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Regular Article

Heapranase role in the treatment of avascular necrosis of femur head

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ABSTRACT

Background: Idiopathic avascular necrosis (AVN) of bone causes significant morbidity in adults although the pathophysiology is unknown. The present treatment options include systemic biphosphonate therapy and local bone drilling decompression, ameliorating the healing process and their by render the weight bearing femur head less vulnerable to collapse. In the present study we demonstrate the involvement of heparanase in AVN and in the acceptable treatments.
Methods: 56 female rats were studied. In 8 control rats AVN was induced by ligamentum teres ligation of the right femur while the left femur remained intact. In the rest of the rats, in addition to right femur AVN, treatment was added by subcutaneous biphosphonate therapy, femoral head drilling or combination of the treatments. All rats were scarified after 6 weeks. Immunostaining of the femur heads were performed to heparanase, tissue factor pathway inhibitor (TFPI), tissue factor (TF) and hematoxylin-eosin.
Results: Staining of heparanase, TFPI and TF were most prominent in the bone-marrow tissue of the femur heads. Staining by hematoxylin-eosin revealed damaged femur heads with prominent heparanase and TFPI staining in the femur with AVN compared to the contra lateral side of the same rat. No difference was found in the TF staining between the two sides. In the drilling and / or biphosphonate therapy groups, in contrast to the control group, femur heads were preserved with no significant difference in heparanase and TFPI staining between the two sides.
Conclusions: Heparanase and TFPI are locally elevated in the process of AVN and are normalized by the acceptable treatments. Inhibition of heparanase by heparins can potentially improve the nowadays therapy modalities.

Introduction

Avascular necrosis (AVN) of bone, also known as osteonecrosis, is an idiopathic process of cellular death of bone tissue due to a disruption of blood supply, micro-infarctions and necrosis [1]. This condition causes significant morbidity in adults and can lead to joint collapse and arthritis. This pathology has the potential to affect any part of the skeletal system; however, the most frequently affected site is the femur head. Risk factors associated with the occurrence of AVN include prolonged corticosteroid use, alcoholism, hemoglobinopathies, Gaucher's disease, pregnancy, hyperbaric exposure, autoimmune diseases and hip trauma, although in many cases the etiology is unknown [2,3]. Activation of the coagulation system with local thrombosis was previously suggested [4,5] as a potential mechanism. Treatment options include drilling of femur head and systemic biphosphonate therapy, supported by our previous publication in rate model, demonstrating significant improvement of femur head stabilization [6]. In the present study we looked for coagulation markers to support the hypothesis that drilling and biphosphonate treatment change the haemostatic balance in the AVN process, giving a potential insight for the mechanism of the treatment options.

Material and Methods

Study Group

The experiment was approved by the Technion Institute Ethic Committee for animal research. The blood circulation of right femur head of 56 female Sprague–Dawley rats weighing 200–250 g was interrupted. The animals were housed in spacious cages to allow ample ambulation and were operated after a minimum of a week for acclimation to the cage. They had free access to water and regular laboratory chow at all times. The rats were anesthetized with an intramuscular injection of ketamine (120 mg/kg) and xylazine (17 mg/kg). They were placed on a heated operating table to prevent hypothermia. After shaving of the skin, antisepsis, draping, and proximally slitting of the cutis by a longitudinal incision over the greater

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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 anterolateral insertion of the articular capsule was transected along the trochanteric ridge, the femur head was dislocated, and the ligamentum teres was cut. With a number 11 blade, the periosteum at the base of the neck of the femur head was incised together with the reflected capsular fibers by circumferentially sweeping the edge of the knife twice, at a 1- mm interval, around the bone. The femur head was relocated. The articular capsule and the gluteal muscles were sutured with vicryl 3–0 stitches. The skin was closed with nylon 2–0 stitches.

Treatment groups 2–6 were treated by daily sub-cuteness Alendronate injections of 200 μg/kg/day and controls were treated with saline, both for a total of 42 days. Groups 2, 4, 6 had drilling of the AVN femur heads, as previously described [6]. For each rat, the right operated head was compared with the left, and the drilling and Alendronate treated groups were compared with the control group.

All rats were sacrificed by CO_2 inhalation on the 42nd postoperative day, both femur heads were harvested. The specimens were fixed in formalin for a week. Following decalcification in EDTA for two weeks, the femur heads were halved at the residue of the ligamentum teres into anterior and posterior parts.

The experiment groups included:

1. AVN, n = 8, Control group.
2. AVN + drilling, n = 8.
3. AVN + Alendronate (after AVN for 6 weeks), n = 11.
4. AVN + drilling + Alendronate (after AVN for 6 weeks), n = 9.
5. AVN + Alendronate (before AVN for 1 week + after AVN for 6 weeks), n = 9.
6. AVN + drilling + Alendronate (before AVN for 1 week + after AVN for 6 weeks), n = 11.

Antibodies

Antibody 733 was raised in rabbits against a 15 amino acid peptide that maps at the N-terminus of the 50 kDa heparanase subunit. The 733 antibody preferentially recognizes the 8 + 50 kDa heterodimer vs. the 65 kDa latent proenzyme [7]. Polyclonal anti-rat tissue factor (TF) and polyclonal anti-rat tissue factor pathway inhibitor (TFPI) antibodies were purchased from Santa Cruz (Santa Cruz, CA).

Immunohistochemistry

Staining of formalin-fixed, paraffin-embedded 5-micron sections was performed. Slides were deparaffinized with xylene, rehydrated and endogenous peroxidase activity was quenched for 30 min by 3% hydrogen peroxide in methanol. Slides were then subjected to antigen retrieval by boiling (20 min) in 10 mM citrate buffer, pH 6. Slides were incubated with 10% normal goat serum in PBS for 60 min to block non-specific binding followed by incubation (20 h, 4 °C) with anti-TF, anti-TFPI, or anti-heparanase antibodies, diluted 1:100 in blocking solution. Slides were then extensively washed with PBS containing 0.01% Triton X-100 and incubated with a secondary reagent (Envision kit; Dako, Glostrup, Denmark) according to the manufacturer's instructions. Following additional washes, color was developed with the AEC reagent (Sigma, St. Louis, MO). The slides were assessed blindly by two persons not knowing the allocated treatment group. Protein's immunostaining representative intensity was rated as follows: none, weak, moderate and intense.

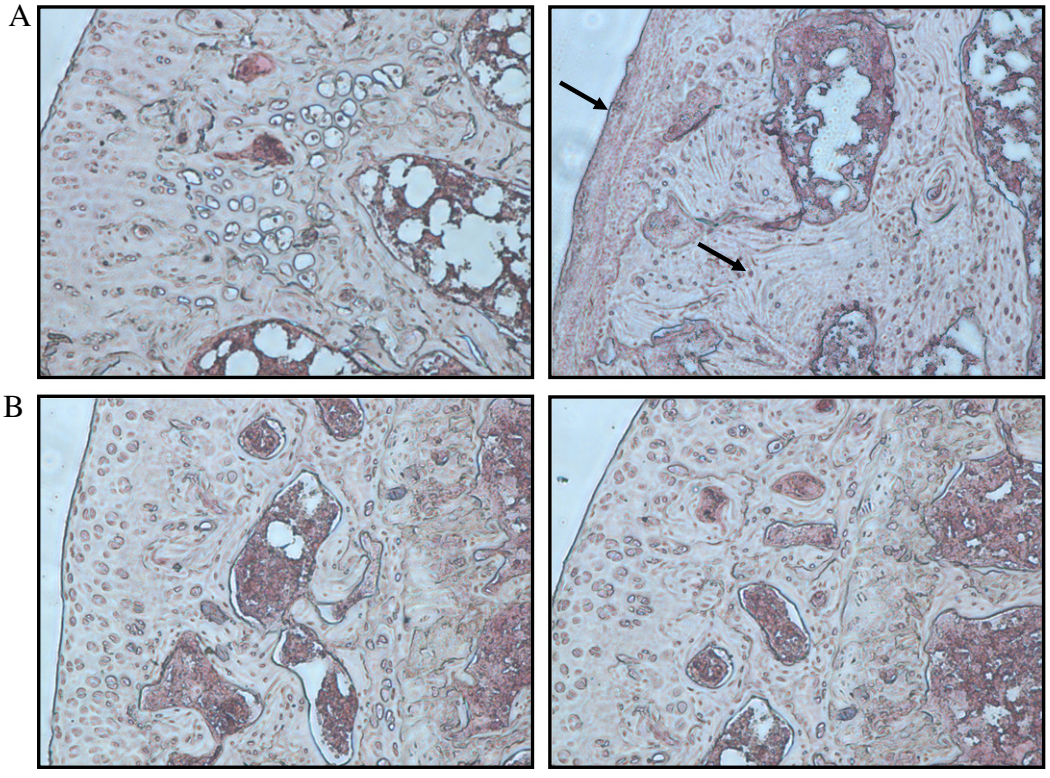

Fig. 1. Histological damage of AVN is protected by treatments of drilling and / or biphosphonate. Staining of femur heads by H&E. A. Left figures. represent the left femur of rats and serve as control. Right figures. represent the right femur of the same rats following AVN. Not the damaged architecture including disappearance of chondrocytes and replacement with thick fibrous tissue in AVN (arrows). B. Representative figures. to the protective effect of drilling and / or biphosphonate treatments. No major difference was observed between the AVN femur head (right) and the control (left). Images (x10 magnification) were visualized through a 10x/0.82 MDC objective lens, captured with a Nikon E995 digital camera (Nikon, Tokyo, Japan).