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REVIEW

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Preclinical Assessment of Inflammatory Pain

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SUMMARY

While acute inflammation is a natural physiological response to tissue injury or infection, chronic inflammation is maladaptive and engenders a considerable amount of adverse pain. The chemical mediators responsible for tissue inflammation act on nociceptive nerve endings to lower neuronal excitation threshold and sensitize afferent firing rate leading to the development of allodynia and hyperalgesia, respectively. Animal models have aided in our understanding of the pathophysiological mechanisms responsible for the generation of chronic inflammatory pain and allowed us to identify and validate numerous analgesic drug candidates. Here we review some of the commonly used models of skin, joint, and gut inflammatory pain along with their relative benefits and limitations. In addition, we describe and discuss several behavioral and electrophysiological approaches used to assess the inflammatory pain in these preclinical models. Despite significant advances having been made in this area, a gap still exists between fundamental research and the implementation of these findings into a clinical setting. As such we need to characterize inherent pathophysiological pathways and develop new endpoints in these animal models to improve their predictive value of human inflammatory diseases in order to design safer and more effective analgesics.

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Introduction

Under normal conditions, acute inflammation is essential for protecting our bodies from invading pathogens, as well as promoting tissue remodeling and repair. Conversely, chronic inflammation, which lasts for 6 weeks or longer, serves no beneficial purpose and results in tissue damage and pain. Pro-inflammatory mediators such as prostaglandins, cytokines, chemokines, proteases, neuropeptides, and growth factors are released at sites of inflammation and are capable of sensitizing peripheral pain sensing neurones (see Figure 1) [1,2]. Three organ systems that are particularly susceptible to the development of inflammatory pain are the skin, joints, and gut. Atopic dermatitis, arthritis, and inflammatory bowel disease all pose a serious global burden, and evidence suggests that the prevalence of each is bound to rise in the future if preventative measures are not taken [3–5]. Fortunately, our understanding of the mechanisms that underlie chronic inflammation is constantly growing. Clinical observations have undoubtedly contributed to how we treat inflammatory disease; however, most of the fundamental discoveries regarding inflammatory mechanisms have come from animal models. *In vivo* models have helped us to elucidate the endogenous molecules involved in initiating and resolving inflammation, in addition to providing us with a better understanding of inflammatory pain. Furthermore, animal models are paramount to testing the efficacy and safety of new chemical entities that have the potential to become novel antiinflammatory analgesics. While animal models are primarily used to help us understand human disease, it is important to be mindful that these discoveries are also applicable to the veterinary field and the treatment of our pets and livestock. The following is a discussion concerning the role animal models play in understanding inflammatory disease and pain. We have also listed different species and tests that can be used in different animal models to study inflammatory pain (see Table 1), as well as the advantages and limitations of these models (see Table 2).

Models of Cutaneous Pain

Capsaicin-Induced Pain

Capsaicin is the active irritant found in chili peppers, which belong to the genus *Capsicum*. Capsaicin is responsible for the hot and zesty taste sensation that we experience from eating spicy foods that contain chili peppers. In addition to its culinary uses, capsaicin has been used as a tool in preclinical and clinical studies,

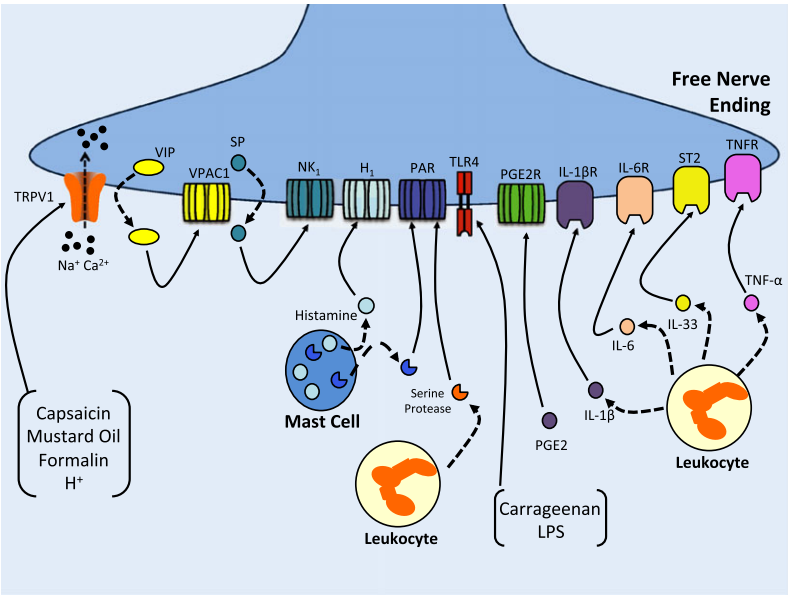

Figure 1 A schematic illustrating peripheral sensitization of a free nerve ending by various inflammatory mediators and irritant substances. Capsaicin, mustard oil, acid, and formalin increase the sensitivity of neurones by opening transient receptor potential vanilloid type 1 (TRPV1) ion channels, which leads to the release of neuropeptides such as substance P (SP) and vasoactive intestinal peptide (VIP). Locally released SP binds to neurokinin 1 (NK1) receptors and VIP binds to VPAC1 receptors. Histamine released from mast cells acts on neuronal H1 receptors. Leukocytes and mast cell release serine proteases which subsequently cleave various proteinase-activated receptors (PAR) leading to downstream nociceptor modulation. Toll-like receptor 4 (TLR4) is activated by exogenous substances like carrageenan and lipopolysaccharides (LPS), while leukocyte-derived cytokines like tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-17 (IL-17) bind to their respective receptors to enhance pain transmission. Prostaglandin E2 (PGE_2) mainly activates the PGE_2 receptor and sensitizes sensory neurones to environmental stimuli.

to understand thermal pain mechanisms [6]. When 1% of capsaicin is administered locally, C fiber firing is enhanced [7]. Intra-arterial administration of capsaicin over the dose range 2–200 μg also activates C polymodal fibers [8], contributing to hyperalgesia [9–11]. Capsaicin administration activates the nonselective cation channel, transient receptor potential vanilloid-1 (TRPV1) [9,12], which is localized on nociceptive free nerve endings and promotes the peripheral release of inflammatory neuropeptides such as calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and substance P (SP). This phenomenon, by which the peripheral nervous system induces tissue inflammation, is called neurogenic inflammation [13–16]. Moreover, blocking neurokinin 1 (NK1) results in the inhibition of capsaicin-induced spontaneous pain, suggesting a role for neurogenic inflammation in capsaicin-induced nociception [17]. Inflammatory cytokines are also known to contribute to neurogenic inflammation induced by capsaicin. One particular study observed that intraplantar injection of capsaicin induced acute nociception and an increase in tissue levels of TNF-α, IL-1β, and IL-6 [18]. Other studies found that injection of TNF-α, IL-1β, IL-6, and IL-8 produces mechanical hyperalgesia in animals [19,20].

Multiple studies have consistently shown that capsaicin alters the thermonociceptive threshold of skin [7,21,22]; however, it should be noted that the effects of capsaicin on nociception depend on the dose and route of administration [6]. Acute application of capsaicin (onset 1 min and duration >1 h) results in an initial firing of nociceptors followed by an increased sensitivity to thermal and mechanical stimuli [23–26]. In human volunteers, transdermal application of capsaicin resulted in mechanical hyperalgesia and allodynia, which persisted for 30 min at the site of administration, as well as in the untreated surrounding skin, suggesting that local capsaicin induces both primary and secondary hyperalgesia [25]. The investigation by Simone et al. [27] observed dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin (0.1–100 μg). Further investigation by Torebjork et al. [28] showed that intradermal injection of capsaicin and nerve electrical microstimulation in humans results in central sensitization, which is responsible for the secondary hyperalgesia. Central sensitization refers to an increase in the firing of neurones located in the central nervous system [29] as a result of altered membrane excitability and increased synaptic efficacy [30]. This increased responsiveness of neurones primarily results in a hyperalgesic response within an uninjured area. In light of these pro-nociceptive properties of capsaicin, it has been mainly used to study primary and secondary hyperalgesia. Additionally, this chemical has been used to screen the effect of novel compounds on TRPV1 ion channels [31]. This ion channel has been implicated in osteoarthritis pain, dental pain, migraine, chronic inflammatory pain, and neuropathic pain, indicating wide utility of this model [32]. Moreover, compounds such as gabapentin, lamotrigine, topiramate [33], diclofenac, hydromorphone, and cannabinoids have been screened for their potential analgesic activity using this model [34,35]. Conversely, repeated dosing or high concentrations of capsaicin deplete thinly myelinated and unmyelinated nociceptive nerve fibers, which results in a loss of responsiveness to painful stimuli [36–38]. This

Table 1 Different experimental models, species and tests that can be used for studying inflammatory pain in skin, joint and gut

Organ	Experimental model	Species	Measurement of pain
Skin	Capsaicin-induced pain	Rat [7,21,22]	Electrophysiological recordings from skin nerves [22]
		Mice [10]	Response to heat stimuli [22]
		Human [23,34]	Pressure algometry [23]
			von Frey hair algesiometry [11]
			Grimace scale [159]
	Mustard oil-induced pain	Mice [41]	Grimace scale [159]
		Rat [40]	Electrophysiology [39]
			Response to heat or cold stimulation [44,45]
			von Frey hair algesiometry [45]
			Response to electrical stimulation [45]
	Formalin-induced pain	Rats [52,54]	Grimace scale [159]
		Mice [57,59]	Time spent in licking/lifting of paw, number of flinches [54]
		Cats [52]	Electrophysiology [54,55]
			Ultrasonic vocalization [53,59]
	Acid-induced pain	Rat [71,73]	Electrophysiology [71]
		Human [72,75]	von Frey hair algesiometry [72]
Joint	FCA-induced hyperalgesia	Rat [86,88,89,93]	Grimace scale [161]
		Mice [80,91,92]	Hargreaves test [91]
			von Frey algesiometry [83,92]
			Weight bearing [81,84]
			Gait analysis [82,86]
			Electrophysiology [85]
			LABORAS [171]
	Kaolin-carrageenan-induced pain	Mice [105]	Grimace scale [161]
		Rat [98,107]	von Frey algesiometry [98,105]
		Cats [96,97]	Weight-bearing [105]
			Guarding behavior [107]
			Vocalization [108]
			Electrophysiology [97,109]
			Hargreaves test [106]
	Collagen-induced arthritis pain	Mice [111,112]	Hargreaves test [114]
		Rat [111]	von Frey algesiometry [114]
			LABORAS [114]
Gut	Capsaicin-induced visceral pain	Mice [121,126]	Measurement of spontaneous behavior (licking, stretching, squashing of abdomen) [121]
		Human [122]	von Frey algesiometry [121]
			Electrophysiology [186]
	Mustard oil-induced visceral pain	Mice [121,127]	Measurement of spontaneous behavior (licking, stretching, squashing of abdomen) [128]
			von Frey algesiometry [127]
			Open-field test [128]
			Electrophysiology [130]
	Acetic acid-induced writhing	Mice [134,138]	Grimace scale [159]
		Rat [134]	Electrophysiology [184]
			Vocalization [184]
			Hot plate [184]
			Writhing response [137]

loss of sensory innervation is irreversible when capsaicin is injected into neonatal animals, whereas in adults there is an eventual recovery of afferent nerve density [37]. Interestingly, birds are insensitive to capsaicin despite expressing TRPV1 receptors, suggesting structural differences between mammalian and avian TRPV1 [39].

Mustard Oil-Induced Pain

Mustard oil is derived from mustard seeds and has a pronounced pungent taste characteristic of the mustard family of plants. It is a chemical irritant that mainly induces inflammation by neurogenic mechanisms [40,41]. Topical application of mustard oil (0.5–20%)

Table 2 Advantages and limitations of different experimental models of inflammatory pain

Organ	Experimental model	Advantages	Limitations
Skin	Capsaicin-induced pain	1. Can study both thermal and mechanical hyperalgesia 2. Good translatability 3. Used to study peripheral and central mechanisms of pain in animals and human 4. Contribution of neurogenic inflammation to pain can be studied 5. Used to study desensitization mechanisms	1. Can lead to Bezhold-Jarrisch reflex 2. Higher doses produce anti-nociceptive effect 3. Neurotoxic at higher doses
	Mustard oil-induced pain	1. Can study both thermal and mechanical hyperalgesia 2. Contribution of neurogenic inflammation to pain can be studied	1. Moderate translatability 2. Infrequently used in pain studies
	Formalin-induced pain	1. Used to study peripheral and central mechanisms of pain 2. Rapid screening of compounds is possible 3. Natural pain response is recorded as animals are unrestrained 4. Biphasic response could be used to differentiate inflammatory and non-inflammatory pain	1. NSAIDs and mild analgesics only work at higher doses in this model [198] 2. Reliability is moderate 3. Less translatability
	Acid-induced pain	1. Used to study heat hyperalgesia 2. Used to study contribution of ASICs to the development of heat and mechanical hyperalgesia	1. Moderate translatability 2. Less reproducibility
Joint	FCA-induced hyperalgesia	1. Good model to study mechanical hyperalgesia and allodynia 2. Produces persistent pain and mechanical hyperalgesia 3. Closely resembles postoperative pain and persistent injury pain seen in humans 4. NSAIDs show good efficacy in this model	1. Polyarthritic animals can become quite sick 2. Minimal involvement of immune system 3. Less reliable for mice
	Kaolin-carrageenan induced pain	1. Produces robust inflammatory pain in cats, primates, and rodents 2. Debridement of articular cartilage and synovitis 3. Contribution of neurogenic inflammation in inducing pain can be studied 4. Reproducible 5. Induction is easy	1. Pain response generated can be severe 2. Pain response typically lasts only 24 h
	Collagen-induced arthritis pain	1. Chronic inflammatory pain 2. Pathology is close to human RA patients 3. Progression of pain is gradual 4. Induction is easy and reproducible	1. Polyarthritis; makes it difficult to assess behavioral pain 2. Time required for arthritis to develop is long 3. Incidence and severity varies among animals 4. Technically demanding
Gut	Capsaicin-induced visceral pain	1. Spontaneous behavioral pain 2. Easy to induce 3. Reproducible 4. Used to study mechanical hyperalgesia 5. Used to study wide range of analgesic compounds	1. Moderate translatability 2. Intervariability within subjects is high 3. Pain can be severe
	Mustard oil-induced visceral pain	1. Robust pain response 2. Used to study spontaneous pain 3. Used to study primary and secondary hyperalgesia 4. Induction is easy	1. NSAIDs show moderate efficacy in this model
	Acid-induced writhing	1. Produces characteristic stretching behavior 2. Good sensitivity to central and peripheral analgesics 3. Induction is easy and reproducible 4. Good translatability 5. Screening of multiple compounds in short period is possible	1. Poor specificity for drug development 2. Interpretation of results can be problematic

onto the ears of mice produces skin inflammation, which is mainly characterized by vasodilatation, increased plasma extravasation, and edema. These changes are blocked by treatment with an NK1 antagonist [41]. Similarly, mustard oil application on rat paw skin also triggers local cutaneous inflammation [40]. Interestingly, local denervation of nociceptive terminals, using a neurotoxic dose of capsaicin, attenuated mustard oil-induced inflammation; confirming a neurogenic inflammatory mechanism

[40,41]. However, a study by Wong et al. [42] indicated a possible involvement of a nonneurogenic mechanism in mustard oil-induced inflammation. This study assessed the effect of local anesthetic blockade on edema induced by mustard oil in the temporomandibular joint. It was observed that local anesthetics, which act directly on the neuronal membrane, failed to prevent the development of mustard oil-induced edema, suggesting a direct action of mustard oil on immune cells or vascular smooth muscle to cause inflammation.

Topical application of mustard oil activates sensory nerve endings (onset 5 min, duration >1 h), which decreases their excitation threshold and hypersensitizes them to thermal and mechanical stimuli [26]. Similar to capsaicin, topical application of mustard oil also induces a burning pain sensation in the applied area (primary hyperalgesia) and pain in the surrounding unaffected area (secondary hyperalgesia) [25]. Application of mustard oil to oral mucosa is responsible for induction of burning sensation and thermal hyperalgesia [43–45]. Several lines of evidence suggest that TRPA1 is the sole ion channel responsible for this action [46,47]. TRPA1 is involved in inflammatory pain and increases thermal and mechanical sensitivity [47]; however, in a recent study comparing TRPV1-knockout with wild-type mice it was reported that mustard oil also engages the capsaicin receptor TRPV1 to initiate acute pain responses [48]. This model has been mainly utilized to study compounds having an effect on TRPV1 or TRPA1 ion channels [46–48]. It has been shown that adenosine reduces secondary hyperalgesia by mustard oil in human subjects [49]. Mustard oil has also been used to promote orofacial inflammation following injection around temporomandibular area [50]. Furthermore, injection of mustard oil into craniofacial muscles resulted in nociception, which was significantly reduced by treatment with morphine [51].

Formalin-Induced Pain

The formalin test has been in existence since 1977, and has been routinely used for testing pain and hyperalgesia [52]. This test involves an injection of formalin (0.5–5%) into the plantar surface of an animal paw and produces specific behaviors like paw lifting, flinching, licking, and vocalization [52–54]. Furthermore, formalin injection produces a biphasic response where in phase I (0–5 min) pain results from the direct activation of primary nociceptive afferents, and in phase II (10–40 min) pain involves, at least in part, inflammation-induced central sensitization in the dorsal horn of the spinal cord [54–56]. Prostaglandins (PGs) are involved in phase II of the formalin model, and cyclooxygenase 2 (COX2)-specific nonsteroidal antiinflammatory drugs (NSAIDs), which block PG production, have been shown to reduce formalin-induced pain during phase II [57,58]. A recent study described a role for IL-33, a pro-inflammatory cytokine, and its receptor, ST2, in mediating formalin-induced nociception [59]. In this study, IL-33 induced pain in both phases of the formalin test, and an ST2-targetted antibody blocked these effects, highlighting the importance of IL-33 in modulating nociceptive changes both peripherally and centrally in formalin-treated animals [59]. For phase II of the formalin test, there are reports which underscore the importance of ongoing primary afferent input for the development and perpetuation of a pain response in animals [60–62]. In light of these reports it is still under debate whether central sensitization and/or primary afferent input is responsible for the pain response during this phase of the formalin model. An earlier study looked into pain responses from 2 h to 4 weeks after injection of formalin. It was observed that the hyperalgesic response lasted until 4 weeks reaching a second peak from day 7–10 [63]. The researchers speculated the possibility of spinal microglial activation and peripheral inflammation leading to central sensitization, which would be responsible for the maintenance of this long-lasting hyperalgesia [63]. In addition to the release of various inflammatory mediators, formalin can also activate TRPA1 directly to elicit pain and inflammation [54]. This test has been successfully used to assess the efficacy of a variety of compounds like morphine, oxyphenylbutazone, acetylsalicylic acid, corticosteroids [64], diflunisal [65], diacerhein, carbamazepine, topiramate, and gabapentin [66]. Antagonists targeting TRPA1 ion channels [54], NMDA receptors [67], and neurokinin-1 receptors [68] have also been examined. The formalin test has also been used to study orofacial pain, as subcutaneous injection into the lip produces nocifensive behaviors in rats [60,69]. One of the limitations of the formalin test is that it has little translational relevance. Unlike other models of cutaneous sensitization (e.g., capsaicin, UVB, acid), intradermal injection of formalin is not carried out in human volunteers. This model is restricted to animal experiments and is mainly used for studying pain mechanisms rather than analgesic evaluation as it is both reproducible and relatively straightforward to perform.

Acid-Induced Pain

Inflammatory and ischemic conditions both lower tissue pH [70]. During inflammation, several factors promote tissue acidity, including intracellular components released following cell lysis, inflammatory mediators, and pumping of lactic acid by leukocytes [71,72]. A study by Steen et al. [73] found that protons are capable of stimulating cutaneous neurones on their own. Additionally, chemical mediators released during inflammation, combined with the low pH environment, conspire to produce a synergistic effect, resulting in prolonged nociceptor activation [74]. Protons produce pain by activating TRPV1 and acid-sensing ion channels (ASICs) on sensory free nerve endings [75]. To date, four different types of ASIC channels have been identified (ASIC1-4), which are located in the central and peripheral nervous systems [76]. Each of these ASIC channels has specific pH sensitivity *viz.* ASIC1: pH5.8–6.8, ASIC2: pH4.5–4.9, and ASIC3: pH6.4–6.6 [76]. These ASICs play an important role in the development of chemogenic inflammatory pain [77], neuropathic pain [78], postoperative pain [79], and neurological disease pain [76].

Models of Joint Pain

Freund's Complete Adjuvant-Induced Hyperalgesia

Freund's complete adjuvant (FCA) is a routinely used model to study chronic inflammatory pain in rodents [80–82]. Intra-articular injection of FCA (heat-killed *Mycobacterium tuberculosis* in paraffin oil) results in localized edema, as well as mechanical and

thermal hyperalgesia [83–86]. Immunological reactions develop 7 days after injection of FCA, which leads to tissue swelling. Therefore, pain studies usually follow 2 treatment regimens: 0–8 days (prophylactic regimen) and post day 8 (therapeutic regimen) [87]. Interestingly, FCA also produces articular hypoemia, which nicely models the hypoxic environment found in rheumatoid arthritic joints [88]. It has been shown in previous studies that FCA injection elicits the production and release of various inflammatory mediators such as PGE2, nitric oxide, leukotriene B2, TNF-α, IL-2, and IL-17 [89]. These pro-inflammatory mediators cause synovitis, polyarticular inflammation, bone resorption, periosteal bone, and proliferation and can result in joint degeneration [87]. In addition, these pro-inflammatory cytokines play an important role in inducing joint pain by causing sensitization of neurones either directly or by indirect means, that is via release of prostaglandins [1]. Extra-articular manifestation resembles those of RA patients. However, the cartilage damage observed in this model is less severe than human RA and is therefore not recommended for the study of this aspect of the disease. The inflammatory mediators released into the joint are also responsible for sensitizing articular nociceptive afferents, resulting in arthritic pain. Injection of FCA also causes activation of TRPV1 ion channels that participate in the joint inflammation process [90]. A study by Keeble et al. [91] investigated the effect of intra-articular FCA in wild-type and TRPV1-knockout mice. Knee swelling and vascular hyperpermeability were significantly reduced in TRPV1-knockout mice, as compared to wild-type mice. Interestingly, leukocyte accumulation and TNF-α levels were similar in WT and TRPV1-knockout mice. To explain this effect, the authors argued that TRPV1 may be acting as a modulator instead of primary contributor. The mechanical hyperalgesia was correlated with knee swelling and was reduced in TRPV1-knockout mice. A further investigation by Fernandes et al. [92] identified a role for TRPA1, as well as TRPV1, in FCA-induced monoarthritis; however, the contribution of TRPA1 was moderate. The inflammatory neuropeptide substance P also plays an important role in FCA-induced joint inflammation and pain. When animals were treated with an NK1 antagonist, FCA-induced hyperalgesia and inflammation were significantly reduced [93]. Based on these results, it is clear that FCA induces the expression of several inflammatory mediators, which promote joint pain. Because of the robust nociceptive responses observed in this model, FCA is one of the better models for studying inflammatory joint pain. It should be noted that the model described here is distinct from the hindpaw FCA injection model, which is used to assess cutaneous inflammatory pain. Both models, however, are highly utilized in academic and industrial settings to study novel antiarthritic compounds and potential analgesics. Several compounds such as dexamethasone, methotrexate, NSAIDs, and biological agents [94,95] which are used clinically had previously shown good efficacy in the FCA model.

Kaolin–Carrageenan-Induced Monoarthritis

The kaolin–carrageenan arthritis model is a widely used monoarthritic model of acute joint inflammation. This model produces a well-defined pattern of inflammation and pain and has been established in primates, cats [96,97], and rodents [98,99]. Kaolin, also known as China clay, is an inorganic substance that is composed of hydrated aluminum silicate ($H_2Al_2Si_2O_8.H_2O$). When injected into the joint, as an aqueous suspension, it produces fine debridement of the articular cartilage and irritates the synovial membrane. λ-Carrageenan type IV (carrageenan) is a sulfated polysaccharide, derived from Irish moss, and so named after the Irish town of Carragheen. Carrageenan produces an acute inflammatory response by first activating cell surface expressed Toll-like receptor 4 (TLR4). TLR4 activation leads to the phosphorylation of BCL10, which then activates nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB). This chain of events results in leukocyte infiltration and the release of cellular mediators that promote local inflammation [100].

Kaolin–carrageenan-induced joint inflammation has a well-defined time course, where signs of inflammation are apparent 1–3 h following injection, peak and plateau around 5–6 h, and are maintained for 7 days or more [101]. This inflammatory phase is characterized by hyperemia, edema and immune cell infiltration [102–104]. In addition to inflammation, this model also produces a number of behavioral and neuronal responses that are characteristic of joint pain. Animals show decreased weight bearing on the inflamed limb, decreased withdrawal thresholds to von Frey hairs [105], noxious heat stimuli [106], increased guarding [107], and pain-induced vocalizations [108]. Furthermore, articular afferents and dorsal horn neurones show increased electrical activity following intra-articular injection of kaolin–carrageenan [97–99,109,110]. Because of the well-defined and consistent pain and inflammation that this model produces, it is frequently used to test new antiinflammatory and analgesic compounds.

Collagen-Induced Arthritis

Collagen-induced arthritis (CIA) is the most commonly used model for studying novel disease modifying drugs for rheumatoid arthritis (RA) in rodents. In this model, an emulsion is made using type II collagen and FCA which is injected into the base of the tail [111,112]. This FCA emulsion acts as an antigen repository which causes slow systemic leakage of antigen thereby triggering an autoimmune response leading to deposition of immune complexes onto the surface of joint tissues. Others features of this model include symmetrical joint involvement which is less observed in the adjuvant monoarthritis model, prominent synovitis, bone resorption, and periosteal proliferation [87]. The cartilage damage and lesions produced after injection of CIA are comparable to those observed in human RA [87]. However, extra-articular manifestations are less apparent in this model as compared to the adjuvant model. For many years, this model was used for screening the antiinflammatory potential of compounds; however, studies conducted in the past few years have shown utility of this model for testing analgesics. Inflammation observed in this model primarily involves T and B cells which promote synovial hyperplasia or thickening, cartilage erosion, swelling, and reduced mobility of the joints [113].

Inglis et al. [114] have shown a correlation between inflammation (paw swelling and clinical score) and mechanical and thermal hyperalgesia in the CIA model. A positive correlation exists between inflammation and hyperalgesia up to 10 days after the onset of arthritic symptoms. Simultaneously, the expression of

TNFα and IL-1β also decreased around that time, which highlights the role of these mediators as algogens. It has been suggested that different cytokines cause sensitization of neurones by receptor-associated kinases, by promoting ion-channel phosphorylation, or by altering downstream signaling [1]. Astrocytes are known to play a supportive role in maintaining chronic pain [115], and CIA-injected animals have increased levels of lumbar spinal cord astrocytes [114]. Finally, the expression of activation transcription factor-3 (ATF-3), a marker of nerve damage, was found to be increased in DRGs supplying collagen-injected ankles indicating peripheral neuropathy in this inflammatory model [114]. Compounds from different drug classes such as corticosteroids, NSAIDs, methotrexate, and biologics have shown better efficacy in this model compared to other models of joint inflammation [94,95]. Alternative compounds that modify different cellular targets such as Janus kinase [116], histone deacetylase 6 [117], MAPK [118], and chemokine receptors [119] have also been successfully screened using the CIA model.

Models of Visceral Pain

Capsaicin-Induced Visceral Pain

Patients suffering from inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis, frequently experience visceral pain. Assessing visceral pain is challenging, as the primary afferent neurones in the gut run closely with the enteric nervous system raising the possibility of interaction between the two nervous systems [120]. The visceral pain observed in response to intraluminal capsaicin injection is poorly localized much like human visceral pain [121,122]. Attempts have been made to develop models of visceral pain, which would allow the evaluation of inflammatory mechanisms and potential therapies. It has been suggested that hypersensitization of visceral afferent neurones is one of the major factors responsible for chronic pain states in IBD patients. As such, capsaicin has been used to gain insights into the mechanism which contribute to visceral pain. Studies have shown that intraluminal administration of capsaicin causes an increase in behavioral response by animals, which is blocked by TRPV1 antagonists highlighting the role of capsaicin-sensitive afferents in mediating visceral pain [123]. Other reports have shown great promise of TRPV1 as a pharmacological target for the treatment of visceral pain [124,125]. Chemostimulation of the colon using capsaicin (0.03–0.3%) has been used to induce visceral pain *in vivo* [121]. Intracolonic administration of capsaicin evokes spontaneous pain behaviors, which are characterized by writhing, stretching and licking of the abdomen, as well as reduced self-grooming [126]. Morphine dose dependently blocks these nociceptive responses [121]. Intracolonic administration of capsaicin also induces mechanical hyperalgesia, which results in increased responsiveness to von Frey hairs (force, 1–32 mN) applied to the abdomen [121]. In addition to a hyperalgesic response, capsaicin also produces low-grade inflammation in the colon, which was evident by increased plasma extravasation. This pro-inflammatory effect of capsaicin was neurogenic, as denervation of the colon abolished this response. Furthermore, NK1-receptor-knockout mice failed to show any behavioral signs of pain, suggesting that tachykinins are involved in capsaicin-induced gut inflammation [121,126].

Mustard Oil-Induced Visceral Pain

Similar to capsaicin, intracolonic injection of mustard oil (0.25–2.5%) produces visceral pain, referred pain, and inflammation [121]. Mustard oil injection causes stimulation of peripheral nociceptors and spinal dorsal horn neurones to evoke the primary pain response and referred hyperalgesia, respectively [126,127]. In a recent study, the activity of two NSAIDs, ketorolac and ketoprofen, as well as morphine were tested against mustard oil-induced nociception. Morphine was able to block spontaneous pain behaviors and referred hyperalgesia tested using von Frey hairs [121]. The NSAIDs, however, were only able to block mustard oil-induced spontaneous pain behaviors. NSAIDs also reduced plasma extravasation, indicating an antiinflammatory effect of these drugs in this model [127]. The algesic and inflammatory effects of intracolonic mustard oil have been found to be TRPV1 dependent, suggesting a common pathway with capsaicin [128]. Intracolonic administration of mustard oil results in the release of various pro-inflammatory cytokines, chemokines, and neuropeptides, which suggests its utility as a model of colonic inflammatory pain [129]. This model produces visceral hypersensitivity and permanent sensitization of dorsal horn neurones, which are also observed in patients suffering from irritable bowel syndrome (IBS) [130,131]. For example, a recent study has shown that targeting TNF-α produces a modest reduction in pain scores in patients suffering from Crohn's disease [132].

Acetic Acid-Induced Writhing

Intraperitoneal injection of chemical irritants has been used for over 40 years to induce visceral pain in animals [133–135]. Substances such as phenylbenzoquinone or acetic acid produce characteristic writhing responses following injection [133,134]. These writhing responses include abdominal stretching, flinching, licking, and motor incoordination [135]. For induction of a writhing response in unanesthetized animals, an intraperitoneal injection of dilute acetic acid (0.6–9%V/V) or phenylquinone (0.3–0.9%) is made at a fixed dose (0.2 ml/mouse or 0.5–2.5 ml/rat) or in some cases a weight-adjusted dose (10 ml/kg) is used [136]. Cytokines [137], prostaglandins [138], and bradykinin [139] are all released following intraperitoneal injection of acetic acid and are capable of sensitizing visceral nociceptive afferents. Inhibition of the pro-inflammatory cytokines TNF-α and IL-1β results in the inhibition of visceral hyperalgesia produced by injection of acetic acid [140]. Furthermore, administration of antiinflammatory cytokines (IL-4, IL-10 and IL-13) also produces an antinociceptive effect by down-regulating the release of eicosanoids in this model [141]. A study conducted by Pavao-de-Souza. [142] looked at the activation of different downstream signaling pathways in the spinal cord after acetic acid injection [142]. Intrathecal administration of inhibitors of extracellular signal-regulated kinase (ERK), Jun N-terminal Kinase (JNK), p38, phosphatidylinositol 3-kinase (PI3K) reduced nociceptive responses in a dose-dependent manner [142]. This model has been mainly used as a screening tool to characterize analgesic activity of opioid compounds [143] and NSAIDs

[144,145]. Some of the limitations of this test include lack of specificity as some nonanalgesic drugs also show efficacy. Irritation of nongastrointestinal tissues has also been reported in this model [136].

Assessment of Inflammatory Pain

von Frey Hair Tactile Sensitivity

One method that is used to test peripheral and central sensitization associated with inflammatory pain is von Frey hair mechanosensitivity. von Frey hairs are a graded series of thin, calibrated filaments that bend to exert a specific mechanical force [146]. Normal animals show a paw withdrawal response when stimulated by a von Frey hair applied to the plantar surface of the paw. von Frey hairs with a low bending force elicit a tactile withdrawal in naïve animals; however, filaments with higher forces could produce a nocifensive response. Inflamed tissues are hypersensitive to von Frey hairs, leading to a reduction in withdrawal threshold (i.e., they respond to a hair which normally does not evoke a withdrawal response). In the periphery, inflammation can result in changes in voltage-gated sodium and calcium channel expression and a heightened firing frequency of nociceptors [147]. Furthermore, changes within the CNS have also been attributed to heightened von Frey hair sensitivity following inflammation. Central sensitization can involve altered pain signal processing in the spinal cord [148] and brain [149], insufficient descending inhibitory signals [150], excessive descending facilitatory signals [151], and changes in synaptic plasticity in the anterior cingulate cortex [152]. Measuring the changes in sensitivity to plantarly applied von Frey hairs allows researchers to investigate referred pain during inflammation, and how this secondary pain response develops and responds to drug treatment. There are some limitations to this technique including the potential of experimenter bias, that is, the withdrawal response nocifensive or a startle reflex. An additional limitation is that paw withdrawal thresholds have the propensity to vary between animals because of repeated application of multiple of hairs. To overcome these limitations, electronic von Frey hairs with a calibrated force transducer and dynamic plantar aesthesiometers have been developed. These instruments are capable of recording force and latency to withdrawal following application of a single fine metal monofilament with increasing force, thus avoiding the need for repeated application of multiple filaments. Also, the rate of increasing force is applied at a constant ramp speed which reduces experimenter bias [153].

Hindlimb Weight Bearing

Spontaneous pain is a characteristic of many inflammatory diseases [1]; therefore, measuring nonevoked painful behaviors in animal models is essential in understanding this clinically relevant phenomenon. One technique used to measure spontaneous-like pain is hindlimb incapacitance, in which the difference in weight bearing between a quadruped's hindlimbs is assessed [154]. Normally, rodents distribute their weight equally between both hindlimbs; however, following the induction of unilateral hindlimb inflammation, rodents tend to favor the noninflamed leg. This shift in weight bearing can be measured in stationary animals (static weight bearing; SWB), as well as freely mobile animals (dynamic weight bearing; DWB). SWB requires that the animal rear on both hindlimbs while standing on two force plates, one under each hindpaw. When measuring DWB, the animal is placed in a chamber carpeted with a pressure sensitive flooring pad, which allows weight bearing to be assessed during movement. In both techniques, the weight borne by each hindlimb is measured, and the difference between the two is calculated. Hindlimb incapacitance measurements are useful in testing weight bearing changes in models of inflammatory arthritis, osteoarthritis, neuropathic pain, hindpaw dermatitis, and bone cancer pain [154,155]. Potential limitations of this technique are that it only permits the assessment of hindlimb inflammatory pain, some animals may experience restraining stress, and repeated testing could modify behavior [156].

Grimace Scale

Facial expression is an important element of communicating pain between individuals. In 1978, Ekman and Friesen developed a facial action coding system, a tool to characterize distinct emotions in human [157]. Facial expressions have also been effectively used for the detection of pain in noncommunicative humans such as neonates and patients with cognitive impairment [158,159]. The expression of pain facially is not only restricted to humans, but is conserved in many other sentient species [159,160]. This physical aspect of nonhuman pain has been developed into a grimace scale using a standardized behavioral coding system to measure spontaneous pain in rodents [159,161]. In this test, five facial features (orbital tightening, nose bulge, cheek bulge, ear position, and whisker position change) are scored by blinded coders on a three-point scale, where 0 = not present, 1 = moderately present, and 2 = severe. To test the reliability of this scale, various nociceptive assays were performed and pain severity was compared to the grimace score. It was observed that the grimace scale produced consistent and reliable pain scores across all behavioral assays tested. Grimace has been successfully used to demonstrate analgesia in rodents where a linear correlation was observed between dose of morphine and a reduction in grimace score following intraplantar or intra-articular zymosan and cyclophosphamide-induced bladder cystitis [159,161].One of the potential disadvantages of the grimace score is that it can be very time-consuming; however, this limitation has been mitigated by the use of Rodent Face Finder® software, which automatically generates photographs from video thereby saving a significant amount of processing time. Another limitation of this technique is that it is only effective in assessing acute pain, as animals with chronic pain learn to adapt and do not produce facial grimacing [161]. Nevertheless, as this test is capable of capturing spontaneous pain, it could be used for prioritizing compounds in the later stages of drug discovery.

Hargreaves Test

Hypersensitivity to noxious thermal stimuli is a commonly observed during cutaneous inflammation, and the Hargreaves test was designed to assess this response in rodents. In this test, a movable heat source is placed under a glass floor upon which the

animal is standing. The thermode is then positioned underneath the plantar surface of the hindpaw and radiant heat is gradually increased at a fixed rate until the animal feels pain and withdraws its paw [162]. This test allows researchers to measure thermal hyperalgesia in an unrestrained animals, therefore providing less stressful testing conditions for the animals [163]. One limitation of the Hargreaves test is that repeated measurements can affect withdrawal latencies [163], since rodents learn rapidly and quickly adapt to a changing environment [163].

Randall-Selitto Paw Pressure Test

The Randall-Selitto paw pressure test is used to measure the cutaneous mechanical hyperalgesia in rats [164]. In this test, the paw or tail of the rat is placed on a circular platform and a linearly increasing force is applied to the tissue with a dome-shaped plastic tip [165]. A similar system has been developed to assess mechanonociception in experimental models of arthritis where calibrated forceps are oriented along the joint line and an increasing compression force is applied. When the mechanical force reaches a painful threshold, the rat either withdraws its hindlimb or vocalizes. At this point, the force application is stopped and the nociceptive withdrawal threshold is recorded. The entire procedure is repeated 2–3 times and a mean value is calculated. Care must be taken not to exceed the maximum force as this could result in tissue injury and inflammation. It is possible to discriminate between left and right hindlimb responses using this test, and it also allows multiple measurements within the same animal. Since the test involves manually restraining the animal, stress-induced analgesia could diminish the magnitude of the pain _measured [166]. While this test is practicable for assessing inflammatory pain in the extremities, it not useful for measuring visceral pain.

Locomotor Activity

Most of the tests that are designed to study pain mechanisms and evaluate analgesic activity of compounds are based on evoked responses to an external stimulus, involve restraining animals, and are very much experimenter-dependent [167]. In an attempt to overcome these issues, Matson et al. [168] developed the reduction in spontaneous activity by adjuvant (RSAA) test, which assesses spontaneous animal behaviors in a novel environment. The animals are placed in a Plexiglass box, and the walls of this box are fitted with sensors to detect changes in locomotion. Two activity measures, total distance travelled and vertical activity (rearing), are recorded before and after the administration of an inflammatory agent. It has been shown that administration of these agents results in a decrease in locomotor activity and rearing. Moreover, treatment with morphine, ibuprofen, rofecoxib, celecoxib, piroxicam, and dexamethasone all reversed these locomotor deficits, suggesting a usefulness of this technique to evaluate analgesic activity [168,169]. This test allows animals to be unrestrained which helps in reducing stress and there is no need for subjective behavioral scoring [168]. Repeated testing may not suitable as this test relies on the novelty of the environment and the exploratory nature of rodent animals [167].

Another useful tool used to study pain-induced behavior changes in rodents is the Laboratory Animal Behaviour Observation, Registration and Analysis System (LABORAS). This is an automated system which is capable of detecting changes in the behavior and locomotor activity of rodents by assessing changes in vibration signatures produced by their movement [170]. This system discriminates between different behaviors such as eating, drinking, grooming, climbing, resting, and locomotion, which are all compromised during pain [170]. This system has been successfully used to detect pain behaviors after induction of collagen-induced arthritis [114] and adjuvant-induced cutaneous inflammation [171].

Changes in burrowing activity is another behavioral readout designed to capture spontaneous-like pain in experimental animals. Burrowing is a natural phenomenon in rodents in which the animals dig holes or tunnels to create a domicile or a place of refuge. This behavior can be quantified by measuring the difference in the known weight of bedding placed in a habitation tube and the weight which has been excavated during a period of burrowing [172,173]. This assay has been successfully used to predict the analgesic efficacy of compounds in models of knee joint inflammation [174,175], osteoarthritis [176], colitis [177], and peripheral nerve injury [178]. This assay offers good sensitivity, is easy to perform, inexpensive, and requires minimum experimenter training [172,173]. High variability between animals is one limitation of this method; however, this can be somewhat mitigated by training the animals prior to recording. Furthermore, it is uncertain whether changes in burrowing activity are attributable to pain or whether the animal is demonstrating anxiety or depression.

Electrophysiological Recording of Nerves

Tissue inflammation leads to an increase in neuronal excitability and a decrease in activation threshold in response to environmental stimuli. Therefore, studying the electrical activity of nociceptors using exquisite electrophysiological techniques can offer an objective means of assessing nociception. Nerve recordings may be carried out in the periphery (primary afferent neurones, dorsal root ganglia), in the dorsal horn of the spinal cord, or in supraspinal regions involved in pain perception (e.g., thalamus, somatosensory cortex, amygdala). Nerve recordings can be either extracellular or intracellular with extracellular recording being the most commonly used approach for pain studies. Extracellular recordings are carried out *in vivo* and can take the form of either single unit recordings from discrete axon bundles, or compound action potentials from whole nerve preparations. *In vitro* intracellular recordings can be carried out on intact or dissociated neurones, intact dorsal root ganglia, or isolated tissue slices. *In vivo* intracellular recordings are possible with whole neurones, single axons, or dorsal root ganglia. These electrophysiological approaches have dramatically advanced our understanding of the neuronal mechanisms responsible for the generation of evoked and spontaneous pain.

The superficial location of cutaneous afferents make them an attractive and accessible population of nerve fibers from which to record. Following a focal injury, skin develops two zones of inflammatory pain: the first is restricted to the precise site where

the injury occurred (primary hyperalgesia) while the second extends into the area surrounding the initial insult (secondary hyperalgesia). Primary hyperalgesia is attributable to the sensitization of nociceptor nerve endings in the skin whereas secondary hyperalgesia arises due to plasticity changes occurring in the central nervous system. Thus, skin is an appealing organ in which to assess inflammatory pain at different levels of the pain pathway.

Electrophysiological recordings have also been performed on animal knee joint primary afferents [179–182]. Single unit extracellular recordings provide persuasive subjective information regarding joint nociceptor biology and responsiveness to inflammatory mediators. Numerous studies have shown that articular administration of pro-inflammatory chemicals causes a reduction in afferent mechanical threshold and increased firing in response to joint movement [183].

Electrophysiological approaches have also been utilized to investigate the mechanisms of visceral pain. Electrophysiological recordings from nociceptive dorsal horn neurones have been used to give insights into the neuronal mechanisms involved in the generation of abdominal pain in irritable bowel syndrome and colitis [130]. Intraperitoneal injection of noxious agents such as acetic acid or infectious agents such as *Citrobacter rodentium* can sensitize visceral extrinsic neurones leading to increased afferent firing that encodes gastrointestinal pain [184,185]. The generation of referred hyperalgesia is an important characteristic of visceral pain. A study by Sanoja et al. [186] assessed the electrical activity of "ON-like" cells in the rostral ventromedial medulla (RVM) following intracolonic instillation of capsaicin. Electrophysiological recording of "ON-like" RVM neurones showed a long-lasting sensitization phenomenon and this altered firing pattern encodes referred hyperalgesia.

In the spinal cord, recordings are made from neurones located in laminae I and II of the dorsal horn. Electrophysiological measurements can be performed on an *in vivo* preparation or on slices of spinal tissue, although these latter experiments must be carried out within hours of tissue excision and plating. Spinal recordings are useful to determine the effect of inflammatory mediators on central sensitization mechanisms and their effect on central neurotransmission. Supraspinally, pan cortical recording of the somatosensory cortex has been carried out in response to peripheral inflammation [187]. In order to gain insight into the affective aspects of inflammatory pain, *in vivo* recordings have also been carried out on specific nuclei of the amygdala [188]. Thus, recording from different regions of the brain allows us to explore how inflammatory pain signals are processed into a negative sensory experience.

Reasons for the Translational Gap between Animal Models and Human Inflammatory Pain

Animal models have successfully been able to predict clinical efficacy of commonly used treatments for inflammatory pain including acetaminophen, ibuprofen, morphine, corticosteroids, and biologics [51,87,189–195]. However, there are still a number of compounds which show great efficacy in preclinical models but fail during clinical trials. For example, substance P antagonists [196] and an endocannabinoid hydrolysis inhibitor [197] showed great promise in animal models of disease, but were found wanting in the clinic. These reports imply that there are profound limitations with animal models which question their translational veracity. Some of the reasons for the discrepancy between humans and animal models include differences in how we assess pain, mismatches in the time required for pain development, and interspecies variation in pharmacokinetic/pharmacodynamic properties of drugs.

There are intrinsic differences in perception of pain between animals and humans. Animals lack the ability to describe their pain intensity and can only manifest nociception in behavior or show withdrawal reflexes to noxious stimuli. As such, current animal models of inflammatory pain and our interpretation thereof are primarily based on generating disturbances in neurosensation. On the other hand, human pain is multifaceted encompassing emotional, cognitive, affective, and psychosocial components which can impact pain reporting [198,199]. Clearly, there is a need for the development of new endpoints or animal models which are capable of recapitulating multiple features of human pain states. Comorbidities are also a prominent part of human disease which are often overlooked in animal models. Patients suffering from rheumatoid arthritis, for example, are at higher risk of cardiovascular disease and gastrointestinal inflammation. A greater focus should therefore be placed on animal models that incorporate comorbidities across different species.

Our interpretation of pain in animals and humans is different. In preclinical models, pain is mainly assessed by recording changes in the withdrawal threshold to mechanical or thermal stimuli. In contrast, the severity and quality of human pain is predominantly assessed by using subjective rating scales like the visual analog scale (VAS), verbal rating scale (VRS), or the numerical rating scale (NRS) [200]. These rating scales essentially are a series of questions designed to capture self-reported clinical pain which is subjective and inherently leads to variability. Therefore, pain responses captured in animals will not mimic the expressed feelings of pain in humans. Another important difference between animal and human pain is the time required for the pain to develop. In the case of animal models, it usually takes from hours to weeks whereas in humans persistent pathological pain lasts for months or years [199]. Factors such as cost and ethics preclude long-term pain studies in animals and as such it is impractical to model the precise temporal aspects of chronic human pain.

Another important characteristic that has largely been underestimated is the disparity in pharmacokinetics/pharmacodynamics (PK/PD) between species. Pharmacokinetic studies generate information about the drug concentrations at target and nontarget sites, metabolism and elimination pattern. Pharmacodynamic studies mainly assess the pharmacological effect of drug at available concentrations. It should be noted that a correlation exists between these two disciplines and they are interdependent [201]. However, several studies have shown that pharmacokinetic parameters differ between species which makes extrapolation of animal data to humans challenging [202,203]. These differences could impact the pharmacological efficacy and safety of a drug in different species. For example, analgesics such as celecoxib and indomethacin exhibit differences in dose, plasma exposure, and bioavailability between rat and human [204]. It is essential, therefore, to recognize important pharmacokinetic and pharmacodynamics parameters between species and between different pain

states. This consideration would improve the predictive capacity and translatability of future preclinical studies.

Concluding Remarks

Patients suffering from chronic inflammatory conditions live in a considerable amount of pain over which we have limited control. The establishment of experimental models and different techniques to assess pain have played a significant role in advancing our knowledge about peripheral and central mechanisms of pain. Furthermore, these models have been successfully used to discover new molecular and cellular targets which form the basis of future analgesic drugs. These inflammatory models should be continually refined so as to improve their translatability to human conditions. In addition, novel ways of assessing and interpreting pain in animal models are required to help complement and supplement the current battery of techniques used to detect nociception.

Conflict of Interest

The authors declare no conflict of interest.

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