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EDITORIAL

Animal models of osteoarthritis

THE BENEFITS AND COSTS OF REDUCING VARIABILITY

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Induced and naturally occurring animal models of disease are a cornerstone of mechanistic discovery and therapeutic development research across the spectrum of medical disciplines.[1,2] The musculoskeletal system and associated orthopaedic and rheumatological conditions such as osteoarthritis (OA) are no exception.[3-6] Studying animal physiology and disease pathophysiology to inform human health, so-called 'comparative medicine' has been practised for over 2,000 years.[1,2] The concept of animal welfare and legislated regulation of animal use in research, on the other hand, did not really emerge until the mid-to-late 19th century with the passing of the Cruelty to Animals Act in the UK (1876), followed nearly 100 years later by the Animal Welfare Act in the USA (1966).[2] At about the same time as the USA Act, the concept of 'animal ethics' developed, and the "3 R's" of animal research (Refinement, Reduction, Replacement) were conceived and published.[7] In the 60 years since, legislation, mandated guidelines for the responsible and ethical use of animals in research, and national, state, and/or institutional animal ethics committees with oversight and governance powers have been developed and introduced worldwide, and continue to evolve.

Through this ongoing evolution in humane and regulated animal-based medical research, the 3 R's have become globally accepted principles underpinning and guiding animal use in research and ethics committee protocol evaluation. A paper by Hu et al[8] in the current issue of *Bone & Joint Research* highlights the importance of ongoing efforts to embrace the 3 R's through exploring refinement of even the most widely used and validated animal models, to enable future reduction in animal numbers required to appropriately power a given experiment. The authors studied the impact on structural and symptomatic OA of performing unilateral destabilization of the medial meniscus (DMM) surgery in young male C57Bl6 mice with or without the aid of a stereomicroscope. The surgeries were conducted by a single operator with over five years of experience in performing DMM with or without microscopy, and researchers conducting pain and histology outcomes were blinded to the surgical methodology and study purpose. The two surgical approaches resulted in no difference in mean OA cartilage pathology and synovitis at 16 weeks post-surgery, and resulted in the same average tactile allodynia, reduced static weightbearing, and gait abnormality in the operated limb measured from week 8 to 16. However, the variability between individual mice in all of the measured parameters was statistically significantly greater when surgery was done without the aid of microscopy. The authors quite rightly conclude that microscope-assisted DMM induced more consistent structural and symptomatic OA. As such, the use of microscopy is recommended, with the reduction in variability decreasing animal numbers required for statistically valid and powered research.

While it may seem self-evident that improving surgical visualization of anatomical structures would be beneficial, it is interesting that the impact was in consistency (reduced variance) rather than overall severity (mean score/measure) of both OA pathology and pain. Nevertheless, there were more mice with higher cartilage pathology scores in the non-microscope-assisted group compared with the microscope group (n = 10 vs 5 and 13 vs 8 with scores ≥ 12/14 in the femur/tibia, respectively), as might be expected with less surgical precision and thus greater iatrogenic trauma and/or inflammation at the time of

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surgery contributing to worse long-term OA pathology. This hypothesis is consistent with the greater variability in pain outcomes being driven largely by some mice in the non-microscope group having markedly worse allodynia and reduced weightbearing, particularly at earlier postoperative times (eight to ten weeks). Somewhat counterintuitively however, there were also mice in the non-microscope group with much less severe 16-week OA pathology than their microscope-assisted counterparts (n = 6/7 with scores < 7 in the femur/tibia in the non-microscope cohort vs none with scores this low with microscope-assisted surgery). This might suggest incomplete/inconsistent meniscotibial ligament transection in some animals and/or poorer joint reconstruction and postoperative patella subluxation in the absence of surgical microscopic assistance, both of which can lead to reduced medial femorotibial OA pathology in the DMM model. While no correlation analysis was presented to determine if reduced OA pathology was associated with less severe pain in individuals, animals with the least allodynia and weightbearing derangement at 16 weeks were in the non-microscope group.

This present study highlights a common issue that contributes to an apparent lack of reproducibility in animal model research: inconsistent methodology exacerbated/hidden by poor reporting.[9] Even seemingly small experimental differences such as the source of "the same" inbred mouse strain, diet, caging (size, animal density, enrichment provision, rack position), and experience and sex of animal handlers can alter structural and symptomatic OA disease onset, progression, and severity.[3,6,9] The study by Hu et al[8] confirms that minor alterations in surgical technique, even by a single experienced operator, can contribute to outcome variability in DMM and likely other OA animal models. Unfortunately, the study only evaluated a single late-stage pathological end point (16 weeks), and pain measures only from eight weeks when joint-wide OA pathology and chronic pain is well established in the DMM model.[10–12] It is therefore not possible to determine if despite similar late-stage OA, the onset and trajectory of structural pathology and symptom progression may have been different with or without microscopy. Temporal analysis combined with correlation studies would shed light on the unanswered questions regarding potential biomolecular and/or mechanical causes of increased pain and pathology variance in the non-microscopy group. Is there increased synovitis in the immediate/early postoperative period, and does this contribute to a more severe or distinct pain phenotype early postoperatively, and the trajectory of OA onset? Alternatively, might early pain and altered load-bearing actually be protective and reduce structural OA progression, as suggested in female mice subjected to DMM?[13] Would providing access to a running wheel or adding enforced exercise, which increase DMM-induced OA and reduce variability,[14] moderate the with-or-without-microscopy differences? Would similar with-or-without-microscopy differences in pathology and/or pain be seen in other mouse strains, in females, and/or in older animals, all of which are inherent patient/animal population factors that have known effects on OA disease trajectory, severity, and ultimately response to treatment?[3,6,9,15]

Defining why DMM surgery with or without microscopic assistance alters OA pathology and pain variability has important implications beyond those of experimental reproducibility and reduction in animal use. While joint injury is a well-recognized risk factor for patients to develop OA, it remains unclear why some patients with apparently similar injuries develop OA and others do not.[16] Incident OA increases with time post-injury, but ultimately only 30% to 50% of individuals with anterior cruciate ligament (ACL) rupture or meniscal injuries develop radiological OA (irrespective of treatment or surgery),[16–19] unlike the 100% expected in animal models. Marked variability in outcome from apparently similar joint injury is therefore a clinically relevant feature in human patients, and defining potentially modifiable factors that underlie this variable OA risk is of significance. Preclinical OA models such as DMM that mimic human joint injuries offer an unprecedented opportunity to explore this issue. There is excellent animal model:human concordance in the effect on injury-induced OA of known risk factors, such as joint loading and cartilage impact, diet, obesity and metabolic syndrome, synovitis and intra-articular bleeding, and older age.[20] Greater inherent variability in structural and symptomatic OA following joint injury in animals, while 'problematic' from an experimental design and cost (ethical and financial) perspective, may in fact more faithfully model the human condition. Capitalizing on inherent variability or outcome diversity in animal models through correlation analyses, for example, enables exploration of divergent OA pathophysiological mechanisms and pathology-pain relationships.[11,12] Rather than the enemy of research discovery, perhaps "the treasure is hidden in diversity".[21]

Defining divergent OA mechanisms as discussed above may help in development of tools to better stratify patients for specific therapeutic approaches. The idea of OA as a single "disease" is being replaced with the more nuanced concept of different cellular, molecular, and biomechanical pathways that lead to a similar final end-stage pathology.[3–6,9,20] The pain and disability that constitutes the "illness" of OA[22] also differs with regard to severity, progression, pain type/patient experience, and underlying pathophysiology. This variability in presentation and progression of OA disease and illness is now recognized as different 'phenotypes', while divergence in pathophysiology between and within phenotypic clusters represents variable 'endotypes'.[23,24] Better stratifying OA patients into different phenotypes, and even more importantly endotypes, is key to effective patient-specific treatment for this erstwhile recalcitrant condition.[25] As preclinical models remain a crucial component of therapeutic development, improving their predictive utility is paramount. In part, optimal translation of statistically significant experimental

findings to clinically meaningful patient outcomes arises from aligning the preclinical model pheno-/endo-type with that of the target human subpopulation.[1,3–6,26–30] This model-patient alignment should include known disease risk variables, such as age, sex, metabolic status, and induced disease/illness characteristics such as degree of joint inflammation, cartilage, and/or bone pathology, and severity and type of pain.[6] Highly uniform progressive preclinical models arguably best represent/align with individuals with substantial progressive cartilage loss and pain, who make up less than 15% of all knee OA patients.[31] Research findings in animal models with the most robust pathological and symptomatic disease might only be translatable to this small 'progressor' subgroup rather than the wider, more heterogenous patient population.

Refining preclinical models to improve reproducibility and reduce animal use is a central tenet of best scientific and ethical research practice. While pursuing this, we must keep sight of why we use animal models: they presently remain the best way to investigate whole organism disease/illness pathophysiology and to develop and test treatments prior to human trials. The study by Hu et al[8] reminds us that we need to control sources of model variability where we can, and where we know they exist. The unquestionable experimental and ethical benefits of the resulting more tightly controlled animal models may, however, come at a cost of restricting their broader translatability. This is not an argument against animal model refinement, but rather a reminder of the need to more thoughtfully and selectively interpret findings from their use. Ultimately, the best predictive and translational utility of preclinical animal research will come from repeatable findings in multiple reproducible models representing different disease pheno-/endo-types and patient characteristics.[1,6,9,15,29,30,32]

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