Q3. Can/should we define a minimum time-course over which pain (and associated joint pathology) outcomes should be evaluated, and does this vary with the pre-clinical model?

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RESPONSE/RECOMMENDATION:

YES. To comprehensively understand the mechanisms that generate and maintain OA pain, it is recommended that preclinical animal studies include multiple time-points. This approach will allow for tracking the progression of pain behavior, monitoring pathological changes over time, and identifying critical transition points in disease progression. The number of time-points may vary depending on how long it takes to develop pain behavior and pathology in each model, the availability of resources, and the rationale for the study. When multiple timepoint studies are not feasible, researchers should clearly define the timepoint studied and provide detailed descriptions of both pain behavior and the extent of pathological change, either in their own experimental design or cited from the broader literature.

LEVEL OF EVIDENCE: Strong

RATIONALE:

Methodology
To broadly capture studies that explored the time-course of changes in pain behavior and/or pathology across many preclinical animal models of OA, we performed PubMed searches for the following terms: “time-course” AND each of the following animal models “MIA” or MIA-induced OA”, “DMM” or “DMM-induced OA”, “PMx” or “partial meniscectomy” or “PMx-induced OA”, “ACLr” or “ACL rupture” or “ACLr-induced OA”, “spontaneous OA” or “OA in companion animals”. We also reported studies that were not captured using these specific search terms, but were identified using backward and forward citation searching. The searches were carefully curated for each model to identify the most relevant studies that included high quality data about pain and/or pathology, and to highlight especially those that explored the time-course within individual studies. Only original studies were included.

Summary of findings
This summary synthesizes current understanding of the time-course of pain behavior and joint pathology across commonly used preclinical animal models of OA. The models fall into three broad categories: chemically induced models, surgically induced or post-traumatic models, and spontaneous models that arise either through genetics or natural ageing.

Chemical model: MIA-induced OA
Monosodium iodoacetate (MIA)- induced OA is one of the most common chemical models used to study OA pain. It develops rapidly in rodents and is useful for studies requiring predictable and robust pain phenotypes over a short timeframe. Evoked pain behaviors (eg. increased mechanical and thermal sensitivity), reductions in weight bearing and joint pathology emerge in association with inflammation in the soft tissues early in disease progression (from days 1-3), evolve quickly and reproducibly, and often persist for up to and beyond days 14, 28 or 42 (typical endpoints of reported studies)[1-8]. Structural damage to cartilage and subchondral bone begin from around days 7-14, reflecting a transition from an acute inflammatory phase to a degenerative phase[8]. Spontaneous pain-like behaviors, including reductions in natural digging and diminished rearing or locomotor activity, also appear in the first 5-7 days[8-10]. There is clear evidence that pain behavior differs across time points[11]. In addition, the magnitude and duration of pain behavior is dose-dependent[8, 12-14], and so the time-course can be titrated to specific study requirements. Molecular mechanisms that contribute to the pain also evolve dynamically, and act at different stages and in different joint compartments to drive pain in the MIA-induced OA model[15-18].

Surgical and post-traumatic models: DMM, PMx, ACLT and ACL rupture-induced OA
Models of surgically induced or post-traumatic OA advance more slowly than chemical models and are often used when the aim is to model the prolonged progression of OA following joint injury. Destabilization of the medial meniscus (DMM) and partial meniscectomy (PMx) produce a gradually progressing pain phenotype[19, 20]. Whilst pain behavior and cartilage degeneration is modest early (weeks 2-4)[19, 21], the most substantial and reproducible pain-like behaviors and pathology emerge later (weeks 8-16)[19-26]. Pain behaviors include increases in mechanical sensitivity at the joint early in disease progression, and both persistent increases in mechanical sensitivity, and impaired digging behavior and rotarod performance late in disease progression. Many of these studies show that the type of pain behavior observed changes over time[19, 20, 23, 24]. For PMx-induced OA, some studies further report reduced weight bearing on the affected limb from around 8–10 weeks[27], whereas others do not[23, 25]. Joint pathology at the late timepoint includes substantial cartilage breakdown with exposure of subchondral bone, osteophyte formation, and bone marrow lesions[23]. For DMM-induced OA, there is a more severe phenotype

observed over time in males than females[28]. Anterior cruciate ligament transection (ACLT) and combined ACLT with meniscal injury (ACLT+MMx) applied to rats produce changes in mechanical sensitivity of the joint and footpad up to week 12, and persistent weight-bearing asymmetry that does not reliably emerge until at least 20 weeks post-injury, with significant differences from sham controls maintained for up to 40 weeks[29]. For DMM, PMx, ACLT and ACLT+MMx, surgery often affects surrounding tissues. In the ACL rupture model (ACLr), there is no obvious damage to surrounding tissues. Joint changes in ACLr are characterized by early synovitis, followed by cartilage degeneration, subchondral bone remodeling and osteophyte development as early as day 14 post-rupture, but few studies have explored the pain phenotype in detail[22, 30, 31].

Spontaneous OA: genetic models, guinea pigs, rabbits and companion animals

Spontaneous models that develop OA with age are attractive for their translational relevance because they replicate slow, non-traumatic disease progression. Several genetically modified mouse strains develop age-related joint degeneration. The STR/ort mouse shows early proteoglycan loss and cartilage fibrillation but does not have a clear pain phenotype[32]. The Col9a1$^{-/-}$ mouse shows joint degradation by three months of age, and both increased mechanical sensitivity and gait alterations by nine months[33, 34]. The Dunkin-Hartley guinea pig naturally develops OA from 2-4 months of age, with moderate to severe degeneration of the joint by 12-18 months[35-37]. Rabbits are also prone to developing spontaneous OA with age, but the onset is much later with some animals developing mild degenerative changes by 3-4 years, with more apparent pathology in later years (6-9 years)[38]. Pain behavior in the guinea pig and rabbit is not well documented. Companion animals provide a naturally occurring model of OA that closely mirrors the human condition in terms of chronicity, comorbidity and functional impact, and pain behavior can be described by owners[39-41]. Such naturally occurring ‘models’ are readily available, and may better reflect the complex genetic, environmental, temporal and physiological influences present in humans. Most knowledge about canine and feline OA relates to established disease in older animals. At this stage affected pets have decreased limb use[42], compromised function[43, 44] and/or decreased activity[45]. The inherent complexity of spontaneous OA in companion animals, including variable time of onset, comorbidities and inter-individual heterogeneity, is similar to human OA, which makes these models particularly valuable for evaluating analgesic therapies when the therapeutic context and trial duration are informed by the agent under investigation. However, whilst these models naturally mimic disease progression in human OA, they are resource-intensive and present challenges for testing due to long timeframes and variable onset. This has limited the use of models of spontaneous OA for thorough explorations of OA pain phenotypes over time.

Key considerations and implications for future work

The temporal dissociation between early inflammatory pain signals and later degenerative drivers of pain is a recurrent theme across models. Rapidly progressing chemical models are favored for pharmacological experiments that require strong, predictable outcomes within short windows, whereas surgical and spontaneous models are better suited to address mechanisms and treatments relevant to chronic, slowly evolving OA. When planning comparative pharmacological studies, investigators should balance the need for rapid readouts against translational relevance, resource constraints, and animal welfare considerations. Surgical and spontaneous models demand long-term husbandry and careful age- and sex-matching, increasing cost and complexity, but they also provide a platform that may better predict clinical efficacy of therapies directed against chronic OA pain.

Many studies adopt an early versus late timepoint strategy for pathology and pain behavior, but relatively few map continuous trajectories or multiple time points over the course of disease. This is perhaps not surprising given they are so difficult to resource. However, it is unclear exactly how ‘early’ and ‘late’ timepoints relate to the evolution of OA in humans, especially when they differ for most of the models we have reviewed here. More extensive temporal mapping of both pain and pathology will strengthen translational relevance and improve the predictive value of preclinical OA research. At the least, it may be relevant to assess both pain and pathology at each timepoint tested so that a better understanding of how OA pathology relates to pain can be achieved.

Conclusion

There remains an unmet need for novel, well-tolerated and effective analgesics for chronic OA knee pain. Addressing this gap requires robust methods for the longitudinal assessment of pain behavior in animal models, characterization of the time-course across models, and judicious selection of model and endpoint to align with the clinical scenario being modelled. Multi-timepoint studies or clearly defined single-timepoint investigations are essential for advancing our understanding of OA pain mechanisms and improving translational outcomes.

References

1. An, et al., Osteoarthritis and Cartilage, 2021. 29: 380–8.
2. Fernihough, et al., Pain, 2004. 112: 83–93.
3. Bove, et al., Brain Res Rev, 2009. 60: 187–201.
4. Morgan, et al., Pain, 2022. 163: 390–402.
5. Morgan, et al., Front Endocrinol (Lausanne), 2022. 13: 1047943.
6. Pomonis, et al., Pain, 2005. 114: 339–46.
7. Bove, et al., Osteoarthritis and Cartilage, 2003. 11: 821–30.
8. Song, et al., J Pain Res, 2025. 18: 5507–22.
9. Pattison, et al., Proceedings of the National Academy of Sciences of the United States of America, 2024. 121: e2410653121.
10. Pattison, et al., Pain, 2024. 165: 1761–73.
11. Bohic, et al., Neuron, 2023. 111: 2811–30 e8.
12. Udo, et al., Osteoarthritis and Cartilage, 2016. 24: 1284–91.
13. Guingamp, et al., Arthritis and Rheumatism, 1997. 40: 1670–9.
14. Nagase, et al., Journal of Pharmacological and Toxicological Methods, 2012. 65: 29–36.
15. Minnema, et al., Front Neurosci, 2022. 16: 738976.
16. Morgan, et al., Osteoarthritis and Cartilage, 2023. 31: 1342–52.
17. Morgan, et al., Bone, 2024. 189: 117260.
18. Aso, et al., Eur J Pain, 2014. 18: 174–81.
19. Miller, et al., Arthritis Rheumatol, 2017. 69: 1429–39.
20. Ai, et al., Arthritis Rheumatol, 2023. 75: 352–63.
21. Glasson, et al., Osteoarthritis and Cartilage, 2007. 15: 1061–9.
22. Gilbert, et al., Dis Model Mech, 2024. 17.
23. Knights, et al., Pain, 2012. 153: 281–92.
24. Driscoll, et al., Arthritis Rheumatol, 2016. 68: 857–67.
25. Goodwin, et al., Arthritis research & therapy, 2025. 27: 67.
26. von Loga, et al., Osteoarthr Cartil Open, 2020. 2: 100101.
27. Batchelor, et al., Osteoarthritis and Cartilage, 2022. 30: S381.
28. Hwang, et al., Osteoarthritis and Cartilage, 2021. 29: 728–38.
29. Kuo, et al., Front Pharmacol, 2025. 16: 1560265.
30. Gilbert, et al., Journal of Orthopaedic Research, 2018. 36: 2118–27.
31. Bergman, et al., Osteoarthritis and Cartilage, 2024. 32: 1060–73.
32. Poulet, et al., Arthritis Rheumatol, 2014. 66: 1832–42.
33. Hu, et al., Arthritis and Rheumatism, 2006. 54: 2891–900.
34. Allen, et al., Arthritis and Rheumatism, 2009. 60: 2684–93.
35. Bendele and Hulman, Arthritis and Rheumatism, 1988. 31: 561–5.
36. Wang, et al., Osteoarthritis and Cartilage, 2013. 21: 574–81.
37. Huebner, et al., Osteoarthritis and Cartilage, 2002. 10: 758–67.
38. Arzi, et al., Lab Anim (NY), 2011. 41: 20–5.
39. Enomoto, et al., Sci Rep, 2024. 14: 2827.
40. Enomoto, et al., Veterinary Record, 2021. 189: e516.
41. Lascelles, et al., Veterinary Surgery, 2010. 39: 535–44.
42. Lascelles, et al., Veterinary Surgery, 2010. 39: 71–7.
43. Robertson-Plouch, et al., Sci Transl Med, 2019. 11.
44. Gruen, et al., PLoS One, 2015. 10: e0131839.
45. Stevens, et al., Front Vet Sci, 2025. 12: 1503009.