Animal Models of Osteoarthritis

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Abstract: The complex pathobiologic changes of human joint disease, particularly osteoarthritis (OA), normally take several decades to develop and may be influenced by a multitude of genetic and environmental factors. The need to clarify the molecular events that occur in joint tissues at the onset and during the progression of OA has necessitated the use of models, which, although imperfect, can exhibit many of the pathologic features that characterize the human disease. *In vitro* studies have proven invaluable in defining specific molecular and cellular events in degradation of joint tissues such as cartilage. However, to fully understand the complex inter-relationship between the different disease mechanisms, joint tissues and body systems, studying OA in animal models is necessary. Models of inflammatory arthropathies have proven predictive of clinical efficacy, with therapies that are beneficial in animals having significant benefit in treatment of rheumatoid arthritis in humans. While none of the available animal models of OA can truly be said to be predictive, as no anti-OA therapies have yet proven to be disease modifying in human trials, this approach represents a cornerstone for discovery of new anti-OA therapeutic targets and drugs. In this paper the available species and models of OA are reviewed and their potential utility discussed.

A. INTRODUCTION

The pathology of osteoarthritis (OA) is characterised by changes in most tissues of the joint, including cartilage, bone, synovium, synovial fluid, ligaments, tendon and joint capsule [1]. Cartilage initially becomes fibrillated in regions of high contact stress with concomitant bone sclerosis and thickening of the synovium and capsule. Recently ligament laxity has been suggested as an early or even causative factor in spontaneous OA development in guinea pigs [2] but whether this is a significant factor in idiopathic human OA is unknown. Clearly with post-traumatic partial or full-thickness tearing of ligaments, joint instability and secondary OA are common sequelae.

The pathophysiology and/or molecular mechanisms of the degenerative process has been defined for only a few of the joint tissues, although it is becoming increasingly obvious that most if not all of the listed tissues are involved, particularly when the clinical manifestations of stiffness and pain as well as structural alterations of the joint are taken into account. Cartilage detritus released from degenerating joint surfaces inflames the synovium, disrupting normal synthesis of synovial fluid components such as hyaluronan. The resulting fluid in OA joints is less viscoelastic [3] and therefore less able to function as a lubricant for the articular cartilage and ligaments. Increased stress on these tissues is transferred to subchondral bone, where impaired intra-osseus vasculature [4] leads to ischemia and compromised bone metabolism. A link between pain in patients with OA of the knee, and capsular fibrosis [5] implicates the important of the capsule in OA but there has been little research studying this tissue in animal models. Furthermore, in persons with knee OA, knee pain severity was associated with subarticular bone attrition, bone marrow lesions, synovitis/effusion, and meniscal tears [6], thus demonstrating that the pain of OA is probably generated by multiple tissues within the joint.

The relationship between the various joint tissues during the initiation and progressive stages of disease is still unclear, most particularly so in human OA, where lack of tissue from early/preclinical disease restricts more detailed research. There are suggestions that the pathogenesis of OA may be different depending on the precipitating, often unknown initiating factor, whether it be biomechanical, immunological or genetic in origin. OA disease development is difficult to study in humans. The time course of disease onset is slow and progression is extremely variable, genetic variability is too great, and humans are subject to a plethora of genetic and environmental factors (hormonal status, exercise, occupation, lifestyle, body mass index, etc) that influence the pathology. Current valid diagnosis is relatively insensitive to disease pathology without invasive procedures. It is very rare to see early OA in patients, with presentation to health care providers usually only occurring at more advanced to end-stage disease, after cartilage is degenerated sufficiently to visualise the changes by joint space narrowing. There is also a lack of availability of age-matched diseased and normal tissues and specific biomarkers for OA.

In light of the problems associated with studying pathophysiology and treatment of OA in humans, animal models remain a cornerstone of new anti-OA drug discovery. Before a promising new therapy can be initiated in the clinical environment, thorough testing in an alternate species is mandatory to ascertain optimal route of administration, dosage, safety and toxicity. Animal models are also required to validate and improve detection methods for OA to aid diagnosis and provide potential means to detect pathology at an earlier stage than is currently possible.

B. THE IDEAL ANIMAL MODEL

An ideal animal model has the following properties:

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1) The model should induce consistent reproducible disease that occurs in a suitable time frame to allow reasonably high throughput studies. These properties contrast with human OA, where the time frame is extremely variable but almost always considerably longer for both development and progression. For example, meniscectomy in sheep produces distinct osteoarthritic changes in the articular cartilage that can be visualised at 3 months post-surgery in 100% of animals [7, 8]. In humans, meniscectomy or ACL injury will cause osteoarthritis in 50% of cases by up 10 to 20 years post-surgery [9]. Recapitulating the human disease time frame even with increased incidence is untenable for an animal model. A suitable time frame of disease for optimum drug development would be from one to twelve months from induction to clinically significant disease. The fact that animal models are generally more rapidly progressive than in humans is also cause for caution when interpreting the efficacy of therapy. Compounds that are not effective in rapidly progressive animal disease may in fact prove to be efficacious in human OA.

2) The induced disease should be universally progressive in the time frame of the study to allow investigation of early, mid and late pathophysiology and treatment effects (both prophylactic and therapeutic for existing disease). Animal models that are too aggressive or rapidly progress to end stage do not provide sufficient "dynamic range" to detect therapeutic effects. Models where the disease reaches a plateau are also not suitable, as outcomes from long-term studies may be misleading with an early therapeutic benefit not being evident at later time points. Models where some animals develop the disease early and some do not develop disease at all are less than ideal for determining therapeutic responses. In an animal model, response to drugs should be similar between individual animals, occur at similar times in similarly treated animals and the effects be measurable both structurally and functionally. Unfortunately, in OA patients, therapies often take years to have a significant effect on any clinical outcome, particularly joint space narrowing (JSN).

3) The animal should be a mammalian species that is tractable, inexpensive, easy to house and manage, large enough to allow multiple analyses/outcome measures (e.g. radiology, magnetic resonance imaging (MRI), synovial fluid analysis, body fluids for potential biomarker analysis, regional histopathology and gene expression studies, biomechanics etc), allows genome wide micro-array analysis (as currently available for mouse and human) and proteomic analysis, sequencing etc. Ideally the model should enable clinical outcomes to be assessed and allow quantitative measurement of symptoms such as pain, gait abnormalities and swelling. There are very few reported animal models of OA where pain has been validated as a clinical outcome. Iodoacetate intraarticular injections in rats were used to induce OA-like changes with concomitant hind paw weight distribution changes [10-12]. The pain could be decreased using common analgesics such as acetaminophen, morphine or nonsteroidal antiinflammatory drugs. Similarly, medial meniscal transection in rats has been reported to alter hind limb weight bearing and is amenable to pharmacological control [13]. Force plate analysis and evaluation of ground reaction forces has been used to evaluate pain in OA models in dogs [14].

   The ability to correlate structure and function (molecular and histological changes with clinical outcomes) is also desired to aid in understanding which of the myriad changes in OA joints truly relate to clinical disease. Even the correlation between molecular alterations and histological grading is unresolved, although often reported for OA models [15, 16].

4) The disease process in the animal recapitulates the human pathology in all tissues of the articulating joint. This is one of the most difficult properties to define as the pathology of the human disease is not completely understood in all tissues, so it is not feasible to assume replication by an animal model. The progression of late stage degeneration in human articular cartilage, bone, synovial fluid and synovium [1] has been well documented, however early OA changes and the pathophysiology of other tissues (entheses [17, 18], ligaments [19], tendon, capsule, meniscus) of the joint during the development of OA has been under-reported. Further comparison and validation of changes in tissues of animal models to those occurring in the human disease is required. Genomic analysis of cartilage from ACLT rats has demonstrated changes in expression of many genes that are similar to those found in human OA cartilage [20]. These microarray studies, however, generate many other potential gene candidates that have yet to be confirmed as relevant to human disease.

   Many comparative human to animal model studies have concentrated on a single joint tissue and neglected the joint as an organ. In addition, as debate continues regarding whether human OA pathogenesis is a cartilage-driven or bone-driven process, use of models that demonstrate temporal differences in bone or cartilage pathology [21] cannot be said to mimic the human etiology. There may also be major genetic differences present between the species used in the animal model and humans that will alter the pathology of the OA disease process. Examples are the finding that adult rats and mice do not express the collagenase, matrix metalloproteinase(MMP)-1[22] and the aggrecan core protein of rodents does not have an extended keratan sulfate binding region present in humans and other species [23]. Whether these inherent differences will diminish the ability to extrapolate findings in rodents to man remains to be established. Undoubtedly other significant differences (that are yet to be defined) exist between humans and many of the commonly used experimental species, and these must be borne in mind when interpreting results from animal studies.

5) The model should be predictive of therapeutic disease modification in humans. In simple terms, what works in the animal, works in patients. Unlike studies on

drugs for modification of rheumatoid arthritis (RA), many therapies that have had a profound effect in OA animal models have lacked efficacy in human patients, and vice versa. Exceptions are diacerhein [24] and doxycycline [25], where positive effects in various OA models have been recapitulated in clinical trials. Models should also be developed to simultaneously answer questions relating therapeutic modulation of structure (of the joint organ, tissues and cells) to biomechanical and hence functional deficiencies in OA joints.

Many good models of OA in animals exist, however none of the existing models fulfil all of the above criteria. In order to optimise research into this disease, it is extremely important to match the question asked to the model chosen. Careful and critical thought is required about the primary aim of the study and whether an available model is the most suitable for answering the question presented.

C. CURRENTLY AVAILABLE OA INDUCTION METHODS

For brevity sake, we have restricted this review to animal models of appendicular articulating joint osteoarthrosis, although we acknowledge that models of spine facet and temporomandibular joint OA exist, where the disease pathology is most likely similar. A variety of species and induction mechanisms have been reported and previously reviewed in detail [26]. These are summarised briefly in Table **1**, along with a few key references.

Induction of pathologic changes by intraarticular injection of a variety of agents, including enzymes (papain, collagenase, trypsin, hyaluronidase [49]), cytokines (interleukin (IL)-1[50], transforming growth factor (TGF)-β[51]) and chemicals (monosodium iodoacetate (MIA) [52]). Many of these agents induce a significant acute local inflammation at the site of injection and thus may not replicate the naturally occurring sequence of the disease process of human OA. Injection of agents that selectively degrade components of the ECM (e.g. collagenase, papain, chondroitinase etc) may not be globally relevant to modelling human OA as the processes that precede and induce proteolysis in human OA have not occurred. Furthermore, the reagents used may deviate significantly from those that exist in human disease (e.g. extracellular glycosidases) as well as potentially increasing inflammation above that which is generally observed in the human condition. Intraarticular injection of MIA has been widely used [26], however as MIA is an inhibitor of glycolysis and induces significant chondrocyte apoptosis and joint inflammation, its utility as a model to investigate pathophysiology of OA may be questioned, despite its validation as a model of pain. Furthermore, as the disease process induced by MIA does not fully mimic the OA process, use of this model even to investigate pain in OA may be misleading.

Immobilization induces atrophic changes within articular cartilage that superficially mimic OA pathology [53]. There are, however, differences in cellular morphology induced by immobilisation that are not observed in OA chondrocytes, such as necrosis and lack of cell cloning. Nutritional deprivation of the cartilage, not necessarily a typical feature of OA, is compromised in immobilization models due to the absence of loading and cyclical fluid movement [54].

OA occurs spontaneously in various genetically modified (GM) mice (topic covered in a separate article in this issue and discussed previously [26]). Rather than being global models to investigate pathophysiology and treatment of OA, GM mice with both increased spontaneous OA or reduced spontaneous and/or induced OA, provide valuable information elucidating the role of specific molecules in the disease process.

Spontaneous OA occurs in various inbred strains of mice, guinea pigs and macaques. OA in the Duncan-Hartley guinea pig is well characterised and widely used in localities where these animals are available. These animals have the advantage of naturally occurring disease, however there is concern that the underlying mechanism for the particular strain of animal developing the disease may not necessary be the same as for OA in humans. The prevalence of OA in the guinea pig appears to be linked to laxity in the cruciate ligaments [2], suggesting a biomechanical driving force for OA development, and that this species is a model for secondary rather than primary disease. Another disadvantage of using the spontaneous OA models is the reduced incidence of naturally occurring disease (not 100% as for many surgically-induced models) and a more variable and extended time frame of disease progress, particularly for the larger animals.

Surgically induced destabilisation of joints is the most widely used induction method, where the underlying initiating mechanism is altered mechanical loading, one of the most common causes of secondary OA in humans. Many induction methods actually copy known injuries in humans, such as ACLT and meniscal injury (partial or complete meniscectomy, meniscal transection and meniscal destabilization). An advantage of these models is temporal control of disease induction (compared to spontaneous animal OA and to the human disease) and, in general, these models follow a predictable progressive disease onset. Surgically-induced destabilization models of OA also appear to mimic the molecular pathology (at least in cartilage e.g. ADAMTS cleavage of aggrecan, collagenase cleavage of collagen, chondrocyte early hypertrophic response etc) and histopathology that is observed in humans. There are, however, limited clinical outcome measures currently available in many of the species used.

D. SPECIES CHOICE

Advantages and disadvantages of species commonly used for models of OA are listed in Table **2**. While certain specific genetic differences are recognised (eg no MMP-1 in rodents), there may be as yet unrecognised species related differences in response to OA-related stimuli and therapies. For example, the response of chondrocytes to different IL-1 isoforms varies with species - equine and human chondrocytes are far more sensitive to IL-1β while bovine and porcine chondrocytes respond better to IL-1α [55, 56].

Table 1. Methods of Inducing Appendicular OA in Animals

OA Induction Method	Species	Comments	Ref.
Spontaneous	Mouse (DBA/1, STR/ORT, C57BL/6, C57) Guinea pig (Hartley, Duncan-Hartley) Macaque (Rhesus, Cynomolgus)	Incidence varies with strain and sex. Well established OA by 3 months. Long life span (disease slow and variable).	[27] [28] [29, 30]
Genetic modification	Mouse	OA induced by a specific gene change not same as human OA	[26, 31]
Intraarticular injection (steroids, collagenase, papain, iodoacetate, cytokines)	Mouse Rat Rabbit Horse	Usually induce significant local inflammation; enzyme injections may not recapitulate human disease	[26, 32]
Immobilization	Rat Rabbit Dog	Atrophy, necrosis of chondrocytes rather than cloning.	[33]
Surgical Induction/Destabilisation			
ACL transection (ACLT)	Rat Guinea pig Rabbit Cat Dog (mongrel, foxhound, beagle)	Speed of onset and severity of disease higher than in humans after same injury	[34, 35]
Meniscectomy	Rat Guinea pig Rabbit Dog (mongrel, greyhound) Sheep Monkey (Grivet)	Maybe partial or total, medial or lateral excision, unilateral or bilateral. Speed of onset and severity of disease in animals usually higher than in humans after same injury	[26, 36, 37]
Meniscal destabilisation	Mouse	Unproven with therapeutic intervention as yet	[38, 39]
Combination surgery	Mouse Rat Guinea pig Rabbit	Various combinations of ACL and/or MCL transection with or without meniscectomy or meniscal destabilisation	[40] [41] [26]
Impact loading; cartilage scarification	Rabbit Dog	Direct acute trauma to joint	[42, 43]
Osteochondral chip and exercise	Horse	Mild post-traumatic cartilage changes	[44, 45]
Ovariectomy	Rat Sheep Macaque	Postmenopausal OA; maybe secondary to weight gain and/or bone changes	[46] [47] [48]

With all the surgical models in Table **1** and in all the species in Table **2**, the age, gender and maturity of animals, as well as levels of exercise/activity can modulate severity and time frame of disease progression. It is also important to recognise that the same surgical procedure in different species may have widely variable effects. For example, ACLT in the dog elicits a strong early cartilage hypertrophic response, followed by cartilage degradation and erosion in a matter of months [57, 58]. In contrast, the same ACLT operation in sheep and goats results in no effect in the same time frame, and little detectable cartilage damage even after 18 months [59-61]. This likely relates to anatomical, and hence biomechanical, differences between the species as sheep and goats have tibial plateau with a very horizontal orientation, whereas the tibial plateau of the dog slopes forward markedly. It is also worth remembering that different joints within the one species of animal respond differently to molecular stimuli e.g ankle *vs* knee in human [62].

E. OUTCOME MEASURES

OA is not specifically a cartilage disease, although many studies have focussed on cartilage changes as a primary outcome measure and most researchers have depended heavily on cartilage histology in evaluating animal models of OA. Despite this, even for cartilage histopathology, standard scoring systems have not been universally available. Even with the most commonly used histopathology grading systems based on that described by Mankin [63], the relationship between the pathological parameters scored and clinically relevant disease is not well established. More recently

Table 2. Advantages and Disadvantages of Species Used in Models of OA

Species	Advantages	Disadvantages
Mouse	Tractable Easy Management Low cost Speed of disease onset is rapid Genetic modifications possible Full genome available Microarray available Low amounts of drug required	Size – limits tissue discrimination and availability Known molecular differences e.g. MMP-1 Limited synovial fluid harvest possible Ratio of cell volume to matrix in cartilage high No regional pathology possible Limited clinical outcomes (MRI, pain etc) Surgical induction more difficult
Rat	As for mouse plus Pain models available	As mouse although surgery is easier
Guinea pig	Tractable Easy Management Spontaneous disease	Size for regional analysis Not available in all countries Lack of full genome
Rabbit	Easy to dose Readily available in most countries Surgically suitable Genetically pure	Prevalent disease Articular cartilage degrades more easily and repairs readily (unlike human) Lack of complete genome
Cat	Size (tissue/fluid collection) Regional tissue analysis possible Full genome available Intraarticular therapy possible	Emotional attachment and ethically controversial in many countries ± tractable Management difficult/costly Genetic variability No microarrays available
Dog	Size (tissue/fluid collection) Tractable and trainable Regional tissue analysis possible Intraarticular therapy convenient Clinical outcome measures well published Full genome available (beagle) Microarray available	Emotional attachment and ethically controversial in many countries Management difficult/costly Genetic variability Lack of complete genome for other than beagle
Goat	Size (tissue/fluid collection) Regional tissue analysis possible Cartilage thickness closer to human's	Cost Lack of complete genome No microarrays available Not monogastric (oral therapy) Housing/management difficult
Sheep	Size (tissue/fluid collection/approximates human) Regional tissue analysis possible Housing and availability (country dependent) Control of strain genetics Clinical outcome measures well published Intraarticular therapy convenient	Lack of complete genome No microarrays available (could use bovine) Not monogastric (oral therapy)
Primates	Closest genetically to humans Size (tissue/fluid collection) Regional tissue analysis possible Genome available for some species Rhesus monkey array available Intraarticular therapy possible	Full genome not available for some Housing and management difficult Cost Emotional attachment and ethically controversial in many countries
Horse	Size (tissue/fluid collection) Regional tissue analysis possible Clinical outcome measures well published Cartilage thickness closer to human's Intraarticular therapy convenient Genome and microarrays available	Cost Management, housing (size) Drug amounts required for systemic therapy may be prohibitive Specialised anaesthesia/surgical facilities required

alternative scoring systems have suggested that only the degree and area of cartilage structural damage should be evaluated [64], but long-term analysis of this new methodology in humans and various animal species is still required.

The gold standard for non-invasive measurement of disease progression and therapy in OA in humans is JSN by plain radiograph. This technique is useful only at late stage disease; requires a minimum of 1-3 years to observe progres-

sion; is difficult to standardise across clinical units and is relatively insensitive to change. There are few published studies on the use of JSN in animal models of OA [65, 66]. Other imaging techniques such as micro-computed tomography and MRI are being increasingly used in both animal models and humans. Many of these methods have not been standardised within different models/species and hence difficulties arise when trying to compare structural findings. New techniques such as gadolinium-enhanced MRI of cartilage (GEMRIC) has been used to measure glycosaminoglycan loss from articular cartilage [67], however these new techniques have yet to be proven quantifiable and be validated to assess disease progression in any animal model.

Despite this progress, the relationship between cartilage pathology and clinically relevant disease progression (pain, range of motion, functionality, quality of life) is not well defined. Attempts to find universal but specific biomarkers for OA in joint and/or blood fluids have been extensive [68, 69] but have yet to be proven for routine and reliable diagnostic or prognostic purposes. Promising candidate molecules such as cartilage oligomeric matrix protein and collagen type II and aggrecan degradation products including specific proteolytically-derived neoepitopes, continue to be validated. However, systemic markers do not yield details of the specific joint or joints involved and do not distinguish between decreasing levels due to disease amelioration and decreasing levels due to extensive loss of articular cartilage.

In the search for outcome measures to monitor disease progression it is important to evaluate other aspects of the joint, including changes in non-cartilaginous tissues and the role of neural and vascular elements in disease progression and their relationship to clinical symptomology. Nowhere is this likely to be more important than in evaluating the potential use of stem cells in OA therapy. These pluripotent precursors are likely to have wide ranging therapeutic effects on all the tissues of the articulating joint. To date, there have been limited studies utilising stem cells in animal models of OA as opposed to their evaluation in partial or full thickness cartilage or tendon defect repair studies. To the best of our knowledge only one study has examined the utility of stem cells to treat OA *per se* [70]. Interestingly, in this report, intraarticular injection of stem cells six weeks after combined ACLT/meniscectomy surgery in goats resulted in early but not late reduction of osteophytosis, subchondral sclerosis and cartilage destruction. Significantly enhanced meniscal regrowth was observed in stem cell treated joints and was associated with reduced OA change. Importantly, stem cells in this model were found to engraft into the synovial capsule, the fat pad, the meniscus and intraarticular ligaments but not articular cartilage, consistent with the idea that any chondroprotection was secondary to effects in other joint tissues.

There are current trials of pure autologous mesenchymal stem cell delivery being performed in patients with knee OA (eg Chondrogen by Osiris Therapeutics). However, as pluripotent cells usually require special stimuli (growth factors, cytokines) to differentiate into matrix-producing connective tissue cells, future trials of stem cell delivery with these additives will no doubt require extensive testing in animal models before being permissible for human use. The environment in an OA joint is hostile, with increased cytokines, chemokines, degradative enzymes and other signalling molecules. If stem cells are to integrate and form new healthy tissue, they will probably have to be supplied in an appropriate milieu to not only stimulate them to become the correct cell type but also calm and restore the local diseased environment into which they are being injected.

F. CONCLUSION

These studies highlight the importance of considering the joint as an organ and the need to evaluate clinical outcomes in these animal models as well as structural and molecular changes in various tissues. Despite numerous available animal models for OA, there is no one gold standard model that can be truly defined to represent human etiology. Each mode of induction and species has distinct advantages and disadvantages, and there are still many gaps in our knowledge of both the human disease and the relationship between documented degenerative molecular changes and functional and clinical deterioration.

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