Experimental models of osteoarthritis: usefulness in the development of disease-modifying osteoarthritis drugs/agents

This work aims at reviewing the experimental animal models capable of reproducing osteoarthritis (OA) changes as seen in humans. Relevant studies in which compounds/agents have been tested for potential effect as disease-modifying OA drugs in large-to-small OA animal models are reviewed. Special attention is given to studies in which compounds were investigated in a translational fashion: from preclinical models to humans. This review is by no means comprehensive, but does cover the majority of therapeutics and disease-modifying OA drugs/agents tested in OA animal models. Findings from these studies may provide insight into the suitability of these models for the evaluation of therapeutic agents that could be effective in humans.

KEYWORDS: animal models ■ disease-modifying osteoarthritis drug ■ osteoarthritis ■ therapeutic agents ■ therapeutic targets

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Osteoarthritis (OA) is by far the most frequent musculoskeletal disorder to affect the majority of people in the second half of their lifespan, often having a significant negative impact on their quality of life. It represents an increasing burden from a medical, social and economic point of view. OA, which mainly affects the diarthrodial joints, is a chronic disease that develops progressively over decades, making it very difficult to precisely identify the different etiological and risk factors that influence the onset of the disease. The primary etiological factors may be numerous; thus, in the majority of patients, the disease is considered to be idiopathic. It is widely believed that aging, a complex process we still know very little about, is the most common predisposing factor for the development of OA and, in certain patients, genetic factors could very well be hidden within what is commonly considered to be related to aging.

This article examines the most relevant studies in which compounds have been tested for disease-modifying OA drug/agent (DMOAD) activity in animal models. It focuses on a number of animal models based on their size, starting with the larger animals – the dog and sheep models, followed by the medium-sized rabbit models, and finally the guinea pig, rat and mouse models. Findings from these studies are of particular interest as they provide insight into the evaluation of therapeutic agents that may be effective in humans.

DMOAD studies in dog & sheep models

The dog anterior cruciate ligament (ACL) model of OA is created by experimentally transecting the ACL in normal mongrels using a closed surgical procedure (stab incision) [1] or an open surgical procedure [2]. The main OA models in sheep are also surgically induced, with the most common involving partial or total meniscectomy. These models have been used to test numerous drugs and agents that have the potential of modifying the progression of OA. The several classes of agents tested can be subdivided as follows.

■ Anti-inflammatory agents
Corticosteroids

Corticosteroids are considered to be the most potent anti-inflammatory drugs. Oral or intra-articular administration has been found to reduce the development of joint structural changes, including cartilage lesions and osteophyte formation, in the OA dog model (closed surgery) [3,4]. To address the possible DMOAD effects of corticosteroids in humans, a double-blind clinical study was conducted exploring the effects of repeated injections of triamcinolone hexacetonide (40 mg every 4 months) compared with placebo (saline solution) for a period of 2 years in knee OA patients [5]. The treatment with corticosteroids was found to significantly improve patient symptoms. However, no difference was found between the two treatment groups in the loss of joint-space width (JSW) as seen on knee x-rays throughout the study period. The conclusion was that in knee OA patients, corticosteroid treatment has a neutral effect on the progression of cartilage loss with no negative effect detected. This study contrasts

with those performed in the dog ACL model as it did not confirm the protective effects of corticosteroids. Many explanations could account for these *in vivo* differences. It could be that the number of patients included in the clinical trial was too small, the drug dosages were not high enough, and/or the imaging technology used (x-rays) may not have been sensitive enough to detect a possible protective effect of the treatment.

Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of symptomatic OA. Several have been tested in the dog ACL model as well as in clinical studies for possible DMOAD effects. Indomethacin, a potent NSAID, has been shown to have a deleterious effect on OA cartilage explants from dogs [6] and in a human knee OA study [7], in which it was tested against paracetamol and tiaprofenic acid. In the latter study, indomethacin was found to significantly accelerate the loss of JSW assessed by x-rays, whereas tiaprofenic acid had a neutral effect comparable to that of paracetamol. Similar findings were reported in a study in hip OA patients, in which indomethacin was also found to accelerate the

loss of JSW [8], again reflecting a negative effect on cartilage. Therefore, with regard to indomethacin, the data from the preclinical and clinical studies are in accordance.

In a recent study, licofelone, an NSAID with dual inhibitory activity against both 5-lipoxygenase and cyclooxygenases, was found to markedly reduce the development of experimental dog OA (closed surgery) (FIGURE 1A) [9]. This effect was mediated through the inhibition of the synthesis of several catabolic factors in cartilage, such as matrix metalloproteinases (MMPs), cathepsin K, a disintegrin and metalloproteinase domain with thrombospondin motifs inducible nitric oxide (iNOS) and IL-1β [9–11]. This drug was also found to reduce calcified cartilage and subchondral bone remodeling/resorption, which may have contributed to the efficacy of the treatment [10]. A 2-year, multicenter, double-blind study was conducted in knee OA patients to examine the potential DMOAD effect of licofelone vis-à-vis naproxen, in which both x-rays and MRI were used to evaluate its effect on OA cartilage [12]. The data showed that treatment with licofelone significantly reduced the loss of cartilage, measured by MRI, compared with naproxen (FIGURE 1B). A similar trend was observed on x-rays, where
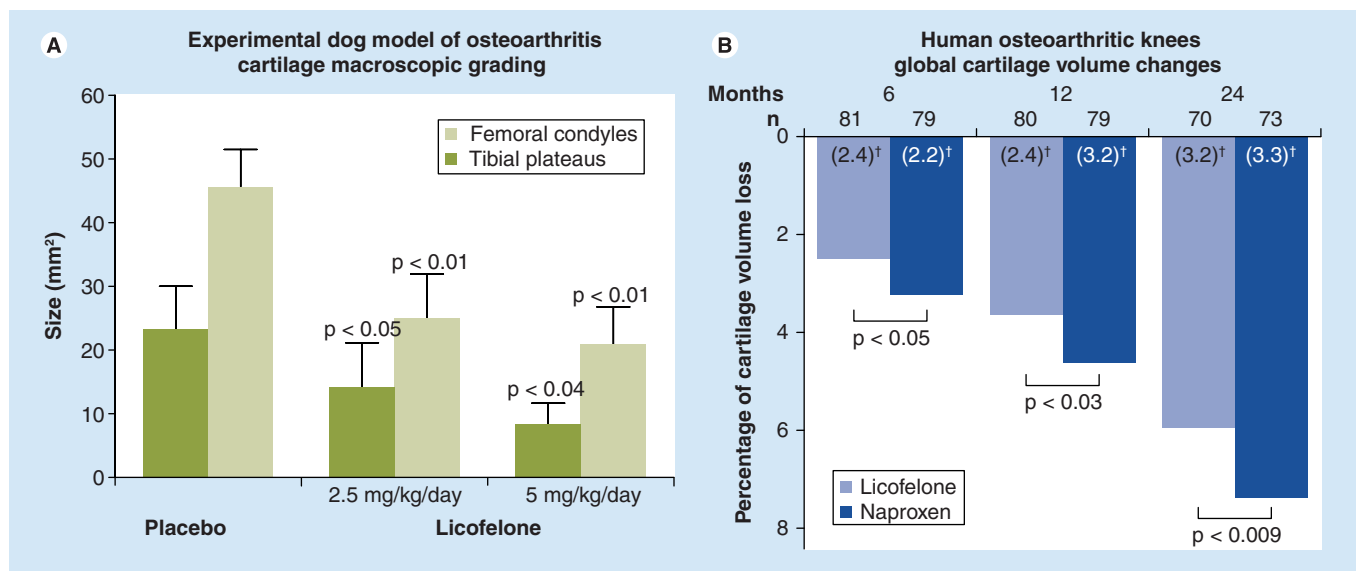
Figure 1. Disease-modifying osteoarthritis drug/agent effect of licofelone. (A) Macroscopic grading of osteoarthritic cartilage from femoral condyles and tibial plateaus of dogs receiving placebo or licofelone at the indicated dosages. The drug was administered orally. Macroscropic lesions were graded as the area of the articular surface changes and expressed as mm^2. Values are the mean ± standard error of the mean, and p-values are versus the placebo group and are assessed using the Mann–Whitney U test. **(B)** Mean percentage of global cartilage volume loss, as assessed by MRI, in knee osteoarthritis patients treated with licofelone (200 mg twice a day) or naproxen (500 mg twice a day) at 6, 12 and 24 months for the according-to-protocol population. p-values were assessed by a two-sample t-test.
[†]Indicates standard deviation, which is in parentheses.
n: Number of patients.
Data taken from [9,12].

the loss of JSW was also found to be less in the licofelone-treated group; however, the difference did not reach statistical significance. These findings are interesting as they point to the superiority of MRI over x-rays in detecting DMOAD effects of a drug as well as the good correlation between the data from preclinical *in vivo* animal studies and human clinical trials.

■ Targeting inflammatory mediators
Cytokine inhibitors

Proinflammatory cytokines, particularly IL-1β, induce excess production of catabolic factors involved in the genesis of OA structural changes [13]. Among the drugs and agents demonstrated to inhibit the synthesis/biological activity of IL-1β in the dog ACL model (closed surgery), and that are relevant to OA, is the oral treatment with tenidap, a potent anti-inflammatory drug with IL-1β inhibitory activity, which was found to reduce the progression of the disease in this model [14], and to be effective at reducing the signs and symptoms of the disease in knee OA patients [15,16].

Another drug, the IL-1 receptor antagonist (IL-1Ra), a competitive inhibitor of IL-1β at the receptor level, showed, in preclinical studies using this animal model, that local administration or increased local production of IL-1Ra inhibits the development of structural changes associated with the disease [17]. In humans, although an open-label study has demonstrated the clinical effectiveness of intra-articular injections of IL-1Ra in patients with painful knee OA [18], a double-blind investigation performed by the same author, demonstrated that a single intra-articular injection of IL-1Ra was not effective for relieving the symptoms of knee OA at 12 weeks. However, significant short-term pain relief, as assessed by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscore, was shown at 4 days with the highest concentration compared with placebo. A number of reasons, some of which are highlighted by the authors of the study [19], and others [20], suggest this is not definite proof that IL-1Ra is ineffective for the treatment of OA.

Diacerein, a drug of the anthraquinone class, has been shown to decrease disease severity in the dog ACL model (open surgery) [21,22]. In the unilateral meniscectomy sheep model, oral treatment with diacerein over a period of 9 months showed an improvement in cartilage and subchondral bone parameters in the lesional areas [23]. In knee OA patients, it was demonstrated *in vitro* to reduce the synthesis of IL-1β in synovium and cartilage [24] and *in vivo* to reduce the level of IL-1β in the synovial fluid [25]. In hip OA patients, a double-blind, placebo-controlled study demonstrated that oral treatment with diacerein significantly reduced the loss of JSW observed on x-rays over a period of 36 months [26], as well as decreasing the level of cartilage degradation biomarkers [27]. These findings correlate well with the preclinical data.

Peroxisome proliferator-activated receptor-γ agonist

Peroxisome proliferator-activated receptor (PPAR)-γ, which belongs to the family of ligand-activated nuclear factors, has been suggested as an attractive target in the treatment of OA. In addition to its classical role on lipid and glucose homeostasis, *in vitro* experiments have shown the ability of PPAR-γ to suppress IL-1β-induced NO and MMP production as well as IL-1β-induced decrease in proteoglycan synthesis. One study explored the effects of pioglitazone, a synthetic agonist of PPAR-γ, in the dog model (closed surgery) [28]. Data showed that this drug reduced the development of OA lesions as well as the synthesis of major proteolytic enzymes and other catabolic pathways through the inhibition of MAPK. Similar findings were also reported in a rabbit ACL model with a specific MAPK inhibitor of ERK1/2 [29].

iNOS inhibitor

The overproduction of NO in OA tissues has been found to contribute to the development and progression of joint structural changes, including synovitis. In the dog ACL model [30], treatment with a specific iNOS inhibitor markedly reduced the level of joint inflammation and the synthesis of a large number of catabolic factors such as MMPs and proinflammatory cytokines. The results from these and other studies in animal models have supported the launch of an important Phase II–III study exploring the potential DMOAD effect of a selective iNOS inhibitor in knee OA patients, which is presently underway.

Enzyme inhibitors

Matrix metalloproteinases have been shown to be among the most important contributing factors to the degradation of articular cartilage in OA [13]. A number of MMP inhibitors have been tested in both preclinical and clinical studies; however, most failed to complete human clinical evaluation owing to significant side

effects that developed during the trials, probably caused by the broad spectrum of MMPs targeted by these drugs. Of the different chemical and biological MMP inhibitors tested for DMOAD effects, doxycycline has probably undergone the most extensive exploration for chondroprotective effects. Doxycycline has been shown to reduce the levels of active MMPs (collagenase and gelatinase) and the synthesis of iNOS [31–33]. Its protective effect was found *in vivo*. Indeed, doxycycline treatment markedly reduced the incidence and progression of joint disease in the dog ACL open surgery accelerated model [34] (FIGURE 2A). The DMOAD effect of doxycycline was examined in obese women with knee OA for a period of 30 months in a randomized, double-blind, placebo-controlled trial [35]. Knee x-rays were taken at baseline, 16 and 30 months to evaluate the joint-space narrowing in the medial tibiofemoral compartment. Treatment with doxycycline significantly reduced the loss of JSW at 16 and 30 months compared with placebo (FIGURE 2B). These data are, again, in accordance with the results from preclinical studies.

In summary, based on the information available today, there is some correlation between the results obtained with anti-inflammatory agents in the larger animal model studies and human clinical trials, although the information is derived from a relatively small number of studies.

■ Targeting bone
Bone antiresorptive agents

Much attention has been devoted to the potential role of subchondral bone in OA pathophysiology [13]. A number of studies have explored the effects of drugs/agents that modulate bone metabolism, mainly targeting the resorptive phase, on the development of joint structural changes that occur during OA.

The bisphosphonates are among the most commonly used drugs for the treatment of diseases involving excessive resorption of bone, such as osteoporosis. In the dog ACL model, the morphological changes that take place at the subchondral bone level, particularly in the early stages of the disease, are predominantly resorptive in nature [10]. Therefore, when targeting the subchondral bone, an antiresorptive compound is a logical choice. A study in the dog ACL model (open surgery) [36], in which NE-10035, a bisphosphonate, was administered via subcutaneous injection, showed that, over the 3 months following the surgery, the prophylactic
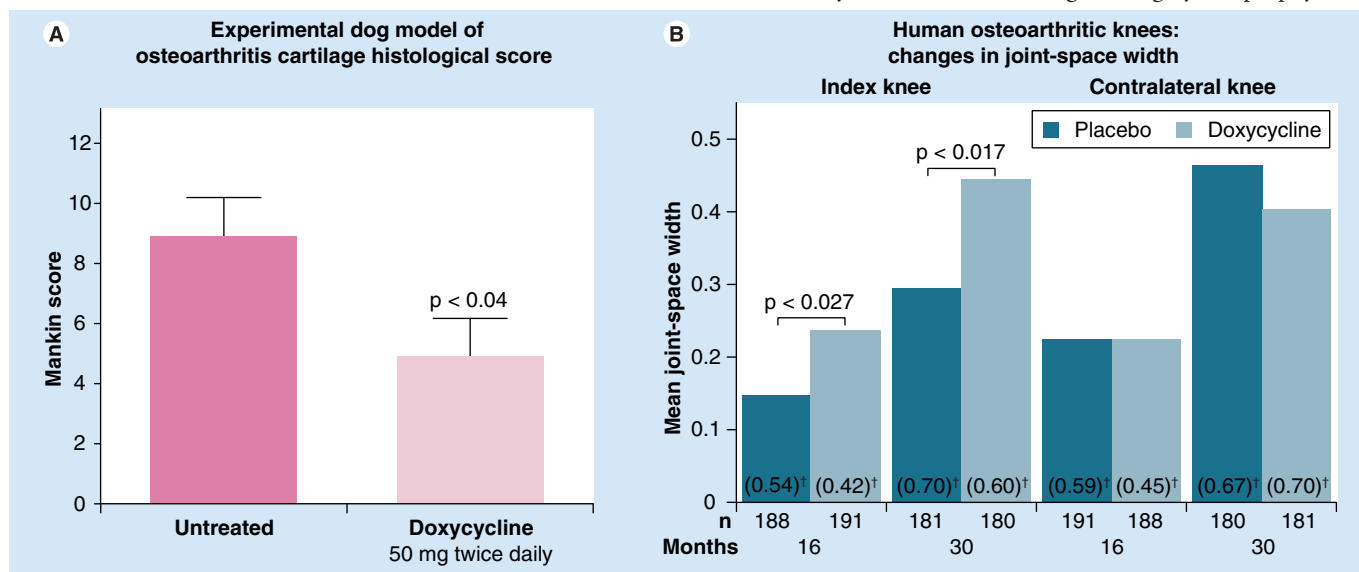
Figure 2. Disease-modifying osteoarthritis drug/agent effect of doxycycline. (A) Histological changes in osteoarthritic cartilage of dogs receiving placebo or doxycycline at the indicated dosage. The drug was administered orally. Histology was performed according to the Mankin score. Values are the mean ± standard error of the mean, and p-values are versus the untreated group and assessed using the Wilcoxon 1-tailed rank sum test. **(B)** Mean joint-space width changes in knee osteoarthritis patients treated with placebo or doxycycline (100 mg twice a day). The p-values for group comparisons are from repeated-measures models with treatment group, location, baseline covariates, visit, treatment group–location interaction, and treatment for up-visit interaction as the independent variables. Baseline joint-space width and knee pain were covariates for analysis of changes in joint-space width in the index knee; baseline BMI was the covariate for the analysis of changes in joint-space width in the contralateral knee.
†Indicates standard deviation, which is in parentheses.
n: Number of patients.
Data taken from [34,35].

treatment effectively reduced the turnover and resorption of subchondral bone in the osteoarthritic joint. However, the treatment had no effect on osteophyte formation or the severity of cartilage changes.

Calcitonin is a well-known bone antiresorptive agent whose efficacy has been demonstrated in the treatment of osteoporosis and Paget's disease. Studies in the dog ACL model (closed surgery) [37,38] under therapeutic conditions showed that subcutaneous injections of calcitonin can reduce the progression of OA cartilage and subchondral bone changes, as well as the level of serum markers of bone resorption up to 4 months after surgery.

The data on bisphosphonates from dog studies are in accordance with those obtained from a human clinical trial in which oral treatment with risedronate, another bisphosphonate used in the treatment of osteoporosis and Paget's disease, failed to show any DMOAD effect in knee OA patients [39]; although this compound appears to reduce the level of a marker of cartilage degradation in humans [39,40]. With regard to calcitonin, it is our understanding that a Phase II–III clinical trial is presently underway to test the DMOAD effect of this drug in knee OA patients.

Hyaluronan
Hyaluronan, used for the treatment of OA symptoms, has been generated from different sources and has a wide range of molecular weights [41]. In a recent study in the dog ACL model (open surgery), hyaluronan was injected repeatedly over time into the knee joint under both prophylactic and therapeutic conditions [42]. At the time of sacrifice, 32 weeks after the surgery, no effect of treatment was shown on the severity of lesions or the biochemistry of the diseased cartilage. Therefore, treatment with hyaluronan did not alter the progression of experimental dog OA. These results confirmed data from a previous study conducted by the same group [43] and are in line with a preliminary study on the DMOAD effect of hyaluronan in knee OA patients, which was inconclusive [44].

The effect of intra-articular injections of hyaluronan on the progression of lesions in the sheep model has been extensively explored [45–48]. Studies using the unilateral medial meniscectomy model [47,48] showed that hyaluronan treatment under therapeutic conditions (when lesions were already present) markedly improved the severity of histological lesions on the femur and tibia, and prevented

bone remodeling. Sheep treated with hyaluronan injections demonstrated a bone histomorphometry similar to that found in the unoperated control. However, the mechanisms through which a positive effect is exerted in subchondral bone remain speculative. It is possible that the improvement in the quality of the cartilage matrix indirectly protects the subchondral bone. Under similar experimental conditions, hyaluronan treatment was also found to improve gait and decrease lameness [45]. Interestingly, hyaluronan treatment showed no effect on the OA cartilage matrix macromolecule content, although it reduced the level of proteoglycans released from OA cartilage [46].

Nutraceuticals
Avocado/soybean unsaponifiables (ASU) have shown a beneficial effect on clinical symptoms of knee and hip OA with an after effect lasting beyond termination of treatment [49–51]. Another study also showed ASU to reduce the progression of joint-space narrowing in a subgroup of hip OA patients with advanced joint-space narrowing [52]. This clinical study, however, was conducted on only a small number of patients and is, therefore, considered a pilot study. These findings are corroborated by those from *in vivo* preclinical studies. In both the rabbit and dog, ASU were shown to exert an anabolic effect by stimulating TGF-β1 [53,54]. In the ACL dog model, ASU treatment reduced the severity of macroscopic and histological cartilage lesions and, by reducing the level of iNOS and MMP-13, significantly improved the subchondral bone volume and calcified cartilage thickness [55].

In a study in sheep, in which knee OA was induced by bilateral meniscectomy followed by oral treatment with either ASU or placebo for 6 months, ASU was shown to increase the cartilage thickness and proteoglycan content, and to prevent the remodeling (sclerosis) of the subchondral bone [56].

DMOAD studies in rabbit models
Targeting bone
Bone morphogenic protein
Rabbit OA models have also been used for testing agents with a primary effect on bone. In a cruciate deficiency model, in which bone morphogenic protein (BMP)-7 (also known as osteogenic protein-1) was delivered to the stifle joint 4 weeks after surgery, OA progression was reduced [57]. Semi-quantitative reverse transcriptase PCR data showed greater

expression levels of aggrecan, and less of MMP-3 and MMP-13 in the treated cartilage and synovium, suggesting a potential therapeutic role for BMP-7. The same authors demonstrated similar effects at 6 weeks [58]. In another study using the same model, rabbits were given BMP-7 at three doses intra-articularly on a weekly basis following surgery and were studied for 12 weeks. Data showed that, at doses of 500 and 5000 ng, BMP-7-treated rabbits had an improved histological score, reduced level of synovial fibrosis, and less ectopic bone and osteophyte formation [59].

Bone antiresorptive agents

Additional bone active agents studied include the antiresorptive agents, salmon calcitonin [60] and bisphosphonates [61,62]. Salmon calcitonin at a daily dosage of 7 IU was administered intramuscularly for 1–8 weeks or from weeks 8–16 in the cruciate deficiency rabbit model [60]. The calcitonin-treated groups demonstrated a smoother cartilage with minimal or no ulcerations, smaller osteophytes, and hypercellularity where mild OA was present, implying regeneration. The authors suggest that calcitonin worked in both prophylactic and therapeutic stages of OA.

Risedronate, a bisphosphonate, at 0.01 mg/kg was administered subcutaneously daily for 6 weeks in the cruciate deficiency rabbit model of OA [62]. There was some loss of bone; however, the loss was minimal in the periarticular region. Angiogenesis, osteophytes and cartilage were not different from the untreated animals. In a post-chymopapain model of OA, zoledronate at 10 µg/kg, intra-articularly, three-times weekly for 28 or 56 days, partially protected the joint, as observed by gross and microscopic examinations of cartilage, including methylene blue staining for proteoglycan, and urinary bone and cartilage markers for collagen [61].

Hyaluronan

Although intra-articular hyaluronates were not very effective in the dog OA model, they have shown promise in the rabbit, in which NO was reduced, as was chondrocyte apoptosis, as assessed by the TUNEL method [63]. In another study using the hemi-meniscectomy rabbit model, the effect of intra-articular high-molecular-weight sodium hyaluronate was compared with an NSAIDs [64]. Data revealed that the hyaluronate-treated animals had improved weight bearing and cartilage integrity in contrast to the untreated animals, suggesting an analgesic and DMOAD effect. In this study, although the NSAID-treated animals had improved weight bearing, the anatomy of cartilage was not improved. Moreover, using one or two courses of intermediate-weight intra-articular hyaluronate resulted in successful disease modification in a 26-week cruciate deficiency rabbit model [65].

Nutraceuticals

As with the dog and sheep, a variety of agents have been studied using the rabbit OA models. A few of them are discussed later, with emphasis on those that have undergone or are undergoing human clinical trials.

There have been several sugars, particularly sulfated sugars belonging to the nutraceutical agents, demonstrated to modify the progression of OA. In a study using the partial meniscectomy rabbit model, glucosamine sulfate, 100 or 200 mg/kg/day, was orally administered to 6-month-old New Zealand white rabbits for 12 weeks following surgery [66]. Gross examination revealed significant cartilage disruption at 12 weeks in the untreated animals, with partial preservation of cartilage surfaces in treated animals. Microscopic (histological) examination was consistent with the gross findings showing Mankin scores for meniscectomy without therapy (9.8 ± 1.7, standard deviation) and glucosamine 100 mg/kg/day (3.7 ± 0.8) and 200 mg/kg/day (4.9 ± 2.0). There was a reduction in cartilage MMP-1 and -3, but not MMP-2 or -9. In a separate study with glucosamine hydrochloride, 100 mg/day for 8 weeks, following cruciate transection of the stifle, the increase in subchondral trabecular bone turnover was reduced [67]. However, only partial cartilage preservation was reported 8 weeks after cruciate ligament transection with glucosamine hydrochloride treatment, 100 mg daily starting at week 3 after the surgery (therapeutic conditions) [68].

Other sugars that have been studied include calcium pentosan polysulfate [69], cyclodextrin polysulfate [70] and glycosaminoglycan peptide [71]. In contrast to sodium pentosan polysulfate, calcium pentosan polysulfate can be administered orally. In a short-term inflammatory model induced by a polycation complex of poly-D-lysine and hyaluronan, calcium pentosan polysulfate preserved cartilage proteoglycans and reduced serum levels of IL-6 [69]. The 8-sugar ring sulfated molecule, γ-cyclodextrin polysulfate (also called cycloamylose), 0.25 or 1 mg/kg subcutaneously weekly, was studied in

the cruciate deficiency rabbit model in a 12-week study [70]. There was a reduction in cartilage scores and osteophytes with the higher doses tested. However, higher doses of cyclodextrin were found to be nephrotoxic in preliminary studies [70]. Glycosaminoglycan-peptide, an extract of bone marrow and cartilage purported to contain sulfated sugars and growth factors, was demonstrated, in both a prophylactic and therapeutic hemi-meniscectomy model at doses of 0.05 to 0.5 mg/kg twice weekly, to reduce OA lesions in a dose-dependent manner [71]. Active, but not total, MMP was reduced and hydroxyproline preserved, suggesting reduced collagenolytic activity.

■ Others

Other agents that have been tested in the rabbit model of OA include basic FGF [72], resveratrol [73], a selective MEK-1/2 inhibitor [29] and IL-1 converting enzyme (ICE), also named caspase-1 [74].

Owing to the short half-life of basic FGF in the joint [75,76], this factor was released slowly into the articulation by administration in gelatin hydrogel microspheres [72]. [I^{125}]-labeled basic FGF was injected intra-articularly once every 3 weeks, from 4 weeks post-ACL surgery, in a 10-week study [72]. A protective effect on OA changes was demonstrated by macroscopic and histological examinations, and reverse transcriptase PCR for proteoglycan core protein messenger RNA.

Resveratrol is a phytoalexin in the skin of grapes and in red wines that inhibits nuclear factor-κB and cyclooxygenase-2 [73]. Resveratrol 10 µmol/kg in dimethylsulfoxide was administered intra-articularly, daily, for 2 weeks from 5 weeks post-ACL surgery, in an 8-week study. There was a reduction in cartilage destruction seen by histology and proteoglycan staining (Safranin O), without improvement in synovial inflammation in animals treated with resveratrol compared with controls.

MEKs are substrates for several protein kinases, which act at an integration point for multiple biochemical signals. An inhibitor of MEK-1/2, PD 198306 given orally at 10 and 30 mg/kg/day, was evaluated in an 8-week study of the cruciate-deficient rabbit model of OA [29]. The higher dose of PD 198306 demonstrated a reduction in cartilage lesions, osteophyte size and synovial hyperplasia. Immunohistochemical analysis detected lower levels of phosphorylated ERK1/2 and MMP-1 in the cartilage.

There has been considerable interest in the inhibition of IL-1β. This cytokine is released extracellularly by ICE. A pan-caspase inhibitor, benzyloxycarbonyl-Val-Ala-Asp fluoromethylketone at 25 µg/ml, was injected intra-articularly in the cruciate-deficient rabbit model of OA [74]. The drug treatment was found to reduce cartilage degradation, as measured macroscopically and microscopically, while increasing the numbers of chondrocytes. This improvement was modest and was not seen with specific inhibitors of caspase-3 or -8, emphasizing the role of caspase-1 and further suggesting that inhibition of IL-1β may have therapeutic value.

DMOAD studies in guinea pig, rat & mouse models

Several drugs targeting different pathways have been tested, using small animal models of OA. The classes of drugs/agents tested range from those traditionally used for OA treatment, such as anti-inflammatory drugs, to those that modify key OA mediators or bone biology.

■ Anti-inflammatory drugs
Corticosteroids & NSAIDs

Many steroidal drugs and NSAIDs have been tested with variable results depending on the model used. The corticosteroid dexamethasone and the classical nonselective NSAIDs nabumetone, diclofenac and ibuprofen, were tested in the mono-iodoacetate (MIA) rat model and all demonstrated improvement in OA lesions [77]. However, another NSAID, meloxicam, showed a neutral effect in a spontaneous ankle OA rat model [78]. Triamcinolone hexacetonide, corticosteroids administered as a single intra-articular dose (0.40 mg/kg) in the guinea pig MIA model [79] induced a dose-dependent protective effect against the development of OA lesions, in which there was a decrease in the cartilage fibrillation, less prominent osteophytes, and less extensive chondrocyte loss and extracellular matrix degradation.

■ Targeting inflammatory mediators

As mentioned earlier, IL-1β is one of the key proinflammatory cytokines involved in OA pathogenesis. In small animal models of OA, its role was evaluated by blocking either its synthesis, with the use of diacerein, or its activation via inhibition of its converting enzyme ICE.

Diacerein was evaluated in two small animal models of OA: the spontaneous OA guinea pig model with joint instability created

by surgical transection of the quadriceps tendon and removal of the right patella [80], and the rat meniscectomy model [81]. Histological evaluation of the knee demonstrated that such treatment prevented the development of OA structural changes and reduced joint stiffness.

Inhibition of ICE was performed by the use of pralnacasan, an orally bioavailable prodrug and a potent inhibitor of ICE. In the spontaneous and the collagenase-induced knee OA mouse models [82], pralnacasan was well tolerated and globally reduced joint lesions in both models, but predominantly in the collagenase induced model. The main improvements were noticed in the medial compartment. In the STR/ort mice with spontaneous OA, it dose dependently reduced the histological scores of lesions and the urinary marker of collagen degradation. Clinical development of the drug was stopped because of its safety profile.

Other possible targets include the inhibition of prostaglandin E2 activity. A recent study in the MIA guinea pig model evaluated a new antagonist of prostanoid receptor MF498 [83], which selectively inhibits the EP4 receptor. The study demonstrated the effectiveness of this compound at blocking pain and inhibiting swelling of the paw.

Another pathway, that of PPAR-γ, which, upon activation, has anti-inflammatory activity, was also tested. In the meniscectomy guinea pig model, pioglitazone, a PPAR-γ agonist, demonstrated a significant protective effect on cartilage by significantly reducing the severity of OA cartilage lesions, as well as decreasing the production of MMP-13 and IL-1β, which are key mediators of cartilage catabolism [84].

■ MMP inhibitors

An orally active selective inhibitor of collagenase was tested in the spontaneous STR/ort mouse model [85]. This study showed that the inhibition of this MMP prevented structural changes in both the cartilage and the subchondral bone. Other studies performed in the surgical meniscal tear and MIA rat models demonstrated that broad spectrum MMP inhibitors were also able to reduce cartilage degradation [86,87].

More recently, an MMP inhibitor with a preferential inhibitory activity against MMP-13 was tested in the guinea pig meniscectomy model and data showed no effect on cartilage degradation [88]. The lack of efficacy was believed to be related to the persistent activity of aggrecanases, raising the concept

that a better strategic approach would be a dual inhibitor of both aggrecanases and MMP-13. However, recently, a new class of potent MMP-13 inhibitors was identified [89], which are nonhydroxamic acid-containing compounds. *In vivo*, in a rat meniscectomy model, the inhibitor exerted chondroprotective effects and, interestingly, without observable musculoskeletal toxicity. At approximately the same time, another class of MMP-13 inhibitors was discovered; one of which was demonstrated, in the same rat model, to reduce cartilage degradation biomarker levels and exert cartilage protective effects [90].

Two groups of investigators also evaluated the *in vivo* efficacy of doxycycline on the Dunkin Hartley guinea pig spontaneous OA model [91,92]. The report of de Bri *et al.* [91] showed that doxycycline did not offer any protection from cartilage loss. By contrast, Bowyer *et al.* demonstrated that doxycycline treatment resulted in partial protection from the loss of cartilage on the medial tibial plateau, as determined by MRI analysis [91]. This finding was associated with a complete inhibition of MMP-13 and -8, and partial reduction of MMP-9 activity. Protocol differences between these two studies, including the age of the animals at study initiation, the route of compound administration and the outcome measurement, could have contributed to the differences in findings. However, the Bowyer *et al.* [91] data correlate well with those from a DMOAD clinical trial by Brandt *et al.* [35].

■ Targeting bone
antiresorptive agents
Bisphosphonates

Alendronate, an effective inhibitor of bone resorption, tested in the rat ACL model, demonstrated chondroprotective effects with osteophyte formation inhibition and reduction in the levels of cartilage degradation biomarkers [93]. Different results, however, were obtained with this drug in the spontaneous guinea pig model [94]. In the latter model, alendronate was demonstrated to increase bone mineral content and density, which was associated with an acceleration of cartilage degradation, throughout the duration of the study (17 weeks follow-up in 6.5-month-old guinea pigs).

Risedronate, another bisphosphonate, was tested in the spontaneous guinea pig model, and was shown to slow the progression of OA by reducing the severity of cartilage lesions and limiting osteophyte formation [95]. In this

study, the comparison between risedronate and alendronate showed the former to be more effective. Furthermore, a comparative study of different bisphosphonates revealed that only those compounds containing nitrogen and pyridinyl side chains positively affected cartilage structure [96].

Osteoprotegerin

Bone remodeling is tightly regulated by the molecular triad osteoprotegerin (OPG)/receptor activator of nuclear factor-κB (RANK)/RANK ligand (RANKL). RANKL, localized on osteoblasts, enhances osteoclastogenesis via its interaction with its receptor RANK, which is localized on osteoclasts; whereas OPG, produced by osteoblasts, inhibits osteoclastogenesis by binding to RANKL. Therefore, OPG functions as an antagonist receptor that prevents the biological effects of RANKL. Recent evidence has shown that human OA subchondral bone alterations involve a disequilibrium in the OPG:RANKL ratio [97].

In addition, exogenous OPG was tested as an inhibitor of osteoclastogenesis in a surgically induced (ACL combined with meniscectomy) mouse model of OA [98]. This study showed that endogenous OPG prevented cartilage degradation and that the administration of exogenous OPG prevented chondrocyte apoptosis and, at the same time, reduced disease progression.

Estrogen

Treatment with estrogen and a selective estrogen-receptor modulator was tested in ovariectomized rats [99]. Both drugs were found to suppress the increase in cartilage turnover and reduce the progression of cartilage lesions. Moreover, the treatment with estrogen normalized the levels of biomarkers from both bone and cartilage, rCTX-I and rCTX-II, while the selective estrogen-receptor modulator reduced only the level of rCTX-II.

■ Hyaluronan

The effect of hyaluronan was evaluated in guinea pigs with spontaneous OA [100]. In this study, high-molecular-weight hyaluronan was injected intra-articularly into both knees, once a week, consecutively, for 5 weeks, starting at the age of 6 months. The guinea pigs received no other injection until they reached 9 months, when the administration of the treatment was repeated for an additional 5 weeks. These animals were sacrificed at 12 months. Hyaluronan reduced the development of cartilage lesions on the femoral condyles and almost completely preserved the cartilage structure on the tibial plateaus, as well as protecting against subchondral bone remodeling, and improving bone volume and subchondral plate thickness. In the latter tissue, there was no difference in collagen or mineral content. The authors hypothesized that hyaluronan treatment, through its action on the subchondral bone, reduces the stress applied to cartilage during impact loading.

■ Nutraceuticals

Glucosamine sulfate or hydrochloride was tested by subcutaneous administration in spontaneous OA STR/ort mice [101], and in the MIA and ACL rat models [81,102,103]. These studies demonstrated interesting and complementary results. Glucosamine delayed the appearance of OA changes in the mice, and presented structure-modifying effects in the MIA and ACL rat models by reducing cartilage damage [81] and by maintaining proteoglycans, as well as inhibiting type II collagen degradation and enhancing type II collagen synthesis in cartilage [103]. However, the exact mechanism of action of this drug remains to be determined.

■ Others

FGF18 was shown to play a central role in skeletal growth and development, and has an anabolic effect on articular cartilage. FGF18 was shown to stimulate chondrogenesis and cartilage repair in the rat meniscectomy model [104]. At present, there are two clinical trials in human OA of the knee: a Phase II in Europe and a Phase I in the USA.

A number of other molecules that have been tested in small animal OA models have been demonstrated to exert effects on joint structural changes. Among these, cilostazol, a selective phosphodiesterase type III inhibitor, was evaluated in the MIA rat model [105]. Cilostazol acts by increasing the intracellular level of cAMP by blocking its hydrolysis. Orally administered, cilostazol reduces cartilage surface irregularities, matrix loss, chondrocyte apoptosis and cartilage iNOS expression, and inhibits MMP expression through its activity on the p38/c-jun N-terminal kinase signaling pathway [105]. These effects suggest a protective effect of cilostazol against cartilage degradation.

Some other compounds were shown to have deleterious effects on joints. These include ofloxacin [106,107] and quinolone [108], which both induced arthropathy in normal guinea pigs.

Conclusion

In conclusion, interesting information regarding the OA disease process has been generated from studies using animal models. Although, in general, animal OA models are injury-induced compared with naturally occuring (idiopathic) OA in human clinical trial patients, the larger OA animal models present disease pathways that are strikingly similar to the natural human disease. The various parameters that can be evaluated in the larger animals and their use in DMOAD studies make them appropriate models. Smaller animal models may be particularly useful in the very early developmental phase of a new drug/agent, when the amount of active substance required is small. However, one must remember that OA animal models are, for the most part, driven by mechanical factors. In idiopathic OA, various etiological factors join forces to bring about the structural and molecular changes typical of the disease. Therefore, it is likely that some factors contributing to the natural human disease may not be affected in certain experimental animal models.

Interestingly, there is generally a good correlation, in both positive and negative findings, between the DMOAD effects of certain drugs/agents in studies using the larger animal models and in human clinical trials. Obvious limitations of the preclinical studies arise from the variability in experimental protocols from one study to another. Furthermore, the level of effectiveness of the treatment is rather difficult to compare between human and animal model studies owing to the methodological differences. For the most part, preclinical studies have been of short duration, which is an additional limitation.

In the medium-sized animal, the rabbit, several promising agents have shown disappointing results in human studies. The discrepancies in findings between the rabbit models and human disease could be due to the therapeutic agent being ineffective in humans, problems with dosing and/or the rabbit models may only reflect subsets of human OA.

Studies in animal models remain very useful for establishing therapeutic targets and assessing the potential of new DMOADs. To date, the results obtained with at least two drugs/agents tested in the dog ACL model of OA were translated to human clinical trials [12,35].

Future perspective

Animal models of OA have been used successfully for decades to explore the structural changes and pathophysiological pathways of OA. Although the use of animal models obviously presents limitations and is subject to criticism, it is of interest to note that they are widely used by a large number of key expert investigators in the field. In addition to helping to improve our knowledge regarding the disease process, these models have also proven to be extremely valuable in identifying major therapeutic targets that have helped bring forth a number of major drug development programs in the field of OA.

Although the data from preclinical research cannot always be extrapolated to clinical trials, many drugs/agents studied in animal models have been found to be potential DMOADs. As experience with these models evolves, and our in-depth knowledge and understanding of their limitations improves, the use of such models in the development of new DMOADs will become increasingly meaningful.

Executive summary

- Experimental animal models of osteoarthritis (OA) reproduce a number of natural changes associated with the disease, which are of importance in the evaluation of therapeutic agents.
- Animals of different sizes are used for OA models: small animals, the guinea pig, rat and mouse; medium sized, the rabbit; and larger models, the dog and sheep.
- There is no clear evidence of the superiority of one particular species over another when using experimental animal models of OA for testing disease-modifying OA drug effects. However, there is a general belief that larger animals can provide for a more global exploration. Of note, in larger animal models of OA, the disease-modifying OA drug effects of two drugs showed a good correlation between preclinical findings and clinical trials in humans.
- The rabbit model of OA has also proven to be a valuable model to explore the potential disease-modifying OA drug therapies.
- Small animal models of OA are often used to establish the comparison between spontaneous versus induced models and are useful in the very early developmental phase of a new drug.

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