femur harvested from 2 dogs that were not included in the study. The articular cartilage and remaining soft tissues were removed from the metaphyseal parts of the long bones. We morselized these bone segments with a standard bone mill (Biomet, Warsaw, IN), resulting in 1- to 3-mm bone chips. The bone allograft chips were kept unwashed, to preserve the fat content in order to optimize the mixing with raloxifene. At the time of allograft harvest, 1.1-g aliquots of allograft were placed individually in small sterile plastic tubes. Although the gap volume is only approximately 0.7 cm^3, we prepared an amount of bone graft equivalent to a volume of 1 cm^3 (1 mL), corresponding to 1.1 g. This was due to the irregularities in the trabecular bone architecture neighboring the gap, which allows more allograft bone to be impacted than can be assumed from the precise gap volume.

Raloxifene was added to the allograft under sterile conditions. We prepared the allograft for the control side first to ensure that no raloxifene would contaminate the control graft. Mixing of raloxifene with allograft was done 1 portion at a time. Each 1.1-g aliquot of morselized allograft was spread on a clean plain surface covering an area of roughly 2×2 cm. The raloxifene (10.0 mg) was evenly sprinkled over each portion of allograft and mixed with separate instruments. The allograft-raloxifene mixture was placed in sterile tubes and frozen at –20°C for no longer than 5 days.

Surgery

We performed all surgical procedures under general anesthesia and under sterile conditions. The tibial insertion site was exposed through a 4-cm incision on the proximal anteromedial side of the tibia. The periosteum was elevated and a 2-mm guide wire was inserted in the bone 1.6 cm distal to the tibiofemoral joint, perpendicular to the bone surface. Using a cannulated drill (diameter 11 mm), we created a 12-mm deep hole in the metaphyseal bone. Before implant insertion, the drill hole was irrigated with 10 mL of 0.9% saline. We inserted

the cylindrical porous titanium implant with attached 11-mm bottom-washer into the hole, using a specially designed hollow instrument that fitted in the drill hole precisely. The bottom-washer ensured uniform central placement of the implants, creating a circumferential gap of 2.5 mm from the porous implant surface to host bone. Thawed, untreated control allograft was first packed in the control gap in 3 portions, each portion undergoing vigorous impaction with the impaction tool in order to ensure homogeneous distribution of the graft. Finally, after filling the gap, we mounted an 11-mm top-washer on the implant in order to close the allograft-filled cavity. After ensuring hemostasis, the soft tissue was closed in layers and finally local anesthesia was administered at the incision sites (1 mL Marcaine and 1 mL 0.9% saline per implant site). The same procedure using raloxifene-treated allograft was performed on the contralateral tibia. We rotated randomly between the right and left tibia, but the control implant was always inserted first in order to eliminate the risk of raloxifene contamination in the control group.

Following recovery from anesthetic, analgesics and antibiotics were administered for a minimum of 3 days. All animals were fully weight bearing within 2 days of surgery, and had been started on a daily exercise program. After an observation period of 28 days, the animals were sedated with Acepromazine (0.5 mg/kg) and—while under general anesthesia with Proporfol (4 mg/kg)—they were killed with an intravenous injection of 10 mL Beuthanasia-D Special (Shering-Plough Animal Health Corp., Union, NJ). The tibial bones were immediately harvested and stored at –20°C.

2 additional time-zero implants were inserted into 2 proximal tibias harvested from the animals that were used as bone allograft donors. Implants were inserted by the same surgeon to enable evaluation of the change in allograft volumes from the time of impaction to the end of the observation period. These 2 implants underwent the same preparation and analysis as all the other implants.

Specimen preparation

We divided bone-implant blocks by transverse sections in 3 parts using a water-cooled circular diamond-saw (Accutom-50; Struers, Ballerup, Denmark). The outermost 2 mm, including the top-washer, was cut and discarded. The next 3.5 mm was stored at –20°C pending a mechanical push-out test. The remaining innermost 5–5.5 mm was fixed in 70% alcohol, dehydrated in graded alcohol (96%–100%), and embedded in methylmethacrylate (Merck). After alignment with the long axis of the implant, we rotated the embedded blocks around the long axis of the implant in order to achieve vertical uniform random (VUR) sections. Four serial histological sections from each bone-implant block, 50–60 µm in thickness, were made using a glycerol-cooled, diamond-blade microtome (MeProTech, Leiden, the Netherlands). We surface-stained the sections with 0.1% toluidine blue (pH 7) (Sigma-Aldrich).

Mechanical testing

Mechanical push-out testing was performed on an MTS Mini Bionix testing machine (model 858; MTS, Eden Prairie, MS). We placed the specimens on a metal support jig, centralizing them over a 7-mm opening. A cylindrical metal test probe (5 mm in diameter) was located centrally above the implant. Specimens were positioned with their superficial/cortical side upwards. We defined contact position as a preload of 2N by the metal test probe. Displacement rate during the test was set to 5 mm/min. From the load-displacement curve and with posttest measure of section thickness and implant diameter, we derived 3 parameters with the purpose of quantifying mechanical implant fixation. These parameters were maximum shear strength (in MPa), apparent shear stiffness (in MPa/mm) and total energy absorption (in J/m^2). The parameters were defined as the peak (strength), the maximum slope (stiffness), and the area under the displacement curve (energy absorption) before implant failure, respectively.

Histomorphometry

Histomorphometric evaluation was performed using the stereological software newCAST (version 3.4.1.0; Visiopharm, Hoersholm, Denmark) in combination with a light microscope (Olympus BX51), and we performed quantitative analysis of surface and volume fractions of new woven bone, lamellar allograft bone, fibrous tissue, and marrow space. New bone was recognized as being dark purple, less organized in its structure, with visible osteocytes. The bone allograft was brighter purple, had empty lacunae, and showed clear evidence of a previously remodeled lamellar organization (Figure 2). Fibrous tissue was seen as dense, organized areas of fibrillated soft tissue containing spindle-shaped nuclei mainly near the implant surface. The surface area covered by any of the 4 tissue types was assessed using a line-interception technique (Baddeley et al. 1986), while volume fractions were evaluated by point-counting technique (Gundersen et al. 1988). These techniques provide highly reliable results with negligible bias (Baas 2008). We defined the region of interest within the grafted gap as the innermost 2000 µm, beginning from implant surface. After counting all samples, a re-count was conducted on 4 randomly chosen specimens to determine intra-observer variability.

Statistics

Statistical analysis was performed using STATA version 11.2. All mechanical push-out data were normally distributed and were analyzed parametrically by paired t-test (2-tailed). The histomorphometric data were also normally distributed and were analyzed parametrically, except for the surface and volume fractions of fibrous tissue. This was primarily because of many zero values and few high values. Thus, we analyzed the surface and volume fractions of fibrous tissue non-parametrically by Wilcoxon signed rank test. Any p-values less than 0.05 were considered statistically significant.
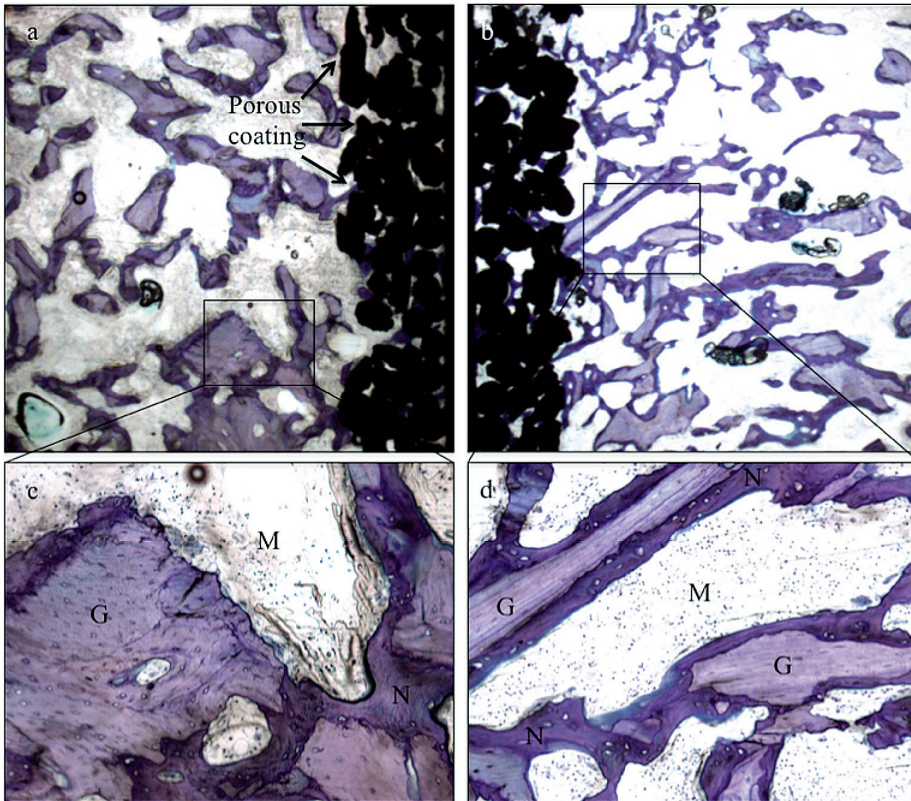
Figure 2. Representative specimens from the histomorphometric evaluation of the control (panels a and c) and the raloxifene group (panels b and d). Magnification was ×1.25 (panels a and b) and ×10 (panels c and d). N: new bone; G: allograft bone; M: marrow space.

testing and for histomorphometry—was also accomplished without any complications. All implants were available for analysis.

Mechanical push-out test revealed inferior mechanical fixation in the raloxifene group relative to the untreated allograft control group (Figure 3), with reduced maximum shear strength (p < 0.001) and reduced apparent shear stiffness (p = 0.001). The same trend was seen for energy absorption, but it was not statistically significant.

Histomorphometric analysis showed that ongrowth of new bone was similar in both groups. Mean values (SD) of new bone covering the implant surface were 9.0% (0.04) in the control group and 9.0% (0.05) in the raloxifene-treated group. Fibrous tissue was present in less than half of the specimens—only to a very limited extent, and evenly distributed between the 2 groups. The raloxifene-treated group showed more new bone formation (p = 0.02) and better allograft resorption (p = 0.03) than the control group (Table). Time-zero values of allograft volume were 45% of total volume in the grafted gaps, which is comparable to previous studies using the same model.

Adding the volume fractions of new and allograft bone together showed similar total amounts of bone tissue in the 2 groups (data not shown).

Results

All animals recovered within 2 days postoperatively and no complications occurred during the period of observation. The preparation of specimens after killing—both for mechanical
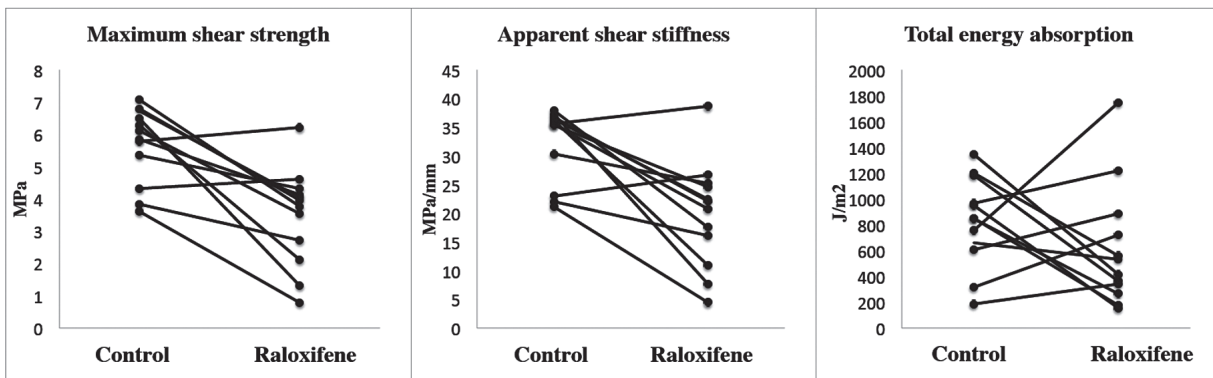

Figure 3. Diagrams showing the results of each parameter from the mechanical push-out test. The control and intervention implants in each animal are connected by a line to illustrate the differences seen in each dog. Mean values (SD) for maximum shear strength were 5.7 (1.2) MPa for the control group and 3.5 (1.5) MPa for the raloxifene-treated group (p < 0.001). Apparent shear stiffness was 32 (6.5) MPa/mm for the control group and 20 (9.3) MPa/mm for the raloxifene-treated group (p = 0.001). Total energy absorption was 823 (346) J/m^2 for the control group and 616 (471) J/m^2 for the raloxifene-treated group (p = 0.3).

Measurements of the volume fractions of new bone, allograft bone, and fibrous tissue within the innermost 2000 μm of the grafted gap

	Total volume fractions (%)		
	Controls	Raloxifene	p-value
New bone [a]	15 (0.02)	18 (0.04)	0.02
Allograft [a]	16 (0.04)	13 (0.06)	0.03
Fibrous tissue [b]	1 (0.02)	1 (0.04)	0.8
Marrow space [a]	67 (0.05)	67 (0.06)	0.9

[a] Mean (SD)
[b] Medium (interquartile range)

Intra-observer variation for the parameters assessed was between 0.1% and 2.8%, with lowest variation in the new bone and allograft parameters and largest variation in the fibrous tissue parameter, due to the small prevalence.

Discussion

Stable uncemented titanium implants were used in a well-established unloaded grafted gap model shown to be useful for investigating different interventions in the setting of impaction bone grafting (Søballe 1993). A limitation of this model is that implants are not exposed to direct weight-bearing conditions or joint fluid, but this is also an advantage since it creates a more controlled environment with less variance. Since this was a paired study with animals functioning as their own controls, the biological variance between the 2 groups was reduced.

We had been expecting that raloxifene would preserve the allograft while not impairing new bone formation. However, we found the opposite. The use of raloxifene-treated allograft had a stimulating effect on bone formation and bone turnover in this setting. The inferior fixation was not due to less bone in the raloxifene group, since the total bone volume (new bone + allograft bone) within the grafted gaps was similar. It is possible that the higher raloxifene fraction of newly formed, woven bone tissue may be less mature and therefore less mechanically competent than the untreated allograft. A longer evaluation period would be required to evaluate whether increased maturity of new bone can raise mechanical interface integrity.

Equal amounts of graft were inserted around the control and intervention implants, due to the preparation methods. In addition, being a finely divided powder, raloxifene became distributed in the cavities between the allograft bone chips and did not increase the graft volume in the intervention group. Washing of morselized graft material before application is a common procedure thought to remove several immunological factors that could harm the interaction between donor and host bone. On the other hand, it may also eliminate possible growth factors from the graft that might act advantageously. It has been shown that washing of autograft prior to application negatively affects the fixation of implants, while the opposite appears to apply to allograft—although the results are not fully consistent (van der Donk et al. 2003, Toms et al. 2004, McNamara 2010, Barckman et al. 2013). Despite this, we kept the allograft bone unwashed, to preserve the fat content in order to optimize the mixing with raloxifene. Fresh or unwashed bone graft is not commonly used, and the amount of fat available in freeze-dried and synthetic bone graft probably differs from the former and could cause different results the graft types among.

In its quantitative assessment of the impact of local raloxifene on bone formation and resorption, the present study is the first of its kind. Early reports dealing with raloxifene stated that there was maintenance of bone mineral density (BMD) and strength (Evans et al. 1994, Turner et al. 1994). The majority of in vitro studies have shown that raloxifene inhibits both osteoclast development and activity (Taranta et al. 2002). It increases both mRNA and protein levels of OPG (Viereck et al. 2003, Michael et al. 2007), whereas it reduces RANKL mRNA transcription (Cheung et al. 2003, Sliwinski et al. 2009) lowering the RANKL/OPG ratio and thereby inhibiting receptor-mediated activation of the osteoclasts. These results suggest that raloxifene increases BMD by an anti-catabolic mechanism. The major concern with these in vitro studies is that they do not include physiological feedback and interactions as in the living organism. Moreover, differences in ER expression in cultured osteoblasts and osteoblasts residing in living organisms make the results difficult to extrapolate to the living organism. However, newer in vivo studies in rats (Yan et al. 2010, Luvizuto et al. 2011) and in postmenopausal women (Messalli et al. 2007, Fernandez-Garcia et al. 2008) have confirmed that there is a similar anti-catabolic mechanism of action. A few studies have focused on the effect of raloxifene treatment on osteoblast functioning, indicating a probable positive effect of raloxifene on osteoblasts (Taranta et al. 2002, Viereck et al. 2003).

While being conducted in a living organism, our results contradict the majority of earlier findings both in vitro and in vivo that raloxifene inhibits bone resorption, at least for allogenic bone grafts. We found that raloxifene stimulates new bone formation and accelerates resorption, i.e. bone turnover. The same condition has been shown from the use of bone morphogenic proteins (BMPs) in allograft (Jensen et al. 2002, McGee et al. 2004, Baas et al. 2008). The reason for the accelerated resorption when using anabolic therapy appears to be caused by the close interaction between formation and resorption.

Raloxifene is thought to imitate the actions of estrogen on bone, since they both occupy the same cellular receptors. In physiological concentrations, estrogen inhibits osteoclast development and activity (Compston 2001). However, studies using high-dose estradiol implant therapy have provided direct histological evidence that high-dose estrogen has anabolic skeletal effects by stimulating osteoblast activity (Wahab et al. 1997, Khastgir et al. 2001). Despite the estrogen being

administered systemically in these trials, it is not unlikely that these findings would explain our results. So far, no similar studies have been conducted in which raloxifene is locally administered.

When treating osteoporosis in humans, an oral dose of 60 mg per day is considered standard treatment. In previously completed in vivo studies using rodents, orally administered doses ranging from 1 mg/kg/day (Luvizuto et al. 2011) to 3 mg/kg/day (Turner et al. 1994) for a period varying from 1 to 12 months have been used. Considering the different sizes of the animals (rodents vs. dogs) and the different rates of metabolism, we used a dose of 10 mg raloxifene distributed in 1.1 g of allograft bone. It is possible that we may have arrived at a dose of raloxifene at which it acts similarly to high doses of estrogen, thereby explaining the anabolic effects of the local treatment of 10 mg raloxifene. Taranta et al. (2002) showed increased osteoblast proliferation when treating cultures with low concentrations of raloxifene (10^{-11} M), while Viereck et al. (2003) demonstrated higher levels of osteoblast differentiation markers at higher raloxifene concentrations (10^{-7} M).

The dogs used for this experiment were all male. It has been thoroughly shown that raloxifene works in women, while this is not as well-established or as well-validated in men. Even though the level of estrogen in men is much lower than in women, the presence of functional ER in men is essential when considering the growth and composition of bone (Smith et al. 1994). As our results were obtained in male dogs, we would expect them to be valid in females, while the opposite assumption would be more questionable. We were not able to conduct the study in both sexes because of cost and ethical considerations.

Conclusion

While we aimed at preservation of allograft bone, we found the opposite situation—with accelerated graft resorption and more new bone formation. Our results are not consistent with current theory regarding the mechanism of how raloxifene counteracts the decrease in BMD after systemic treatment. Instead of it being solely anti-catabolic, as generally believed, we found an anabolic side to raloxifene after local treatment. Stimulation of bone formation by direct actions on osteoblasts could explain the increased amount of new bone, and the tight coupling to osteoclastic activity could account for the accelerated allograft resorption. Additional studies quantifying bone metabolism are needed to confirm our findings. Lower-dose treatment could possibly preserve the allograft as primarily intended here, but high doses of raloxifene in combination with bisphosphonates, for example, might also be promising. This and other studies with longer observation periods will help to define a possible role of Raloxifene in improving fixation of grafted implants. Furthermore, conduction of the study in female dogs would reveal any possible gender differences.

Baas J. Adjuvant therapies of bone graft around non-cemented experimental orthopedic implants stereological methods and experiments in dogs. Acta Orthop Suppl (330) 2008; 79: 1-43.

Baas J, Elmengaard B, Jensen T B, Jakobsen T, Andersen N T, Soballe K. The effect of pretreating morselized allograft bone with rhBMP-2 and/or pamidronate on the fixation of porous Ti and HA-coated implants. Biomaterials 2008; 29 (19): 2915-22.

Baddeley A J, Gundersen H J, Cruz-Orive L M. Estimation of surface area from vertical sections. J Microsc 1986; 142: 259–76.

Barckman J, Baas J, Sørensen M, Bechtold J E, Søballe K. Rinsing of allograft bone does not improve implant fixation. Acta Orthop 2013; 84: 307-13.

Bashir A, Mak Y T, Sankaralingam S, Cheung J, McGowan N W, Grigoriadis A E, et al. Changes in RANKL/OPG/RANK gene expression in peripheral mononuclear cells following treatment with estrogen or raloxifene. Steroids 2005; 70 (13): 847-55.

Cheung J, Mak Y T, Papaioannou S, Evans B A, Fogelman I, Hampson G. Interleukin-6 (IL-6), IL-1, receptor activator of nuclear factor kappaB ligand (RANKL) and osteoprotegerin production by human osteoblastic cells: comparison of the effects of 17-beta oestradiol and raloxifene. J Endocrinol 2003; 177 (3): 423-33.

Compston J E. Sex steroids and bone. Physiol Rev 2001; 81 (1): 419-47.

Crotti T N, Smith M D, Findlay D M, Zreiqat H, Ahern M J, Weedon H, et al. Factors regulating osteoclast formation in human tissues adjacent to peri-implant bone loss: expression of receptor activator NFkappaB, RANK ligand and osteoprotegerin. Biomaterials 2004; 25 (4): 565-73.

Evans G, Bryant H U, Magee D, Sato M, Turner R T. The effects of raloxifene on tibia histomorphometry in ovariectomized rats. Endocrinology 1994; 134 (5): 2283-8.

Fernandez-Garcia D, Munoz-Torres M, Mezquita-Raya P, de la Higuera M, Alonso G, Reyes-Garcia R, et al. Effects of raloxifene therapy on circulating osteoprotegerin and RANK ligand levels in post-menopausal osteoporosis. J Endocrinol Invest 2008; 31 (5): 416-21.

Gundersen H J, Bendtsen T F, Korbo L, Marcussen N, Møller A, Nielsen K, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988; 96: 379–94.

Jensen T B, Overgaard S, Lind M, Rahbek O, Bunger C, Soballe K. Osteogenic protein 1 device increases bone formation and bone graft resorption around cementless implants. Acta Orthop Scand 2002; 73 (1): 31-9.

Khastgir G, Studd J, Holland N, Alaghband-Zadeh J, Fox S, Chow J. Anabolic effect of estrogen replacement on bone in postmenopausal women with osteoporosis: histomorphometric evidence in a longitudinal study. J Clin Endocrinol Metab 2001; 86 (1): 289-95.

Leibrandt A, Penninger J M. RANK(L) as a key target for controlling bone loss. Adv Exp Med Biol 2009; 647: 130-45.

Luvizuto E R, Dias S S, Okamoto T, Dornelles R C, Okamoto R. Raloxifene therapy inhibits osteoclastogenesis during the alveolar healing process in rats. Arch Oral Biol 2011; 56 (10): 984-90.

McGee M A, Findlay D M, Howie D W, Carbone A, Ward P, Stamenkov R, et al. The use of OP-1 in femoral impaction grafting in a sheep model. J Orthop Res 2004; 22 (5): 1008-15.

McNamara I R. Impaction bone grafting in revision hip surgery: past, present and future. Cell Tissue Bank 2010; 11 (1): 57-73.

Messalli E, Mainini G, Scaffa C, Cafiero A, Salzillo P. Raloxifene therapy interacts with serum osteoprotegerin in postmenopausal women. Maturitas 2007; 56: 38-44.

Michael H, Harkonen P L, Kangas L, Vaananen H K, Hentunen T A. Differential effects of selective oestrogen receptor modulators (SERMs) tamoxifen, ospemifene and raloxifene on human osteoclasts in vitro. Br J Pharmacol 2007; 151 (3): 384-95.

Overgaard S. Danish Hip Arthroplastic Register—Annual report 2010. Webadress: http://www.dhr.dk/Ny%20mappe/rapporter/DHR%20Aarsrapport_2010%20t_web.pdf

Sliwinski L, Folwarczna J, Nowinska B, Cegiela U, Pytlik M, Kaczmarczyk-Sedlak I, et al. A comparative study of the effects of genistein, estradiol and raloxifene on the murine skeletal system. Acta Biochim Pol 2009; 56 (2): 261-70.

Smith E P, Boyd J, Frank G R, Takahashi H, Cohen R M, Specker B, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 1994; 331 (16): 1056-61.

Soballe K. Hydroxyapatite ceramic coating for bone implant fixation. Mechanical and histological studies in dogs. Acta Orthop Scand (Suppl 255) 1993: 1-58.

Taranta A, Brama M, Teti A, De luca V, Scandurra R, Spera G, et al. The selective estrogen receptor modulator raloxifene regulates osteoclast and osteoblast activity in vitro. Bone 2002; 30 (2): 368-76.

Teitelbaum S L. Osteoclasts: what do they do and how do they do it? Am J Pathol 2007; 170 (2): 427-35.

Toms A D, Barker R L, Jones R S, Kuiper J H. Impaction bone-grafting in revision joint replacement surgery. J Bone Joint Surg (Am) 2004; 86 (9): 2050-60.

Turner C H, Sato M, Bryant H U. Raloxifene preserves bone strength and bone mass in ovariectomized rats. Endocrinology 1994; 135 (5): 2001-5.

van der Donk S, Weernink T, Buma P, Aspenberg P, Slooff T J, Schreurs B W. Rinsing morselized allografts improves bone and tissue ingrowth. Clin Orthop 2003; (408): 302-10.

Viereck V, Grundker C, Blaschke S, Niederkleine B, Siggelkow H, Frosch K H, et al. Raloxifene concurrently stimulates osteoprotegerin and inhibits interleukin-6 production by human trabecular osteoblasts. J Clin Endocrinol Metab 2003; 88 (9): 4206-13.

Wahab M, Ballard P, Purdie D W, Cooper A, Willson J C. The effect of long-term oestradiol implantation on bone mineral density in postmenopausal women who have undergone hysterectomy and bilateral oophorectomy. Br J Obstet Gynaecol 1997; 104 (6): 728-31.

Yan M Z, Xu Y, Gong Y X, Liu J M, Lu S Y, Huang L, et al. Raloxifene inhibits bone loss and improves bone strength through an Opg-independent mechanism. Endocrine 2010; 37 (1): 55-61.