Changes in Bone Structure, Biomarkers and Density in Collagen-Induced Arthritis

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INTRODUCTION
The purpose of this study was to evaluate the effects of arthritis induced by collagen on bone-related parameters in rats. Those parameters were the bone density using Dual X-ray Absorptiometry (DXA), osteoclast-derived tartrate-resistant acid phosphatase form 5b (TRACP-5b) in serum and tissue samples, and osteoclast cellularity with immunostaining.

MATERIALS AND METHODS
On Day 1, 10 rats (female Lewis rats, 8- to 9-weeks old) the dorsal area at the base of the tail was shaved. Rats assigned to the CIA group were immunized with Type II bovine collagen (Elastin Products Company, Owensville, MO, #CJ385) by 2 intradermal injections at the base of the tail (contralaterally). A boost immunization was performed following the same regimen on Day 7. Rats assigned to the non-disease control group received saline injections on both days.

RESULTS
The following parameters and end points were evaluated: clinical signs, body weights, disease score (clinical score, paw thickness and volume), bone densitometry of the tibiotarsal joint and lumbar spine by DXA (Hologic Discovery A), marker of bone resorption TRACP-5b in serum and tissue samples, and osteoclast cellularity with immunostaining.

Table 1: Experimental Design

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Group Name</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Non-Disease Control</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>CIA</td>
<td>12</td>
</tr>
</tbody>
</table>

On Day 16, lower gains in bone mineral content (BMC) and bone mineral density (BMD), which is considered a systemic effect of arthritis, were observed in the CIA group when compared to the Non-Disease group. There was no observable change in the tibiotarsal area. The effect of arthritis on bone was progressive and on Day 25, statistically significant differences were observed in all DXA parameters (area, BMC and BMD) between Non-Disease animals and CIA animals at both lumbar spine and tibiotarsal joint except for BMC at the tibial area (Table 2).

Table 2: Bone Densitometry by DXA

<table>
<thead>
<tr>
<th>Group</th>
<th>Area (cm²)</th>
<th>BMC (g)</th>
<th>BMD (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>0.00 (0.0)</td>
<td>0.00 (0.0)</td>
<td>0.00 (0.0)</td>
</tr>
<tr>
<td>CIA</td>
<td>0.12 (0.1)</td>
<td>0.10 (0.1)</td>
<td>0.08 (0.1)</td>
</tr>
</tbody>
</table>

In paw extract on Day 26, a 13-fold increase in TRACP-5b was observed in CIA animals (8.76 U/g of protein) when compared to Non-Disease animals (0.624 U/g of protein) (Figure 3b). There was no significant difference between CIA Control animals and Non-Disease animals on Day 26 (Figure 3a).

CONCLUSION
The authors would like to thank the technical team in invivo pharmacology, musculoskeletal/imaging, biomarkers and histopathology groups for their dedicated work.

ACKNOWLEDGMENT

1 INTRODUCTION

2 MATERIALS AND METHODS

3 RESULTS

Clinical Parameters
A decrease in body weights was observed in all arthritic rats for a few days after onset of arthritis, an anticipated effect in this model. Clinical signs of arthritis consisting of swollen, red paws and limited usage of the hindlimbs were observed in CIA animals from Day 4 onwards. The swelling and redness of the paw were confirmed by clinical score measurement. An increase in paw thickness was observed in all arthritic animals starting on Day 14 and was at the maximum observed score of 6 out of 8 from Day 16 until the end of the study. An increase in paw volume and thickness was observed in all arthritic rats from Day 14 onwards with a peak on Days 16/18 and remained elevated until completion of the study (Figure 1).

Bone Densitometry
On Day 10, lower gains in bone mineral content (BMC) and bone mineral density (BMD), which is considered a systemic effect of arthritis, were observed in the CIA group when compared to Non-Disease animals. There was no observable change in the tibiotarsal area. The effect of arthritis on bone was progressive and on Day 25, statistically significant differences were observed in all DXA parameters (area, BMC and BMD) between Non-Disease animals and CIA animals at both lumbar spine and tibiotarsal joint except for BMC at the tibial area (Table 2).

Bone Resorption Marker-TRACP-5b
An increase in serum TRACP-5b of 53% was observed on Day 16 in CIA animals when compared to Non-Disease animals. There was no significant difference between CIA Control animals and Non-Disease animals on Day 26 (Figure 3a).

Histopathology
H&E and Cathepsin K (Abcam ab19027) immunostained sections of tibiotarsal joints.

Table 3: Histopathological Score – Mean (SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Inflammation</th>
<th>Damage</th>
<th>Cartilage</th>
<th>Periosteal Exostotic Changes</th>
<th>Composite Score</th>
<th>Osteoclast Cellularity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>0.08 (0.08)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>1.83 (0.21)</td>
<td>1.83 (0.21)</td>
</tr>
<tr>
<td>CIA</td>
<td>4.00 (0.00)</td>
<td>4.00 (0.00)</td>
<td>3.83 (0.11)</td>
<td>3.92 (0.08)</td>
<td>19.58 (0.19)</td>
<td>4.00 (0.00)</td>
</tr>
</tbody>
</table>

In the Non-Disease group, osteoclasts were always present, generally found in small number and adjacent to the growth plate of the distal tibia, calcaneum and metatarsus. In contrast, this score was very low in the Non-Disease group (range: 0-1), inflammation being observed only in a single animal (Table 3, Figure 4).

4 CONCLUSION

In the rat type II collagen-induced arthritis model, increases in clinical score, paw volume and thickness have been observed systematically. The addition of bone-associated endpoints is also relevant in the evaluation of arthritis as demonstrated by the increase in serum TRACP-5b at disease onset, decreased BMD and increased tissue TRACP-5b at completion of study, as well as an increased histopathological arthritis score and osteoclast cellularity. Integration of these endpoints could be useful in the evaluation of potential compounds for rheumatoid arthritis.

5 ACKNOWLEDGMENT