

Epiphyseal Bone, Subchondral Bone Plate and Epiphyseal Trabecular Bone in Surgically and Chemically Induced Rat Models of Osteoarthritis

Jukka Morko, ZhiQi Peng, Jukka Vääräniemi, Katja M Fagerlund, Jukka P Rissanen, Jenni Bernoulli, and 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 and the preclinical efficacy of OA drug candidates has been studied by using 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 assessment of OA. 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 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.

Materials and Methods

Animal experimentation: The study was conducted using male Lewis rats that were 3 months of age at the beginning of the study (body weight 330-380 g). Unilateral OA was induced in their knee joints by applying the following four OA animal models: 1) MIA at 1 mg; 2) MMT + MCLT; 3) ACLT + pMMx; and 4) ACLT. Body weight and knee joint discomfort/pain were assessed during the study. Knee joints were harvested at two time points in each model as follows (n = 6-8 per model per time point): at 2 and 4 weeks post-injection in the MIA model, at 3 and 6 weeks post-surgery in the MMT+MCLT model, at 4 and 8 weeks post-surgery in the ACLT+pMMx model, and at 5 and 10 weeks post-surgery in the ACLT model. This experimental protocol was approved by National Animal Experiment Board, Regional State Administrative Agency for Southern Finland, Hämeenlinna, Finland.

Knee joint analyses: Knee joint discomfort/pain was analyzed as static weight bearing and static secondary mechanical allodynia. The weight bearing was determined as hind paw weight distribution using Incapacitate Tester (Linton Instrumentation, Norfolk, UK) and the mechanical allodynia as paw withdrawal threshold using von Frey monofilaments (0.02-15.0 g; North Coast Medical, Morgan Hill, CA, USA). Histological OA assessment was performed according to the recommendations of the OARSI histopathology initiative followed by the analysis of 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 and all rats in OA groups were compared with their healthy control rats (CONT). If the assumptions of parametric tests (i.e. the continuous nature of data, the normality of data distribution and the homogeneity of variances) were fulfilled as such or after data transformations, one-way analysis of variance and Dunnett's test were applied for evaluating statistical significances. Otherwise, statistical significances were evaluated using non-parametric Kruskal-Wallis and Mann-Whitney U tests.

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 width (mm)	0.27 ± 0.11	0.67 ± 0.05	1.04 ± 0.09 ***
Cartilage degeneration volume (%)	0.84 ± 0.38	2.36 ± 0.29 *	13.08 ± 3.10 **
Osteophyte width (µm)	186.2 ± 8.79	166.0 ± 15.0	176.7 ± 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 (Tb.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 above growth plate (B.Th; µm)	226.6 ± 7.23	175.1 ± 2.62 ***	185.4 ± 8.51 **

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 (Tb.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 above growth plate (B.Th; µm)	217.0 ± 6.92	187.1 ± 10.2 *	206.2 ± 9.71

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 (Tb.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 above growth plate (B.Th; µm)	217.0 ± 6.92	192.6 ± 4.61	198.3 ± 3.05

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 *	0.28 ± 0.01 *
Trabecular bone area fraction (Tb.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 ± 5.64
Bone thickness above growth plate (B.Th; µm)	217.0 ± 6.92	165.4 ± 5.11 ***	190.6 ± 6.81

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 post-injection.
- 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 post-surgery.
- 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 at 8 weeks post-surgery.
- 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, subchondral bone and epiphyseal trabecular bone at 5 weeks and in the amount of subchondral bone plate at 10 weeks post-surgery.
- This decrease in bone mass was observed together with mild to moderate degenerative changes and a reduction in paw withdrawal threshold.

Conclusions

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

Acknowledgements

We are grateful to Johanna Örling, Anniina Luostarinen, Natalia Hailainen-Kirillov, Riikka Kytömaa, Suvi Suutari and Jani Seppänen for their expert technical assistance.

References

1. Poole R, Blake S, Buschmann M, Goldring S, Lavery S, Lockwood S, et al. Osteoarthritis Cartilage. 2010;18:S10-S16.
2. Martel-Pelletier J, Lukas M, Pelletier J-P. Bone. 2012;51:297-311.
3. Gerwin N, Bendele AM, Glasson S, Carlson CS. Osteoarthritis Cartilage. 2010;18:S24-S34.