

Animal models of osteoarthritis in an era of molecular biology

A.M. Bendele

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

Animal models of osteoarthritis (OA) are used to study the pathogenesis of cartilage degeneration and to evaluate potential anti-arthritic drugs for clinical use. In general, these models fall into 2 categories, spontaneous and induced (surgical instability or genetic manipulation). Animal models of naturally occurring OA occur in knee joints of guinea pigs, mice and Syrian hamsters. 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. Transgenic models have been developed in mice. These models all have potential use in the study of molecular mechanisms associated with OA development via use of immunohistochemistry, biochemistry and molecular probes to identify altered matrix molecules at different stages in disease progression. Testing of specific types of inhibitors developed through evaluation of matrix changes in the disease process will ultimately help identify key processes which initiate and perpetuate the disease and will lead to discovery of new disease modifying pharmaceutical agents for OA patients. This paper will focus on the discussion of several models which are likely to be useful in the molecular dissection of processes involved in cartilage degeneration.

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Instability induced knee OA in mice

Spontaneous OA occurs in the knee joints of various strains of mice¹⁻⁵ with an increased incidence and severity as they age. Unlike the situation in guinea pigs which develop a highly predictable knee OA at a relatively young age⁶⁻⁸, the disease in mice is somewhat sporadic, thus generally precluding use of mice for pharmaceutical testing or pathogenesis studies. In most but not all mouse strains, spontaneous OA tends to develop in the medial aspect of the knee, thus suggesting that mice may preferentially load this compartment of the joint. This led us to investigate the possibility of enhancing the tendency for mice to develop medial compartment cartilage degeneration by destabilizing the knee via damage to the anterior cruciate ligament. In a series of studies in which knee joints of mice were destabilized, highly predictable medial compartment lesions occurred in the cartilage of the medial femoral condyle and medial tibial plateau over a time course which indicated that the lesions were progressive in nature. The potential use of this model in transgenic and knockout mice of various types will allow

investigation of the role of specific mediators in the pathogenesis of lesion development. The model is consistent enough in its development to allow testing of specific inhibitors of cartilage matrix degeneration thus offering another potential approach to dissection of the pathogenesis of disease in a small animal model.

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 pigs⁶⁻⁸ as well as in other strains of guinea pigs⁹. 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 grams⁷. 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.

The disease is progressive so that severe medial compartment degenerative changes are present in 12-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. 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.

Corresponding author: Alison M. Bendele, DVM, Ph.D., BolderPATH, Inc. University of Colorado, MCD Biology, Campus Box 347, Boulder, CO 80309, USA

E-mail: abendele@earthlink.net

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The pathogenesis of naturally occurring knee OA in guinea pigs is not completely understood. However, as is the case in human disease¹⁰, 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 obese, had greatly reduced incidence and severity of knee OA¹¹. Guinea pigs seem to preferentially load the medial aspect of the knee joint as evidenced by the fact that medial 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 knee¹². 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.

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.

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^{13,14}. Guinea pigs, unlike rats and mice, express both collagenase 1 and 3, and this expression occurs at the site of OA lesion development¹⁵. Mechanical forces have been found to increase expression of collagenase 1 mRNA¹⁶ 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 neopeptide marker of OA called 3-B-3(-)¹⁷ which results from a change in the termini of the chondroitin sulfate (glycosaminoglycan) chains of aggrecan^{18,19} and is found in human OA cartilage as well.

Surgically induced osteoarthritis

Rat or guinea pig medial meniscal tear

Unilateral medial meniscal tear in rats or guinea pigs will result in rapidly progressive cartilage degenerative changes characterized by chondrocyte and proteoglycan loss, fibrillation, osteophyte formation and chondrocyte cloning²⁰. 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. Inhibitors of matrix metalloproteinases have been shown to have excellent activity in inhibiting cartilage degeneration and osteophyte formation in this model²¹. Additional testing with spe-

cific inhibitors of various MMPs will allow further dissection of the importance of these mediators at different stages in the disease.

Beagle dog partial medial meniscectomy

Use of the beagle dog for OA model conduct, as with the rat, offers the opportunity to generate efficacy data in a species commonly used for toxicology testing. 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. Lesions are reasonably consistent with respect to location and severity. Small osteophytes or areas of peripheral fibrous tissue proliferation are present on the medial aspect of the joint but are rare in the patellar groove area or lateral side of the joint. Subchondral bone thickening is quite prominent at 1-3 months post surgery. Synovial membrane changes are relatively mild and generally consist of papillary proliferation.

Besides the obvious advantage of being able to generate efficacy data in a species in which toxicology testing is likely to be done, there are several other advantages to using this model. Limited data are available on testing of inhibitory agents in this model or the molecular analysis of matrix changes.

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 injury²²⁻²⁴. 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. Lesions may occur in any location on the medial and lateral tibial plateaus and femoral condyles and severity of lesion is often associated with 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 disease²⁵ as well as studies documenting the presence of cytokines such as TNF- α or other mediators. Both canine models offer the opportunity to obtain reasonable quantities of cartilage for RNA or other biochemical analysis.

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