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

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1 INTRODUCTION

The purpose of this study was to evaluate the effects of arthritis induced by collagen on different bone-associated 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.

2 MATERIALS AND METHODS

On Day 1, for all 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.

Table 1: Experimental Design

Group No.	Group	No. of Animals
		Females
1	Non-Disease Control	12
2	CIA	12

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 turnover (TRACP-5b; RatTRACP™ Assay by ImmunoDiagnostics Systems Limited)) and histopathologic examinations of H&E and Cathepsin K (Abcam ab19027) immunostained sections of tibiotarsal joints.

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 14 onwards. The swelling and redness of the paw were confirmed by clinical score measurement. An increase in score 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 16, lower gains in bone mineral content (BMC) and bone mineral density (BMD), which is considered a systemic consequence of inflammation, were noted at the lumbar spine for CIA animals 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 in all DXA parameters (area, BMC and BMD) were noted between Non-Disease animals and CIA animals at both lumbar spine and tibiotarsal jointm except for BMC at the tibiotarsal area (Table 2).

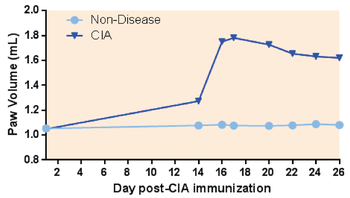

Figure 1a: Group Mean Paw Volume

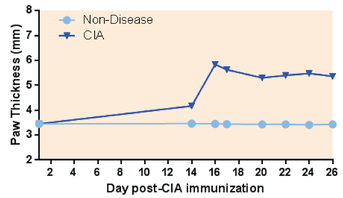

Figure 1b: Group Mean Paw Volume

Table 2: Bone Densitometry by DXA

		Lumbar spine			Tibiotarsal		
		Area (cm2)	BMC (g)	BMD (g/cm2)	Area (cm2)	BMC (g)	BMD (g/cm2)
Pre-Immunization	ND	0.910	0.126	0.138	0.271	0.061	0.226
	CIA	0.922	0.127	0.137	0.269	0.060	0.224
Day 16	ND	1.290	0.215	0.166	0.305	0.079	0.259
	CIA	1.242	0.194	0.156	0.314	0.080	0.254
Day 25	ND	1.378	0.243	0.176	0.319	0.085	0.267
	CIA	1.253	0.199	0.159	0.351	0.083	0.235

Bone Resorption Marker-TRACP-5b

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).

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).

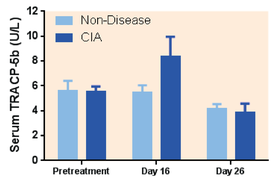

Figure 3a: Group Mean Serum TRACP-5b

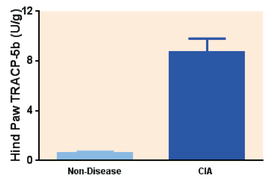

Figure 3b: Group Mean TRACP-5b from Paw Extract

Anatomical Pathology

Rats responded strongly and consistently to collagen administration. All CIA animals had a composite histopathologic arthritis at or close to the maximum possible score of 20 (range: 18-20). Inflammation, cartilage erosion, synovial hyperplasia, bone resorption and periosteal/exostotic changes were observed in all CIA animals. 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).

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 comparison, large number of osteoclasts was noted in CIA rats, in which the osteoclast population was most abundant at bone resorption sites. Small tarsal bones were the most severely affected bones but bone resorption also involved the talus, calcaneum, distal tibia and/or metatarsal extremities (Table 3; Figure 4).

Table 3: Histopathological Score – Mean (SEM)

Group	Inflammation	Cartilage Damage (Erosion)	Pannus (Synovial Hyperplasia)	Bone Resorption	Periosteal/ Exostotic Changes	Composite Score	Osteoclast Cellularity
1	0.08 (0.08)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.08 (0.08)	1.83 (0.21)
2	4.00 (0.00)	4.00 (0.00)	3.83 (0.11)	3.83 (0.11)	3.92 (0.08)	19.58 (0.19)	4.00 (0.00)

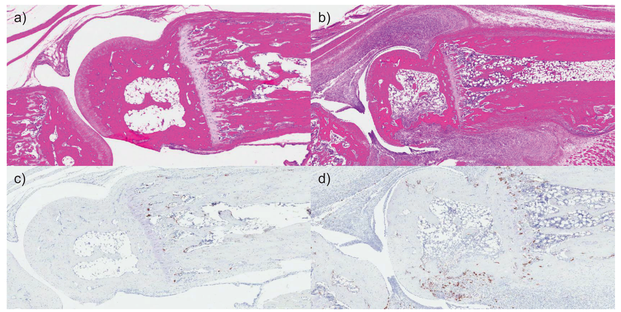

Figure 4: Metatarsus area; a) non-disease control rat, H&E (4X); b) CIA rat, H&E (3X); c) non-disease control rat, Cathepsin K (4X); d) CIA rat, Cathepsin K (4X)

4 CONCLUSION

In the rat type II collagen-induced arthritis model, increases in clinical score, paw volume and thickness have been observed historically. The addition of bone-associated endpoints is also reliable 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 increased histopathological arthritic score and osteoclast cellularity. Integration of these endpoints could be useful in the evaluation of potential compounds for rheumatoid arthritis.

5 ACKNOWLEDGMENT

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