Changes in epiphyseal bone, subchondral bone plate and epiphyseal trabecular bone in surgically and chemically induced rat models of osteoarthritis

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Introduction

Several experimental animal models have been developed for human osteoarthritis (OA) and used to study the preclinical efficacy of disease and symptom modifying OA drug candidates in various species[1,2]. The preclinical efficacy has been determined by numerous microscopic scoring systems and joint pain assessments. Recently, the histopathology initiative of Osteoarthritis Research Society International (OARSI) presented recommendations for histological OA assessment in different species in order to standardize preclinical efficacy studies. Recommendations for rat samples focus on articular cartilage, synovium, joint capsule, and growth plate[3]. When studying treatment effects on osteoarthritic rat bone, the histological OA assessment should be completed with the analysis of epiphyseal bone, subchondral bone plate and epiphyseal trabecular bone.

Aim of the study

This study characterized changes in epiphyseal bone, subchondral bone plate and epiphyseal trabecular bone and assessed knee joint discomfort, pain and degenerative changes in four rat OA models, induced by intra-articular mono-iodoacetate (MIA, 1 mg), medial meniscal tear combined with medial collateral ligament transection (MMT + MCLT), anterior cruciate ligament transection combined with partial medial meniscectomy (ACLT + pMMx), and ACLT alone.

Methods

Animal experimentation: Unilateral OA was induced in the knee joints of 3-month-old male Lewis rats (body weight 330 - 380 g) using the following rat OA models: 1) MIA at 1 mg, 2) MMT + MCLT, 3) ACLT + pMMx, and 4) ACLT. Body weight, static weight bearing and static secondary mechanical allodynia were followed during the in-life phase of the study. Knee joints were harvested at two time points in each model, as follows: at 2 and 4 weeks in the MIA model, at 3 and 6 weeks in the MMT + MCLT model, at 4 and 8 weeks in the ACLT + pMMx model, and at 5 and 10 weeks in the ACLT model.

Knee joint analyses: Static weight bearing was determined as hind paw weight distribution by Incapacitance Tester (Linton Instrumentation, Norfolk, UK) and static mechanical allodynia as paw withdrawal threshold by von Frey filaments (0.02-15.0 g; North Coast Medical, Morgan Hill, CA, USA). Histological OA assessment was performed by OARSI rat scoring system[3] and histological bone analysis separately in epiphyseal bone, subchondral bone plate and epiphyseal trabecular bone in three coronal sections obtained from the weight-bearing area of medial tibial plateau at 200 μm intervals and stained in Toluidine blue.
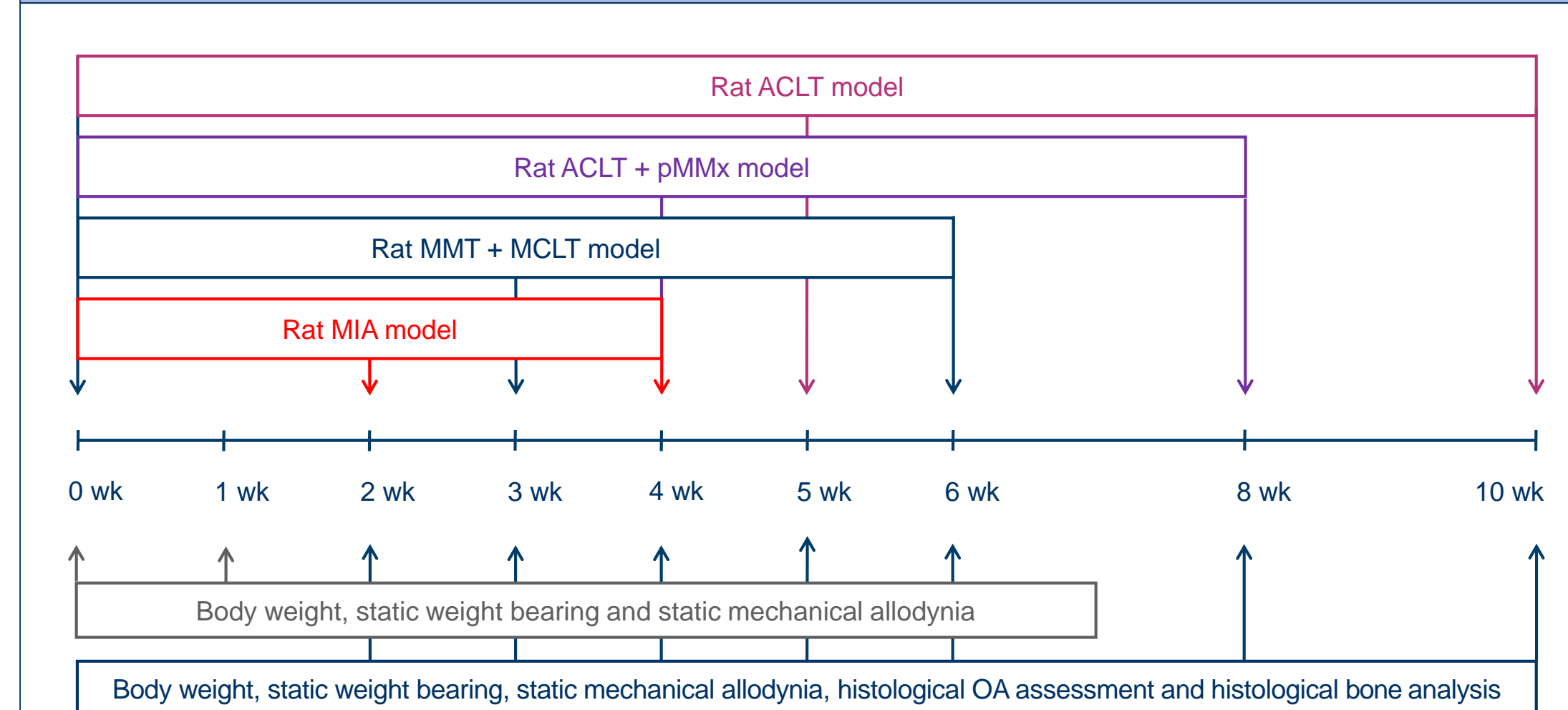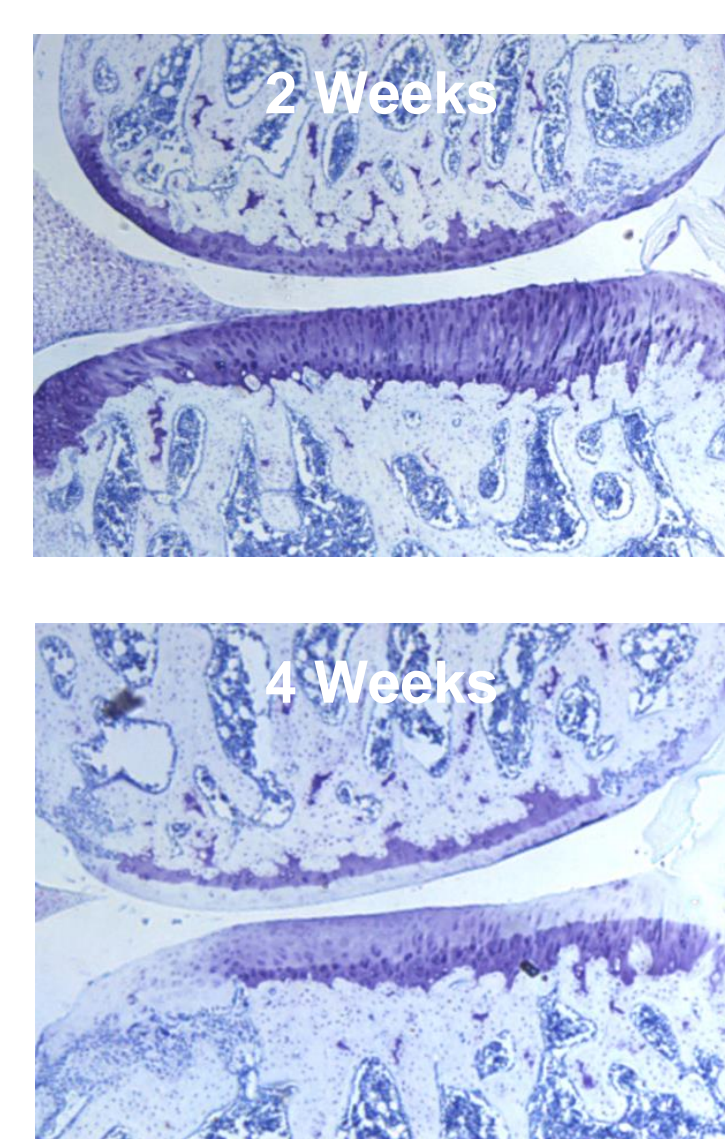
Study design

Fig. 1. This study included four rat OA models. Body weight, static weight bearing, static secondary mechanical allodynia and degenerative knee changes including changes in osteoarthritic bone were analyzed as presented above.

Rat MIA Model

Follow-up of body weight and OA symptoms	1 Week		4 Weeks	
	Control	MIA (1mg)	Control	MIA (1 mg)
Body weight (g)	349.1 ± 4.2	343.8 ± 2.5	394.1 ± 5.0	393.9 ± 5.9
Hind paw weight distribution (%)	51.4 ± 1.8	50.3 ± 1.9	53.6 ± 2.3	50.4 ± 2.2
Paw withdrawal threshold (g)	15.0 ± 0.0	6.1 ± 0.7 ***	15.0 ± 0.0	11.3 ± 1.6 *

| End-point measurements	Control	Intra-articular MIA (1 mg)	
		2 Weeks	4 Weeks
Histological OA assessment			
Total joint score (score 0-33)	0.50 ± 0.11	1.58 ± 0.37 *	4.10 ± 0.79 **
Tibial score (score 0-24)	0.50 ± 0.11	0.75 ± 0.27	3.81 ± 0.70 **
Cartilage degeneration width (mm)	0.27 ± 0.11	0.65 ± 0.05 **	1.04 ± 0.09 ***
Cartilage degeneration volume (%)	0.84 ± 0.38	2.36 ± 0.29 **	13.09 ± 3.10 **
Osteophyte width (μm)	186.2 ± 8.8	166.0 ± 15.1	176.8 ± 10.8
Synovial membrane inflammation (score 0-4)	0.00 ± 0.00	0.83 ± 0.14 ***	0.29 ± 0.15
Histological bone analysis			
Epiphyseal bone area fraction (B.Ar/T.Ar; %)	68.4 ± 1.1	59.0 ± 1.4 ***	60.3 ± 1.4 **
Subchondral bone area (B.Ar; mm²)	0.47 ± 0.01	0.33 ± 0.01 ***	0.38 ± 0.01 ***
Trabecular bone area fraction (B.Ar/T.Ar; %)	49.5 ± 0.9	41.1 ± 1.3 ***	39.7 ± 2.0 **
Trabecular number (Tb.N; mm⁻¹)	3.30 ± 0.08	3.52 ± 0.09	3.27 ± 0.09
Trabecular thickness (Tb.Th; μm)	151.3 ± 5.1	117.5 ± 4.3 ***	121.8 ± 6.7 **
Bone thickness proximal to growth plate (B.Th; μm)	226.6 ± 7.2	175.1 ± 2.6 ***	185.4 ± 8.5 **

Rat MMT + MCLT Model
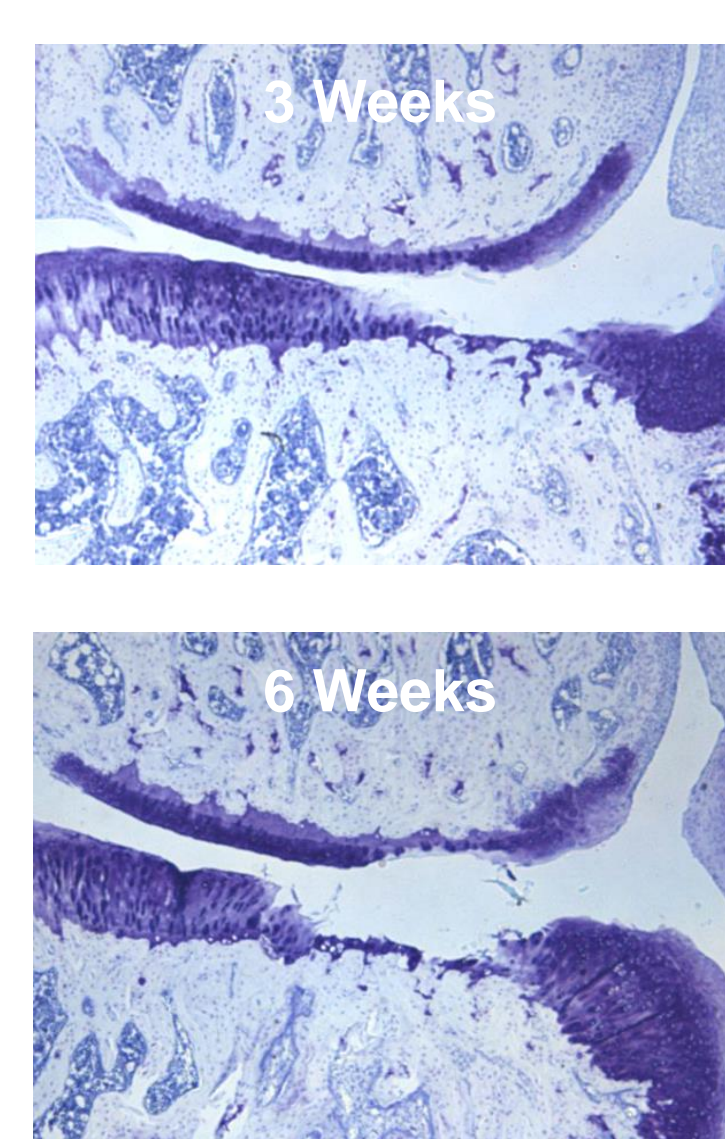
Follow-up of body weight and OA symptoms	1 Week		6 Weeks	
	Control	MMT + MCLT	Control	MMT + MCLT
Body weight (g)	349.1 ± 4.2	335.4 ± 2.9 *	415.9 ± 5.4	403.0 ± 6.5
Hind paw weight distribution (%)	48.6 ± 1.8	44.4 ± 1.0 *	51.8 ± 2.6	45.9 ± 3.4
Paw withdrawal threshold (g)	15.0 ± 0.0	7.8 ± 1.0 ***	15.0 ± 0.0	6.5 ± 0.8 ***

| End-point measurements	Control	MMT + MCLT	
		3 Weeks	6 Weeks
Histological OA assessment			
Total joint score (score 0-33)	0.67 ± 0.12	16.43 ± 0.34 ***	17.57 ± 1.32 ***
Tibial score (score 0-24)	0.67 ± 0.12	11.71 ± 0.39 ***	13.19 ± 0.91 ***
Cartilage degeneration width (mm)	0.39 ± 0.09	1.29 ± 0.10 ***	1.58 ± 0.10 ***
Cartilage degeneration volume (%)	1.52 ± 0.35	27.91 ± 2.87 ***	30.77 ± 4.18 ***
Osteophyte width (μm)	185.3 ± 8.9	475.2 ± 53.9 ***	576.5 ± 62.7 ***
Synovial membrane inflammation (score 0-4)	0.00 ± 0.00	2.14 ± 0.12 ***	2.05 ± 0.31 ***
Histological bone analysis			
Epiphyseal bone area fraction (B.Ar/T.Ar; %)	61.6 ± 0.9	68.7 ± 1.2 ***	72.3 ± 1.8 ***
Subchondral bone area (B.Ar; mm²)	0.41 ± 0.03	0.50 ± 0.02 *	0.58 ± 0.03 **
Trabecular bone area fraction (B.Ar/T.Ar; %)	42.0 ± 0.8	48.3 ± 1.2 **	50.9 ± 2.4 **
Trabecular number (Tb.N; mm⁻¹)	3.14 ± 0.08	3.31 ± 0.08	3.51 ± 0.16
Trabecular thickness (Tb.Th; μm)	133.9 ± 5.8	146.5 ± 5.7	146.9 ± 8.7
Bone thickness proximal to growth plate (B.Th; μm)	217.0 ± 6.9	192.6 ± 4.6 *	198.3 ± 3.0 *

Rat ACLT + pMMx Model
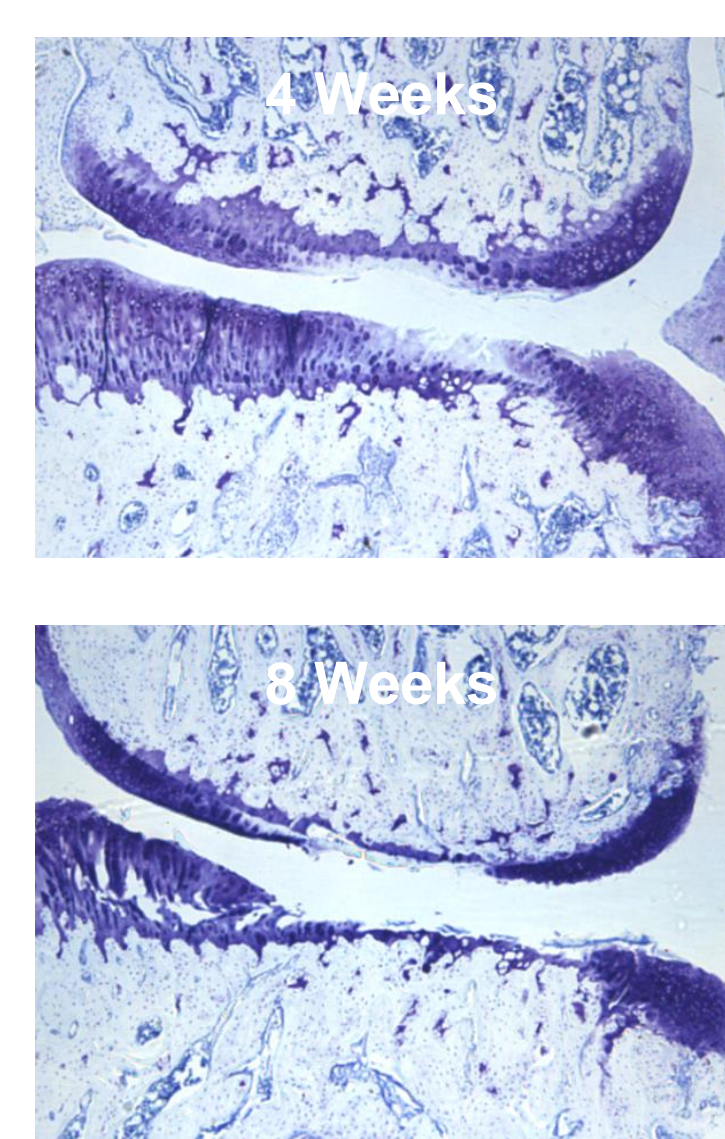
Follow-up of body weight and OA symptoms	1 Week		8 Weeks	
	Control	ACLT + pMMx	Control	ACLT + pMMx
Body weight (g)	349.1 ± 4.2	332.9 ± 3.0 **	436.7 ± 5.9	416.5 ± 5.5 *
Hind paw weight distribution (%)	48.6 ± 1.8	40.5 ± 1.3 **	48.5 ± 1.8	47.4 ± 1.9
Paw withdrawal threshold (g)	15.0 ± 0.0	7.3 ± 0.9 ***	15.0 ± 0.0	12.4 ± 1.3 *

End-point measurements	Control	ACLT + pMMx	
		4 Weeks	8 Weeks
Histological OA assessment			
Total joint score (score 0-33)	0.67 ± 0.12	14.17 ± 2.08 ***	15.89 ± 2.56 ***
Tibial score (score 0-24)	0.67 ± 0.12	9.88 ± 1.81 ***	12.06 ± 2.00 ***
Cartilage degeneration width (mm)	0.39 ± 0.09	1.34 ± 0.11 ***	1.56 ± 0.20 ***
Cartilage degeneration volume (%)	1.52 ± 0.35	24.26 ± 6.02 **	28.47 ± 8.43 **
Osteophyte width (μm)	185.3 ± 8.9	423.2 ± 56.6 **	462.3 ± 31.8 ***
Synovial membrane inflammation (score 0-4)	0.00 ± 0.00	2.17 ± 0.20 ***	1.44 ± 0.20 ***
Histological bone analysis			
Epiphyseal bone area fraction (B.Ar/T.Ar; %)	61.6 ± 0.9	65.8 ± 2.8	71.7 ± 2.6 **
Subchondral bone area (B.Ar; mm²)	0.41 ± 0.03	0.51 ± 0.06	0.46 ± 0.05
Trabecular bone area fraction (B.Ar/T.Ar; %)	42.0 ± 0.8	44.9 ± 2.6	47.5 ± 2.5 *
Trabecular number (Tb.N; mm⁻¹)	3.14 ± 0.08	3.34 ± 0.08	3.34 ± 0.19
Trabecular thickness (Tb.Th; μm)	133.9 ± 5.8	136.6 ± 10.5	142.0 ± 4.4
Bone thickness proximal to growth plate (B.Th; μm)	217.0 ± 6.9	187.1 ± 10.2 *	206.2 ± 9.7

Rat ACLT Model
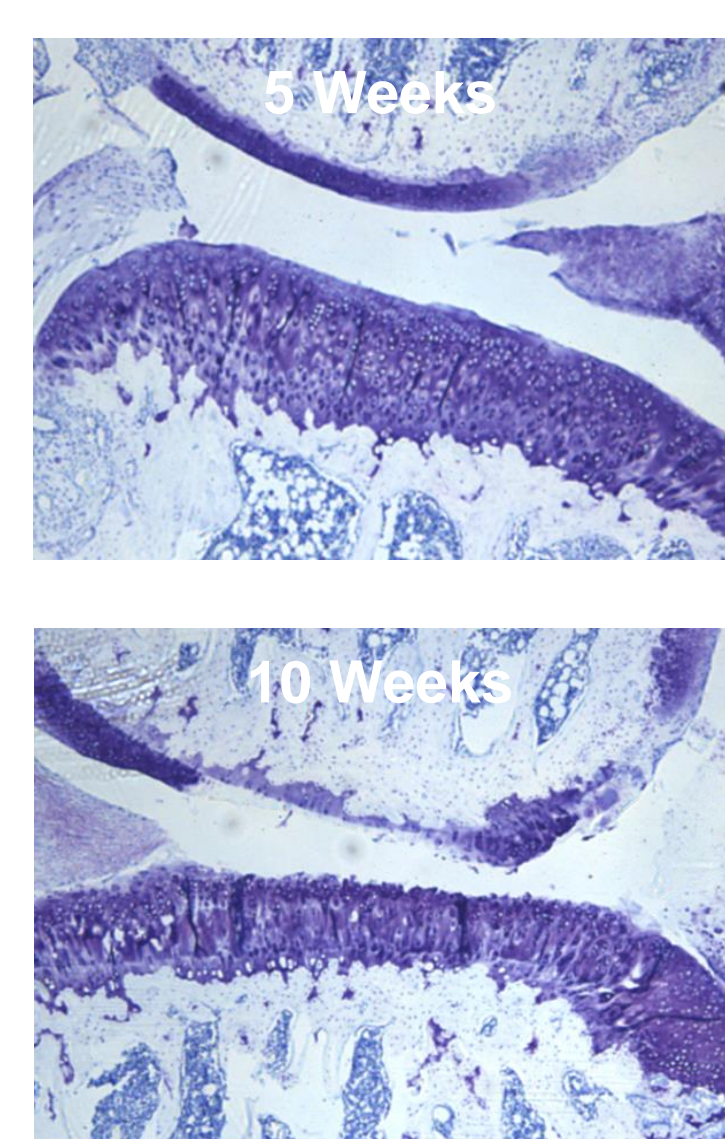
Follow-up of body weight and OA symptoms	1 Week		10 Weeks	
	Control	ACLT	Control	ACLT
Body weight (g)	349.1 ± 4.2	337.7 ± 2.6 *	453.9 ± 5.5	449.4 ± 5.9
Hind paw weight distribution (%)	48.6 ± 1.8	36.5 ± 2.0 ***	51.2 ± 2.2	47.8 ± 2.3
Paw withdrawal threshold (g)	15.0 ± 0.0	7.9 ± 0.9 ***	15.0 ± 0.0	12.9 ± 1.1 *

End-point measurements	Control	ACLT	
		5 Weeks	10 Weeks
Histological OA assessment			
Total joint score (score 0-33)	0.67 ± 0.12	3.29 ± 0.87 *	11.53 ± 4.41 *
Tibial score (score 0-24)	0.67 ± 0.12	2.33 ± 0.68 *	8.27 ± 3.54 *
Cartilage degeneration width (mm)	0.39 ± 0.09	0.66 ± 0.08 *	1.20 ± 0.21 **
Cartilage degeneration volume (%)	1.52 ± 0.35	5.40 ± 3.42	19.47 ± 10.8
Osteophyte width (μm)	185.3 ± 8.9	256.8 ± 20.7	335.8 ± 47.6 *
Synovial membrane inflammation (score 0-4)	0.00 ± 0.00	0.95 ± 0.29 *	1.00 ± 0.26 **
Histological bone analysis			
Epiphyseal bone area fraction (B.Ar/T.Ar; %)	61.6 ± 0.9	55.9 ± 2.1 *	67.0 ± 4.5
Subchondral bone area (B.Ar; mm²)	0.41 ± 0.03	0.27 ± 0.03 **	0.28 ± 0.01 **
Trabecular bone area fraction (B.Ar/T.Ar; %)	42.0 ± 0.8	36.8 ± 2.0 *	47.4 ± 4.8
Trabecular number (Tb.N; mm⁻¹)	3.14 ± 0.08	3.63 ± 0.17 *	3.65 ± 0.25
Trabecular thickness (Tb.Th; μm)	133.9 ± 5.8	102.0 ± 4.4 **	129.2 ± 5.6
Bone thickness proximal to growth plate (B.Th; μm)	217.0 ± 6.9	165.4 ± 5.1 ***	190.6 ± 6.8 *

Summary

➢ Rat MIA model exhibited a reduction in the amount of epiphyseal bone, subchondral bone plate and epiphyseal trabecular bone at 2 and 4 weeks.

➢ This reduction in bone mass was associated with mild to moderate degenerative changes and a reduction in paw withdrawal threshold.

➢ Rat MMT+MCLT model demonstrated an increase in the amount of epiphyseal bone, subchondral bone plate and epiphyseal trabecular bone at 3 and 6 weeks.

➢ This gain in bone mass was observed together with moderate to severe degenerative changes and a reduction in paw withdrawal threshold.

➢ Rat ACLT+pMMx model showed an increase in the amount of epiphyseal bone and epiphyseal trabecular bone at 8 weeks.

➢ This increase in bone mass was associated with moderate to severe degenerative changes and a reduction in paw withdrawal threshold.

➢ Rat ACLT model exhibited a reduction in the amount of epiphyseal bone and epiphyseal trabecular bone at 5 weeks and in the amount of subchondral bone plate at 5 and 10 weeks.

➢ This decrease in bone mass was associated with mild to moderate degenerative changes and a reduction in paw withdrawal threshold.

Conclusions

The amount of epiphyseal bone, subchondral bone plate and epiphyseal trabecular bone decreased in the chemically induced rat OA model (MIA) and in the surgically induced rat OA model (ACLT) exhibiting mild to moderate OA changes. The amount of epiphyseal bone, subchondral bone plate and/or epiphyseal trabecular bone increased in the surgically induced rat OA models (MMT+MCLT and ACLT+pMMx) exhibiting moderate to severe OA changes.

References

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