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Editorial

Adding insult to injury: synergistic effect of combining risk-factors in models of post-traumatic osteoarthritis

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"Synergy" is the interaction of two or more factors to produce a combined outcome greater than the sum of their individual effects. It is interesting to consider synergism in the context of osteoarthritis (OA) as a "whole-joint disease", and how the combination of individual events or risk-factors that contribute to OA initiation and/or progression, may have more than just additive effects on joint-wide OA pathology and its systemic symptomatic consequences. This idea is highlighted in an interesting paper by McCulloch *et al.* in the current issue (REF####), where the authors studied the effect of concomitant focal cartilage injury on the severity and progression of post-traumatic (pt)OA joint pathology and pain induced by destabilization of the medial meniscus (DMM) in mice.

Accelerated ptOA model or different disease phenotype?

Surgically induced meniscal injuries, and DMM in mice in particular, are among the most widely used pre-clinical models of ptOA[1]. This reflects the consistent, robust and reliable OA induction that is seen across species with disruption of meniscal function, mimicking the pivotal role of meniscal injury alone or combined with anterior cruciate ligament (ACL) rupture, as the key risk factor for development of ptOA in patients following knee injury[2,3]. In their well-controlled pre-clinical study, McCulloch and co-authors had a single surgeon (to minimize variation) induce one of three injuries in one knee of young adult (10 week-old) male C57Bl6 mice: DMM, medial tibial plateau cartilage injury (3 partial thickness cartilage scratches: "CS"), or DMM followed by CS ("DCS"). The contra-lateral knee had an arthrotomy alone and was used as a non-OA ("Sham") control. Groups of mice were sacrificed 7, 14 and 28-days post-surgery and OA pathology (medial tibial and femoral cartilage damage; medial tibio-femoral compartment synovitis; medial tibial plateau subchondral bone volume (BV/TV); knee joint osteophyte number, size/volume, and BV/TV) quantified using micro-CT and histopathology. A cohort of mice had dynamic weight-bearing of all four limbs quantified 14-days post-surgery as an indirect measure of pain. This comprehensive evaluation enabled the authors to demonstrate, albeit at a relatively early stage in the disease course, that a localized cartilage injury significantly enhanced/accelerated the severity/progression of broad OA joint pathology and its clinical sequelae. Of note and consistent with the concept of the "joint-organ", they demonstrated significant positive correlations between various aspects of early (day-14) pathology, such as synovitis with cartilage damage ($R = 0.77$), osteophyte number with cartilage damage ($R = 0.59$), and osteophyte number with synovitis ($R = 0.49$).

The authors quite rightly conclude that the DCS combination provides a novel model of accelerated ptOA that mimics the increased OA-risk in patients with multi-tissue injury following naturally occurring knee joint trauma[2,3]. Specifically in this case, that the presence of cartilage injury/damage at the time of surgery for meniscal[4] or ACL injury[3,5] significantly increases the risk of patients subsequently developing radiographic and symptomatic knee OA. Exactly why this is the case remains unclear, with arguments ranging from as simple as starting from an already lower baseline (additive cartilage damage), to the presence of chondropathy being evidence of a more severe or energetic joint trauma that is more likely to have accompanying occult injury to other tissues such as subchondral bone that then increase OA-risk (additive joint tissue injury), or that cellular and molecular changes in the damaged cartilage activate additional pathophysiological pathways that drive not only progressive cartilage damage but pathologic change in other joint tissues (synergistic joint pathology).

In the study by McCulloch *et al.* evidence for both additive and synergistic OA-pathology with DCS is presented. In some tissues such as subchondral (and metaphyseal) bone, there was limited effect with the increased osteosclerosis compared with Sham for the most part indistinguishable in DMM, CS and DCS, and the latter not increased compared with DMM alone. In the case of osteophyte development, the effect of combining DMM and CS appeared to be additive, particularly in terms of number rather than size or bone volume. However, the enhancement of cartilage damage (not only in the tibia but the otherwise uninjured femur) and synovitis histopathology scores in DCS suggested a synergistic rather than additive effect, particularly at 14 days but still evident at day-28. Perhaps even more interesting than the enhanced joint tissue pathology, was the effect on pain (as measured by limb-loading) in DCS compared with DMM or CS alone. While data from naïve animals or mice pre-operatively was not available to determine the effect of arthrotomy (Sham), it was very clear that DCS had a significantly different/worse effect on limb use than Sham, DMM or CS alone. Interestingly this enhanced pain response, at least for front paw loading, was significantly correlated with increased osteophyte number ($R = 0.66$).

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A number of questions remain to be addressed in the DCS model such as: whether the enhanced ptOA compared with DMM persists in the longer term, if effects are also seen in the lateral tibio-femoral and patello-femoral compartments, whether inducing the cartilage damage in joint regions or compartments other than the medial tibia has similar OA augmenting effects? Nevertheless, the present study showing synergistic pathology and a unique pain profile argue that beyond simply being an accelerated ptOA model, DCS, as with meniscal injures accompanied by chondral damage in patients, may be a specific disease endotype with activation of distinct pathophysiological pathways. It would be of considerable interest to explore this question in future studies, by for example comparing mRNA and/or μRNA expression profiles in different joint tissues after DMM, CS and DCS[6,7]. Similarly, comparing OA outcomes following DMM vs DCS in genetically modified mice could provide evidence of distinct molecular pathophysiology (disease endotype), as has been done with collagenase-induced OA vs DMM and spontaneous age-associated OA[8,9].

Adding insult to injury: modeling OA risk in patients

The McCulloch study is not alone in evaluating the outcome from combining known clinically-relevant OA risk-factors in a pre-clinical model (Table I). As with the DCS model, these previous studies often demonstrate synergistically accelerated joint-wide ptOA onset and progression when a primary ptOA-inducing joint injury (increased loading, intra-articular fracture, ACL rupture, meniscal injury) is accompanied by a variety of second-hit insults: injury to other joint supporting/stabilizing structures[10], forced exercise/loading[11], cartilage impact[12], obesity/metabolic-syndrome[13–18], synovitis[19], intra-articular blood/hemorrhage[20,21], local or regional neural damage[22–25], and older age[7]. It is important to recognize that while we have deliberately focused the examples in Table I with joint injury as the 1° event, it is equally valid to consider this as the "2° insult" that for example is necessary to accelerate diet-induced OA progression[26]. In this scenario, the inciting joint injury may not even need to be severe enough to result in progressive OA on its own.

Joint injury, especially of the knee, has long been recognized as a key risk-factor for developing OA[2]. While damage to specific structures (ACL, meniscus) and combinatorial multi-tissue damage in particular increase OA risk, it remains unclear why some patients with apparently similar injuries to these structures do develop OA and others do not. The odds of incident OA increase with time post-injury but ultimately 30–50% of individuals with ACL rupture or meniscal injuries requiring resection will develop radiographic OA, irrespective of treatment or surgery[2–5]. Defining factors that identify at-risk patients would be of significant benefit, and pre-clinical ptOA models offer an unprecedented opportunity to explore this issue. Available evidence (Table I) suggests a strong concordance in pre-clinical models with factors (insults) known to increase long-term OA risk in joint-injury patients (e.g., multi-tissue injury, chondral injury, synovitis, age)[2–5], as well as others far less studied such as neural deficits and prior mild joint trauma[27,28]. These latter insults may be related as patients with a higher frequency of injury have worse sensitization and hypoalgesia indicative of a neuropathy-like-syndrome[29]. While it is tempting to assume that poor neuromuscular and proprioceptive control lead to injury, there is evidence of worsening neurological

Table I
Pre-clinical models where a secondary insult has been shown to increase the progression and/or severity of a primary OA-inducing joint injury

1° Injury	2° Insult	Species	Outcome of injury-&-insult relative to injury alone	Ref.
Cartilage	Obesity/metabolic syndrome	Rat	Increased cartilage damage, aggravated synovitis, increased osteophyte formation and volume; no effect on subchondral bone parameters (12 weeks post-surgery)	13
ACL	Other stabilizing structures	Mouse	Accelerated and more severe cartilage degeneration, increased osteophyte formation	10
	Neuropathology	Dog	Accelerated and more severe cartilage degeneration, increased osteophyte formation, periosteal bone formation, reduced sensory responses	23–25
	I/A haemorrhage	Dog	Increased cartilage hypertrophy, chondrocyte cloning, fibrillation and changes in tangential zone chondrocytes, greater incidence of synovitis and synovial iron deposition (10–12 weeks post-surgery)	21
ACL + Meniscus	Forced exercise	Rat	Accelerated cartilage degeneration and greater joint deformation, worse subchondral bone plate failure, sclerosis and enlarged marrow spaces, greater osteophyte formation	11
	Neuropathology	Rabbit	Accelerated onset of cartilage degeneration (but end stage severity similar)	22
	Obesity/metabolic syndrome	Rabbit	Increased synovial inflammation; cartilage pathology not affected (12 weeks post-surgery)	15
Meniscus	Cartilage scratch	Mouse	Accelerated and more severe cartilage damage, increased synovitis, more osteophytes, worse pain, minimal change in subchondral bone parameters	OAC
	Cartilage impact	Rabbit	Lower glycosaminoglycan fraction in cartilage at the impact site, reduced proteoglycan depth across femoral condyle (12 weeks post-surgery)	12
	Obesity/metabolic syndrome	Mouse	Increased synovial inflammation, greater osteophyte severity, heterotopic ossification, decreased bone volume (28 weeks post-surgery)	18
	Age	Mouse	Cartilage pathology more severe, reduced articular cartilage area and thickness; sham operated older mice also displayed signs of mild OA (8 weeks post-surgery)	7
Meniscus + MCL	Joint inflammation	Rat	Increased cartilage damage, greater osteophyte maturation (increased synovial inflammation as part of model; 35 days post-surgery)	19
	Obesity/metabolic syndrome	Mouse	Accelerated and more severe cartilage pathology, increased bone volume, larger osteophytes, more progressive meniscal calcification; sham knees also presented with small osteophytes	17
I/A fracture	Obesity/metabolic syndrome	Mouse	Increased cartilage damage, increased synovitis, more cancellous bone loss (8 weeks post-fracture)	16
Cyclic joint loading	Obesity/metabolic syndrome	Mouse	Increased cartilage damage; no effect on subchondral bone parameters	14
Forced limb loading	I/A blood	Dog	Increased proteoglycan release, increased cartilage damage, increased synovial inflammation (10 weeks after the final I/A injection)	20

ACL = anterior cruciate ligament; MCL = medial collateral ligament; I/A = intra-articular; Neuropathology = induced via intra-articular neuro-toxin, neurectomy or dorsal root ganglionectomy. Single time point studies where evaluation of effect of insult on disease onset or acceleration are not possible have the time post-injury indicated.

status with time post-ACL injury[30]. Thus prior mild injury could induce neurological damage which increases the risk for subsequent critical injury and OA should such an injury occur. Using existing and developing further combined injury-&-insult preclinical models, will enable this and even more complex clinically relevant ptOA-risk questions to be addressed.

Disease complexity provides a clearer pathway to better treatment

OA has long been considered a single "disease", and while end-stage pathology may be similar there is burgeoning evidence that there are different cellular and molecular pathways to this final destination. Whether as debated at the 2019 OARSI World Congress we accept these as "OA phenotypes", these different pathophysiological paths to a common endpoint do represent variable potential therapeutic targets and approaches. The economic model for pharmaceutical companies developing OA therapies may suffer from greater disease subdivision and reduction in suitable patient-pool for any one drug/treatment. However, improved selection of candidates for clinical trials of new therapies can only improve and streamline drug development, and ultimately lead to effective therapies and improved patient outcomes. The DCS and other injury-&-insult models that combine risk-factors to better mimic individual patients, will be an important component of this improved therapeutic development pathway.

Author contributions

All authors contributed to the planning, writing, reviewing and editing of this manuscript. All authors approved the final submitted version of the paper and take shared responsibility for the accuracy of the data presented therein.

Conflicts of interest

The authors have no potential or apparent conflicts of interest to declare with regard to this work. The manuscript has not been submitted or is not simultaneously being submitted elsewhere and has not been published in proceedings or transactions of meetings or symposium volumes.

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