The AAPS Journal, Vol. 15, No. 2, April 2013 (© 2013)
DOI: 10.1208/s12248-013-9454-x

Review Article

Theme: Human and Veterinary Therapeutics: Interspecies Extrapolations and Shared Challenges
Guest Editor: Marilyn Martinez

Animal Models of Osteoarthritis: Challenges of Model Selection and Analysis

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Received 19 July 2012; accepted 4 January 2013; published online 18 January 2013

ABSTRACT. Osteoarthritis (OA) is the most common musculoskeletal disease, affecting millions of individuals worldwide. New treatment approaches require an understanding of the pathophysiology of OA and its biomechanical, inflammatory, genetic, and environmental risk factors. The purpose of animal models of OA is to reproduce the pattern and progression of degenerative damage in a controlled fashion, so that opportunities to monitor and modulate symptoms and disease progression can be identified and new therapies developed. This review discusses the features, strengths, and weaknesses of the common animal models of OA; considerations to be taken when choosing a method for experimental induction of joint degeneration; and the challenges of measuring of OA progression and symptoms in these models.

KEY WORDS: animal; model; osteoarthritis.

INTRODUCTION

Osteoarthritis (OA) is the most common musculoskeletal disease, affecting millions of individuals worldwide. A chronic disease, OA develops progressively over a span of decades with joint failure as its final outcome: cartilage loss, synovial inflammation, subchondral bone sclerosis and cyst formation, osteophytosis, loss of range of motion, and pain. Factors which may contribute to OA development include inflammation (1,2), trauma (2), aging (2), obesity (2,3), chondrocyte differentiation (4), and genetic predisposition (2). Recent reviews on contributing factors in OA pathophysiology are referenced in the preceding list. An excellent review of OA as a whole organ disease of the joint was recently published by Loeser *et al.* (5). Identifying and understanding the interplay between the various contributing mechanisms will lead to better treatment options.

For the pharmaceutical industry, there are three critical targets in the treatment of OA: pain, function, and disease progression. Disease modifying OA drugs are being actively sought to attenuate OA progression by targeting the biologic and biomechanical causes of OA, but none have yet been successfully translated for human use. Currently available therapies address only symptoms, and still there remains a significant unmet need for treatments to further decrease pain and increase function. Such treatments could extend the time until joint replacement surgery as well as provide symptomatic relief in patients who will never be candidates for this operation for medical reasons. Given the scale of the global burden of OA disease, either disease modifying or new symptomatic OA therapies will find wide markets when discovered.

The development of these treatments, however, requires an understanding of the pathophysiology of OA; its biomechanical, inflammatory, genetic, and environmental risk factors; and the ability to model these conditions using *in vivo* animal models. As our understanding of OA pathophysiology improves, our ability to develop more accurate disease models will also improve. Given the rapid expansion of our understanding of the disease processes in OA, it is an ideal time to evaluate and compare our available animal models. The purpose of animal models of OA is to controllably reproduce the scale and progression of joint damage, so that opportunities to detect and modulate symptoms and disease progression can be identified and new therapies developed. An ideal animal model is of relatively low cost and displays reproducible disease progression with a magnitude of effect large enough to detect differences within a short period of time. If the model progresses too rapidly to end-stage degeneration, intermediate time points, which are representative of OA pathophysiology, may not be obtainable and in the absence of this information, subtle effects of potential

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interventions may be missed. Recognizing that OA is an end-stage phenotype, the result of an interaction of mechanical and biochemical processes, animal models allow these factors to be studied in a controlled environment.

While spontaneous OA progression has been described in a number of laboratory animal species, including guinea pigs, hamsters, and certain mouse strains (6), OA development in these animals proceeds slowly. For that reason, surgically induced joint instability and adjuvant injection models have been developed to create faster disease progression models. A valid concern with these induced models is that while they may be reflective of inflammatory or posttraumatic OA, they may not be entirely representative of spontaneous, naturally occurring human OA. Determining what animal model or models accurately represent OA development and symptoms is essential for the process of translating therapies from laboratory to clinical applications. Animal data provide a foundation of expectation upon which plans for clinical trials are built, and the success of these trials is dependent upon the use of appropriate preclinical models of disease. The purpose of this review is to discuss the features, strengths, and weaknesses of the common animal models of OA; considerations to be taken when choosing a method for experimental induction of joint degeneration; and the challenges of measuring of OA progression and symptom modulation in these models.

COMMON ANIMAL MODELS

In October, 2010, *Osteoarthritis and Cartilage* published the OAC Histopathology Supplement (6), which provides a review of the published models and guidelines for histopathologic assessment of osteoarthritis progression in the mouse, rat, guinea pig, rabbit, dog, sheep, goat, and horse. Each reference includes a review of the published methods of OA induction for these species models, and this supplementary volume represents an expert consensus recommendation for gross and histologic assessment of OA in the commonly used animal models. This work is an important contribution toward standardizing and validating the outcomes of animal OA investigations, although this has not yet been accomplished. Table I is derived from this supplement and lists a summary of age at skeletal maturity, methods of OA induction, and special considerations for each of these animal models. The referenced supplement contains detailed histology preparation instructions and illustrated outcome metrics to be used for histologic assessment of OA progression in each species model (6). There still remains a need for a similar set of consensus guidelines for imaging and molecular biomarkers, pain, function, and gait measures in OA animal models.

As seen in Table I, three general categories exist for *in vivo* OA models: naturally occurring OA (including genetically modified animals), the initiation or acceleration of joint degeneration using surgery or other trauma, and the intra-articular injection of chondrotoxic or pro-inflammatory substances. Animal subjects range in size from mice to horses. For reasons of cost, ease of handling and greater availability of facilities for housing, smaller animal species (mice, rats, guinea pigs, and rabbits) are most often used for early investigations, with subsequent verification of findings in larger animal models (dogs, sheep, goats, horses, and pigs). Joint mechanics, cartilage thickness and histology, and OA disease progression rates are variable among the species and joints being studied. As large mammals, the larger animal models generally have cartilage morphology and responses to injury that are more similar to humans, but these models are limited by slower disease progression, higher cost, and larger housing requirements.

Notably, the Osteoarthritis Research Society International (OARSI) supplement does not address the use of the mini pig for OA studies. In our experience, anterior cruciate ligament (ACL) transection in the mini pig, with or without reconstruction, has provided a useful model for the study of OA changes in a large animal model (7). The anatomy of the pig knee is similar enough to human anatomy to allow for the study of surgical interventions including arthroscopy, cartilage resurfacing, and ACL reconstruction (8–11). Mini pigs reach skeletal maturity at 18–24 months (8). The mini pig is preferred because the smaller size allows easier handling of the animals and avoids the rapid growth phase of other domestic pig breeds. Unlike goats and sheep, pigs are not ruminants and therefore would also provide advantages in studying the effects of orally administered therapies. A disadvantage of using the mini pig, compared to sheep or goats, is that these animals cost more. Obtaining juvenile specimens is relatively easy, but late adolescent and adult specimens are more difficult to find and require advance planning in order to request a number of specimens of the same age.

Determining which model is best remains an open question in OA research. Each model has limitations, and for each new investigation, considering the mechanism of action of the intervention may be helpful for guiding model choice. For example, surgically induced OA models are more representative of posttraumatic OA. Other considerations that should influence the choice of model include the length of time available to conduct the study, the need to study OA disease symptoms *versus* tissue damage, and the amount and type of tissue (synovium, meniscus, cartilage, etc.) needed for analysis. And equally as important as the choice of an appropriate model for the induction of OA disease is the selection of an appropriate control group for comparison. When a unilateral intervention is used to induce OA disease, it is tempting to use the contralateral limb as a control. However, it is important to note that changes in gait to allow for offloading of the injured limb may induce inflammation or altered cartilage stresses in the contralateral limb, making it an imperfect control. An illustration of the effects of unilateral joint injury on subsequent pathology is the finding of increased progression to contralateral cranial cruciate ligament (an alternate name for the anterior cruciate ligament) rupture in a longitudinal study done of client-owned dogs with initial unilateral cranial cruciate ligament rupture (12). A non-operated or sham surgery control group provides a more true control, accepting that this then adds cost for a study. But even defining an appropriate control presents decisions to be made, since any surgical procedure, even a sham such as a capsular incision, will cause inflammation, pain, and possibly altered gait by comparison to a nonoperative control.

SURGICAL OA INDUCTION

In all animal models, surgical methods of OA induction work through a combination of joint destabilization, altered

Table I. Common Animal Osteoarthritis Models

Species	Age at skeletal maturity	Spontaneous OA	Surgical OA	Injection OA	Other OA	Special considerations
Mouse (6)	10 weeks	+	ACLT	Collagenase	Transgenic knock out and knock in strains available	Murine cartilage is very thin and does not have distinct superficial, transitional, and radial zones
Rat (6)	3 months	+	MMT ACLT pMMx ACLT + pMMx Ovariectomy	Iodoacetate Papain Collagenase Immunotoxin		OA progression may be accelerated with exercise protocols
Guinea pig (6)	6 months	+	ACLT pMMx MCLT LCLT ACLT + pMMx ACLT + MCLT + pMMx Osteotomy Ovariectomy Patellectomy Sciatic neurectomy+/− pMMx	Iodoacetate Papain Collagenase Copper II bisglycinate LPS CMP	Impact loading	Animals in paired housing have significantly greater OA progression compared to single-caged animals
Rabbit (6)	8–9 months	−	ACLT MMx ACLT + MMx ACLT/PCLT + MMx Patellectomy	Iodoacetate Chymopapain Collagenase Trypsin Il-1B Chondroitinase ABC Vitamin A Fibronectin fragments	Immobilization Repetitive loading Impact	Lateral compartment is predominantly loaded in the knee. OA progresses more rapidly in older animals following surgical OA induction.
Dog (6)	9–18 months	+	ACLT MMx Abrasion Groove Valgus osteotomy Cartilage defect Pelvic osteotomy	Iodoacetate Papain Calcium Pyro-phosphate crystals	Impact Oral quinolones Patellofemoral loading	Certain breeds are chondrodystrophic (beagles, dachshunds) and should not be used.
Sheep (6)	2 years	−	MMx LMx Bilateral LMx ACLT ACLT + MCLT ACLT + MMx			ACLt alone causes only limited OA damage in both sheep and goats
Goat (6)	2 years	+	MMx MMx + MCLT			Lentivirus infection may cause synovitis and joint degeneration.

articular surface contact forces, and intra-articular inflammation. These methods create models that are intended to represent posttraumatic OA. The surgical models include meniscectomy, ACL transection, and osteotomy. An essential consideration when choosing any surgical OA model is to understand the biomechanics of the joint chosen. Knee joints are most commonly used in animal surgical OA models, but load distribution and gait mechanics for this joint vary by species. In particular, in humans and many animal species, greater load is transmitted through the medial compartment of the knee. Since the area of greatest load transmission is usually the site of the earliest and most consistent degenerative changes, surgical intervention, such as meniscectomy, should be performed on the medial compartment. An important exception to this is in the rabbit knee, where loading is greater in the lateral compartment. In the rabbit knee, the lateral compartment is the site of early arthritic changes and should be the site of intervention and early analysis (13).

Another important point to recognize is that published surgical OA induction procedures may be different from the clinical injuries which occur in humans but share the same name. The standardization of surgical OA induction procedures is also imperfect. An example of the need for clarity is found with the rat medial meniscal tear model, medial meniscal transection, and partial meniscectomy. The rat medial meniscal tear model has been described in the literature (13) as a procedure in which the medial collateral ligament is transected just below its attachment to the meniscus and the meniscus is then reflected toward the femur and cut at its narrowest point. The abbreviation used for this procedure is "MMT." Another procedure described in the literature is medial meniscal transection in the rat knee (14), which is also abbreviated as MMT, but in this procedure, the medial collateral ligament is left intact after the meniscus is transected. In contrast, in clinical medicine in humans, meniscal tear refers only to an injury to the meniscus and usually does not include an injury to the medial collateral ligament (15). In the animal model, accelerated disease progression may be achieved through destabilizing the joint by transecting the medial collateral ligament in addition to compromising meniscal function (13). This distinction is relevant for interventions that aim to investigate interventions for chondroprotection. It should be noted that each condition presents a unique biomechanically challenging environment.

Apart from meniscal transection, partial or complete meniscectomy is another procedure for which the animal model differs from the clinical entity. In one sense, a partial or complete meniscectomy can be understood as the removal of either part or all of the meniscus. A typical partial meniscectomy is described in animal studies as removing the anterior one third to one half of the meniscus through an anterior arthrotomy (16). However, because the meniscus functions by distributing load through hoop stresses, detaching the anterior meniscus functionally achieves a similar result to complete meniscectomy (17). In contrast, partial meniscectomy in humans is used to describe arthroscopic debridement of the inner aspect of the meniscus to remove the loose edges of a meniscal tear. The horns of the meniscus are left attached, which maintains at least partial function (18).

Anterior cruciate ligament transection (ACLT) is another commonly used method for surgical induction of OA

Table I. (continued)

Species	Age at skeletal maturity	Spontaneous OA	Surgical OA	Injection OA	Other OA	Special considerations
			ACLT			
			ACLT + MMx			
Horse (6)	2 years	+	Carpal fracture	Filipin	Impact	Osteochondral fragment exercise model is the most common published model in horses.
			MCPLT	Sodium MIA		
			Osteochondral Fragment + exercise	Amphotericin		
				E. coli LPS		
				Il-1		
				Polyvinyl alcohol foam particles		
				Carrageenan		
Mini pig (8–11)	18–22 months		Chondral and osteochondral defects			Mini pig strains are preferred to domestic strains due to growth velocity and adult size.
			ACLT			
			ACL reconstruction			

Compiled from reviews of published animal OA models found in the OAC Histopathology Supplement (6) and also includes references addressing the use of the mini pig OA model (8–10)
ACLT anterior cruciate ligament transection, *MMT* medial meniscal tear, *pMMx* partial medial meniscectomy, *MCLT* medial collateral ligament transection, *LCLT* lateral collateral ligament transection, *LPS* lipopolysaccharide, *CMP* chondromucoprotein, *Mx* meniscectomy, *MMx* medial meniscectomy, *LMx* lateral meniscectomy, *MCPLT* metacarpophalangeal ligament transection, *MIA* monoiodoacetate

(6,16). Among the surgical methods of OA induction, ACLT has the greatest effect on joint instability. Meniscectomy or medial collateral ligament transection may be performed at the same time as ACLT to further compromise joint mechanics and accelerate OA progression (6). ACLT as a procedure for OA induction in animal models is also not directly comparable to the human injury. Notably, in the clinical injury, the anterior cruciate ligament is ruptured by a combination of mechanical forces external to the joint which may result in injury to other joint structures involved in OA disease progression (i.e., blunt trauma to the cartilage, menisci, and subchondral bone). In the animal model, the anterior cruciate ligament is transected after an arthrotomy incision is made and the patella is retracted to allow access to the femoral notch. In the ACLT model, if the retinaculum is incompletely repaired or fails to heal, there may be patellar maltracking or dislocation of the patella, which will affect the progression of joint degeneration, and the procedure may irritate the patellar fat pad, causing additional intra-articular inflammation which is not entirely representative of human anterior cruciate ligament injury. As for all surgical models, ACLT procedures introduce elements of postsurgical inflammation, incisional pain, and uniquely altered joint and gait mechanics.

When comparing the results of surgical OA induction procedures, differences in the speed and sites of OA progression are expected, since each surgical model has its own unique effects on joint mechanics and inflammation. Highlighting these potential differences, a 2012 study by Moody *et al.* found significant variability in the morphology of OA progression between ovine ACLT/medial collateral ligament transection, meniscectomy, and ACL core models, as measured by differences in the parametric scores assigned during modified Mankin grading (19). The variability in these results embodies the challenge in selecting any one animal surgical OA model as the one most representative of OA. When selecting a particular animal OA model, it is essential to be familiar with the expected progression of disease, including the timeline for progression, the expected severity of OA lesions at harvest endpoints, and the location and number of sites within the joint to be used for histologic or other analysis. When choosing an animal model, it is best to identify its limitations relative to the endpoints of interest. As a reference for the comparison and selection of animal models of OA, the OARSI histopathology supplement provides an important guide (6).

INJECTION METHODS OF OA INDUCTION

Injection methods of OA induction act by stimulating intra-articular inflammation, direct matrix damage, or chondrocyte toxicity (13). These methods are useful for studying matrix degeneration but are limited in that the bulk death of chondrocytes is not representative of either spontaneous or posttraumatic OA progression, since a single episode of severe inflammation and chondrocyte necrosis is not the precipitating event for OA progression in most cases. Injections which provoke an inflammatory or autoimmune response are probably more appropriate for simulating inflammatory arthritides. An important concern for using injection models is whether the end-stage joint damage produced in these models provides a valid model of OA. In support of this concern, a recent study comparing transcriptional profiles of human OA cartilage and cartilage from rat knee joints treated with monosodium iodoacetate demonstrated total gene overlap of less than 4% (20).

ACCELERATION OF OA PROGRESSION

The addition of forced mobilization has been studied as a means of accelerating OA progression in joint destabilization OA models. High-intensity exercise has been associated with accelerated OA progression in several animal models (21–23). However, it has been found that mild to moderate exercise is protective against cartilage damage in the rat ACLT model (21). These data are consistent with the clinically observed benefits of mild to moderate exercise on OA pain and function (24). Although accelerating OA progression with forced exercise may be beneficial in reducing the time, and therefore the cost, associated with a study, it should be recognized that inclusion of exercise in the postoperative regimen introduces another variable in the disease process being modeled. The effects of exercise on cartilage metabolism, and the mechanisms by which joint mobilization result in either chondroprotection or damage, are themselves not fully understood and are an area of research interest.

Obesity is another factor that may accelerate OA progression in animal models. In humans, obesity is a known risk factor for weight bearing and non-weight bearing joint OA, the effects of obesity being mediated by biomechanical, inflammatory, and behavioral mechanisms (25). In animal models, the effects of obesity in accelerating OA progression have been studied most often in mice (26–28). The Dunkin–Hartley guinea pig, a model of spontaneous OA, is heavier than other guinea pig strains, and this model has also been proposed as a possible model of obesity-induced OA (28). Illustrating the complex influence of individual factors on OA development and progression, wheel exercise has been found to be protective against cartilage damage in an obese mouse OA model (29). In contrast to surgical or injection models intended to produce pathology on which to test pharmaceutical interventions, animal OA models that incorporate proposed risk factors such as activity level and obesity may provide an opportunity to study behavioral interventions to modify OA risk and symptoms.

IMAGING BIOMARKERS

Radiographic OA progression using the Kellgren–Lawrence grading system was historically been used to define and follow OA disease progression in humans (30). The application of this scale in animal models has not been standardized and validated. As our understanding of OA development and progression has improved, it has come to be recognized that OA disease is incident long before X-ray changes are evident. Newer imaging techniques, in particular magnetic resonance imaging and microCT, provide the opportunity to noninvasively follow early OA changes including cartilage thickness and matrix alterations, synovitis, effusion, and structural changes in the bone and soft tissues of the joint. Advanced imaging in animal studies may enable the use of fewer

animals and improve our understanding of early structural changes in OA development. Animal models also provide the opportunity to refine these techniques for application in human studies to develop techniques for earlier diagnosis, prognosis, and outcome studies. MRI has been used on animal models as small as the rat (31) and guinea pig (32) to evaluate cartilage and periarticular tissue alterations accompanying OA progression.

Cartilage-specific MRI imaging techniques are being refined to identify imaging biomarkers for preradiographic OA disease. Chan and Neu have written a 2012 review of the current quantitative MRI techniques under development to evaluate structural and biochemical alterations in articular cartilage: T2 mapping, delayed gadolinium enhanced resonance imaging of cartilage, T1ρ mapping, magnetic resonance elastography, and sodium MRI (33). Alongside the development of these advanced imaging techniques, studies to correlate MRI findings with pain scores and radiographic OA grade in human patients have been conducted, and a systematic review of the validity of MRI biomarkers was undertaken in 2011 by Hunter and colleagues (34). MRI biomarker studies aim to develop sensitive techniques for the clinical diagnosis of preradiographic disease and the noninvasive monitoring of early interventions for the prevention of OA. MRI surveillance of joint damage provides noninvasive information on the location and extent of cartilage tissue alterations, as well as other features of OA progression including synovitis, effusion, meniscal damage, subchondral edema, and ligament integrity. As yet, no gold standard for imaging biomarkers exists for studying OA disease in human, or for that matter in animals. Incorporating advanced imaging into animal OA studies provides further opportunities to refine these techniques and correlate the results of imaging studies with other measures of OA progression including histology, cartilage mechanical properties, and gait functionality measures.

MOLECULAR BIOMARKERS

In 2011, the OARSI/Federal Drug Administration (FDA) Initiative working group published its consensus document for the application of *in vitro* biomarkers for the development of drugs for OA (35). This important document was created to summarize and guide the application of *in vitro* biomarkers for the characterization of OA and the development of drug therapies to treat this disease. The report includes an important paradigm for OA progression, dividing the disease into three stages: molecular, preradiographic, and radiographic. The report also makes note of the fact that current OA human clinical trials rely upon the American College of Rheumatology clinical and radiographic criteria for the diagnosis of hip, knee, and hand OA, effectively eliminating patients with molecular and preradiographic OA from clinical trials of OA therapies. The inclusion of biomarkers in diagnosing and characterizing the OA disease process will be essential in identifying patients at risk of developing OA and studying therapies to prevent or mitigate progression in this disease.

Biomarkers in synovial fluid, serum, and urine potentially provide noninvasive or minimally invasive measures of disease initiation and progression. The Burden of disease, Investigative, Prognostic, Efficacy of intervention, Diagnostic, and Safety classification has been proposed to categorize human markers of OA (35,36). Alternative classification systems use breakdown products of collagen or proteoglycan or levels of inflammatory mediators as biomarkers of disease initiation and progression. Since OA represents a process of matrix damage, turnover, and attempted repair, the use of multiple biomarkers, both anabolic and catabolic, will likely be most accurate in characterizing OA disease. The 2011 OARSI/FDA consensus report on biomarkers includes a table with a recommended panel of informative commercially available biomarkers qualified for the study of OA outcomes, as well as a summary of published OA clinical trial biomarker data (35).

Ultimately, a detailed understanding of the changes in biomarkers that characterize OA progression will serve multiple purposes in animal models. The goals of biomarker analyses are to improve our understanding of OA disease risk, incidence, progression, and response to treatment. Biomarker profiles from animal OA models can also be compared to human profiles and symptoms to determine how accurately a given model represents disease. When selecting biomarkers for analysis, it is important to note that urine and serum measurements represent total body levels of the marker of interest and may be affected by processes external to the joint of interest. This is particularly true in skeletally immature animals, in which matrix turnover and tissue remodeling occur as a part of normal growth (6). Synovial fluid analysis has the advantage that this fluid represents the local environment of the joint. A disadvantage of synovial fluid is that repeated joint aspiration, with or without lavage, may influence the course of intra-articular inflammation, and the amount of synovial fluid is very limited in small animal models. When joint lavage is used, biomarker levels should be corrected for dilution using serum and lavage fluid urea levels (37). Another published method for obtaining small quantities of synovial fluid is the use of paper or alginate to collect synovial fluid from a joint after harvest followed by digestion of the alginate or paper to isolate the synovial fluid constituents (38). The limited quantity of synovial fluid available for analysis necessitates careful choice of biomarkers to be included in studies using small animal OA models.

PAIN, FUNCTION, AND GAIT

Pain measurement may include the assessment of several types of pain: primary hyperalgesia (pain at the site of tissue damage or inflammation), secondary hyperalgesia (pain in the distal affected extremity, contralateral joint, or referred elsewhere in the body), and allodynia (pain provoked by light touch, pressure, or temperature stimuli that would not usually be painful). There is a current unmet need for validated, standardized methods for pain measurement, gait analysis, and functional evaluation in animal models of OA. This is one of the most potentially useful clinical areas for OA therapies given that there are millions of patients with established pathology. In clinical OA, the relationships between gross and histologic OA severity and clinical symptoms are not clear. Characterizing pain and disability in animal subjects is an enormous challenge. Gait alterations

have been used to try to measure pain and disability, but it is important to note that gait alterations may be the results of both pain-driven avoidance or biomechanical joint dysfunction.

Pain measurement relies upon the identification of alterations in animal behavior that reliably indicate the sensation of pain. Measures of pain and joint instability and functional assessment are important correlates of gross and histologic damage. A review by Little and Zaki (39) includes a list of outcome measures that have been used to assess pain in OA animal studies. Interestingly, their review points out that injection OA models are much more frequently used in the study of interventions to reduce OA pain than surgical instability models. Of the 112 studies targeting OA pain included in this review, 67% used injection methods or other methods that induced joint damage that "would not be widely accepted as typical of OA," while only 25% used surgically induced instability (39). This dichotomy, between commonly published animal models of OA pain and those of OA histology, limits the comparison of results between studies of interventions to target OA symptoms and disease progression. A detailed review of methods for pain assessment in OA animal models has been written by Neugebauer *et al.* (40).

Methods for pain assessment in animal models include measures of different types of OA pain, such as primary hyperalgesia measured by joint tenderness on palpation or compression, allodynia measured with temperature application or von Frey filament testing, and static or dynamic analysis of weight distribution between the arthritic and contralateral limbs (40). The translational value of information from animal studies of OA pain is directly related to the ability of animal models to represent the pathophysiology of clinically observed OA pain and our ability to measure their symptoms. As an illustration of the challenges of measuring different types of OA pain, von Frey filament testing is a frequently used method for the assessment of allodynia in rat OA models. There is, however, some variability in the implementation of this technique across studies. In basic practice, a von Frey filament of graduated stiffness (measured in grams) is applied to the plantar aspect of the subject's foot through the wire bottom of a cage (41). The magnitude at which the animal withdraws its foot is reported as the paw withdrawal threshold, and a lower paw withdrawal threshold is taken to represent increased sensitivity and neuropathic pain in the distal limb. Decreased paw withdrawal thresholds have been reported in a number of rat OA models including MMT (42,43), partial medial meniscectomy (44), and monosodium iodoacetate injection (44). When the filament is applied, however, it has been reported that filaments with stiffness greater than 16 g may lift the rat's foot (44). Filament testing with greater than 16 g has been reported (43), but movement of the rat's lower extremity and knee becomes an additional potential source of pain at these values which may be more reflective of hyperalgesia than allodynia under these circumstances.

From an animal behavior perspective, many species have evolved under selection pressure not to appear sick or lame, so measurements of pain and disability in animal models such as the rat present a challenge. Gait analysis offers significant potential for the further assessment of functional alterations with OA progression. Static hind limb weight distribution has been reported in rat OA studies using an incapacitance meter to measure relative weight distribution between hind limbs with the rat in a reared position (45). Dynamic gait analysis adds to this information by measuring gait changes such as symmetry of weight bearing during ambulation (46) and range of motion alterations (47). There is currently no standard method of gait analysis for use in animal OA models. Published methods have included scoring based on apparent lameness (40), measurement of stride length and limb rotation from inked paw prints in rats (48), measurement of dynamic force application using a pressure-sensitive walkway (42,46), and fluoroscopic measurements of hind limb motion (47). In larger animals, gait and functionality measures such as kinematic marker analysis, ground reaction force measurements, and observational gait assessment have been applied to study OA-related changes for conditions including dog hip OA (49), lameness in horses (50), and postsurgical sheep knee OA (51). Of note, all of the commonly used animal models of OA use quadruped animals, with different compensatory gait alterations compared to bipedal humans, and with variable effects on gait according to the affected joint. A review of quadruped gait mechanics, compensatory load distribution strategies, and the measurement of these gait alterations in OA animal models is beyond the scope of this paper, although a systematic review of this topic is much needed. Gait analysis techniques enable more detailed, objective, functional assessments in the presence of behavioral adaptations which obscure pain symptoms in animal models.

CONCLUSION

Although OA and its associated pain and disability are among the most common chronic health problems worldwide, there are few treatments available. Animal models of OA have been developed to study the pathophysiology of OA and to assist with the development of new treatments to modulate OA symptoms and disease progression. As might be anticipated from the multifactorial causes leading to OA development and the clinical variability of OA symptoms, there is no definitive *in vivo* animal model for this disease. The lack of standardization in OA models and outcome measures makes it difficult to compare results between studies. The scaling of studies from small to large animal models, essential for translation of potential therapies to human medicine, presents an additional challenge. As our understanding of the pathophysiology of OA increases, animal models can be refined and improved. Validating and refining outcome measures including biomarkers, advanced imaging techniques, and gait analysis will improve our ability to study OA disease and interventions.

ACKNOWLEDGMENT

This publication was made possible by NIH Grant Numbers RO1-AR056834, R41 AR057276 and 2P20 GM104937 and the Lucy Lippitt Endowment. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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