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Perspective Article

Hylonome

Animal models of osteoarthritis

A.M. Bendele

BolderPATH, Inc., University of Colorado, MCD Biology, Colorado, USA

Abstract

Animal models of osteoarthritis are used to study the pathogenesis of cartilage degeneration and to evaluate potential anti-arthritic drugs for clinical use. Animal models of naturally occurring osteoarthritis (OA) occur in knee joints of guinea pigs, mice and other laboratory animal species. Transgenic models have been developed in mice. Commonly utilized surgical instability models include medial meniscal tear in guinea pigs and rats, medial or lateral partial meniscectomy in rabbits, medial partial or total meniscectomy or anterior cruciate transection in dogs. Additional models of cartilage degeneration can be induced by intra-articular iodoacetate injection or by administration of oral or parenteral quinolone antibiotics. None of these models have a proven track record of predicting efficacy in human disease since there are no agents that have been proven to provide anything other than symptomatic relief in human OA. However, agents that are active in these models are currently in clinical trials. Methodologies, gross and histopathologic features and comparisons to human disease will be discussed for the various models.

Keywords: Osteoarthritis, Mouse, Guinea Pig, Rat, Rabbit, Dog

Introduction

Animal models of osteoarthritis (OA) commonly used in studying the pathogenesis of cartilage degeneration and potential therapeutic modulation of disease are generally either naturally occurring or surgically-induced. Spontaneous OA occurs in the knee joints of various strains of mice[1-5] and transgenic and mutant mouse models of OA have been developed and characterized[6-14]. Spontaneous OA occurs in guinea pigs[15-17], Syrian hamsters[18] and nonhuman primates[19]. Naturally occurring or transgenically-induced disease in these species results in slowly progressive disease and hence the period for drug testing or studies of pathogenesis is long. However, the pathology and pathogenesis (especially spontaneous models) are probably similar to those occurring in the most common forms of slowly progressive human disease.

Surgically-induced instability models of OA have been described in various animal species. Traumatic OA does occur in humans and therefore these models may mimic aspects of the pathogenesis and pathology. One important difference however is that humans with a traumatic injury generally decrease use of the affected limb until restabilization has occurred. Animals (especially rodents) in the same situation generally do not (observation, A. Bendele). Therefore, the disease progression is usually much more rapid in the animal models, thus making it less amenable to therapeutic intervention[20,21]. Since most surgical models use knee joints, an important consideration in the use of surgical instability models is the load-bearing (medial vs lateral) pattern of the species being used. Animals that predominantly load the medial aspect of the joint will develop more severe lesions on the medial side after a medial meniscectomy than on the lateral side after a similar insult and vice versa.

Animal models of OA have been used fairly extensively for testing of potential anti-arthritic agents and disease modifying effects have been reported[22-24] for agents currently used to treat patients with OA. Human clinical documentation of efficacy (other than symptomatic relief) is lacking in large part due to the difficulties in monitoring OA disease progression and the long duration of clinical trials. Since most of the models have been extensively described in the literature and their relevance to human disease is not based on the track record of predictability of drug-induced modification of disease progression but rather histopathological similarities to human disease, this paper will provide a discussion of basic features of spontaneous OA and surgically-induced models in several species. In addition,

Corresponding author: Dr. Alison M. Bendele DVM, Ph.D., BolderPATH Inc., University of Colorado, MCD Biology, Campus Box 347, Colorado 80309, USA. E-mail: abendele@sni.net

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several models of cartilage degeneration will be reviewed.

The approach I took when preparing this perspective on OA models was to try to provide the information that is commonly requested from me when I go to various pharmaceutical companies or academic institutions and am asked questions about research in OA and relevant (to human disease) models.

In this paper, I have covered all the models that are commonly used for pharmaceutical testing in OA and placed emphasis on the ones that are considered to be the most useful for testing the types of agents that are currently being investigated.

Naturally occurring osteoarthritis

Hartley albino guinea pigs

Spontaneous OA occurs in the medial compartment of the knee joint of male and female Hartley albino guinea pigs[15-17] as well as in other strains of guinea pigs[25]. Although it occurs in both males and females, males tend to grow faster, thus reaching greater body weights and therefore tend to have more consistent pathological alterations. The disease is generally bilaterally symmetrical with respect to incidence and severity and the earliest changes can be seen when animals are approximately 3 months old and weigh about 700 grams[15]. The lesions are initially present on the medial tibial plateau in the area not protected by the meniscus and consist of focal chondrocyte death, proteoglycan loss and fibrillation. Usually about 50% of the animals of this age and weight will have minimal focal changes. The underlying chondrocytes do not exhibit cloning at this stage nor are there morphologic changes in subchondral bone, menisci or synovial membranes. Histopathologic sections must be prepared in the frontal plane in order to observe the medial and lateral aspects of the joint and step sections (200 μm) will maximize ability to detect early changes.

When animals are 6 months old and weigh approximately 900 grams, minimal to moderate lesions will be present in 90-100% of the medial tibial plateaus (Table 1). Lesions will generally be bilaterally symmetrical (Fig. 1). Histopathological features include chondrocyte death/loss extending into the upper middle zone, fibrillation and proteoglycan loss. In addition, cloning extends into the middle and sometimes

Treatment Group Animal#	Final Body Wt (grams)	Knee	Medial Tibia Cart. Degen. Score*	Mean % depth of Medial Tibial Lesion**	Medial Tibia Osteophyte Score	Medial Femur Cart. Degen. Score	Medial Femur Osteophyte Score***	Total Joint Score
Group 1. Vehicle Control. Right Knee								
1	948	R	6	63	1	0	0	70
3	859	R	5	58	1	0	0	64
5	973	R	4	38	2	0	0	44
7	974	R	4	54	1	0	0	59
8	915	R	2	70	1	0	0	73
10	990	R	1	40	0	0	0	41
11	938	R	1	50	1	0	0	52
12	970	R	6	43	1	0	0	50
Mean	**945.9**		**3.6**	**52.0**	**1.0**	**0.0**	**0.0**	**56.6**
SE	**15.0**		**0.7**	**4.0**	**0.2**	**0.0**	**0.0**	**4.2**
Group 1. Vehicle Control. Left Knee								
1		L	6	59	1	0	0	66
3		L	3	22	1	0	0	26
5		L	6	67	1	0	0	74
7		L	1	67	0	0	0	68
8		L	6	46	1	0	0	53
10		L	1	66	0	0	0	67
11		L	8	64	1	0	0	73
12		L	6	55	1	0	0	62
Mean			**4.6**	**55.8**	**0.8**	**0.0**	**0.0**	**61.1**
SE			**0.9**	**5.5**	**0.2**	**0.0**	**0.0**	**5.5**

*Lesion Depth (graded 1-5) X area (thirds) was scored for 3 step sections and the mean determined
**Lesion Depth in μm vs cartilage depth to tidemark was measured over 4 approximately equidistant sites on the tibial plateau and the mean determined
***Osteophytes were assigned scores of 0-3 based on-μm measurement from original tide mark

Table 1. Summary of right and left knee pathology in 6-month-old guinea pigs.

deep zones and shifts in toluidine blue orthochromatic (blue) to metachromatic (purple) staining of matrix occur, thus indicating changes in proteoglycan synthesis in areas not affected by severe changes leading to fibrillation/ cartilage loss. Small osteophytes are often present at the outer aspect of the medial tibial plateau. Generally there are no obvious subchondral bone changes, meniscal degenerative changes, femoral cartilage degeneration or synovial inflammation at this stage.

Nine-month-old animals will have mild to moderate medial tibial cartilage degeneration, mild femoral condylar degeneration and tibial osteophytes. Mild degenerative changes may be present in the menisci and synovial membranes may be minimally thickened as a result of synoviocyte proliferation. Early sclerosis of subchondral bone may be apparent.

By the time the animals are 1 year old, cartilage degenerative changes are usually quite profound and involve all aspects of the medial compartment of the knee. Chondrocyte and proteoglycan loss with fibrillation may extend into the deep zone and cloning is prominent. Subchondral sclerosis is often extensive and subchondral bone cysts are present with severe meniscal degenerative changes. Synovial hypercellularity increases and papillary proliferation can be seen. Osteophytes (usually with significant cartilagenous matrix remaining) may be very large and contribute to the marked recontouring of the shape of the medial tibial plateau and medial femoral condyles. Clinical abnormalities in gait and ability to extend the knee joint can be detected at this stage.

Severe medial compartment degenerative changes are present in 18-month to 2-year-old and older animals with dramatic recontouring of medial surfaces, bone sclerosis, bone cyst formation and large osteophytes which have undergone near complete endochondral ossification (Fig. 2). Synovium is thickened as a result of papillary proliferation and mild mononuclear inflammatory cell infiltration. Mild degenerative changes may be present on the lateral side of the joint.

The pathogenesis of naturally occurring knee OA in guinea pigs is not completely understood. However, as is the case in human disease[26], body mass is an important factor. Guinea pigs on restricted diets designed to decrease overall food consumption in an effort to prevent them from becoming sedentary obese creatures, had greatly reduced incidence and severity of knee OA[27]. In a study in which guinea pigs were exercised on treadmills from an early age (approximately 2 months to 5 months), OA lesions were not enhanced as might be expected but rather were decreased in association with body weight decreases (unpublished, A. Bendele).

Guinea pigs seem to preferentially load the medial aspect of the knee joint as evidenced by the fact that medial

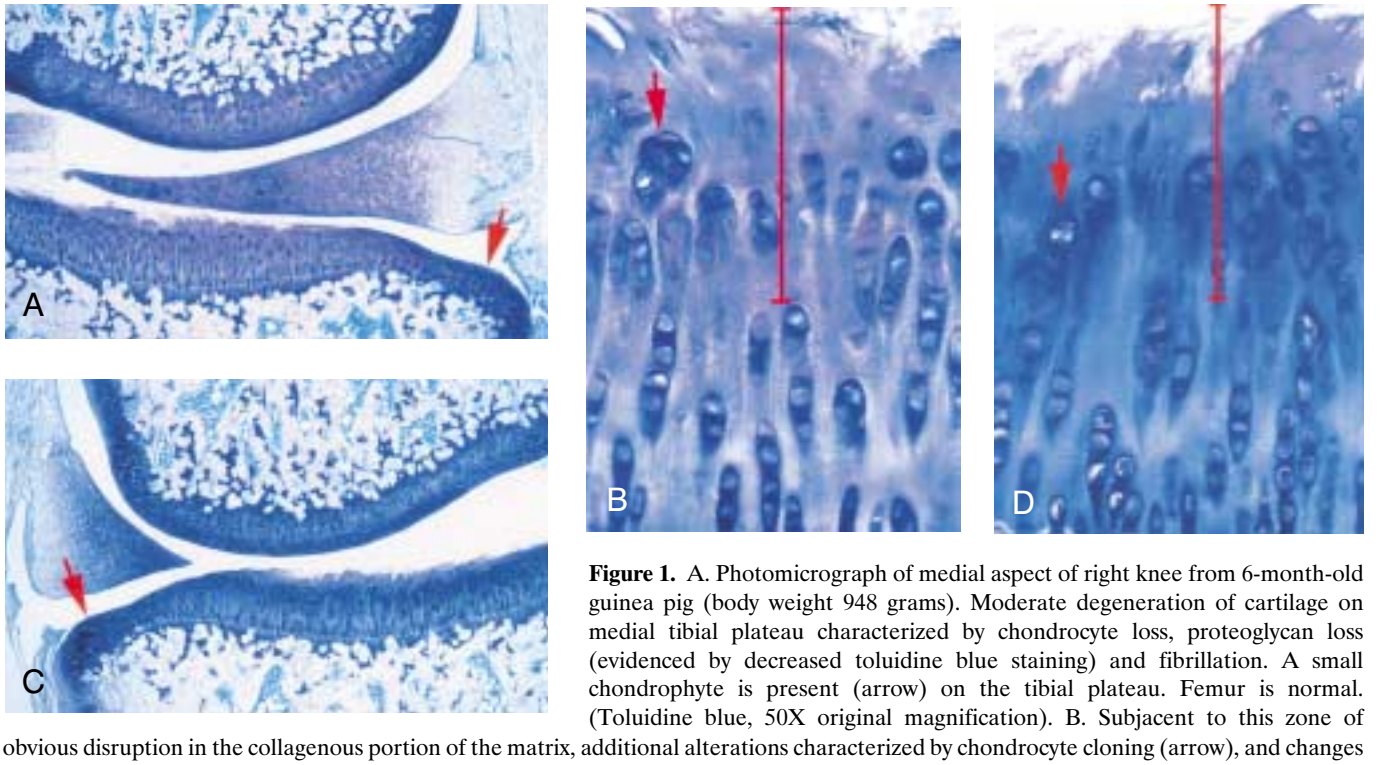

Figure 1. A. Photomicrograph of medial aspect of right knee from 6-month-old guinea pig (body weight 948 grams). Moderate degeneration of cartilage on medial tibial plateau characterized by chondrocyte loss, proteoglycan loss (evidenced by decreased toluidine blue staining) and fibrillation. A small chondrophyte is present (arrow) on the tibial plateau. Femur is normal. (Toluidine blue, 50X original magnification). B. Subjacent to this zone of obvious disruption in the collagenous portion of the matrix, additional alterations characterized by chondrocyte cloning (arrow), and changes in the character of toluidine blue staining, extend 250 μm (bar) from the surface (Toluidine blue, 100X original magnification). **C.** Photomicrograph of medial aspect of left knee from 6-month-old guinea pig shown in figure 1A. Note similarity of lesion severity in this bilaterally symmetrical disease, with small osteophyte (arrow) and normal femur. (Toluidine blue, 50X original magnification). **D.** Higher magnification shows similar degenerative changes extending 250 μm from surface, as was present in right knee. (Toluidine blue, original magnification=100X)

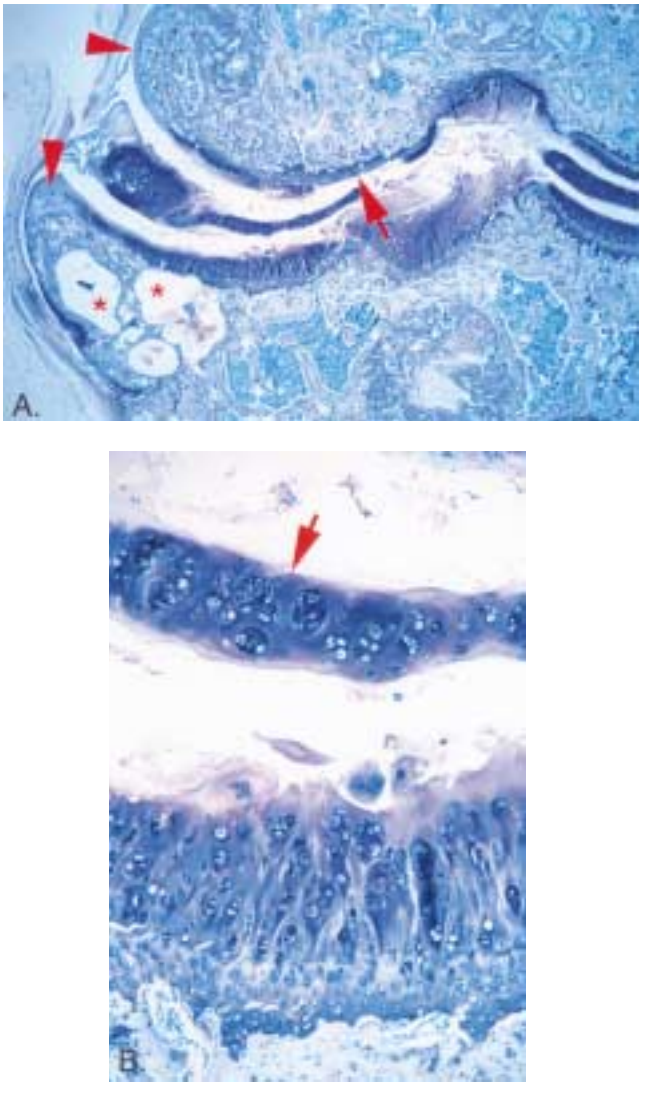

Figure 2. A. Photomicrograph of medial aspect of knee joint from 2-year-old guinea pig with spontaneous OA. Tibial and femoral cartilage degeneration extends into the deep zone and are characterized by proteoglycan loss, fibrillation and chondrocyte cloning. A focal area of full thickness degeneration (to the tide mark) is present on the femur (arrow). Subchondral sclerosis and cyst formation are prominent features*. A large osteophyte is present on the medial tibia and femur and has resulted in extensive recontouring of the surface (arrow heads). Toluidine blue, 25X original magnification. B. Higher magnification demonstrates marked chondrocyte cloning and matrix degeneration in both tibial cartilage and meniscus (arrow). Toluidine blue, original magnification=100X

meniscectomy results in severe lesions and lateral meniscectomy results in mild to no lesions in the respective compartments (unpublished, A. Bendele). This is similar to the situation in humans where approximately 75% of the load (normal conformation) passes through the medial aspect of the knee[28]. Therefore, any additional stress such as increased body mass would add to this predisposition to naturally load this area and possibly contribute to adverse matrix/cellular changes leading to degeneration.

Other theories that have been proposed to explain this increased incidence of disease include the presence of bone cysts in the area where the cruciate ligaments attach, thus leading to instability[29]. While it is possible that these cysts may contribute to the pathogenesis through this mechanism, these types of cysts occur routinely in aging rats, a species that has virtually no spontaneous knee OA.

Because of the very predictable manner in which guinea pigs develop spontaneous knee OA and the obvious similarities to human disease, the model can be used for a variety of purposes including studies of pathogenesis and potential therapeutic intervention.

Prophylactic testing of inhibitors of cartilage degeneration could be evaluated by initiating treatment when animals are 2.5 months old. At this stage the tibial articular/epiphyseal growth plate is still open and the tide mark is not well formed. Generally there are no degenerative changes present. Dosing would have to be done until the animals were 6-7 months old in order to achieve an incidence and severity of changes sufficient for evaluation of protective effects. Evaluation of lesions would be solely on the tibial plateau as the femoral lesions (which lag behind in development) would be in the very early stages of development. Small osteophytes would be present primarily on the tibia. An N=20/group would be appropriate for demonstrating beneficial effects of treatment.

Another possible scenario for use of the model in drug testing is to begin administering potential inhibitors when animals are 4.5 months old. Approximately 75% of them will have minimal to mild tibial degeneration but no femoral degeneration. Dosing for 4.5 to 5 months until animals are 8.5 to 9 months of age would essentially give evaluation of inhibitory effects on both mildly established lesions (therapeutic-medial tibia) and developing lesions (prophylactic-medial femur) in the same animal. Since the lesions are reasonably bilaterally symmetrical, both knees can be evaluated for drug effects. An N=15 is sufficient for testing in these older animals.

Evaluation of histologic changes in guinea pig spontaneous OA should be done on frontal sections of the knee joint. In general, the best approach for section preparation is as follows: At necropsy, trim muscle from the femur and tibia and reflect the patella distally and remove to allow fixative (10% neutral buffered formalin) to enter the joint space. Transect the femur and tibia with a rongeur some distance from the joint to avoid fragmentation of bone into the joint area. Drop the joint into the fixative and allow it to assume a natural degree of flexion. After 2 full days of fixation, and 5-7 days of 5% formic acid decalcifier exposure, trim the ends of the bones and place a forcep in the patellar groove and posterior aspect of the joint. Try to cut the joint (using the collateral ligaments as a land mark) into approximately equal halves (frontal plane), place in megacassettes if necessary due to the size of the animal, and return to 5% formic acid for an additional 24 hours. Wash the trimmed

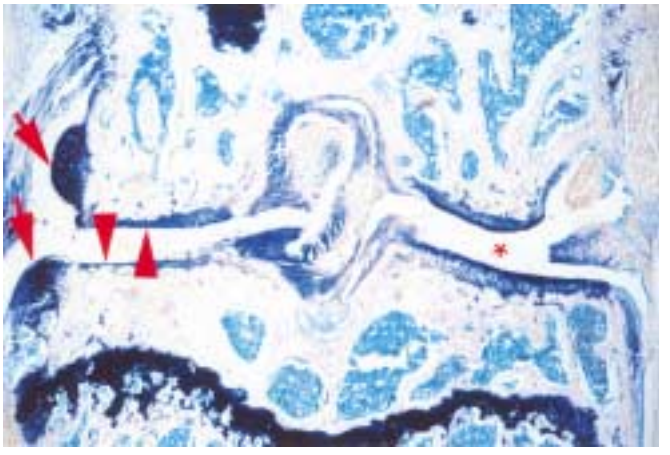

Figure 3. Photomicrograph of knee joint from 15-month-old C57BL6 mouse with spontaneous OA. Medial tibial and femoral cartilage degeneration extends to the tide mark (arrow heads) and large osteophytes are present (arrows) on both. The lateral aspect of the knee is normal*. Toluidine blue, original magnification=50X.

halves extensively, process for paraffin embedding and section at 8 μm for toluidine blue staining. If both halves of the joint are properly embedded, step sections (initial plus 2 at 500 μm intervals) will provide the opportunity to observe all the important aspects of the pathology.

Scoring or evaluation of the lesions should utilize a system that incorporates a depth and area of lesion evaluation and should be simple enough so that it does not require extensive referral to the description of the scoring system. Generally, in the rodent models, it is sufficient to use the worst case scenario of lesions in the various step sections as the ultimate score for the joint under evaluation. Alternatively the scores for 3 sections can be averaged.

Scoring for the various compartments (medial tibia, medial femur) should be kept separate since disease progression is different in the different locations. Synovial reactions and osteophyte size (measured with an ocular micrometer) should also be tabulated separately as they reflect different processes.

When the model is evaluated at the later stages of disease progression, lesions will consist of severe matrix changes/cell and proteoglycan loss with fibrillation extending to a certain depth. The subjacent matrix, while still reasonably intact, will likely have cloning and tinctorial changes in staining that indicate less severe matrix changes extending to additional depths. These various permutations of the lesion need to be incorporated into the scoring system and can be done using an ocular micrometer to determine depth. Measurement from surface to tide mark will give the depth at risk and measurement to depth of fibrillation vs depth to cloning/staining changes can then help delineate the various components of the lesion and effects of treatments on these aspects of the changes. Agents have shown activity in this model[22] and are currently in clinical trials designed to determine safety and disease-modifying activity[30,31].

Recent advancements in the development of matrix metalloproteinase (MMP) inhibitors have raised concerns about the expression of the various MMPs, a family of highly homologous zinc endopeptidases that include the collagenases, stromelysins and gelatinases. Both collagenase 1 (MMP-1) and collagenase 3 (MMP-13) have been implicated in the pathogenesis of arthritis in humans[32-33]. Guinea pigs, unlike rats and mice, express both collagenase 1 and 3, and this expression occurs at the site of OA lesion development[34]. Mechanical forces have been found to increase expression of collagenase 1 mRNA[35] so high levels present on the medial aspect of the guinea pig knee may be in response to increased loading (relative to the lateral side) of this compartment.

Guinea pigs with OA also have expression patterns of an early biochemical neoepitope marker of OA called 3-B-3(-)[36] which results from a change in the termini of the chondroitin sulfate (glycosaminoglycan) chains of aggrecan[37,38] and is found in human OA cartilage as well.

Mice, Syrian hamsters and primates

Other commonly used laboratory animals that develop spontaneous OA include Syrian[18], but not Chinese hamsters, and many strains of mice. Various factors including patellar luxation[1-3], varus or valgus conformational abnormalities[4] and other genetic defects including mutations in the type II collagen gene[6,13,14] have been implicated in the pathogenesis of knee OA in some strains of mice. However, virtually all aging mice have medial compartment cartilage degeneration that in some animals progresses to OA (unpublished, A Bendele). Generally, some evidence of degeneration will be present in the medial compartment of the knee by the time most mice are 6 months of age. This degeneration may become quite severe (Fig. 3) with full thickness cartilage loss and large osteophytes by the time the mice are 15 months old. Scoring of cartilage lesions in mice and hamsters can be challenging since the cartilage has relatively few cell layers compared to the larger species, but if relatively simple scoring systems are used (0-3=none, mild, moderate, severe for cartilage degeneration), reproducible data can be obtained from these models.

Since mice are commonly used in toxicology testing and are being dosed with compounds for various time periods, there is an opportunity to obtain tissues for evaluation of either spontaneous incidence in particular strains or effects of agents being tested. As with the guinea pig, it is important to section the joints in the frontal plane to distinguish medial from lateral.

Spontaneous OA has been described in knee joints of non human primates[19]. Variability in lesion severity and difficulties associated with obtaining adequate numbers of primates for meaningful studies probably preclude general use of this interesting model.

Surgically-induced osteoarthritis

Rat medial meniscal tear

Unilateral medial meniscal tear in 300-400 gram rats will result in rapidly progressive cartilage degenerative changes characterized by chondrocyte and proteoglycan loss, fibrillation, osteophyte formation and chondrocyte cloning. This model is performed by transection of the medial collateral ligament just below its attachment to the meniscus so that when the joint space opens, the meniscus is reflected toward the femur. The meniscus is cut at its narrowest point (away from the ossicles) taking care not to damage the tibial surface. Although aseptic techniques should be used, rats are extremely resistant to infection and this is seldom a post-operative isssue.

Progressive degenerative changes occur and by 3-6 weeks post-surgery, tibial cartilage degeneration may be focally severe with degenerative changes of lesser severity in the surrounding matrix and prominent osteophytes (Fig. 4). Scoring of lesions (depth and area) can be done in similar fashion to that described for spontaneous OA in guinea pigs. When using this model for potential pharmaceutical testing, it may be desirable to pre-dose animals to steady state plasma levels prior to initiating surgical trauma since the progression is so rapid. Duration of testing should be at least 3 weeks post-surgery and an N=20/group is sufficient to account for variability in lesion severity. Lesions are reasonably consistent if the surgical technique is consistent (Table 2). The surgery is fairly difficult since the surgeon is working in a small joint space and rats tend to have a lot of highly vascular adipose tissue and muscle in the medial knee region. Therefore, it is generally preferable to have one person perform all the surgery in a study. Rats resume weight bearing immediately post-surgery and gait analysis suggests little if any change in load-bearing of the operated knee. The rapid progression of cartilage degeneration makes this model an extremely high hurdle test for detecting protective effects. From the perspective of comparative pathogenesis to human disease, the lesions are morphologically similar but occur so much more rapidly, probably due to the inapparent perception of the animal that it should not continue using the unstable joint as would be the case with

Treatment Group Animal#	Knee	Medial Tibia Cart. Degeneration Score*	Tibial Cartilage Degeneration Width (μm)**	Depth Ratio Any Matrix Change***	Medial Tibia Osteophyte Score#	Medial Femur Cart. Degeneration Score*
Group 1. Surgery+vehicle						
1	R	7.7	533.3	0.65	2	0
2	R	9.3	716.7	0.60	3	1
3	R	6.3	550.0	0.60	2.7	0
4	R	8.3	666.7	0.78	1.7	0.3
5	R	8.7	700.0	0.70	3	0.3
6	R	8.3	666.7	0.83	3	0
7	R	8	683.3	0.61	2	0.3
8	R	9.3	933.3	0.71	2	1.3
9	R	9.3	900.0	0.75	3	0
10	R	7.7	816.7	0.52	3	0.3
11	R	6	333.3	0.55	2	0
12	R	7.7	500.0	0.55	1.7	1.3
13	R	9	833.3	0.75	2.7	0.7
14	R	9	800.0	0.65	2.3	0
15	R	7	633.3	0.59	2	1
16	R	5	100.0	0.55	1.3	0
17	R	5.5	566.7	0.67	1	0
18	R	5.5	266.7	0.63	2.3	1.3
19	R	6.5	583.3	0.76	1	4.7
20	R	8.5	933.3	0.67	2.3	0.7
Mean		7.6	635.8	0.66	2.2	0.7
SE		0.31	49.18	0.020	0.15	0.24

*Cartilage degeneration score=depth (1-5) X width (1=1/3,2=2/3,3=3/3 of surface area), mean of 3 step section
**Width of severely compromised cartilage matrix degeneration, mean of 3 step sections
*** Lesion depth in μm vs depth to tidemark was measured over 4 approximately equidistant sites on the tibial plateau and the mean determined
#Osteophytes were assigned scores 1= up to 299 μm, 2= up to 399 μm, mean of 3 sections

Table 2. Summary of right knee pathology in rats with meniscal tears.

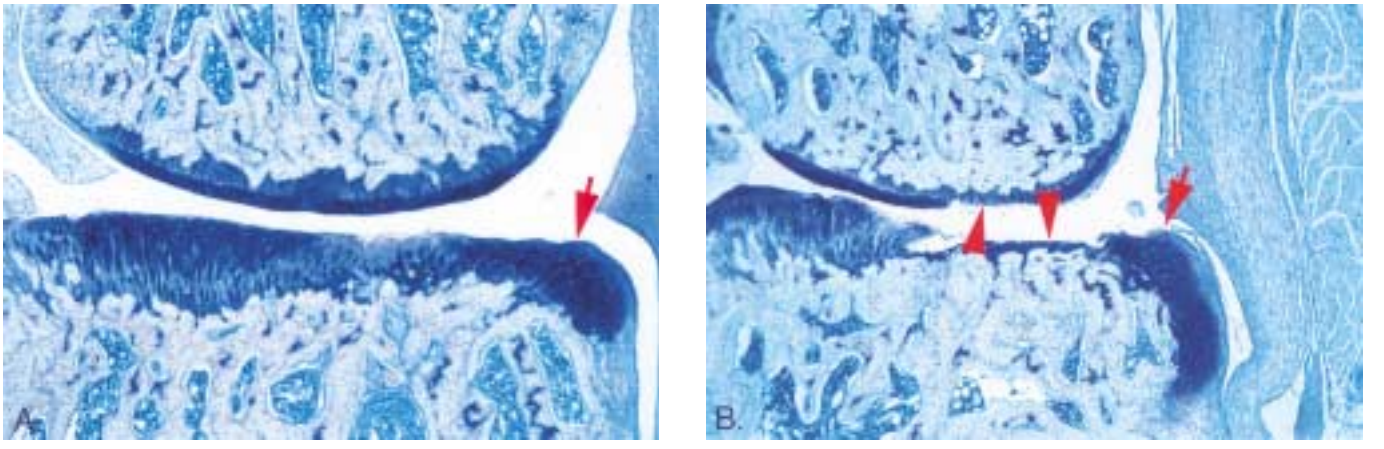

Figure 4. A. Photomicrograph of medial aspect of knee joint from rat which had unilateral medial meniscal tear surgery 2 weeks previously. Focal cartilage degeneration characterized by chondrocyte and proteoglycan loss as well as fibrillation is present on 1/3 of the medial tibial plateau and extends 180 μm deep into the deep zone. Mild cloning response is present and an osteophyte (300 μm) extends from the medial tibial marginal zone (arrow). Toluidine blue, original magnification=50X. B. Photomicrograph of medial aspect of knee joint from rat which had unilateral medial meniscal tear surgery 6 weeks previously. Focal severe cartilage degeneration characterized by chondrocyte and proteoglycan loss as well as fibrillation is present on 2/3 of the medial tibial plateau and 1/2 of the femoral condyle (arrow heads). An osteophyte (280 μm) extends from the medial tibial marginal zone (arrow). Toluidine blue, original magnification=50X.

most humans. Currently there are no published data in this model describing activity of agents that are in clinical trials in humans.

One advantage of using the rat model is that this species is commonly used in toxicology testing of compounds. Efficacy in this species in combination with toxicology evaluation allows for the generation of a therapeutic index for compounds under evaluation. Rats have very little spontaneous degeneration in their knee joints[39] so lesions observed are generally a result of the surgical manipulation only.

Guinea pig medial meniscal tear

Surgical instability from medial meniscal tear, similar to that described for rats, results in cartilage degeneration in knee joints of guinea pigs[40]. Since guinea pigs preferentially load the medial aspect of the knee joint, this procedure must be done on the medial side in order to induce consistent pathological alterations. Similar surgery on the lateral meniscus results in highly variable to no cartilage degenerative changes.

Three-month-old male guinea pigs (with well-formed tibial tide marks) are anesthetized with Isoflurane and the medial aspect of the right knee clipped and scrubbed in preparation for surgery. If surgery is performed on immature animals, lesions will resemble osteochondrosis rather than OA in histological appearance. The medial collateral ligament is transected, the medial meniscus is grasped with a fine toothed hemostat and reflected proximally toward the femoral condyle. The meniscus is then transected with a scalpel or fine scissors. No attempt is made to close the joint capsule and the skin is closed with 3-0 silk sutures. Animals will generally remove the sutures or they can be left in place without problems occurring.

Guinea pigs killed 3 days post-meniscal tear have loss of medial tibial chondrocytes and proteoglycan (as evidenced by decreased toluidine blue staining) only in the superficial and upper middle zone of the articular cartilage. Mild collagen disruption of the superficial layer is also evident. Moderate acute inflammation, edema and fibroblast proliferation is evident in the transected synovium. At 3 weeks post-surgery, cartilage degeneration in guinea pigs extends through 1/3 of the medial tibial articular cartilage and large chondrophytes are present on the tibia. Smaller chondrophytes are evident on the opposing femoral condyle. Inflammation is absent from the synovium, however it is markedly thickened as a result of fibrous tissue proliferation. Animals killed at 6 weeks post-surgery have cartilage degeneration extending into the middle zone of the medial tibial plateau and cloning of chondrocytes was evident in the subjacent cartilage. Large tibial chondrophytes undergoing endochondral ossification are present. By 12 weeks post-surgery, degeneration extends into the deep zone of the cartilage and clones are prominent in guinea pigs (Fig. 5). Chondrophytes exhibit extensive but incomplete ossification. The synovium is still thickened as a result of fibrous tissue proliferation. The importance of load-bearing in the generation of these lesions has been demonstrated in studies in which sciatic nerve transection was done in conjunction with meniscal damage[41]. Animals failed to develop typical OA-like lesions of cartilage degeneration in the absence of loading in short term studies.

Obviously, since guinea pigs develop spontaneous OA, the contralateral non-operated joint can be utilized in the evaluation of effects of various manipulations in this model. One potential scenario would be to perform surgery on 4-month-old animals (spontaneous OA would be minimal to mild) and treat for 3 months until the animals are 7 months

old. Effects of treatment could be evaluated on the operated knee as well as the non-operated knee. Surgically-induced lesions will be severe and as with the rat, represent a high hurdle model from the standpoint of achieving protective effects. Lesions in the contralateral knee are mild, more slowly progressive and hence more representative of what generally happens in the pathogenesis of human OA. An expectation for outcome for a matrix protective agent might be mild effects on the surgical knee (difficult test) but good to excellent effects on the spontaneous. Evaluation of the spontaneous lesion over this time frame (3 months) could only be considered a screening method as lesion variability in animals 7 months of age might preclude critical interpretation. If it is desirable to simply use the surgical model, 3 weeks post-surgery is sufficient to induce lesions of a magnitude and consistency to see treatment effects with an N=12. The surgical procedure in this model is reasonably easy so consistent lesions should be achievable with little iatrogenic trauma to the femoral or tibial surfaces, both of which should be utilized in the evaluation. Since guinea pigs, like rats, resume load-bearing immediately post surgery and hence rapidly progress to marked degeneration, it may be desirable to pretreat to steady state plasma levels prior to surgery in this challenging assay. Currently there are no data in this model describing activity of the agents that are in clinical trials in humans.

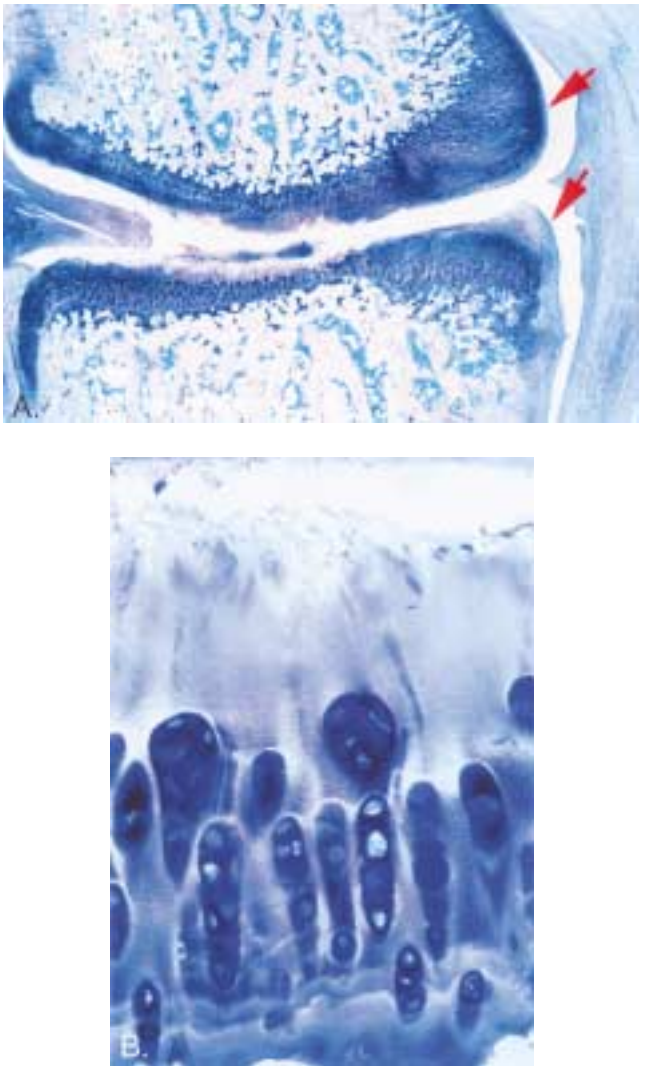

Figure 5. A. Photomicrograph of knee joint from guinea pig which had unilateral medial meniscal tear surgery 12 weeks previously. Focal cartilage degeneration characterized by chondrocyte and proteoglycan loss as well as fibrillation is present on 2/3 of the medial tibial plateau and femoral condyle and extends 300 μm deep into the middle zone. Large chondrophytes are present on the tibia and femur (arrows). (Toluidine blue, 50X original magnification). B. Higher magnification shows marked cloning response and degenerative changes extending to the tidemark. Toluidine blue, original magnification=400X.

Rabbit meniscectomy

Partial meniscectomy surgery in New Zealand White rabbits (approximately 4 kg) results in lesions resembling those occurring in human OA. Rabbits unlike rats, mice and guinea pigs, preferentially load the lateral aspect of the knee joint. Partial meniscectomy surgery on the medial aspect of the joint generally results in relatively mild to moderate degenerative changes and this model has been used extensively for testing of potential chondroprotective agents[42]. Partial lateral meniscectomy induces a very consistent focal degenerative change involving approximately 1/2 of the lateral tibial plateau and femoral condyle[43]. If the surgeon is consistent in removing the same size piece of meniscus from the same location on the lateral side and the histotechnologist is consistent in the sectioning process, the lesions are remarkably similar between animals at 6 weeks post-surgery. The surgical procedure involves transection of the fibular collateral ligament prior to entry into the joint space.

Because of the larger size of the rabbit joint, it is preferable to disarticulate the tibia and femur and visualize the gross lesions on both surfaces. Both tibia and femur (after decalcification) can be trimmed into 3 approximately equal slabs (from anterior to posterior) and embedded with the posterior surface down to give 3 sections that reliably represent the extent of lesions on these surfaces. Unlike the situation in rodents where generally the scoring is the worst case scenario for the various steps, it is best to sum the scores for the 3 tibial and 3 femoral sections to arrive at a total joint score that reflects the area of the lesion.

Although partial lateral meniscectomy offers a model with very consistent (location and severity) lesion development, the lesions progress fairly rapidly, thus making this a challenging test for therapeutic intervention. Therefore, it may be preferable to initiate therapy several days prior to surgery in order to achieve steady state plasma levels. Termination of the study (N=10/group) 6 weeks post-surgery is adequate for evaluation of the effects of compounds on marked to severe focal chondrocyte loss, proteoglycan loss and fibrillation. Osteophyte formation will be fairly striking and subchondral bone on the lateral side

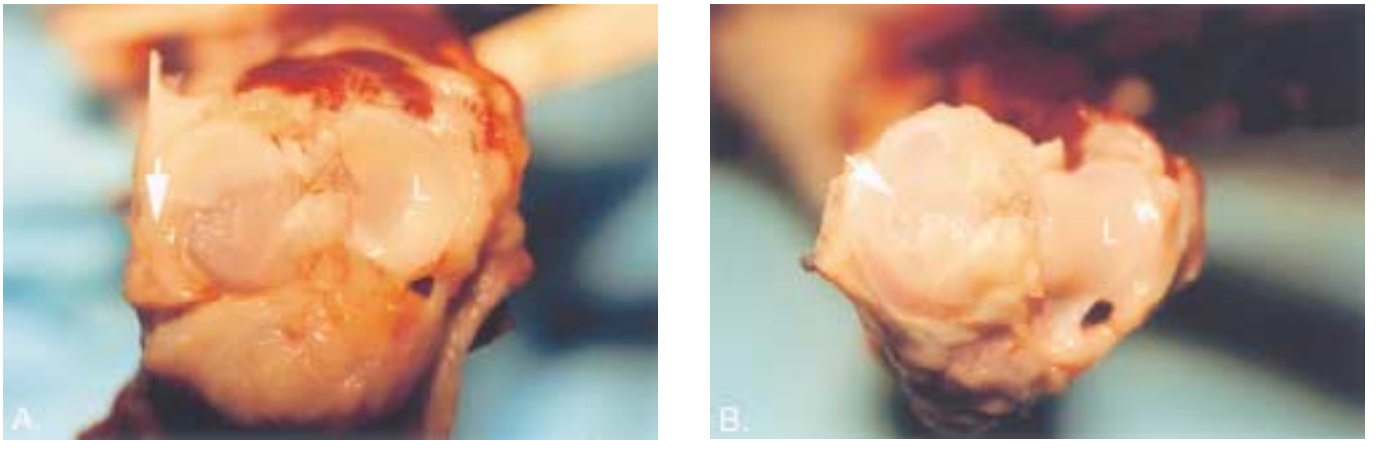

Figure 6. A. Gross photograph of tibial plateau of dog which had 1/2 of the anterior meniscus removed one month earlier. A mild attempt at repair by proliferation of fibrous tissue (arrow) is evident in the area of the transected meniscus. Lateral side (L) is normal. B. Gross photograph of tibial plateau of dog from Figure 6A. Menisci have been removed and a large area of focal degeneration (arrow) is evident on the medial tibial plateau (arrow). Lateral side (L) is normal.

will have obviously thickened trabeculae. The presence of subchondral bone alterations indicates a shift in the loading patterns as a result of the instability created at the time of surgery. In the best case scenario of compound testing, the bone changes would be present thus confirming the conduct of the surgical procedure but the cartilage matrix would be less damaged as a result of protection by the treatment. If protection of the matrix is seen in the absence of bone changes (unless the compound might have effects on bone remodeling) careful examination must be done to ensure appropriate surgical procedure.

Rabbits have very little spontaneous degeneration in their knee joints but have a tremendous capacity to regenerate the transected meniscus with fibrous tissue so often on opening the joint 6 weeks post-surgery, there may be some question of whether the meniscus was actually transected. The presence of subchondral sclerosis will confirm that the surgery was probably done correctly.

Dog meniscectomy

Use of the beagle dog for OA model conduct, as with the rat, offers the opportunity to generate efficacious data in a species commonly used for toxicology testing. When using this model, it is important to house the animals in large runs or enclosures that allow abundant opportunity for exercise. Individual housing in stainless steel cages with intermittent exercise will result in much milder and highly variable pathological alterations. Additionally, surgical proficiency resulting in induction of minimal trauma is important in that it will result in faster recovery to weight bearing time. Closure of the joint capsule followed by subcutis and then buried subcuticular absorbable sutures result in a small surgical wound that draws little attention from the animal and hence no post-surgical complications. Dogs, unlike rodents, alter their gait/load-bearing patterns post-surgically and in general, the more instability that occurs as a result of the procedure, the more/prolonged the alterations will be. In our studies, we routinely treat animals for 3 days with an analgesic and expect them to resume weight bearing in a reasonably clinically normal pattern the morning after surgery is performed. Mature (2 years old or greater) female beagles (7-11 kg) in which approximately 1/2 of the anterior portion of the medial meniscus is removed (with no transection of the medial collateral ligament) consistently (over a 1-3 month period) develop moderate degenerative changes in the tibial and femoral cartilage (Figs. 6, 7). Despite the fact that the animal makes serious attempts to regenerate the meniscus in the area of removal by proliferation of fibrous tissue, lesions are reasonably consistent with respect to location and severity. Disarticulation of the joints allows gross evaluation and photography of the morphological changes. While gross

Figure 7. Photomicrograph of tibial cartilage from dog which had 1/2 of the anterior meniscus removed three months earlier. Focal cartilage degeneration is characterized by chondrocyte loss and cloning of remaining chondrocytes on the medial side (arrow). Toluidine blue, original magnification=100X.

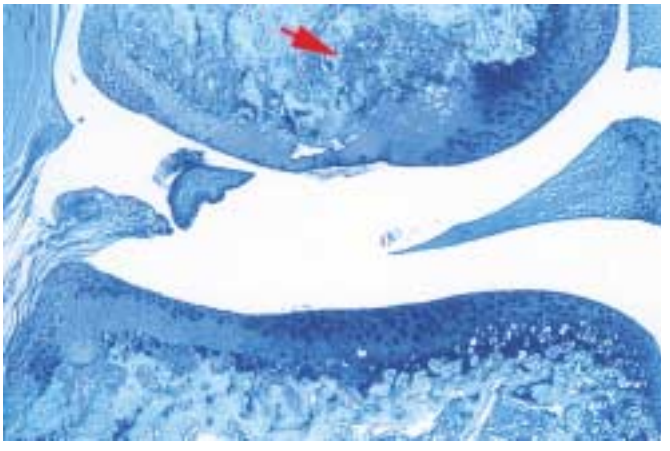

Figure 8. Photomicrograph of knee joint from immature rat which had unilateral iodoacetate injection 2 weeks previously. Focal cartilage degeneration characterized by chondrocyte and proteoglycan loss as well is present on 2/3 of the medial femoral condyle and extends through the entire articular cartilage and into the articular/epiphyseal growth plate cartilage which is thickened. Subjacent bone appears compressed and marrow is fibrotic around the area of cartilage degeneration (arrow). Although proteoglycan and cell loss are severe, fibrillation or fraying of the collagenous portion of the matrix is minor and the surface is smooth. Toluidine blue, original magnification=40X.

evaluation is useful, the ultimate analysis must be histological as depth and morphology have to be taken into account. Sectioning (divide tibia into 3 approximately 0.25 inch thick slabs) should be done as described for the rabbit and attention to detail and consistency here are important to the ultimate interpretation as distinct differences in severity of lesion will be seen at each level of sectioning as a result of lesion location differences. In addition to focal chondrocyte/ proteoglycan loss and fibrillation, subjacent and surrounding chondrocytes often exhibit striking clonal proliferation of chondrocytes and obvious matrix staining color (orthochromatic to metachromatic) changes. Lesions should be scored with attention to depth and area affected with differentiation of lesions of matrix loss vs lesions of abnormal matrix (cloning, metachromasia). Osteophytes will be present on the medial aspect of the joint but are rare in the patellar groove area or lateral side. Subchondral bone thickening will be quite prominent at 3 months post-surgery. Synovial membrane changes remain relatively mild and generally consist of papillary proliferation.

Besides the obvious advantage of being able to generate efficacious data in a species in which toxicology testing is likely to be done, there are several other advantages to using this model. While mature beagles do consistently have minimal spontaneous superficial degenerative changes on the medial tibial plateau in the area not protected by the meniscus, these lesions consist of minimal cell/proteoglycan loss and focal loss of the tangential layer and rarely have deeper fibrillation or evidence of cloning. Beagle cartilage is relatively thick and so from the histological perspective, offers more opportunity to discriminate on the depth of lesions. Finally, the lesions seem to progress much more slowly than do those induced by surgery in rodents or rabbits with lateral meniscectomy. From the standpoint of potential therapeutic intervention, this may be a slightly less difficult hurdle than that induced in the rodent models. The lesions in this model (assuming good surgical technique) are consistent enough to allow testing to occur with 10-15 animals per treatment group and the duration of the model (1-3 months) is acceptable for screening/testing purposes. Complete removal of the medial meniscus resulting in lesions of OA has also been described in beagle dogs[44].

Dog anterior cruciate ligament transection

Transection of the anterior cruciate ligament (via arthroscopic, direct visualization through an incision or blind cut through a stab incision) results in a true instability-induced OA lesion that mimics OA occurring naturally in dogs or humans following traumatic injury[45-47]. Clinically these lesions progress to OA in both species after extended periods of time. So from a pathogenesis perspective, this model offers the opportunity to study developing OA in a slowly progressive situation. Interestingly, transection of the ACL in beagle dogs results in very little overt cartilage degeneration over a 3-month period (unpublished, A. Bendele). However, osteophyte formation in the patellar groove is quite striking and unfibrillated articular cartilage may exhibit striking hypercellularity as a result of chondrocyte cloning. Animals show a greater tendency to favor or carry the limb for prolonged periods post-surgery than with the medial meniscectomy model. Presumably this is because they perceive greater instability/abnormality on load-bearing and attempt to compensate with a reduction in load-bearing. Although it is likely that with more time, they would develop OA lesions, the early changes are extremely mild and variable and so not conducive to efficient compound testing.

ACL transection in larger dogs (various hounds weighing 20-30 kg) results in more severe cartilage degenerative changes. Lesions may occur in any location on the medial and lateral tibial plateaus and femoral condyles and severity of lesions is often associated with the degree of meniscal degeneration. In some animals there may be little or no evidence of meniscal shredding and minimal cartilage degeneration while others have extensive meniscal (mainly medial) shredding/fibrous proliferative attempts at repair and striking cartilage changes. A very prominent feature in this model is the presence of numerous large osteophytes on the outside surfaces of the patellar grooves. Superficial to middle zone chondrocyte and proteoglycan loss with fibrillation are common. Extensive and deep alterations characterized by relatively intact matrix with marked hypercellularity due to cloning and toluidine blue staining changes are often evident. Occasionally pannus-like fibrous tissue extends over the cartilage surface.

Since this model has been used extensively over the years

there are numerous publications describing the effects of various agents in modifying disease[23] as well as studies documenting the presence of cytokines such as TNF-α or other mediators[48].

The obvious advantage of this model is that it offers an opportunity to study OA in a situation where pathogenesis mimics the naturally occurring disease. The disadvantages include the length of time required to generate lesions, variability in location and severity of lesions, and the large size of dog required (housing and compound requirement considerations).

Models of cartilage degeneration

Intra-articular iodoacetate injection

Cartilage degeneration can be induced in virtually any species by the intra-articular injection of iodoacetic acid (IA), an inhibitor of aerobic glycolysis, which kills chondrocytes. Depending on the concentration used and frequency, different degrees of killing and thus degeneration can be achieved. In guinea pigs, 2 intra-articular injections of IA (0.1 ml of 3 mg/ml) at 24-hour intervals will result in the death of all chondrocytes on the tibial plateaus and femoral condyles[50]. The chondrocytes at the far outer margins of the joint (marginal zone) in the area where chondrophytes/ osteophytes form, usually survive this insult and ultimately proliferate to form these structures. This insult in the presence of normal load-bearing results in the progressive loss of proteoglycan as evidenced by decreased toluidine blue matrix staining, and atrophy of the remaining collagenous portion of the matrix. Fibrillation is a late change as is the collapse of the remaining collagenous matrix into partially resorbed and degraded subchondral bone (Fig. 8). Ultimately large osteophytes occur in this model[51].

Potential uses for this model include evaluation of agents designed to inhibit acute matrix degeneration or in the case where chondrocyte killing is incomplete, inhibition of matrix degeneration, induction of repair and evaluation of effects of agents on gait alterations that occur[52]. Since osteophyte formation is a prominent feature, this model could be used to study their induction or inhibition of formation.

Quinolone antibiotic treatment

Quinolone antibiotics are potent broad spectrum antibiotics that target DMA gyrase (bacterial topoisomerase)[53]. All quinolone antimicrobials have the capacity to cause articular cartilage degeneration in growing animals and for this reason their use is contra-indicated in adolescents and pregnant women. Administration of these compounds (Naladixic acid, 350 mg/kg, single sc dose) induces a characteristic blister-like lesion in the mid-zone cartilage of guinea pigs[54-55]. Degenerative changes at 24 hours include focal swelling, decreased toluidine blue staining and obvious chondrocyte death (Fig. 9). Ultimately the viable upper layers desquamate off leaving an area of fibrillation, cell and proteoglycan loss (with later marked cloning) that resembles lesions seen in various models of OA. Generally the deep zone chondrocytes survive the insult. Factors important in the profound matrix degeneration that occurs in this model are unknown but could involve MMPs and if so, this would provide an easy test system for evaluation of inhibitory effects.

Discussion

Virtually all of the spontaneous or surgical animal models of OA ultimately result in morphological changes that

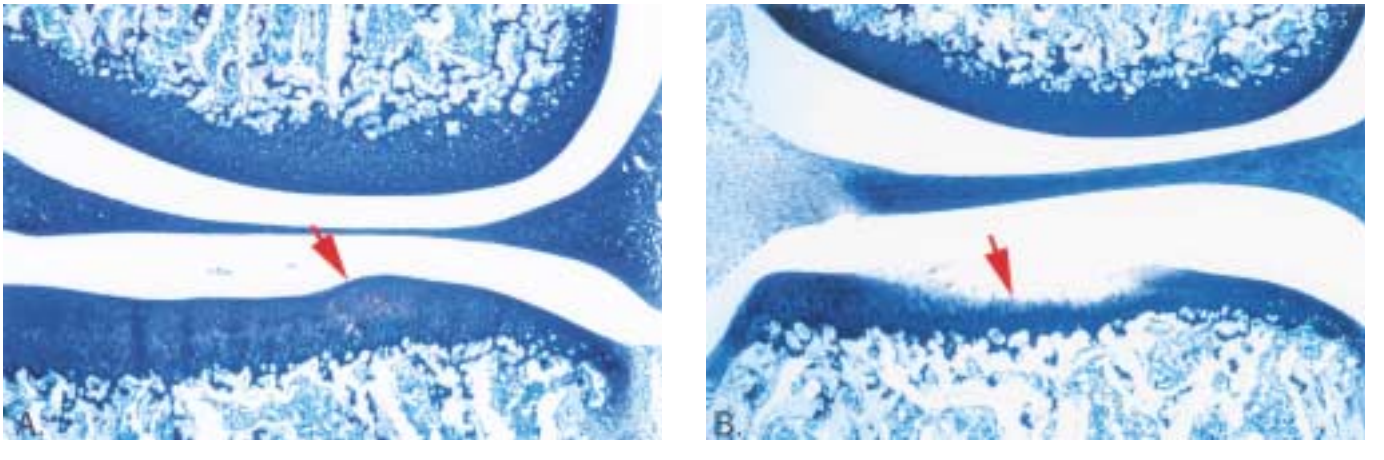

Figure 9. A. Photomicrograph of knee joint from immature guinea pig given naladixic acid 24 hrs previously. Focal mid-zone cartilage degeneration (arrow) characterized by obvious swelling to form a blister-like lesion with chondrocyte death and proteoglycan loss is present on 1/3 of the medial tibial plateau. The deep zone cartilage is unaffected. Toluidine blue, original magnification=40X. B. Photomicrograph of knee joint from immature guinea pig which had naladixic acid 72 hrs previously. Focal mid-zone cartilage degeneration (arrow) characterized by chondrocyte death and proteoglycan loss with fibrillation of the collagenous matrix is present on 2/3 of the medial tibial plateau. The deep zone cartilage is unaffected. Toluidine blue, original magnification=40X.

resemble those occurring in some stage of human OA. Differences are generally in how rapidly they progress. Obviously the spontaneous models such as the guinea pig, mouse, hamster and nonhuman primate offer the best opportunity to study the slowly progressive OA that is most characteristic of human disease.

Surgically-induced models of OA, especially in rodents and rabbits, usually have rapid and severe cartilage degeneration after the instability is created. Generally, the greater the instability, the greater the lesion. However, the load-bearing tendencies of the particular species must be factored in. For example, partial medial meniscectomy in the guinea pig results in severe lesions within a week post-surgery[20,21]. Lateral meniscectomy in this same species produces only minimal to mild changes (unpublished A. Bendele). The opposite is true for rabbits where lateral meniscectomy results in a severe lesion and medial meniscectomy causes milder changes[42,43]. We have performed surgical instability models in rats, guinea pigs, rabbits and dogs. For testing of potential OA drugs, it would seem that the ideal model would be a small species such as the rat or guinea pig that would allow for dosing relatively large groups of animals. The duration of the model should not be longer than 3 months and the disease progression should occur over that duration. In other words, most of the cartilage damage should not occur in the first week. The model that offers the potential to gain the most information in the shortest period of time would seem to be the guinea pig meniscal tear/spontaneous OA combination model. This model allows for evaluation of both a surgically-induced lesion (relatively rapidly progressive) and the spontaneous lesion (slowly progressive, contra-lateral limb) in the same animal and in the same test. As with all of the other models, the predictability for human disease modification remains to be determined.

For studies of pathogenesis of OA, any of the spontaneous disease models would be appropriate, depending on the design of the studies. Obviously, studies requiring the harvest of large amounts of diseased cartilage for mRNA extraction would require the use of a species larger than the mouse.

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