

Differences in the Nature of Osteoarthritis between Surgically and Chemically Induced Rat Models

Jukka Morko, ZhiQi Peng, Jukka Vääräniemi, Katja M. Fagerlund, Jukka P. Rissanen, Jenni Bernoulli, Jussi M. Halleen

Pharmatest Services Ltd, Turku, Finland

E-mail correspondence to Jukka Morko (jukka.morko@pharmatest.com)

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} These animal models have been characterized by numerous microscopic scoring systems and joint pain assessments. The histopathology initiative of Osteoarthritis Research Society International (OARSI) has presented recommendations for histological OA assessment in different species in order to standardize the histopathological evaluation of OA in these animal models. Recommendations for rat samples focus on articular cartilage, synovium, joint capsule, and growth plate.³ As many OA drug candidates affect also bone tissue, the histopathological evaluation of OA should be completed with the analysis of epiphyseal bone, subchondral bone plate and epiphyseal trabecular bone.

Objectives

The aim of this study was to characterize knee joint tissues including articular cartilage, synovium and epiphyseal bone together with knee joint discomfort/pain in four rat OA models, induced by intra-articular monoiodoacetate (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 system³ 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.³

Statistical analyses: All data is presented as mean ± standard error of mean. All statistical analyses were performed as two-sided tests as presented in the study abstract in more detailed. All rats in OA groups were compared with their healthy control rats (CONT).

Study design

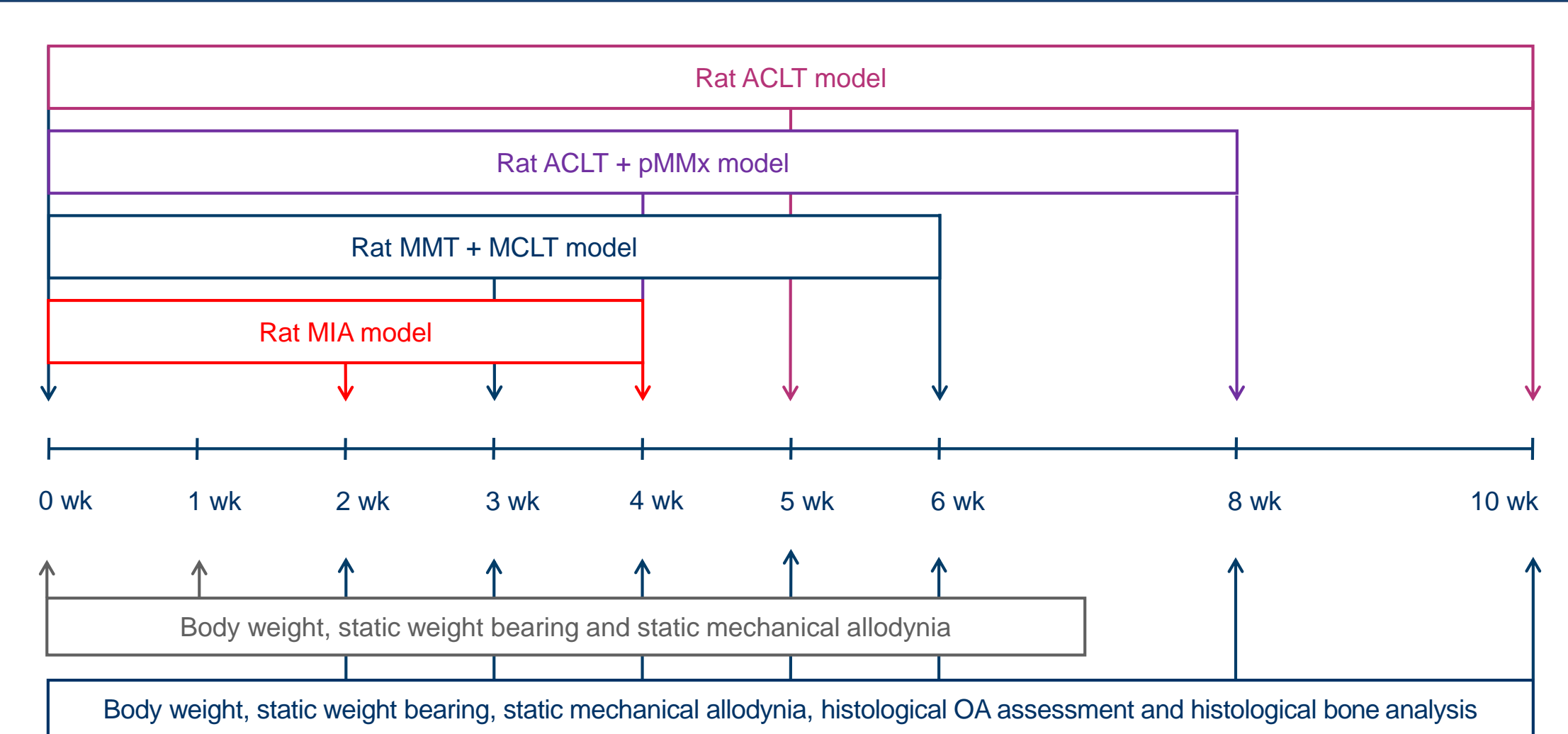
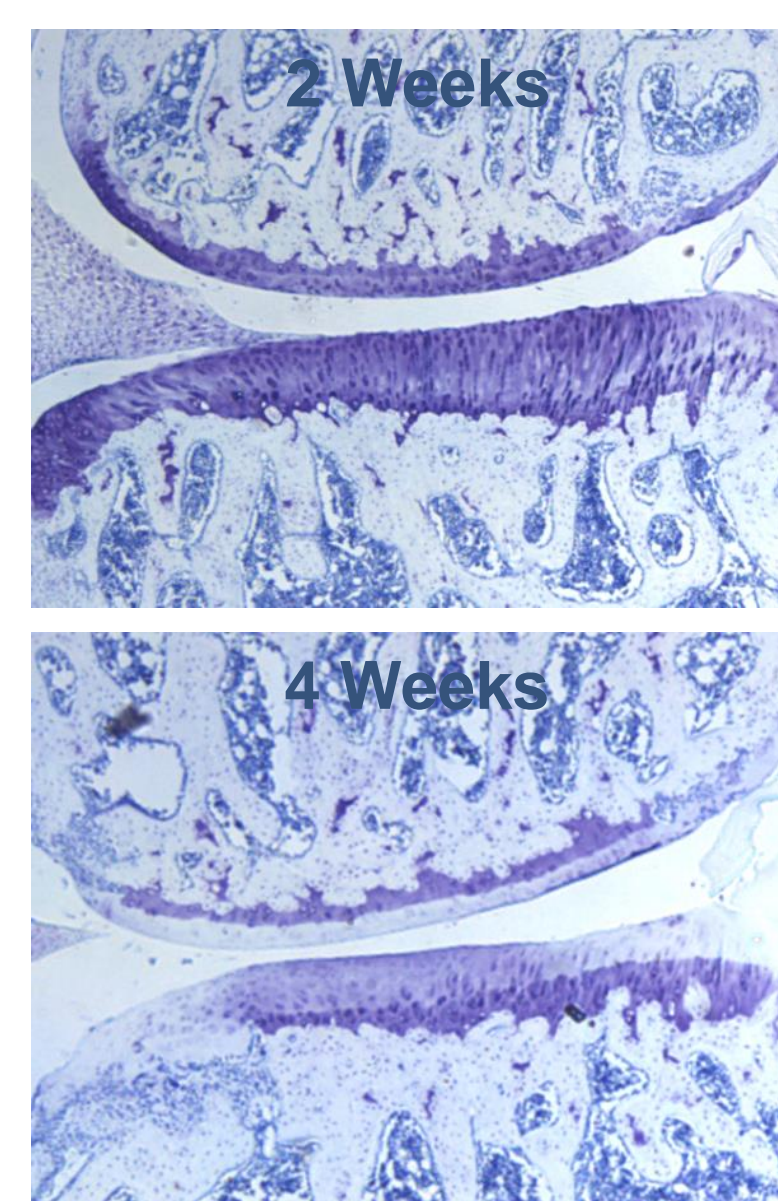


Figure 1. This study included four rat OA models. Body weight, static weight bearing, static secondary mechanical allodynia and degenerative knee changes including changes in articular cartilage, synovium and epiphyseal bone were analyzed as presented above.

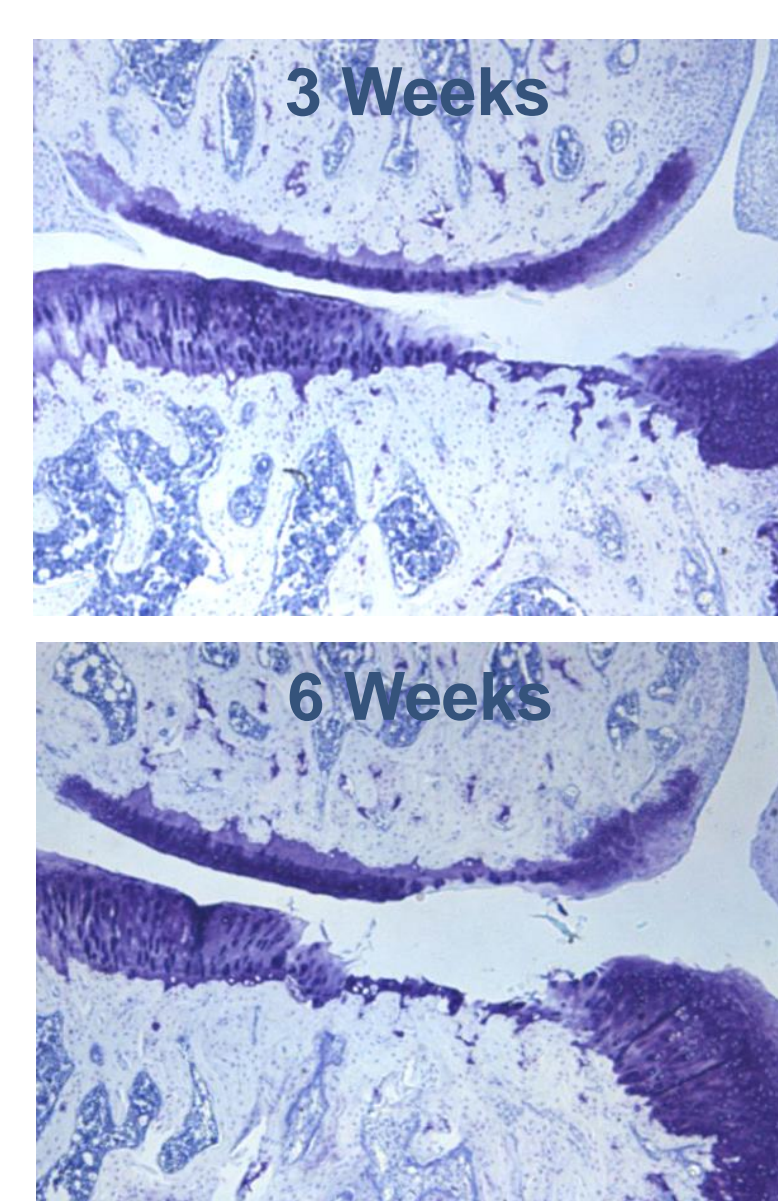
Rat MIA Model



Follow-up of body weight and OA symptoms	1 Week		4 Weeks	
	Control	MIA (1 mg)	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 volume (mm)	0.27 ± 0.11	0.67 ± 0.05	4.04 ± 0.09 ***
Cartilage degeneration volume (%)	0.84 ± 0.38	2.36 ± 0.29 *	13.09 ± 3.10 **
Osteophyte width (µm)	186.2 ± 8.79	165.0 ± 15.0	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 ± 1.14	59.0 ± 1.35 **	60.3 ± 1.37 **
Subchondral bone area (B.Ar; mm ²)	0.47 ± 0.01	0.33 ± 0.01 **	0.38 ± 0.01 **
Trabecular bone area fraction (B.Ar/T.Ar; %)	49.5 ± 0.95	41.1 ± 1.28 **	39.7 ± 2.04 **
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 ± 5.05	117.5 ± 4.31 **	121.8 ± 6.72 *
Bone thickness proximal to growth plate (B.Th; µm)	226.6 ± 7.23	175.1 ± 2.62 ***	185.4 ± 8.51 **

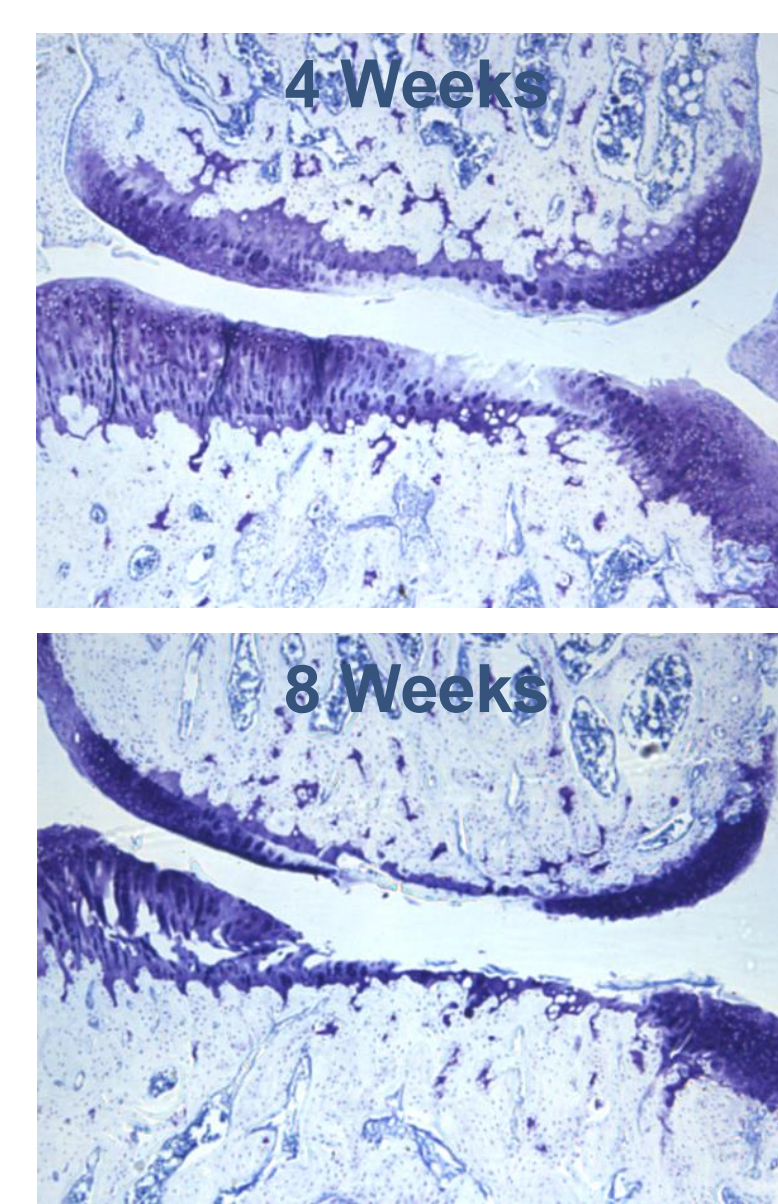
Rat MMT + MCLT Model



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 (%)	51.4 ± 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 ± 8.95	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 ± 0.93	68.7 ± 1.18 **	72.3 ± 1.83 **
Subchondral bone area (B.Ar; mm ²)	0.41 ± 0.03	0.50 ± 0.02 *	0.58 ± 0.03 *
Trabecular bone area fraction (B.Ar/T.Ar; %)	42.0 ± 0.83	48.3 ± 1.16 *	50.9 ± 2.40 *
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 ± 5.78	146.5 ± 5.68	146.9 ± 8.68
Bone thickness proximal to growth plate (B.Th; µm)	217.0 ± 6.92	192.6 ± 4.61	198.3 ± 3.05

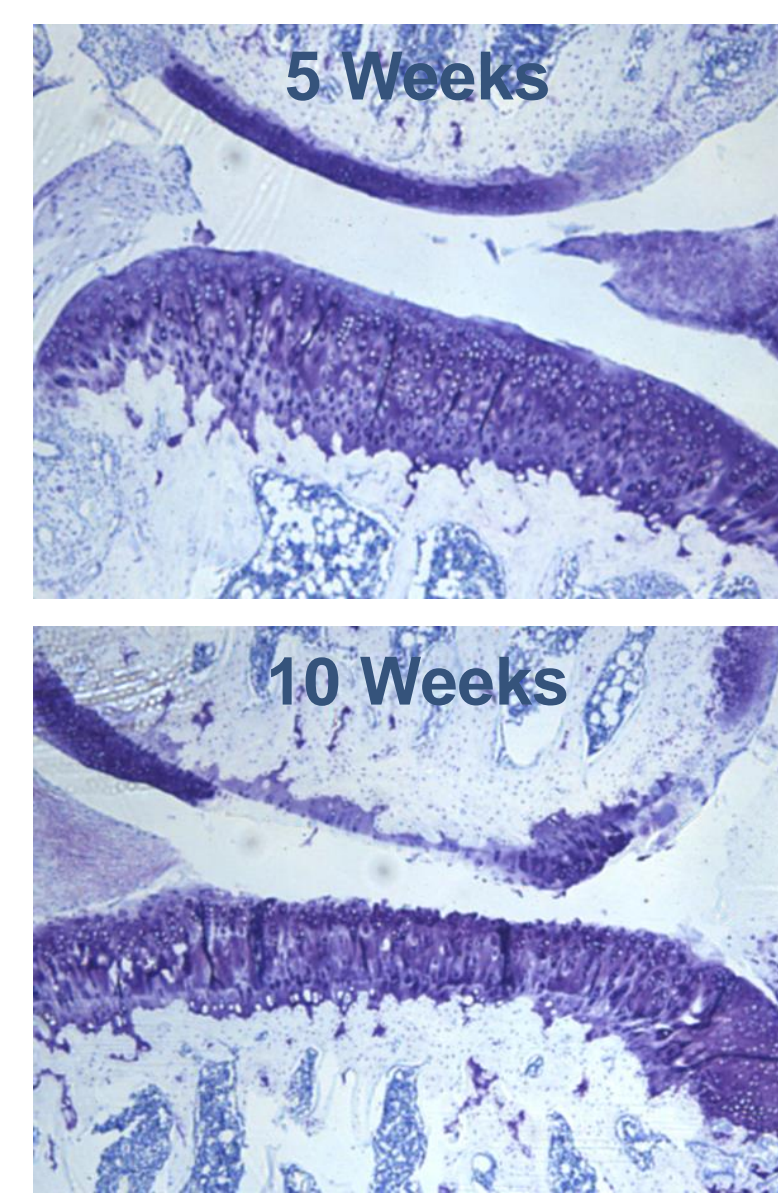
Rat ACLT + pMMx Model



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 (%)	51.4 ± 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 ± 8.95	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 ± 0.93	65.8 ± 2.84	71.7 ± 2.59 **
Subchondral bone area (B.Ar; mm ²)	0.41 ± 0.03	0.51 ± 0.06	0.46 ± 0.05
Trabecular bone area fraction (B.Ar/T.Ar; %)	42.0 ± 0.83	44.9 ± 2.61	47.5 ± 2.49
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 ± 5.78	136.6 ± 10.5	142.0 ± 4.36
Bone thickness proximal to growth plate (B.Th; µm)	217.0 ± 6.92	187.1 ± 10.2 *	206.2 ± 9.71

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 (%)	51.4 ± 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 ± 8.95	256.8 ± 20.0 *	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 ± 0.93	55.9 ± 2.07 *	67.0 ± 4.45
Subchondral bone area (B.Ar; mm ²)	0.41 ± 0.03	0.27 ± 0.03 *	0.29 ± 0.01 *
Trabecular bone area fraction (B.Ar/T.Ar; %)	42.0 ± 0.83	36.8 ± 2.02 *	47.4 ± 4.75
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 ± 5.78	102.0 ± 4.40 **	129.2 ± 6.64
Bone thickness proximal to growth plate (B.Th; µm)	217.0 ± 6.92	165.4 ± 5.11 ***	190.6 ± 6.81

Summary

- Rat MIA model exhibited from mild to moderate degenerative knee changes (including cartilage degeneration and mild synovial inflammation), reduced amount of epiphyseal, subchondral and trabecular bone, and neuropathic pain at 2 and 4 weeks post-injection.
- Rat MMT+MCLT model demonstrated from moderate to severe degenerative knee changes (including cartilage degeneration, osteophytes and synovial inflammation), increased amount of epiphyseal, subchondral and trabecular bone, and neuropathic pain at 3 and 6 weeks post-surgery.
- Rat ACLT+pMMx model exhibited from moderate to severe degenerative knee changes (including cartilage degeneration, osteophytes and synovial inflammation) and neuropathic pain without changes in epiphyseal bone at 4 weeks and with an increased amount of epiphyseal bone at 8 weeks post-surgery.
- Rat ACLT model demonstrated moderate degenerative knee changes (including osteophytes and mild synovial inflammation), reduced amount of epiphyseal, subchondral and trabecular bone, and neuropathic pain at 5 weeks post-surgery as well as more severe degenerative knee changes (including cartilage degeneration), reduced amount of subchondral bone and neuropathic pain at 10 weeks post-surgery.

Conclusions

The study characterized knee joint tissues including articular cartilage, synovium and epiphyseal bone together with knee joint discomfort/pain in four rat OA models used frequently in preclinical efficacy studies. Study results demonstrated apparent differences in the nature of OA between these surgically and chemically induced models. **This study can be used to evaluate and select appropriate rat OA models for testing the preclinical efficacy of disease and symptom modifying OA drug candidates.**

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