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BASIC SCIENCE

Prevention of distortion of vascular deprivation-induced osteonecrosis of the rat femoral head by treatment with alendronate

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Abstract
Introduction In animals with a disrupted blood supply and drainage of the femoral head, the dead epiphyseal bone undergoes osteoclastic osteolysis and is replaced by newly synthesized, immature, and weak bone, which cannot withstand the daily loads and, therefore, the articular surface caves in.
Methods Female Sprague–Dawley rats with interrupted blood circulation of the femoral head were treated with alendronate and compared to controls.
Results There was no distortion of the femoral heads in the alendronate-treated animals.
Interpretation Alendronate medication interferes with osteoclastic activities, slowing down bone turnover. These observations verify our hypothesis that osteoclastic activity is detrimental to the conservation of a hemispherical femoral head because of the rapidly occurring replacement of the dead by living tissues. Hence, halting the activities of the osteoclasts by alendronate stops the hasty new bone formation which is responsible for early femoral capital disfigurement.

Keywords Experimental osteonecrosis · Femoral head · Alendronate · Osteoclasts · Rats · Animal model of human disease

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Introduction

The pathological findings during the repair phases of vascular deprivation-induced osteonecrosis of the femoral heads of untreated rats and animals treated by diverse modalities were reported elsewhere in detail [4, 7, 10–13, 15, 16]. Briefly, necrosis of the hematopoietic-fatty tissue of the bone marrow is evident on the second day after severance of the blood supply and drainage of the femoral head whereas necrosis of the subchondral and trabecular bone of the epiphysis is detected on the fifth postoperative day. The processes of restoration gradually lead to the reconstruction of a viable epiphysis after the second postoperative week. The topic of osteonecrosis of the rats' femoral head has been reviewed [5]. Comparison of the progression of spontaneous repair with treatment-supported restoration implies that the more rapid the replacement of the epiphyseal necrotic bone by the newly formed bone the sooner the distortion of the femoral capital architecture advances to terminate in the postosteonecrotic osteoarthritis-like disorder. It is hypothesized that because recently deposited bone is biomechanically weaker than mature bone, the pace of the bone turnover determines the outcome [6]. In an attempt to validate this theory, we studied alendronate-medicated rats with femoral capital osteonecrosis. This nitrogen-containing bisphosphonate is retained in the bone and specifically targets the osteoclasts, the activity of which is thereby inhibited [17].

Materials and methods

Approval of the Institutional Review Board of the Rappaport Faculty of Medicine was obtained prior to the start of the experiments. The blood circulation of the right femoral

head of 24 female Sprague–Dawley rats, weighing 400–450 g, was interrupted. There were 16 alendronate treated rats and 8 controls. The rats were anesthetized with an intramuscular injection of ketamine (120 mg/kg) and xylazine (17 mg/kg). They were placed on a heated operation table to prevent hypothermia. After shaving of the skin, antisepsis, draping, and proximally slitting open the cutis by a longitudinal incision over the greater trochanter, the gluteus maximus muscle was split in the direction of its bundles and the anterior two-thirds of the gluteus medius muscle were detached from the bone. The antero-lateral insertion of the articular capsule was transected along the trochanteric ridge, the femoral head was dislocated, and the ligamentum teres was cut. With a number-11 blade, the periosteum at the base of the neck of the femoral head was incised together with the reflected capsular fibers by twice circumferentially sweeping the edge of the knife, at a 1-mm interval, around the bone. The femoral head was relocated. The articular capsule and the gluteal muscles were sutured with Dexon 000 stitches. The skin was closed with Nylon 00 stitches. The animals were housed in spacious cases to allow ample ambulation. They had free access to water and regular laboratory chow at all time.

Alendronate tablets were diluted at a concentration of 500 μg/mL of physiological saline and mixed in an electrical stirrer for 90 min. Postoperatively, in the alendronate-treated group, the rats received, at the neck region, daily subcutaneous injections of 200 μg of alendronate per 1 kg of body weight. Eleven and 5 rats were treated for 6 and 4 weeks, respectively. They were all killed by CO_2 inhalation on the 42nd and 28th postoperative day, respectively. Both femoral heads were harvested and the soft tissue excised. The specimens were fixed in formalin for a week. Following decalcification in EDTA for 2 weeks, the femoral heads were halved at the residue of the ligamentum teres into anterior and posterior parts. The paraffin blocks were cut into 4-μm sections and were stained with hematoxylin and eosin. Eight control rats were handled equally except that they were given injections of saline in place of alendronate.

Results

The left femoral heads were normal. Hematopoietic and fat cells fully packed the intertrabecular spaces. The epiphyseal and metaphyseal compartments were separated from one another by a somewhat undulating physeal cartilage. The basophilic articular cartilage was smoothly contoured (Fig. 1).

The vessels-deprived right femoral heads of the animals not medicated with alendronate disclosed a repopulation of the intertrabecular spaces with hematopoietic-fatty tissue

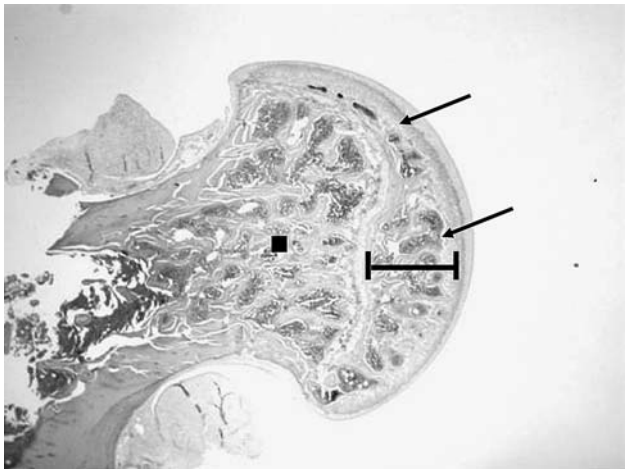

Fig. 1 Normally shaped femoral head. The left normally shaped femoral head. Note the regular hemispherical configuration and the smoothly contoured articular cartilage (*short thick arrow*). Undulating physeal cartilage (*long thin arrow*). The height of epiphysis is within the standard range. Metaphysis (*square*)

and reconstruction of living bone, but the hemispherical configuration was lost, the structurally distorted heads developing any of a number of bizarre shapes, as described in detail elsewhere [10, 11, 13, 15, 16].

First, a decisive difference between the vessels-deprived femoral heads of otherwise untreated in opposition to the alendronate-medicated rats was the preservation of a hemispherical configuration of the femoral heads in the latter group of rats. Even though the signs of an ischemic insult and evidence of reparative activities at an earlier period were overt at high-magnification examination, the overall shape of the femoral heads was well preserved at low magnification (Fig. 2).

Second, the height of the epiphyses, i.e., the distance between the physeal cartilage and the joint cartilage, was essentially of the same magnitude in the control animals and the alendronate-treated rats, and, hence, an explicit collapse of the femoral heads could be excluded.

The physeal cartilage was interrupted at one or more sites, evidencing an episode of irrepairable damage at some prior date. At the 42nd postoperative day, no remains of focally dead physeal cartilage was recognized, but segmental coagulation necrosis of the physeal cartilage was illustrated in preceding experiments in which the rats were killed at earlier intervals after the operation [16]. Consequent to the interruption(s) in the continuity of the physeal cartilage, bony bridges evolved linking the trabeculae of the epiphysis with the trabeculae of the metaphysis (Fig. 2). Additionally, despite the repopulation of the intertrabecular spaces by hematopoietic-fatty tissue, slight architectural irregularity of the epiphyseal bone was evident.

The differences between the comparatively well preserved femoral heads of alendronate-treated animals (Fig. 2) and

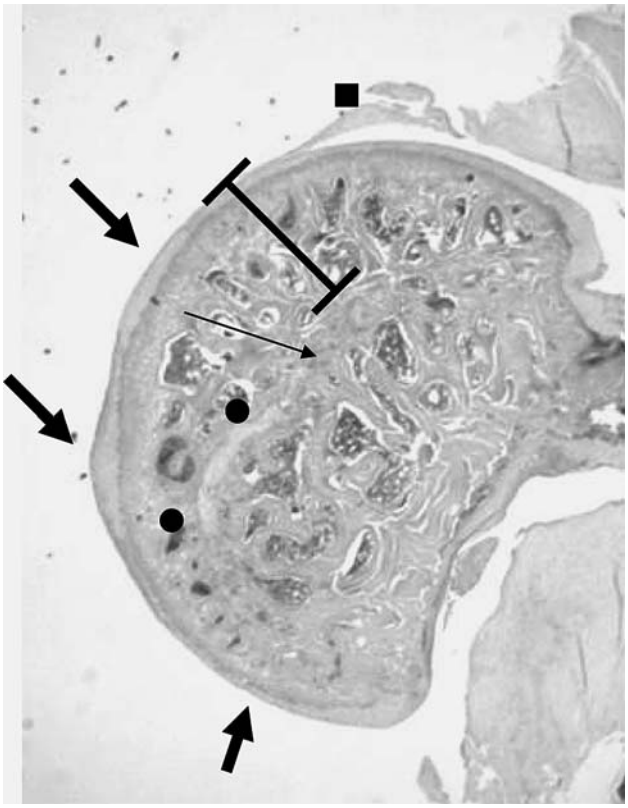

Fig. 2 Femoral head with AVN treated with alendronate after 42 days. The right operated femoral head of an alendronate-treated rat. There are just remnants of the physeal cartilage (*filled circle*). The physis has been breached and epiphyseal-metaphyseal bridges (*long thin arrow*) join the epiphyseal and metaphyseal bony trabeculae with one another. The articular cartilage is of unequal thickness (*thick arrow*). Even so the hemispherical configuration is preserved. The height of epiphysis is within the standard range. Remnants of the ligamentum teres (*filled square*)

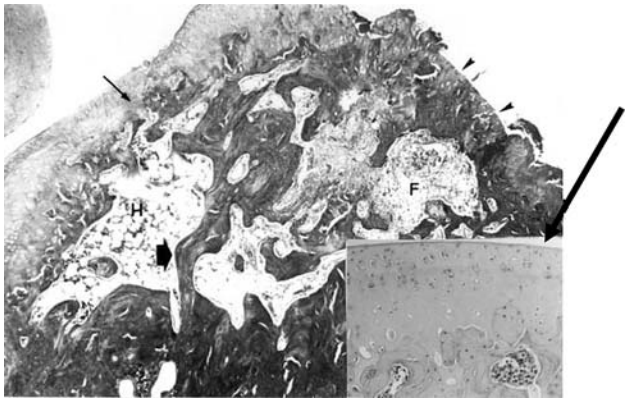

Fig. 3 Jagged articular surface of an almost triangular femoral head. The articular cartilage is degenerated, of varying thickness, and where it is segmentally absent, polished and eburnated bone constitutes the exposed articular aspect (*arrowheads*). The subchondral bone plate is focally interrupted by fibrous tissue (*thin arrow*). Fibrous (*F*) and hematopoietic (*H*) tissues are present between the sparse and atypically structured epiphyseal bony trabeculae. The physeal cartilage has been totally resorbed, and the epiphyseal and metaphyseal bony trabeculae are connected by osseous bridges (*thick arrow*). Inset: The cartilage and smoothly contoured articular surface (*arrow*) of a normal (control) femoral head

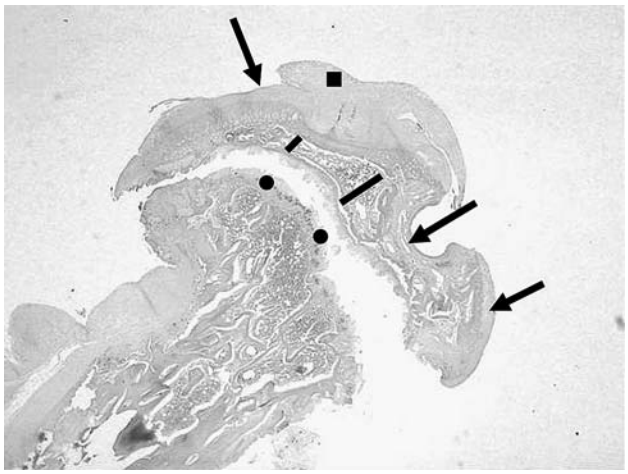

Fig. 4 Untreated femoral head with AVN after 42 days. The right operated femoral head of a rat after 42 days without any treatment-control animal. The sphericity is totally distorted. The physeal cartilage (*short thick arrow*)—there are just remnants of the physeal cartilage (*filled circle*). The epiphysis (*thick bar*) has lost height and the articular cartilage is of unequal thickness (*thick arrow*). Even so the hemispherical configuration is preserved. Remnants of the ligamentum teres (*filled square*)

untreated or enoxaparin-medicated rats (Figs. 3, 4), for that matter, followed-up for a month or so was striking inasmuch as the latter developed an osteoarthritis-like disorder characterized by a tricompartmental remodeling of the joint [5, 10, 11].

Discussion

Having scrutinized over 400 histological sections of vessels-deprived necrotic femoral heads of otherwise untreated rats as well as animals undergoing a variety of treatment modalities, it has become clear that the degree of architectural distortion of the femoral epiphyses depends on the extent of bone turnover leading to resorption of all debris and its replacement by living osseous and soft tissues [4, 7, 10–13, 15, 16]. It is counterintuitive that the more rapid and more extensive the reconstruction of living epiphyses progresses, the smaller is the prospect of reshaping a hemispherical or near-hemispherical femoral head. This might appear paradoxical at first sight. However, the decreased stiffness, strength, toughness, yield strength, and elastic modulus as well as the raised strain-to-failure of recently deposited and mineralized osseous matrix are second-rate to those of mature bones. And so the recently rebuilt epiphyses cannot carry daily transarticular loads without caving in. In fact, the revascularization-related reconstitution of weak bony trabeculae is blamed for the collapse of the

femoral heads only 4–6 weeks after disruption of the venous drainage of the femoral neck of minipigs [18]. It follows that an expedited osteogenesis is detrimental to maintaining the hemispherical shape of the femoral head and thus to an articulation with congruent load-bearing surfaces. If this indeed is the case, the remodeling of the necrotic femoral heads should be delayed, rather than speeded up, as present day paradigm would have it.

Bearing in mind that the dead osseous structures keep their biomechanical attributes for quite a while, a slowed-down new bone formation would favor the gradual replacement of the necrotic by living bone. For that reason, the treatment of patients with osteonecrosis ought to focus on a slowly progressive substitution so that the decline of the bone's biomechanical properties is kept to a minimum. One viable therapeutic mode is medication of inhibitors of the vascular endothelial growth factor [6].

In the investigation presented here, the authors attempted to delay the speedy renewal of living epiphyses by medication of alendronate. Gardeniers has removed a section of goats' femoral heads and reattached them with bone cement, which prevents revascularization. No collapse occurs and mechanical testing of the avascular segment displays no weakening of the bone compared to control specimens. Yet, when revascularization occurs by way of a hole drilled through the cement, revascularization occurs and the femoral head segment collapses. Biomechanical testing has verified the weakening of the revascularized segment [9]. Others have confirmed that necrotic bone retains load bearing capacity [14]. Consequently the death of bone cells per se does not cause structural failure. It is caused by the osteoclasts-mediated resorption of the necrotic bone during or following the revascularization of the necrotic tissue together with new bone formation. Assuming that bone resorption can be inhibited or delayed until sufficient new bone is formed, structural failure and its consequences may hopefully be avoided. Osteoclastic activity may be reduced with bisphosphonates, which bind to the mineralized osseous matrix. During osteolysis, the bisphosphonates are internalized and interfere with the metabolism of the osteoclasts, leading to their apoptosis.

Clinically, 10 mg of alendronate daily or 70 mg weekly are the usually recommended dose for osteoporosis treatment. Yet, in view of the low gastrointestinal uptake, only about 0.07 mg is distributed systemically. In Åstrand and Aspenberg's experiments on lessening osteoclastic activity adjacent to unstable implants, the low dose of 3.8 μg/kg per day has been found to be ineffective in rats. They therefore medicated the animals with doses between 115 and 21,000 μg/kg per day. However, the search for the relevant doses of biphosphonate in humans for the treatment of osteoporosis has been performed on rats. The estimated correlation is that a 10 times higher dose is needed in humans than in rats [2, 3]. In the current study, the rats were medicated 200 μg of alendronate per 1 kg of body weight. Inasmuch as the rats weighed 400–450 g, ~90 μg of alendronate was daily injected subcutaneously. The bisphosphonates do not uniformly distribute to the bones. They bind preferentially to regions with a high turnover, say, the trabecular bone in the animals' proximal tibiae [8]. Consequently, it is reasonable to assume that alendronate is concentrated in the post-osteonecrotic femoral heads, in which osteolysis and osteogenesis reach a peak during the second to fourth post-operative weeks.

Agarwala and his coworkers in a letter to the editor of Rheumatology have communicated their practice of daily medicating their patients with capital femoral osteonecrosis with 10 mg of alendronate and 1 g of calcium. Weight bearing was prohibited. Improvement in clinical parameters and range of motion were evinced in all 14 patients after 12 weeks of treatment. The MRI images remained stable in ten cases and the edema resolved in four cases. Given that they did not perform drill decompression, tissue diagnoses are lacking [1].

In conclusion, in the light of the above-described observations we have confirmed our hypothesis elaborated at length in the paper on "experimentally gained insight-based proposal apropos the treatment of osteonecrosis of the femoral head" [6], namely that the osteoclastic activity is detrimental for the conservation of a hemispherical femoral head because of the rapidly occurring (within 2–4 weeks) replacement of the dead by living tissues. We proposed that halting the activities of the osteoclasts by a biphosphonate would stop the hasty osteoneogenesis, which is responsible for the early femoral capital disfigurement. This premise has herein been shown to hold true for alendronate-treated rats with vascular deprivation-induced osteonecrosis of the femoral head.

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