Osteoarthritis and Cartilage 25 (2017) 369–375

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Review

Factors that influence outcome in experimental osteoarthritis

P.M. van der Kraan*

Experimental Rheumatology, Radboudumc, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 11 May 2016
Received in revised form
23 August 2016
Accepted 1 September 2016

Keywords:
Osteoarthritis model
Age
Gender
Housing
Circadian rhythm

SUMMARY

Objective: Osteoarthritis (OA) is the most common joint disease but an effective pharmacological therapy has not been developed yet. To identify targets for treatment and ways to interfere with OA development and progression both spontaneous and induced OA models are still needed. In this narrative review it is discussed what variables can be identified that lead to variation in OA animal model studies.
Design: Literature was screened (Pubmed) with the following terms; *OA animal models* in combination with *species*, *age*, *strain*, *gender/sex*, *housing*, *diet*, *fighting*, *circadian rhythm*, *transgenic*. Relevant articles were selected and additional papers were searched for and read for specific subtopics.
Results: Studies with OA models are subject to a multitude of variables, stimuli and conditions that can influence the outcome of an animal experiment. Outcome will depend on amongst others; the model used, species and strain, age, gender, diet, housing conditions, circadian rhythm, timing of intervention, stress levels and activity. Variations in these variables can account for discrepancies between OA model experiments, intervention studies and conclusions.
Conclusion: To improve OA animal model research, investigators should be aware of all the stimuli and conditions that can interfere with disease development and disease intervention and take these into account in their study design and execution.

Introduction

Osteoarthritis (OA) is the most common joint disease and an important source of chronic suffering and loss of physical and social functioning of patients. The pathogenesis of OA has become clearer in recent years but is not completely understood yet. Effective interventions, except for joint replacement in end stage disease, have not been developed up till now. Human joint tissues are scarcely available and can be only obtained in sufficient quantities during joint replacement. To overcome this hurdle, animal models have been used to get more mechanistic insight in the disease process and to test potential pharmacological interventions. However, there is no generally accepted animal model of OA and study outcomes in different models can differ. In this narrative review we do not discuss the various animal models of OA but elaborate on the sources of variability between different models and different experimental conditions that can result in variable outcomes. Pubmed was screened with the following terms; *OA animal models* in combination with *species*, *age*, *strain*, *gender/sex*, *housing*, *diet*, *fighting*, *circadian rhythm*, *transgenic*. Most OA models run in the knee, or stifle joint, and this review will be focused on OA models that are carried out in this joint.

Joint anatomy is different in different species

Various species are used as models for human OA, small rodent animal models, such as mice, but also large animal models, like horses. Furthermore, OA is studied in our primate relatives but mainly in its natural appearance. Joint anatomy and specific joint tissue characteristics will influence how OA develops and progresses in a specific joint. Although the relatively dimensions of knee joint structures, ligaments, menisci, cartilage surfaces, amongst human and other mammalian species are relatively similar, structural differences in the ligament attachment sites, meniscus and articular cartilage morphology between species is considerable[1]. Size and morphology of a knee joint, but also its relative dimensions compared to the size of femur and tibia/fibula and muscle strength, influence the forces within a joint. The torque patterns, rotational forces, in the knee joint are unique comparing different species[2]. The differences in forces acting on the articular

* Address correspondence and reprint requests to: P.M. van der Kraan, Experimental Rheumatology, Radboudumc, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Fax: 31-24-354-0403.
E-mail address: peter.vanderkraan@radboudumc.nl.

http://dx.doi.org/10.1016/j.joca.2016.09.005
1063-4584/

cartilage will effect pace and pattern of cartilage degeneration and pathological changes in other joint tissues.

Large differences in knee joint cartilage thickness are present in the various animal species used in OA research, varying from 50 to 100 μm in mice up to 2000 μm in horses[3], the later being in the range of human cartilage thickness (Table I). However, not only cartilage thickness is highly variable, cellularity can be relatively high in small animals while being very low in larger animals. A general rule is larger animals having thicker cartilage and lower cellularity. Also the relative thickness of the calcified cartilage to the non-calcified cartilage varies greatly. In small species, like mice, the calcified cartilage can be thicker than the non-calcified cartilage while in larger species the calcified cartilage is always only a small portion of the total cartilage volume. Besides these parameters, articular cartilage glycosaminoglycan content and chondrocyte gene expression vary widely between species[3]. Moreover, even fiber collagen orientation differs between species[4]. It might be clear that differences in gross joint anatomy, chondrocyte density and metabolic differences will not only influence the outcome of a specific OA model but also the outcome of therapeutic intervention in a specific model.

Method of OA induction

A major problem in the choice of the optimal OA model is the question what are you modeling? It is clear that human OA is not a single disease entity but that OA can be divided in different so-called OA phenotypes, most likely all with different underlying mechanisms. Moreover, even a single end-stage clinical phenotype can have various causal mechanisms. Is your model mimicking post-traumatic OA, age-related, metabolic OA or is even the underlying mechanism of the OA model not fully elucidated. The latter being often the case in spontaneous OA models in relatively young animals, with or without a known genetic defect, or in transgenic animal models, in which the gene defects is known but not how this relates to accelerated OA development.

Age-related OA models

Most OA models that are based on aging of the animal, old age being the prime risk factor for OA, are studied in small animals. This is a logical consequence of the in general short life span of these animals and therefore relative short time span in which these animals develop OA. However, this time span is only relatively short. Different mouse strains develop OA at different ages and also at different locations in the knee joint but in general not before the age of 1,5 year[5,6]. Intervention studies in these models are still laborious and expensive due to the length of these experiments. Moreover, experiments and housing should be strictly controlled during the course of the entire experiment since conditions can easily vary between cages and different experiments performed longitudinally, making the data sometimes hard to interpret.

In addition, mouse strains are used that show OA at an early age. In most cases these mice have a known or unknown genetic defect and/or a metabolic disorder, such as the STR/ort and blotchy mice[7,8]. For instance, many mouse strains with skeletal dysplasias develop early OA[9]. Furthermore, mice that show accelerated aging are used[10]. It can be questioned how representative these strains are for the overall majority of human OA cases. These strains can be useful to detect pathways that are important in maintenance of normal joint physiology but these models should only be used with great caution as a drug screening model. It can even be that a drug that is effective in a specific model is only working in this particular model.

Table I
Skeletal development and cartilage characteristics in different animal species frequently used for experimental OA studies. Numbers can vary by strain, location and age. For comparison characteristics of human cartilage are added

Species	Skeletal maturity	Cartilage thickness (μm) (approximate)	Cellularity 10^{-3} cell/mm^3	References
Mouse	No epiphyseal closure*	50–100	330	[5,6]
Rat	38–48 months*	170	265	[3,6]
Guinea pig	6–7 months*	200	250	[7]
Rabbit	6–8 months	300–400	190	[3,6,8]
Goat	24–36 months	900	130	[3]
Sheep	36–46 months	500–1500	50–60	[4,6,9,10]
Dog	12–24 months	500–1800	40–50	[4–6,8,10]
Horse	5–6 years	1300–2000	20–40	[5,8]
Human	17–20 years	2500	10–30	[6,8]

* Rodents never fully reach skeletal maturation.

Post-traumatic OA

Post-traumatic OA models are the most widely used. The trauma can be induced by (micro)surgery, blunt force or injection of damaging chemicals. A variety of surgically-induced and blunt force induced models has been reported, including partial or total meniscectomy, destabilization of the medial meniscus (DMM), meniscal tear, anterior cruciate ligament (ACL) or posterior cruciate ligament transection, medial and/or lateral collateral ligament transection, creation of cartilage groove(s), osteotomy, transarticular impact, and intra-articular osteochondral fragmentation (reviewed in Refs. 11 and 12).

The most widely used model in mice is DMM[13]. This model is based on a microsurgical procedure that has to be performed by a skilled operator, preferably assisted by a second person. This model has a well known learning curve, experienced operators being able to perform this procedure with less joint damage and intra-articular bleeding than inexperienced ones. The same will hold true for other OA models based on microsurgery techniques. Although not systematically studied, it has become apparent that the magnitude of initial joint damage during operation affects the subsequent severity of cartilage damage, more initial damage means more late damage. Since many of the surgical techniques performed in small but also in larger animals depend on the technical skills of the surgeon this can be a large source for variation between experiments. Furthermore, since the anatomy of different mouse strains is dissimilar, surgery on a specific, for instance small strain, can be more difficult than surgery on another, larger mouse strain. This can all result in different outcomes even if one uses, in theory, the same procedure to induce OA.

Post-traumatic OA has been also induced by intra-articular injection of chemicals that damage joint structures, such as tendons. Injection of bacterial collagenase has been carried out in mice, rats, rabbits and horses[14–18]. Injection of collagenase in the knee joint induces joint laxity but also leads to an initial inflammatory reaction[19]. In this manner it is not very different from surgical methods to induce OA, combining the induction of joint laxity with an (initial) inflammatory reaction. The joint laxity in combination with the amount and type of joint inflammation will together determine the process of OA development and progression in models of post-traumatic OA, being dependent on technical skills, animal species and strain, gender and age of the animals. These can all differ between experiments and be a source for variation between these experiments and interpretation of the outcome of intervention studies.

Variables that affect outcome of OA experiments

Variability between experiments even occurs when the same OA model, the same species and the same strain is used. Variations