



Decalcified Bone Sections in Surgically-Induced Osteoarthritis in Rat

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Introduction

Osteoarthritis (OA) is a degenerative joint disease that causes the breakdown of the joint's cartilage. It is one of the oldest and most common types of arthritis affecting more than 20 million adults in the United States and is estimated by 2030 to affect 70 million Americans. The surgically induced animal model of osteoarthritis in rats is used to study the pathogenesis of cartilage degeneration and to evaluate potential anti-arthritis drugs for clinical use. Surgically creating a medial meniscal tear results in rapid and severe cartilage degeneration that resembles



those occurring in some stages of human osteoarthritis. These morphological changes can only be demonstrated through the histological preparation of the knee joint. Histological evaluation is performed by analyzing the decalcified, paraffin embedded Toluidine Blue sections to determine the severity of cartilage degeneration, osteophyte formation and efficacy of the potential drug candidate.

Figure 1. Photomicrograph of the medial aspect of a knee joint from rat which had medial meniscal tear surgery. Stained with toluidine blue, original magnification = 50X.

Materials & Methods

Animal Model

The surgically induced rat medial meniscal tear is performed on 300 - 400 gram rats. The medial collateral ligament is transected and the medial meniscus is reflected medially toward the femur and cut. The cut is made through the full thickness to simulate a complete tear. As a result rapid cartilage degeneration occurs within 3 weeks post surgery. Testing of potential anti-arthritis drugs is initiated prior to or post surgery and continued for 3 weeks with 20 animals per treatment group to account for variability in lesion severity. At necropsy, the right knee joint is trimmed of muscle, connective tissue and the patella is removed to allow proper fixation of the joint. The femur and tibia are transected with a rongeur some distance from the joint to avoid fragmentation of the bone around the joint area. The joint is then placed in 10% Neutral Buffered Formalin (fixative) and allowed to assume a natural degree of flexion.



Figure 2. Gross photograph of rat knee demonstrating normal flexion. The majority of the connective tissue and muscle have been removed along with the patella to allow penetration of the fixative into the joint.

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Histology

The knee joints are fixed in 10% Neutral Buffered Formalin for 48 hours and then placed into decal (5% formic acid) for 3-6 days. Once decalcified the knee joints are cut into approximately 2 equal halves in the frontal plane, using the collateral ligament as a landmark.

The joints are processed for paraffin embedding and sectioned at 8um for toluidine blue staining. An initial section is cut followed by two step cuts at 200um intervals yielding a total of 3 sections per knee.



Figure 3. A. After several days in decal the ends of the bone are trimmed and the knee joint is placed in a forceps along the patellar groove to hold the orientation while cutting in half. The collateral ligament is used as a landmark to achieve two equal halves, which is the critical step in grossing.

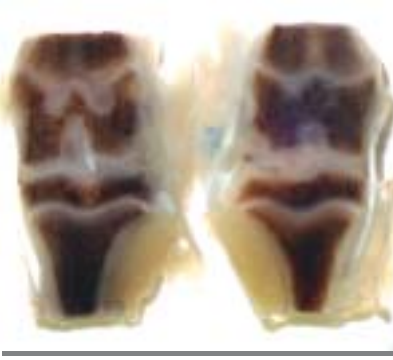


Figure 3. B. Demonstration of what is achieved when using the collateral ligament as a guide to cut the knee joint into two equal halves.

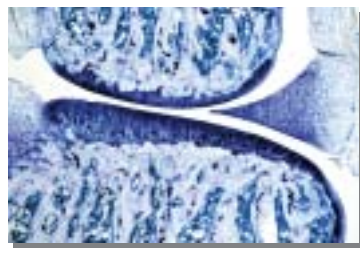


Figure 4. A. Photomicrograph of medial aspect of a normal knee joint. There is no chondrocyte or proteoglycan loss. Toluidine blue, original magnification = 50X.

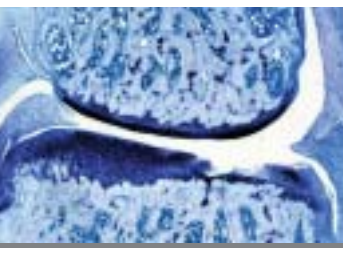


Figure 4. B. Photomicrograph of medial aspect of knee joint from rat which had unilateral medial meniscal tear surgery 3 weeks previously. Focal severe cartilage degeneration characterized by chondrocyte and proteoglycan loss as well as fibrillation is present on the medial tibial plateau. Toluidine blue, original magnification = 50X.

Results

The knee joints are evaluated for severity of the medial femoral and medial tibial cartilage degeneration and osteophyte formation. The primary area that is evaluated for cartilage damage is the medial tibia. The medial tibia is divided into 3 zones (inner, middle, outer), each zone is scored separately. The following system is used to score the cartilage degeneration in each zone.

Initially the depth of chondrocyte and proteoglycan loss with fibrillation is calculated by using the following criteria.

- 1= minimal superficial zone only
- 2= mild extends into the upper middle zone
- 3= moderate well into the middle zone
- 4= marked into the deep zone but not to tidemark
- 5= severe full thickness degeneration to tidemark

Then the area of cartilage degeneration involved is assessed as 1/3, 2/3, or 3/3 of the surface of the section and the score is then multiplied by 1, 2, or 3 to reflect the extent of the tibial plateau that is involved.

For tibial degenerative change, a micrometer measurement is taken across the portion of medial tibia that demonstrates any cartilage degeneration. Then a second measurement is taken of the area of degeneration that has resulted in significant matrix loss (greater than 50% of the cartilage thickness) in an effort to further quantitate the more serious changes.

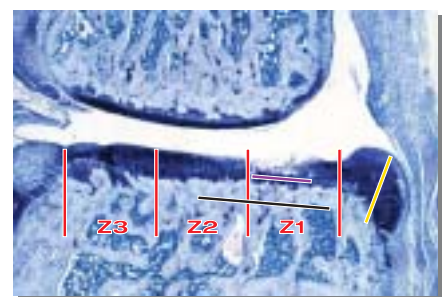


Figure 5. Photomicrograph of knee joint that has been assessed histologically for cartilage degeneration and osteophyte formation. The red lines divide the tibial plateau into 3 zones, Z1, Z2 and Z3. A micrometer measurement is taken across areas of degeneration, the purple line denotes the significant matrix loss. The osteophyte formation measurement is denoted by the yellow line.

Osteophytes are measured and categorized into small, medium and large using an ocular micrometer. Osteophyte Evaluation is determined by the following criteria:

- 1= small up to 299um
- 2= moderate 300 - 399um
- 3= large 400 or greater um

Study Information	Sec: Male	Animal Number: 1	Disposition: Killed Terminal					
Right Knee - level 1	Outside	Medial Tibia	Inside	Score	Medial Femur	Score	Osteophyte	Score
Total Width/Sig. Width (um) - Level 1	1800	200	200	170	250	20	200	
Relative Depth - level 1	0.14	0.5	0.1	0.27				
Right Knee - level 2	2	100	2	1	5	NSL	0	Large
Total Width/Sig. Width (um) - Level 2	50	200	100	200	50	300	30	200
Relative Depth - level 2	0.25	0.5	0.17	0.1				
Right Knee - level 3	2	1000	2	0	4	NSL	0	Large
Total Width/Sig. Width (um) - Level 3	50	300	20	180	20	250	0	300
Relative Depth - level 3	0.25	0.11	0.06	0				
Mean Right Knee (by zone)	2		2.33		1			
Mean Total/Sig. Right Width	1933		133					
Mean Relative Depth	0.35	0.38	0.17	0.24				
Mean level 1			Mean level 2					
Mean level 3			Mean level 4					
Mean Right Medial Tibia	5.3	Mean Right Medial Femur	0.0	Mean Right Osteophyte	3.0			
Total Right Medial Tibia and Right Medial Femur Cartilage Degeneration	5.3	Mean Right Osteophyte Measurement	400					
Total Right Knee	8.3							

Table 1. Individual Animal Histopathology Report. The table is created in Microsoft Excel to generate and tabulate the data taken from the joint sections.

Conclusions

The information acquired from the histopathology allows the evaluation of potential anti-arthritis drugs. The results acquired from this animal model of disease shows the effects of compounds on proteoglycan degradation and osteophyte formation. In addition it may detect activity of metalloproteinase inhibitors and other anti-arthritis compounds. This model mimics human traumatic osteoarthritis since it results in morphological changes with comparative pathogenesis to human disease.

Since the data generated from the histology cannot be captured by any other means it is important that each specimen is handled appropriately. Great care must be taken to assure that the knee joint is bisected into two equal halves, processed and embedded properly so that the subsequent levels demonstrate with consistency the lesion severity.

Vehicle	Medial Tibia	Medial Tibia	Medial Tibia	Medial Tibia	Total Tibial	Significant	Depth Ratio	Medial Tibia	Medial Tibia	Medial Tibia	Total	Total	
Animal #	Cartilage	Cartilage	Cartilage	Cartilage	Cartilage	Tibial	Any Matrix	Osteophyte	Osteophyte	Femur	Joint Score	Joint	
	Score*	Score	Score	Score	Score	Change	Score	Score	Score	Score	Score	Score	
	Zone 1 (Outer)	Zone 2 (Middle)	Zone 3 (Inner)	Zone 3	Width (um)	Depth	Width (um)	Depth	Width (um)	Depth	Score	Score	
1	R	7.87	5.00	2.33	0.33	1787	767	0.39	2.00	313	1.33	8.87	11.00
2	R	5.87	3.87	1.87	0.33	1393	882	0.49	3.00	386	3.67	8.87	10.33
3	R	6.87	2.87	1.87	0.33	2082	582	0.49	2.00	357	1.33	8.87	10.00
4	R	3.87	1.87	0.87	0.33	1387	0	0.21	0.00	330	1.00	8.87	7.87
5	R	5.87	4.87	2.87	0.33	1887	687	0.39	2.00	417	2.00	8.87	10.00
6	R	6.87	4.87	2.87	0.33	1487	687	0.48	2.00	455	1.00	8.87	10.00
7	R	5.87	3.87	1.87	0.33	1393	133	0.24	3.00	450	0.00	8.33	8.33
8	R	5.87	3.87	1.87	0.33	1287	333	0.39	2.00	357	0.00	8.87	9.87
9	R	6.87	2.87	1.87	0.33	1887	782	0.52	3.00	587	0.33	8.33	8.87
10	R	4.87	2.87	1.87	0.33	1393	387	0.33	3.00	486	0.67	7.00	7.67
11	R	3.87	2.87	1.87	0.33	1387	0	0.11	2.00	343	0.00	5.00	5.00
12	R	5.87	3.87	1.87	0.33	1487	687	0.39	1.00	393	0.00	8.33	8.33
13	R	5.87	3.87	1.87	0.33	1487	687	0.48	2.00	357	0.00	7.00	6.33
14	R	0.87	0.87	0.87	0.00	0	0	0.00	1.00	280	0.00	1.00	1.00
15	R	6.87	5.87	3.87	0.33	1133	728	0.65	2.00	327	0.00	8.33	8.33
16	R	3.87	2.87	1.87	0.33	1393	0	0.17	0.00	480	0.00	8.33	8.33
17	R	2.87	0.87	0.87	0.33	1887	0	0.10	0.00	420	0.00	5.00	5.00
18	R	7.87	5.87	3.87	0.33	1133	900	0.67	3.00	400	0.00	10.00	10.00
19	R	4.87	2.87	1.87	0.33	1480	100	0.19	3.00	450	0.67	7.00	8.67
20	R	5.87	2.87	1.87	0.33	1280	0	0.14	0.00	450	0.00	8.87	8.87
Mean		4.87	2.88	1.77	0.33	1318.33	384.17	0.32	2.39	484.88	0.78	7.28	7.95
SE		0.42	0.22	0.16	0.11	67	75	0.06	0.14	18.52	0.23	0.47	0.58

Table 2. Summary of Individual pathology of rats with surgically induced osteoarthritis.

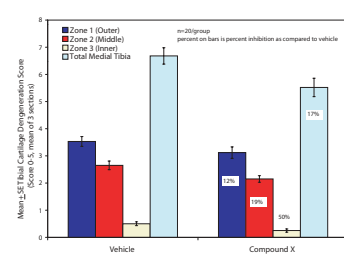


Figure 6A. Graphical representation of the major histopathologic parameters to include the mean, standard error, statistical analysis, and percent inhibition. Cartilage degeneration score of sample vehicle and compound data.

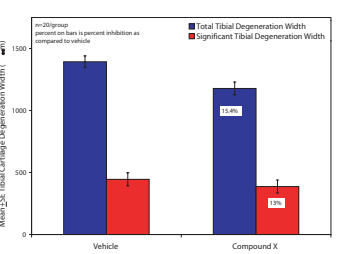


Figure 6B. Cartilage degeneration width of sample vehicle and compound data.

Literature Cited

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