Decalcified Bone Sections in Animal Models of Rheumatoid Arthritis

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Introduction

Rheumatoid arthritis is an autoimmune inflammatory disease that causes pain, swelling, stiffness and loss of function in the joints. Rheumatoid arthritis affects approximately 2.1 million people or 1 percent of the United States adult population. Current therapies have various degrees of efficacy, but toxicity frequently limits their long-term use. Animal models of arthritis are used to help provide basic insights into autoimmune disease and to test novel experimental approaches to the treatment of the disease with potential anti-arthritic drugs. Some animal models of Rheumatoid Arthritis (RA) with a proven track record of predictability for efficacy in humans include adjuvant and collagen-induced arthritis in rats. These experimental models of rheumatoid arthritis induce inflammation, bone resorption, cartilage damage and pannus that resemble the human disease. Clinical or live phase parameters of body weight and paw swelling are used along with the histopathology of the joint sections to predict the efficacy of potential anti-arthritic drugs.

Materials & Methods

Animal Models

Adjuvant-induced Arthritis in Rat

Rat adjuvant arthritis is an experimental model of polyarthritis. Arthritis is induced by the injection of Freunds complete adjuvant (FCA) supplemented with mycobacterium. The hallmarks of this model are reliable onset and progression of a robust polyarticular inflammation with marked bone resorption and periosteal bone proliferation. Cartilage destruction can occur but is disproportionally mild.

The primary live phase endpoints in adjuvant-induced arthritis are body weight, paw swelling and splenomegaly. Histology of the ankle joints are evaluated for inflammation and bone resorption, while the spleen is evaluated for inflammation, increased extramedullary hematopoiesis and lymphoid atrophy.

Type II Collagen-induced Arthritis in Rat

Rat collagen arthritis results when rats are immunized against homologous or heterologous type II collagen. The results of this animal model of disease is also reliable onset and progression of polyarticular inflammation. This polyarticular inflammation includes marked cartilage destruction in association with pannus formation, mild to moderate bone resorption and periosteal bone proliferation.

The primary endpoints for type II collagen arthritis in rats is body weight, paw swelling, and histology of the ankle and knee joints for cartilage damage, inflammation, pannus and bone resorption.

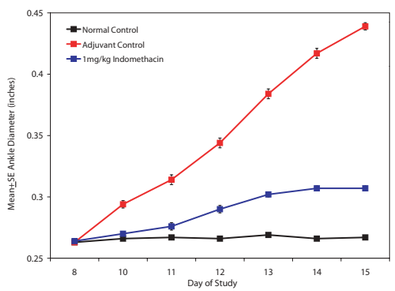

Figure 1. Effects of Indomethacin (po, BID, d0-14) on the live phase parameter of ankle swelling in Developing Adjuvant Disease in Rats

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Histology

Specimen Collection

At necropsy the tibiotarsal joint of both ankles are removed at the medial and lateral malleolus. This can be accomplished with rongeurs or a small pair of pruning shears. Once the paws are weighed and the weights recorded the digits are removed, allowing for better penetration of fixative and subsequent decalcifying solution. To identify the right from the left ankle a thick line is placed on the left ankle with a permanent marker. Both knees are collected by first removing the majority of muscle from the femur and tibia. The patella is also removed to allow fixative to penetrate into the joint space. The tibia and femur are transected away from the knee joint to avoid fragmentation into the joint area with a pair of rongeurs. To identify the left knee from the right knee the left knee is transected longer than the right knee.

Both ankles and knees are placed into 10% Neutral Buffered Formalin allowing each knee to assume a natural degree of flexion. After 48 hours in fixative the specimens are then placed into 5% formic acid for decalcification. The knee joints will take approximately 2-4 days to decal while the ankle joints can take up to 7. When the specimens are sufficiently decalced the ankle joint is transected in the longitudinal plane into two approximately equal halves, using the tibia as a guide. The knee joints are cut into approximately equal halves in the frontal plane using the collateral ligament as a guide. Once the specimens have been grossed in they are placed into fresh decal solution overnight and processed for paraffin embedding the following day.

Sectioning and Staining

Ankles from adjuvant-induced arthritis are sectioned at 5 microns and stained with hematoxylin and eosin. Ankles from type II collagen-induced arthritis are sectioned at 8 microns and stained with toluidine blue. For appropriate evaluation the stained sections must demonstrate the distal tibia with both cortices and abundant distal tibia medullary space.

Knees from type II collagen-induced arthritis are sectioned at 8 microns and stained with toludine blue. For evaluation the stained sections must demonstrate the medial and lateral portion of the knee to include both the femur and tibia and associated inflammation.

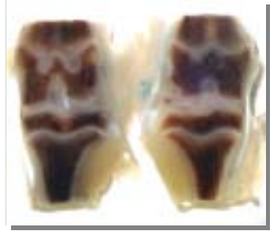


Figure 2. Gross photograph of a what a decalcified knee joint should look like after cutting the two approximately equal halves.

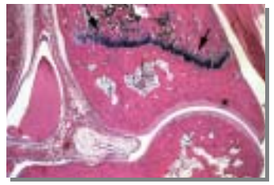


Figure 3. Photomicrograph of a normal rat ankle demonstrating the presence of both tibial cortices and abundant distal tibia medullary space. Growth plate and surrounding bone is intact (arrows). No inflammation is present. Hematoxylin and eosin, original magnification=50X

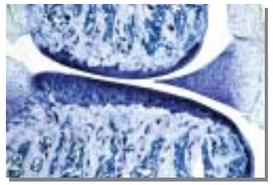


Figure 4. Photomicrograph of the medial portion of a normal rat knee demonstrating a complete section of both the tibia, femur and synovium. Shows intact articular cartilage and non inflammed synovium. Toludine blue, original magnification=50X

Histopathology

Adjuvant Arthritis

Hematoxylin and eosin stained sections of both ankles are given scores of 0 to 5 for inflammation and bone resorption according to specific criteria. Cartilage damage is not scored in the adjuvant model because it has been found to be a minor feature.

Hematoxylin and Eosin stained sections of the spleen may also be evaluated for potential treated related effects. Spleens are evaluated for inflammation, increased extramedullary hematopoiesis and lymphoid atrophy and are also scored 0 to 5 using criteria similar to those used for inflammation.

Type II Collagen Arthritis

Toluidine blue stained slides of both ankles and knees are given scores of 0 to 5 for cartilage damage, inflammation, pannus formation and bone resorption according to specific criteria.

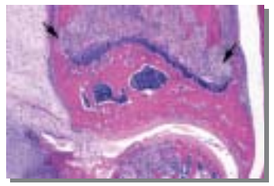


Figure 5.A. Ankle from a vehicle treated animal with adjuvant-induced arthritis. Growth plate has destruction of bone across 100% and into both cortices (arrows). Score for bone resorption is 5. Inflammation is present in the periarticular tissue. Hematoxylin and eosin, original magnification=50X

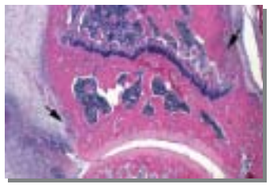
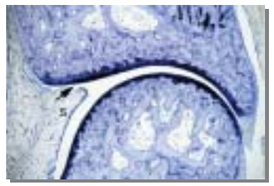
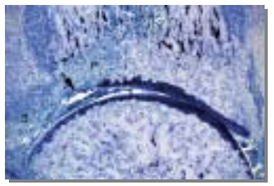
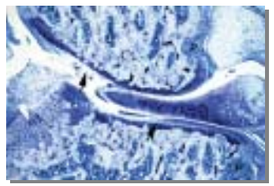


Figure 5.B. Ankle from another vehicle treated animal with adjuvant-induced arthritis. Growth plate has approximately 5% destruction of bone extending in one cortex (arrow) and resorption of epiphysis (arrow). Score for bone resorption is 1. Inflammation is present in the periarticular tissue. Hematoxylin and eosin, original magnification=50X







Clockwise from top left:

Figure 6.A. Ankle from a normal animal. Shows intact articular cartilage (arrow) and non inflammed synovium (S). Toludine blue, original magnification =50X

Figure 6.B. Ankle from a vehicle treated animal with type II collagen-induced arthritis. Shows extensive periarticular and synovial inflammation (S) as well as cartilage damage (arrow) and bone destruction by pannus (P). Score for cartiage damage is 3, pannas 2, inflammation 5 and bone resorption 2. Toludine blue, original magnification=50X

Figure 6.C. Knee from a vehicle treated animal with type II collagen-induced arthritis. Articular cartilage destruction is present (arrows) with bone destruction due to pannus (P). Score for cartilage damage is 3, pannus 2 and bone resorption 1. Toludine blue, original magnification=50X

Results

The histopathologic scores are entered into a excel spreadsheet that will summarize and tabulate means and standard error for each treatment group on each of the parameters scored. Statistical analysis can be performed by using either a two-tailed students t-test or mann-whitney with a significance set at $p \leq 0.05$.

Graphical representation of the data is also prepared in excel. Knee and ankle data is tabulated and graphed separately. Percent inhibition of each of the histologic parameters is calculated by the following equation:

% Inhibition = A - B/A X 100
A = Mean of Disease Control - Mean of Normal Control
B = Mean of Treated - Mean Normal Control

Figure 7. Example of an excel spreadsheet that contains the vehicle control data, to include mean and standard error on all histological parameters for a type II collagen-induced animal model of disease

Vehicle Control	JOINT	INFLAMMATION SCORE	PANNUS SCORE	CARTILAGE DAMAGE	BONE DAMAGE	TOTAL SCORE
Animal Number						
1	Rt Ankle	5	3	3	3	14
	Lt Ankle	5	3	3	3	14
2	Rt Ankle	5	2	2	2	11
	Lt Ankle	5	2	3	2	12
3	Rt Ankle	5	2	3	2	12
	Lt Ankle	5	2	3	2	12
4	Rt Ankle	4	2	3	2	11
	Lt Ankle	5	3	3	3	14
5	Rt Ankle	5	2	3	2	12
	Lt Ankle	5	2	3	2	12
6	Rt Ankle	5	2	3	2	12
	Lt Ankle	5	3	3	3	14
7	Rt Ankle	4	2	3	2	11
	Lt Ankle	4	1	2	1	8
8	Rt Ankle	5	3	3	3	14
	Lt Ankle	5	3	3	3	14
MEAN		4.81	2.31	2.88	2.31	12.31
SE		0.10	0.15	0.09	0.15	0.42

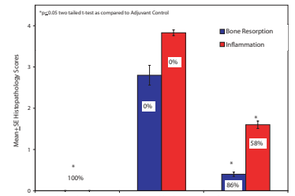


Figure 8. Graphical representation of histopathology scores to include statistics and percent inhibition of the effects of Indomethacin on adjuvant-induced arthritis in rats

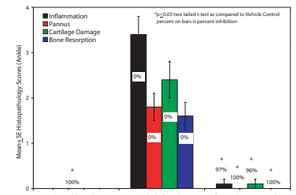


Figure 9.A. Graphical representation of the ankle histopathology scores to include statistics and percent inhibition of the effects of dexamethasone (po, QD) on established type II collagen arthritis in rats

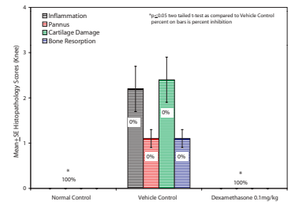


Figure 9.B. Graphical representation of the knee histopathology scores to include statistics and percent inhibition of the effects of dexamethasone (po, QD) on established type II collagen arthritis in rats

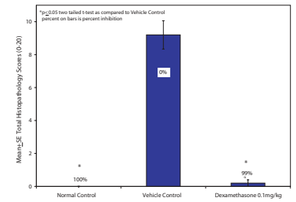


Figure 9.C. Graphical representation of the total histopathology scores to include statistics and percent inhibition of the effects of dexamethasone (po, QD) on established type II collagen arthritis in rats

Conclusions

The collection and histological evaluation of the knee and ankle joints in animal models of rheumatoid arthritis can help determine the efficacy of potential clinical candidates. Granted the live phase parameters of body weight and paw swelling can help determine the effectiveness of a drug candidate. The effects of a compound on bone resorption, pannus and cartilage damage can not be determined by any other means other than the evaluation of these parameters on stained joint sections.

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