Decreased bone strength in HLA-B27 transgenic rat model of spondyloarthropathy

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Objective. To investigate the nature of osteopenia/osteoporosis in spondyloarthropathy, an inflammatory disorder, using the HLA-B27 transgenic rat model.

Methods. HLA-B27 transgenic rats were housed individually and sacrificed at the peak of their disease (8-month-old). The spine and femurs were removed and stored in saline at −20°C until analysis. The bone structure and strength were determined using a micro-computed tomography (micro-CT) device (Scanco Medical) and mechanical testing (Instron 5543). Vertebral bodies and femurs were scanned to determine trabecular structural properties in terms of bone volume (BV/TV), trabecular thickness, and spacing. After scanning, the mid-shaft femurs were subjected to a 3-point bending test (along anterior-posterior direction), the femoral necks were tested in bending, and the vertebral bodies (L4) were tested in compression. Structural (ultimate/yield load, stiffness) and apparent material (ultimate/yield stress, modulus) strength parameters were then determined.

Results. The majority of the bone structural and strength parameters were significantly lower (P < 0.05) in the HLA-B27 transgenic rats as compared with control littermates. Micro-CT data suggested that the transgenic animals had lower BV/TV and trabecular thickness in their vertebral bodies. The poor trabecular structure observed in HLA-B27 rats is also indicative of the poor biomechanical strength properties in the vertebral bodies as well.

Conclusion. The HLA-B27 transgenic rats develop bone fragility similar to that seen in spondyloarthropathy and may be an important model for the study of osteoporosis in spondyloarthropathy.

Key words: Bone, Spondyloarthropathy, Transgenic rat, Femur, Vertebral body, Stiffness, Bone strength.

Introduction

Spondyloarthropathy and related inflammatory disorders are well-known conditions which result in inflammation involving the spine, peripheral joints and entheses [1]. Additionally, the patients with spondyloarthropathy often develop dorsal kyphosis due partly to vertebral compression fractures. It is clear that these patients often develop osteopenia/osteoporosis [2–9]. These osteoporotic changes, especially in spine, pose a significant risk of fractures especially vertebral fracture.

The exact aetiology of the osteopenia/osteoporosis is not clear [2–7]. Well-known factors include corticosteroid use [10], changes in hormones, mechanical weight bearing. The cytokines involved in the underlying inflammatory disease can also lead to bone fragility [7, 11, 12]. Since reduced bone mineral density and abnormal mechanical properties can be seen early in the disease course [9, 13], factors other than medication or mechanical load may also play a significant role in the causation of osteoporosis. For example, increased bone turnover and/or break down of collagen cross-links in these patients may lead to bone loss and skeletal fragility [14, 15]. However, the exact factors involved in the pathogenesis of the osteoporosis remain elusive and further studies are necessary.

Currently, there are no known studies characterizing the biomechanical properties of bone tissue from human or animals with spondyloarthropathy. In a well-established but imperfect animal model of spondyloarthropathy (HLA-B27 transgenic rat) [16], it was demonstrated that alveolar bone loss occurred in both male and female animals [17, 18]. The mechanism for this bone loss has not been established and we do not know if there are other bony changes elsewhere in skeleton.

To better understand the aetiology of osteopenia/osteoporosis in spondyloarthropathy and to characterize the bone fragility in spondyloarthropathy, we investigated whether the HLA-B27 transgenic rat can serve as a model to study the mechanical properties of the skeleton in spondyloarthropathy.

Methods

The transgenic HLA–B27 male rats (n = 8) and their littermates (n = 8) were purchased from Taconic Farms at 6–8 weeks of age. The transgenic HLA-B27 rats were originally developed by Hammer et al. [19]. These animals expressed HLA-B27 and human βt and develop a disorder similar to human with HLA-B27 associated spondyloarthropathy, including spondylitis, inflammatory bowel disease and alopecia, orchitis and others [20]. The animals were housed individually and sacrificed at the age of 8-months. The experiments were performed according to Creighton University animal care-approved protocols, and animals were housed in accordance with the ILAR (Institute of Laboratory Animal Research) Guide for the Care and Use of Laboratory Animals.

Body weights were measured at the time of necropsy and bone specimens were collected and frozen for subsequent physical and biomechanical analyses.

Physical measurements

Physical measurements of femurs and vertebral bodies were made after removing the soft tissue. Femoral lengths and outer (periosteal) mid-shaft widths in both medial-lateral (ML) and anterior-posterior (AP) directions were measured. Vertebral bodies were prepared with flat and parallel ends and their lengths were measured along the caudal-cranial direction.

Micro-CT measurements

Before mechanical testing, both vertebral bodies and the femurs (mid-shaft and distal) were scanned (17 micron resolution) using a micro-computed tomography (micro-CT) device (μCT40, CT40,
Scanco, Medical AG, Bassersdorf, Switzerland). The scan length for vertebral body was ~6 mm (obtaining a total of 350 slices) and for distal femur was 3 mm (176 slices) with an integration time of 80 ms. Vertebral body and distal femur trabecular structure [bone volume to total volume fraction (BV/TV), trabecular thickness, trabecular number, and trabecular spacing], volumetric apparent density (App.D) and tissue density (Tiss.D) were measured. At mid shaft femurs, we measured the second moment of area in the direction (anterior-posterior) of 3-point bending test.

**Mechanical testing**

Vertebral body. Fourth lumbar vertebral bodies (L4) were isolated from the spinal processes and prepared with flat and parallel ends using a diamond wafer saw (Beuhler, IL, USA). The prepared L4 vertebral bodies were tested in compression with loads applied along the cranio-caudal axis [21–23].

Femur shaft. The mid-shafts of femurs were tested in three-point bending using a mechanical testing device (Instron 5543, Canton, MA, USA). During mechanical testing each femur was placed on two lower supports that were 17 mm apart. Load (force) was applied at the mid-diaphysis on the anterior surface such that the anterior surface was in compression and the posterior surface in tension [21–23].

Femur neck. After the mid-shaft bending test, the femoral neck strength (ultimate load) was tested by applying load to the femoral head in a direction parallel to the femoral shaft. The femoral shaft was constrained in a mechanical chuck during the femoral neck test [21–23]. All mechanical tests were done at room temperature using a displacement/stroke rate of 3 mm/min. Load-displacement data was captured by using Merlin software (Instron, Canton, MA) (Fig. 1). Structural strength data such as ultimate load (N=Newton), yield load (N) and stiffness (N/mm) were directly measured from the load-displacement diagram for each specimen. For each vertebral body and femur the apparent material properties were calculated from their cross sectional area properties using the standard engineering equations [22].

**Bone ash measurements**

After mechanical testing, the vertebral bodies were used to measure bone percent ash content (Bone dry weight/ash weight) using standard bone ash techniques (600°C oven) [24].

**Statistical Analysis**

Student’s t-test was used to find differences in all the measured variables between transgenic and littermate control rats. The measured variables were obtained from a three separate and unconnected techniques (mechanical testing, micro-CT and bone ash). Adjusting for multiple comparisons, the conservative level of statistical significance of 0.01 was also used. While P-values <0.05 are marked with the superscript ‘b’, the P-value <0.01 are marked by the superscript ‘a’ in all the tables. The data are reported as mean ± standard deviation (s.d.). All statistical analyses were performed using the statistical package SPSS for Windows (SPSS Inc., Chicago, IL, USA).

**Results**

**Characteristics of the vertebrae from control and HLA-B27 transgenic rats**

HLA-B27 transgenic rats developed full clinical symptoms around 8 months [16]. At time of necropsy, these animals were significantly smaller than their normal male littermates (body weight HLA = 353 ± 42 g vs WT = 382 ± 33 g). However, despite the difference in weight, there was no difference in the vertebral height and cross sectional area between the transgenic rats and their littermates (Table 1). These data indicate that the observed differences described below are not due to the size of the bone.

Compared with the normal littermates (male), HLA-B27 transgenic rats (male) had poor biomechanical strength and structural properties as shown in Table 1. Thus, vertebral body structural strength (ultimate load) and apparent material strength (ultimate stress, yield stress, modulus) were significantly (P < 0.05) lower in HLA-B27 transgenic rats as compared with the controls. Furthermore, the mean ultimate load, yield load, ultimate stress, yield stress and modulus were 36, 33, 46, 43 and 32% lower in HLA-B27 transgenic rats, respectively.

To determine the structural changes that would account for the observed mechanical difference mentioned earlier, micro-CT was used to analyse the vertebra from the control and HLA-B27 transgenic rats. Typical vertebral body scans were shown in Fig. 2. The B27-transgenic vertebra had less bone volume in the trabecular bone and greater spacing between the trabeculae (Fig. 2B as compared with Fig. 2A). The micro-CT data from the experimental groups were summarized in Table 2 which shows that the HLA-B27 transgenic rats had lower BV/TV (23%), trabecular thickness (25%), apparent density (25%) and greater trabecular spacing (50%) in their vertebral bodies. The decrease in mineral content of the vertebral body was demonstrated by the load vertebral body ash to dry weight ratio (6%) in the HLA-B27 transgenic rats (Table 3).

**Characteristics of the femur from the control and HLA-B27 transgenic rats**

To determine whether the weight bearing femur showed similar structural abnormality as the vertebra of the HLA-B27 transgenic rats, mechanical studies similar to those mentioned in the previous paragraph were performed with the femur. Again, there was no difference in the size of the femur in the control and transgenic rats (Table 1). The anterior-posterior and medial-lateral widths of the two groups were similar. In contrast, the structural strength (ultimate load) in the mid-shaft femur (22%, P < 0.01) and femoral neck (26%, P < 0.05) were lower in the HLA-B27 transgenic rats as compared to the controls. On the other hand,
there was no difference in the Yield load and Stiffness of the two groups of animals.

Micro-CT data showed that the HLA-B27 transgenic rats had lower BV/TV (33%, \( P < 0.01 \)), trabecular thickness (13%, \( P < 0.05 \)), apparent density (48% \( P < 0.01 \)) and greater trabecular spacing (67%, \( P < 0.05 \)) in their distal femur (Table 2). Typical distal femoral scans were shown in Fig. 3. The B27-transgenic distal femur had less bone volume in the trabecular bone and greater spacing between the trabeculae (Fig. 3B as compared with Fig. 3A).

**Discussion**

The aim of this project was to investigate the utility of an animal model of spondyloarthropathy to study the mechanism of osteoporosis seen in this disorder. Here, we confirmed that this animal model may be useful for further study of the aetiology of osteoporosis in this disease. Our result indicated that the poor vertebral trabecular structure in HLA-B27 rats was consistent with its poor biomechanical strength and abnormal structural properties (Table 1 and 2). In addition, declining femoral mid-shaft and femoral neck structural strength (ultimate load) provided further evidence that spondyloarthropathy in rats causes deterioration in the overall mechanical properties of bones.

This study demonstrated that the decrease in bone strength was not due to a difference in body weight or bone size of the HLAB-27 transgenic animals. Rather, despite a similar vertebral size (cross sectional area, vertebral height), the HLA-B27 transgenic rats developed a lower BV/TV and lower trabecular thickness. These data supported the finding of lower bone mineral density in the transgenic rats; the lower ash to dry weight ratio (bone mass) rendered further support for this hypothesis.

The structural and strength changes in the vertebral bodies appear to be more severe as compared with that in the mid-shaft femoral bone in the HLA-B27 rat. That is, the structural and mechanical strength properties of vertebral bodies appear to be more susceptible to spondyloarthropathy-related changes than that of femoral bone. There are a number of possible reasons for these findings. The trabecular bone is known to undergo relatively rapid turnover as compared with the cortical bone; therefore, its mechanical properties are more sensitive to environmental influences. The site-specific differences in bone strength may be due to the presence of different types of bone. For instance, while the mid-shaft femur bending strength is due to cortical bone only, the vertebral body strength depends upon both cortical and trabecular bone. Furthermore, the rapid turnover of the trabecular bone in the vertebra probably leads to the additional site-specific differences in the bone strength.
The environmental factors that have an impact on the structure and strength of different bones are not completely elucidated. Recent studies have indicated that systemic inflammatory changes with the production of inflammatory cytokines [16, 19, 20] can affect bone formation and these may influence trabecular bone at a greater rate than cortical bone.

The vertebral body data (both structure and strength) along with the mid-shaft and femoral neck data from this animal model of spondyloarthritis were similar to that seen in patients with spondyloarthropathies [3–5] who, with low bone mass and density, had increased skeletal fragility and hence, increased fracture risk. However, one should be careful in extrapolating rat bone structure and strength data to that seen in humans. Humans do not experience the non-physiological loading conditions (3-point bending in femur, one time high compression load of vertebra) that were used in this study to characterize the bone fragility in femurs and vertebral bodies. Furthermore, unlike the mode of mechanical testing used in this study, human skeleton (femurs, vertebral bodies) experience fatigue loading (in bending and compression) and rarely experience the complete mechanical failure of femurs and vertebral bodies during normal load bearing activities. Nevertheless, humans with spondyloarthritis have also been found to experience bone loss in several skeletal sites including trabecular bone in the spine [9, 13, 25]. Thus, animal models of spondyloarthritis, mimicking the skeletal conditions seen in human subjects are useful tools to further investigate the causative factors in the pathogenesis of osteoporosis in spondyloarthritis.

The mechanism by which spondyloarthritis causes a decline in bone mass/density and skeletal fragility is not known. HLA-B27 gene may contribute directly or indirectly to bone fragility seen in spondyloarthritis as manifested in these animals. In humans, spondyloarthritis can occur in absence of HLA-B27; therefore, it’s (HLA-B27) effect is likely an indirect one.

The HLA-B27 gene-related mechanisms are probably multifactorial including elaboration of inflammatory cytokines, hormonal dysregulation due to orchitis and malnutrition due to the gastrointestinal abnormalities in these animals [16, 19, 20]. In this study, at least, pharmaceutical agents such as corticosteroids are not an issue although in clinical settings that still remains a possibility. Factors such as inflammatory cytokines and mechanical stress can all play a role in causing bone fragility. Further studies using this animal model may yield information which is more difficult to obtain in the clinical setting. For instance, we can manipulate the hormonal status of the animals to determine the substances responsible for changes in the bone strength in the HLA-B27 rats. In addition, we can investigate if there is a deterioration of the true intrinsic material strength properties of the bones from these animals, as measured by nano-indentation techniques. [26, 27]. Recently, a new B27 transgenic rat model has been described, and it appears to have features mimicking even more closely to that seen in humans [28]. This animal model [28] will be very useful in further dissecting the various aetiological factors contributing bone fragility in this disorder.

We observed only one of the transgenic animals showed mild hind paw swelling. Due to the small number, it is difficult to correlate the appearance of arthritis with the parameters of bone strength and health. The recently described model [28] would be a better model to examine this issue.

In conclusion, this study provides basic biomechanical data of the increased bone fragility seen in an animal model of spondyloarthritis. Future work using this or better animal model [28] is needed in order to evaluate bone histomorphometry, immune and inflammatory mechanisms that may be involved in the pathogenesis of osteoporosis. Such studies may shed light on the mechanisms responsible for the bone loss and the resulting bone fragility in patients with spondyloarthritis.

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