J Orthopaed Traumatol (2006) 7:88–92
DOI 10.1007/s10195-006-0127-8

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Effects of alendronate medication on the fate of the necrotic femoral head of rats with or without core decompression

Received: 5 November 2005
Accepted: 14 March 2006
Published online: 20 June 2006

Prof. J.H. Boss, who actively participated to the experimental study throughout, passed away in Haifa on February 15, 2006.

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Abstract After producing bilateral osteonecrosis of the femoral head, rats were medicated alendronate according to one of 6 schedules. In group 1 injection was administered one week prior to the operation of devascularization and thereafter was continued for another 42 days. In group 2, medication started on the day of the operation. The control group 3 was similarly handled except that they were given injections of physiological saline. Group 4 animals were subjected to drilling of the right femoral head and treated with alendronate a week before the operation and 42 days thereafter, while in group 5 medication begun on the day of the operation. Control group 6 was similarly handled, but received physiological saline. The furthest advanced changes occurred in groups 3 and 6 (in which an advanced stage of an osteoarthritis-like disorder were observed) followed by groups 4 and 5 (in which alterations were mild to moderate), and finally by groups 1 and 2 (in which there were but mild changes). In addition, all changes were less pronounced in the animals which had been treated with alendronate for one week prior to the operation than in those given the first dose from the operative day. These findings demonstrate that bisphosphonate medication preserves the shape of rats' femoral heads in the early post-necrotic phase to a greater degree than in the Authors' earlier, diverse attempts to arrest the disastrous progression of the post-necrotic remodeling towards hip osteoarthritis. The presented findings warrant the preclinical trial of bisphosphonate therapy in patients admitted for the management of osteonecrosis of the femoral head.

Keywords Animal model of human disease • Osteonecrosis of the femoral head • Osteoarthritis • Alendronate • Rat • Core decompression

Introduction

Clinical trials of novel treatment modalities for avascular osteonecrosis of the femoral head have been impeded by the lack of an appropriate experimental model of the human disease [1]. To explore the chain of events leading to osteocytic death, investigators should attempt to duplicate the "circulatory deprivation" implied by clinicians' custom of applying the epithet of "avascular" for the human disease. Irrespective of where the blood circulation in the bone is at first disrupted, i.e., at the level of the arteries, veins, capillaries, or sinusoids, the flow in the arteries is eventually arrested. In the final analysis, the

reduced uptake of bone-seeking isotopes implicates that disruption of the blood supply triggers all cases of osteonecrosis. Experimental interruption of the blood circulation in animals with a life-long persisting physis, say rats, mimics Legg-Calvé-Perthes disease in children more than it imitates capital osteonecrosis in adult femoral heads [2]. Norman et al.'s described a model to induce osteonecrosis of rats' femoral heads [3]. Boss and Misslevich [4] reviewed the subject of avascular osteonecrosis in small laboratory animals, while Boss et al. [5] used this model to study diverse therapeutic options. Here, we describe the effects of alendronate medication on the fate of necrotic femoral heads in rats with or without experimental "core decompression", in the form of an intraosseous conduit produced by drilling the rats' femoral heads [6].

Materials and methods

The Institutional Review Board of the Rappaport Faculty of Medicine of Technion (Israel Institute of Technology) granted approval for this study. Sixty-six-month old female Sprague-Dawley rats, weighing about 400 g, underwent interruption of the blood supply of the right femoral head. 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, local antisepsis, and draping, a proximal longitudinal incision was made over the large trochanter. The gluteus maximus muscle was split in the direction of its bundles. The anterior two-thirds of the gluteus medius muscle were detached from the bone. The anterolateral insertion of the articular capsule was transected along the trochanteric ridge. The ligamentum teres was cut and the femoral head was dislocated. The periosteum at the base of the neck was incised together with the reflected capsular fibers by twice sweeping a number 11 blade, at an interval of 1 mm, circumferentially around the bone. The femoral head was relocated at the end of the operation. The joint capsules and gluteal muscles were sutured with Dexon 000 stitches and the skin was closed with nylon 00 stitches. The animals were placed in roomy cages such that their perambulation was relatively unrestrained. They had access to regular laboratory chow and water at all times.

In 30 of the 60 rats, a 21-gauge needle was pushed through the residue of the attachment site of the ligamentum teres and thrust forward in the direction of the neck up to (but not through) the opposite cortical bone. After pulling out the needle, blood seeping from the cylindrical channel was wiped until all oozing stopped [6].

Thirty rats were not subjected to drilling or "core decompression". In 11 rats, group 1 (PT), medication was initiated one week prior to the operation and thereafter continued for another 42 days. In group 2 (T), a second cohort of 11 rats medication was started on the day of the operation. Group 3 (C), 8 control rats were similarly handled except that they were given injections of 0.5 ml of physiological saline instead of alendronate sodium. Thirty rats were subjected to drilling of the right femoral head. Of these 10 animals, group 4 (PTD), were treated with alendronate sodium one week before the operation and 42 days thereafter. In group 5 (DT) 12 rats, the medication was begun on the day of the operation. Finally, the remaining 8 animals, group 6 (CD) control for the drilling femoral heads, were similarly managed except that they were given injections of 0.5 ml of physiological saline instead of alendronate sodium. All the animals were sacrificed by CO_2 inhalation on the forty-second postoperative day (Table 1).

Table 1 Experimental groups

	Additional one week Alendronate pretreatment	6 weeks postoperative Alendronate treatment	Control
Without drilling	*n*=11 Group 1 (PT)	*n*=11 Group 2 (T)	*n*=8 Group 3 (C)
Drilling	*n*=10 Group 4 (PTD)	*n*=12 Group 5 (TD)	*n*=8 Group 6 (CD)

n, number of rats. All the animals were sacrificed on the forty-second postoperative day

Tablets of alendronate sodium (Fosalan) were diluted at a concentration of 500 microgram/milliliter (μg/ml) of physiological saline and blended in an electrical stirrer for 90 minutes. Postoperatively, the rats were medicated with (200 μg of alendronate per 1 kg of body weight) adding physiological saline to a total volume of 0.5 ml by subcutaneous injections at back between the scapulae. The control groups were treated by injecting 0.5 ml of physiological saline at the same site.

At the time of sacrifice (vide infra) both femurs (the left femur serving as a control) were removed together with the joint capsule and adjoining muscles. The specimens were fixed in formalin, decalcified in EDTA, and embedded in paraffin. The blocks were trimmed so that longitudinally oriented, sagittal slices bisected the insertion of the ligamentum teres. Four microns-thick sections were stained with hematoxylin and eosin; they were also analyzed under polarized light to optimally discriminate between the lamellar-fibered and the woven-fibered bones.

Results

All femoral heads of the non-operated left-side hip joints were microscopically normal. Histologically, the femoral heads of the rats pertaining to control groups C and CD (groups 3 and 6) were severely distorted, including osteoarthritis-like features [7], collapse of the epiphysis, pannus formation, and filling of the space left after the

drilling procedure by chronically and mildly inflamed, densely textured fibrous tissue which was polluted by numerous, tiny particles of necrotic bone (Figs. 1–4). Additionally, large chunks of necrotic articular cartilage were haphazardly scattered in the fibrous tissue and, secondly, the cartilage on both sides of the drilling site was hypertrophic (Fig. 2). Evidence of the drilling were easily detectable, as a cylinder-shaped shaft filled with the fibrous tissue (Figs. 3, 4), as a bowl-shaped depression constituting the tangentially cut residue of the drilling procedure (Fig. 2), or as tiny necrotic bone fragments polluting the fibrous tissue (Fig. 3). All hematopoietic and fat cells of the intertrabecular spaces of the epiphysis were replaced by fibrous tissue. More often than not, the cartilage of the physis was focally or entirely absent such that osseous trabeculae of the epiphysis and metaphysis linked with each other, forming so-called epiphyseal-metaphyseal bridges (Fig. 2). The fibrous tissue within the cylinder-shaped shaft was sharply demarcated from the adjacent epiphysis in all cases (Fig. 3).

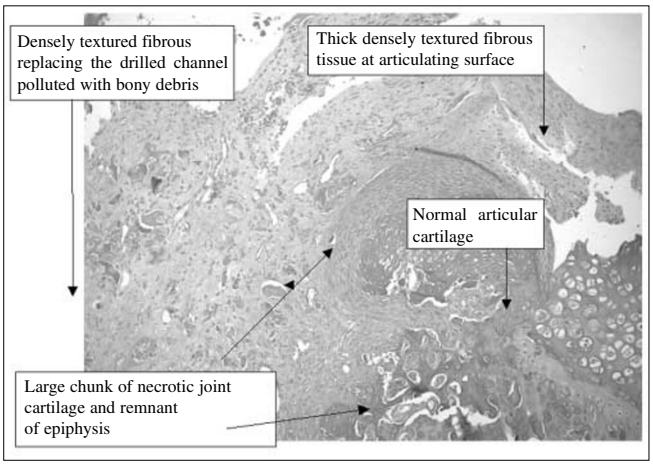

Fig. 1 The severely remodeled proximal epiphysis of the femoral head after a drilling procedure, Hematoxylin and eosin, x 120

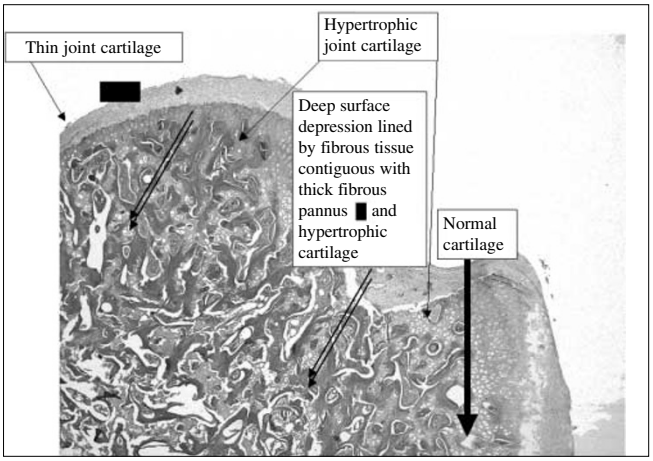

Fig. 2 Deep surface depression and loss of epiphyseal cartilage with formation of epiphyseal- metaphyseal osseous bridges (*double thin arrows*) after a drilling procedure. Hematoxylin and eosin, x 120

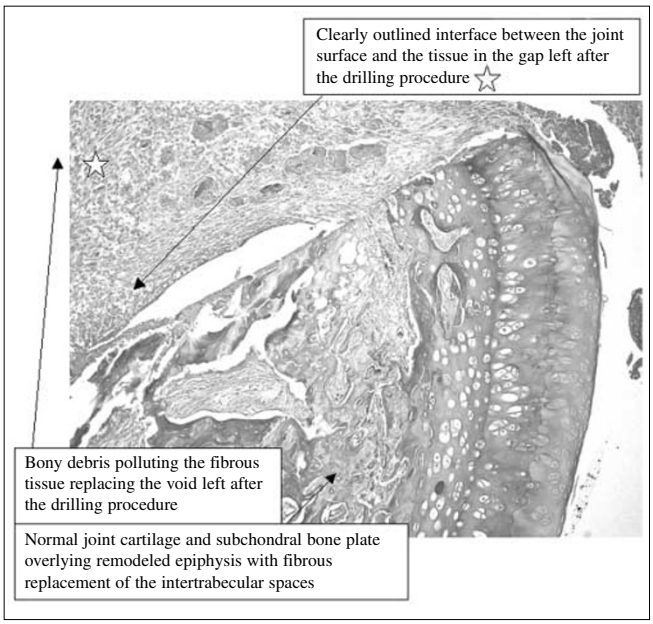

Fig. 3 Clearly outlined interface between the joint surface and the tissue in the gap left after the drilling procedure. Note the bony debris which pollutes the fibrous tissue replacing the void left after the drilling procedure. Hematoxylin and eosin, x 120

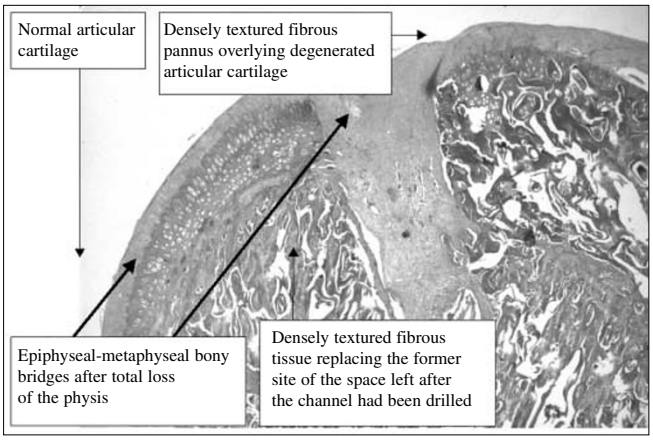

Fig. 4 Densely textured fibrous tissue replacing the space left after the channel has been drilled and epiphyseal-metaphyseal bony bridges after total loss of the physis. Hematoxylin and eosin, x 120

These alterations were encountered in all animals, yet their severity varied from one group to another. The furthest advanced changes occurred in groups C and CD (groups 3 and 6) (saline-treated rats), followed by groups PTD and TD (groups 4 and 5) (mild to moderate changes in the alendronate-treated rats that underwent the drilling), and, finally, groups PT and T (groups 1 and 2) (mild

changes in rats medicated with alendronate only). Unmistakable differences were found in the severity of the changes between the animals that had been treated with alendronate for one week prior to the operation, on the one hand, and the severity of the alterations in the rats treated from the day of the operation, on the other hand, in so far in the latter the evaluated parameters were especially overt.

Discussion

It is by now well known that within a short while of inducing osteonecrosis of rat femoral heads an osteoarthritis-like disorder of the hip ensues [4, 5]. We have previously sought means to hinder or delay the destructive remodeling of the necrotic femoral heads, but our endeavors are to no avail in as much as experimentally accelerating the healing processes hastens the evolution of the osteoarthritis-like disorder [6, 8–11]. We have consequently selected alendronate medication of rats with femoral capital osteonecrosis in the expectation that slowing down the function of the osteoclasts [12–14] would preserve the normal shape of the femoral epiphysis for a longer time period than in the untreated control animals. The stiffness, strength, and toughness of recently deposited and mineralized osseous matrix are inferior to those of mature bones. In fact, revascularization-related reconstitution of weak bony trabeculae is blamed for the femoral capital collapse 4–6 weeks after disruption of the venous drainage of the femoral neck of minipigs [15]. Hypothesizing that preserving bony trabeculae by inhibiting osteoclastic osteolysis would minimize the development of deformity following ischemic necrosis of the femoral head, Kim et al. [16] demonstrated that in piglets treated with ibandronate, a highly potent antiresorptive agent, bone resorption was inhibited. Thereby, the repair process was altered, the shape of the femoral epiphysis was better preserved, and deformity of the femoral heads was prevented during the early reparative phase [16]. It is worthy of note in this context that the most severe changes, say, thinning or total loss of the cartilage associated with the formation of a thick pannus (Fig. 4), were localized to the weight-bearing zone of the articulating surface, similar to the features reported in human osteoarthritis [17].

The aim set at the outset of this study, namely medication with a bisphosphonate in an attempt to preserve the shape of the rats' femoral heads in the early post-necrotic phase has been accomplished by the data presented herein. Therefore, on the basis of this and other experimental studies as well as on the present-day paradigm about the evolution of post-necrotic osteoarthritis-like disorder, a preclinical trial of bisphosphonate therapy in patients with osteonecrosis of the femoral head is warranted.

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