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TRPV1 antagonists in the treatment of osteoarthritis pain

Pain is the dominant symptom of osteoarthritis (OA) and available analgesic treatments offer inadequate pain relief. The capsaicin receptor, TRPV1 is considered to be a promising analgesic target. TRPV1 is expressed in multiple cell types of the joint. A variant of the *TRPV1* gene is associated with the risk of developing symptomatic OA and synovial TRPV1 expression is increased in OA patients undergoing total joint replacement. Potent and selective small molecule TRPV1 antagonists have analgesic effects in preclinical models of OA highlighting the potential utility of TRPV1 antagonists in OA pain management. However, use of these compounds is associated with serious on-target mediated side effects and analgesic efficacy in OA patients in clinical trials remains to be proven. This review article will discuss recent findings in this area and explore the potential utility of TRPV1 antagonists in the treatment of OA pain.

Sara Kelly
Arthritis Research UK Pain Centre,
School of Biosciences, University of
Nottingham, Sutton Bonington Campus,
Nr Loughborough, Leicestershire,
LE12 5RD, UK
sara.kelly@nottingham.ac.uk

Osteoarthritis

Osteoarthritis (OA) is the most common form of arthritis worldwide with an estimated 8.5 million sufferers in the United Kingdom alone [1]. OA is characterized by progressive joint pathology that includes synovitis, cartilage degradation and subchondral bone sclerosis [2]. The most commonly affected joints are those that are load bearing, including the hip and knee. OA leads to functional impairment of the affected joints due to the associated chronic, often intractable pain. The pain symptoms associated with OA are complex and heterogeneous among patients, and include activity-related pain, pain at rest that occurs randomly and without an obvious identifiable cause, and pain referred to distant areas of the body [3,4]. OA pain sensations are commonly described as 'stabbing', 'tearing', 'electric-shock like', 'cramping' and 'pulsating' and more rarely as 'crushing', 'prickling' and 'burning' [3]. The chronic pain of OA impacts upon the individual's ability to carry out normal daily activities, seriously reducing quality of life [4].

In the absence of any OA disease modifying drugs, current licensed treatments aim to improve function and to reduce pain [5]. Despite this, many OA sufferers experience inadequate pain relief and surgical joint replacement remains the only remaining treatment option. Current therapies used to treat pain in OA such as nonsteroidal anti-inflammatory drugs (NSAIDs) are not affective in all patients, at all stages of the disease and can be associated with serious use limiting side effects making them unsuitable for chronic long-term use [5]. Given that the incidence of OA is set to rise in our progressively aging population with increasing levels of obesity, the unmet clinical need of OA pain is set to increase rapidly over the coming decades [6]. As such there is an increasing clinical need for the development of more effective analgesic treatments for OA. This review will discuss recent developments in this area and in doing so will explore the potential of TRPV1 antagonists for the treatment of OA pain. Adverse effects of TRPV1 antagonists will also be addressed.

Future
Medicine part of fsg

Targeting TRPV1 in the treatment of OA pain

There is now a significant body of evidence supporting targeting TRPV1), for the treatment of OA pain [7–10]. The relevance of TRPV1 to OA pain was highlighted by a recent human genetic study that discovered polymorphisms in the TRPV1 gene associated with symptomatic knee OA [11]. Persistent activation of TRPV1 by the agonist capsaicin causes TRPV1 desensitization and inactivation of TRPV1 expressing sensory neurones. These effects account for why TRPV1 agonists can produce paradoxical analgesia. Topical capsaicin is more effective than placebo for a 50% reduction in pain with a number needed to treat of 8.1 [5]. However, the use of topical capsaicin is associated with increased local adverse events and withdrawals due to these events [12]. The TRPV1 agonist 4975 (Adlea™) developed by Anesiva has been shown to cause a reduction in pain scores with no safety concerns following a single intra-articular injection in end-stage OA patients [13]. A recent Phase II clinical trial demonstrated analgesic efficacy of topic administration of civamide (cis isomer of capsaicin) in patients with knee OA pain [14] and has been approved for topical treatment as a 0.075% cream for the relief of pain in OA patients [14]. Qutenza® an 8% capsaicin patch is indicated for the management of neuropathic pain associated with postherpetic neuralgia. The long-term analgesic efficacy of topical capsaicin cream in the treatment of knee OA pain [12] and of a capsaicin patch in postherpatic neuralgia provides clear evidence that the blockade of TRPV1 expressing nociceptive afferents at the periphery is a useful strategy to treat chronic pain, although this does not necessarily inform us of the role of TRPV1 itself. The relatively low effectiveness of capsaicin cream and the need to reapply, along with the associated skin irritation has limited civamides' use. Despite this, TRPV1 remains a potential target for OA pain treatment with accumulating preclinical data suggesting that TRPV1 has an important role in the maintenance of established OA pain [7–10,15]. Several pharmaceutical companies have invested major efforts into the development of TRPV1 antagonists [9,16,17]. Although preclinical findings have been encouraging, the analgesic efficacy of TRPV1 antagonists in clinical trials conducted in OA patients remains to be proven. Moreover, the development of these compounds has been held back by on-target-mediated hyperthermic side effects [18] also observed in rodent models [9,16,17,19].

TRPV1 background

For many years prior to 1997, it was known that plant-derived chemicals such as capsaicin elicit a sensation of burning pain. The molecular basis of these sensations was revealed with the cloning of TRPV1 (then known as VR1) [20] and as such it became the subject of intensive research. TRPV1 is a nonselective cation-permeable ion channel expressed in small sensory C-type neurones and to a lesser extent in A-type neurones of the dorsal root, nodose and trigeminal ganglia [21]. Activation of TRPV1 occurs in response to multiple noxious stimuli including heat, protons, capsaicin and a variety of endogenous lipid ligands [21,22]. TRPV1 activation leads to the influx of calcium, membrane depolarization and the generation of action potentials with the subsequent peripheral and central release of neuropeptides and transmitters [21,22]. In DRG neurones TRPV1 is coexpressed with proteinase-activated receptor 2 [23] and cannabinoid CB1 receptors [24] that have pro- and anti-nociceptive roles in joints respectively [25,26]. A recent transcriptional profiling study of DRG neurones [27] demonstrated that TRPV1 is expressed in distinct subsets of neurones one of which expresses high levels of TrKA and Nav1.8 as well as significant levels of aquaporin 1 and Kv8.1 thought to function as thermosensitive C-fibers. At the level of the dorsal horn of the spinal cord, TRPV1 is localized in both presynaptic afferent neurones where it is coexpressed with the neuropeptide CGRP, and in postsynaptic neurones as well as glia in lamina I and II [10,28,29]. Also with possible relevance to pain, TRPV1 expression has been noted in non-neuronal cells such as fibroblasts [30], macrophages [10] and synoviocytes [31], although its role in these cell types is not well established.

TRPV1 activation *in vivo* has been associated with a number of different pathological pain states such as diabetic neuropathy [32], irritable bowel syndrome [33] and inflammatory arthritis [34–36]. TRPV1 is thought to contribute to hyperalgesic responses via the peripheral release of substances that might sensitize sensory neurones to other physical, thermal and chemical stimuli such as CGRP [21]. TRPV1 itself is sensitized by the neurotrophin NGF bradykinin, certain prostanoids and potentially also CGRP via intracellular signaling cascades mediated by PKC, PKA and PLC [21]. Further, in peptidergic nociceptors the potentiation of TRPV1 activity by algesic substances primarily requires membrane insertion of TRPV1 channels [37]. Studies in TRPV1 knockout mice have demonstrated a critical role for TRPV1 in inflammatory thermal hyperalgesia [38,39]. Furthermore, both thermal and mechanical evoked pain in models of inflammatory hyperalgesia are attenuated by TRPV1 antagonists [17,40,41]. For these reasons, TRPV1 has been seen as a viable target for the treatment of several different pain states and considerable investment by the pharmaceutical industry has been channeled into the clinical development of TRPV1 antagonists.

Development of TRPV1 antagonists

The pharmaceutical industry has invested billions of pounds in drug screening and lead optimization programs that have identified selective and potent small molecule TRPV1 antagonists, some of which have entered clinical trials for pain relief [42]. Capsazepine, a chemical derivative of capsaicin was the first reported TRPV1 antagonist that has been used extensively as a pharmacological tool to assess the role of TRPV1 in inflammatory hyperalgesia [43,44]. Given capsazepine's relative nonselectivity and species-related effect differences [44], as well as low metabolic stability and poor pharmacokinetic properties it was not considered an important candidate for clinical development. These limitations lead to the development of a second generation of potent TRPV1 antagonists with increased selectivity [45]. These additional compounds demonstrated excellent therapeutic potential for pain reduction and were considered to be good candidates for clinical development. The major competitive TRPV1 antagonists discovered to date can be classified as either classical or nonclassical antagonists. The classical antagonists contain a carbonyl group present in the form of a thiourea, urea, ester or amide group. The compounds JYL-1421, A-425619, BCTC, JNJ-17203212 and SB-705498 are examples [45]. Nonclassical TRPV1 antagonists contain a carbonyl group for example, as part of a heterocyclic ring (e.g., AMG-2674). Noncompetitive TRPV1 antagonists are pore blockers that prevent channel opening by the agonist or block the aqueous pore [45]. The trinuclear polyamine compound ruthenium red was the first noncompetitive TRPV1 antagonist to be developed but exhibited poor selectivity that hampered its development as an analgesic compound [45].

It was originally considered the case that the gold standard TRPV1 antagonist should inhibit all modes of TRPV1 activation (protons, heat and capsaicin) as well as reducing inflammation-induced hyperalgesia as is the case for BCTC, A-425619 and AMG-9810 [45] (Table 1). However, it is has now become apparent that blockade of certain modalities of TRPV1 activation are associated with on-target-mediated side effects [46]. Hence, a new generation of TRPV1 antagonists that block only certain modalities are being developed to overcome this issue. CNS penetration is a desirable property of TRPV1 antagonists that would be expected to increase potency in reducing pain under conditions of central sensitization.

TRPV1 in synovial joints

Studies combining selective retrograde labeling of knee joint afferents with immunohistochemistry have demonstrated that synovial joints receive sensory innervation by TRPV1 expressing nociceptors [7,47]. In the mouse knee and ankle joint, approximately 40% of the sensory afferent population express TRPV1 with the majority coexpressing CGRP [47]. In the rat around 54% of knee joint afferents express TRPV1, compared with around 32% of the whole DRG cell population demonstrating that TRPV1 is enriched in knee joint afferents. TRPV1 expressing afferents innervate the periosteum, articular capsule and the posterior aspect of the patellar ligament and terminate in the subsynovial tissue as free nerve endings many of which are closely associated with blood vessels [47]. Cho and Valtschanoff [47] demonstrated in the mouse that many of the TRPV1 expressing fibers innervating the joint capsule in the knee and ankle and the posterior aspect of the patellar ligament terminate in the subsynovial connective tissue as free axonal endings. While the majority of nerve fibers in the knee are TRPV1 expressing [47], only 40–50% of DRG neurones backlabeled from the knee or ankle express TRPV1. This implies that compared with the cell soma, peripheral axons of articular afferents express higher levels of TRPV1. Despite this, the functional role of TRPV1 at the level of articular afferents remains elusive. Endogenous agonists or endogenously released protons produced and released following joint injury/inflammation are assumed to activate TRPV1 under these conditions [48]. In support of this theory, TRPV1 expressing afferents have been shown to have an important role in the pathogenesis of experimental inflammatory arthritis [35,36,38] and may directly contribute to the arthritic process via promoting the release of neuropeptides such as CGRP from afferent fibers innervating the joint. In the carrageenan and complete Freund's adjuvant models of inflammatory arthritis, desensitization [49] or genetic deletion of TRPV1 [35] is analgesic and reduces joint swelling, whereas, activation of TRPV1 by capsaicin in rat and mouse knee joints leads to acute vasoconstriction [36].

There is increasing evidence that the expression of TRPV1 at the level of the joint is not restricted to primary afferent neurones and that other cells and tissues including chondrocytes [50], osteoclasts [51], osteoblasts [52] and synovial fibroblasts [30] all express TRPV1. The role of TRPV1 in these cells is not well understood but given the non-neuronal as well as neuronal expression, it is possible that TRPV1 makes a complex contribution to both structural change and pain in arthritides (Figure 1). Synovial fibroblasts are key cells of the joint involved in the inflammatory response during arthritis and can be exposed to the TRPV1 agonist low pH during inflammation, infection or injury [53]. Agonists of TRPV1 evoke increases in intracellular calcium levels in synoviocytes in culture [31] and as such this channel is suggested to have a role in proliferative and secretory function of synoviocytes during joint inflam-

Table 1. *In vitro* and *in vivo* profiles of select TRPV1 antagonists and their effects on body temperature.

TRPV1 antagonist	Blockade of recombinant rat TRPV1 (IC_{50})			Blockade of native rat TRPV1 (IC_{50})			Analgesic effects in rodent pain models (ED_{50})							
	Caps	H+	Heat	Caps	H+	Heat	Caps	CFA	MIA	Post-op	Bone cancer	Nerve injury	Heat	CBT
ABT-102	√(1 nM)	√(16 nM)	√	√(6 nM)	-	-	√	√(10 μmol/kg)	√(30 μmol/kg)	√(40 μmol/kg)	√	-	-	↑
AMG8562	√(1.8 nM)	↑	X	-	-	-	√	√	-	√	-	-	-	↓(1°C)
AMG9810	√(85.6 nM)	√(294 nM)	√(21 nM)	√(9 nM)	-	-	-	√	√	√	-	-	√	↑(0.6°C)
BCTC	√(0.5 nM)	√(0.65 nM)	√(0.6 nM)	√	√	-	√	√	-	√	-	√(10.6 mg/kg)	√	↑(1.4°C)
SB366791	√(7.5 nM)	√	-	√(652 nM)	-	-	√	√	-	√	√	-	-	X
JNJ17203212	√(102 nM)	√(16 nM)	-	-	-	-	√	√	√	-	√	√	√	↑(0.8°C)
I-RTX	√(3.8 nM)	√(0.55 nM)	√(1 μM)	-	-	-	√	-	-	-	√	-	X	↓(partial agonist)
JYL-1421	-	↑	-	√(8 nM)	√(partial)	√(60% @ 4 μM)	√	-	-	-	-	-	-	x
A-425619	√(25.9 nM)	√(20.1 nM)	-	√(10 nM)	√(13 nM)	√(58 nM)	√(5 mg/kg)	√(40 mg/kg)	√(>100 μmol/kg)	√(65 μmol/kg)	-	√(>100 μmol/kg)	√(>100 μmol/kg)	↑(1.0°C)

IC_{50} and EC_{50} values are stated if reported.
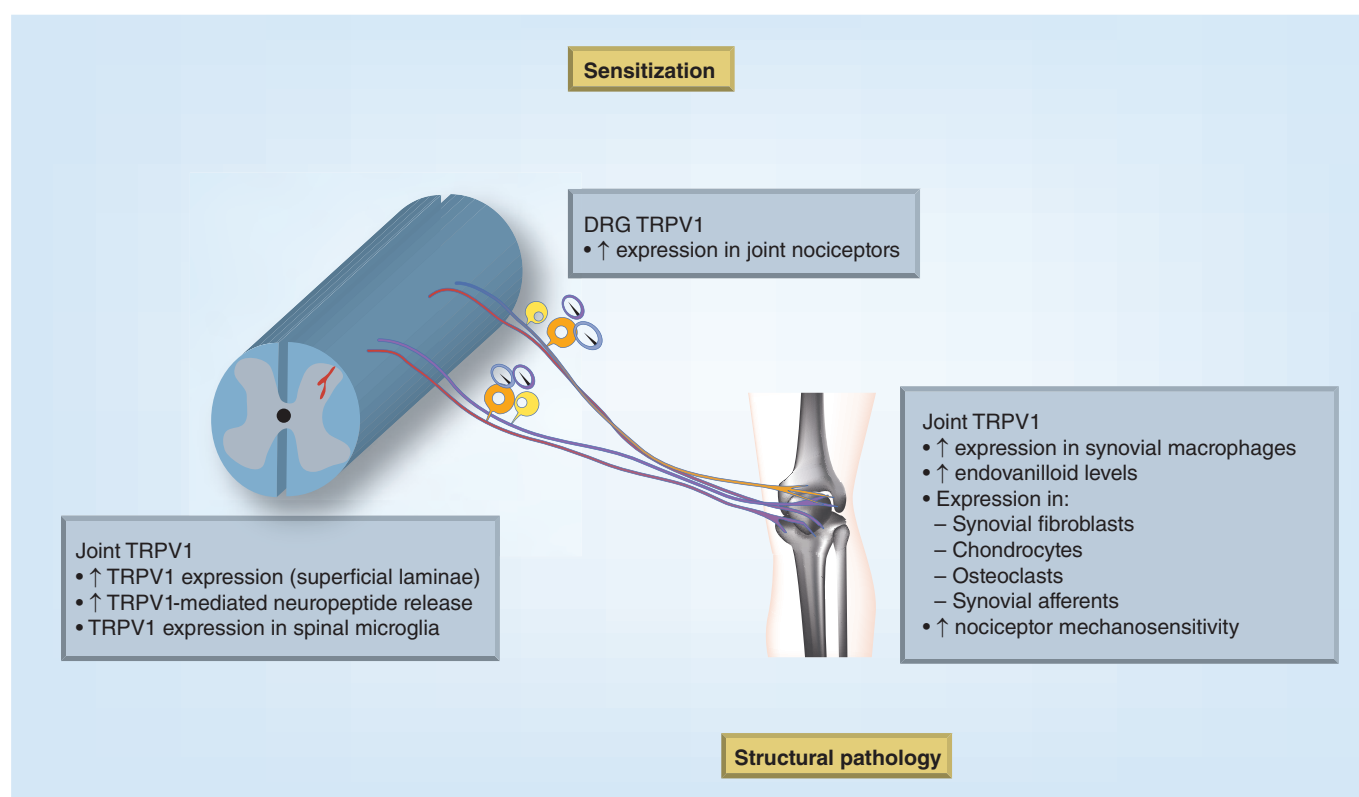CBT: Core body temperature; -: Not reported; x: No effect.

Figure 1. Overview of the peripheral contribution of TRPV1 to arthritis pain. TRPV1 is expressed in non-neuronal as well as neuronal cells and evidence suggests that TRPV1 may make a complex contribution to both structural change and pain in arthritides.

mation. Capsaicin activation of TRPV1 in these cells increases the mRNA and protein expression of IL-6 in synovial fibroblast cell supernatants [30]. As IL-6 is a known modulator of joint nociceptor function [54] these findings suggest that TRPV1 may be involved in non-neuronal to neuronal cell communication that contributes to aberrant peripheral nociceptive inputs during arthritic conditions.

TRPV1 is not only expressed by nociceptors inner-vating bone [55] but also by the bone resorbing osteo-clasts themselves [51]. The TRPV1 agonists capsaicin and resiniferatoxin increase intracellular calcium levels in human osteoclasts a response blocked by antagonism of TRPV1 [51]. Persistent TRPV1 activation increases expression and activity levels of tartrate-resistant acid phosphatase and cathepsin K, enzymes involved in maintaining and amplifying osteoclastic bone resorp-tion [51]. The TRPV1 antagonist capsazepine inhibits osteoclast differentiation *in vitro* [56]. For these rea-sons, TRPV1 is thought to play an important role in modulating osteoclast function and activation [51] and in driving bone pain mechanisms [57,58]. Due to the multiple cell types within synovial joints that express TRPV1 and its known role in nociceptive transduction mechanisms and hyperalgesia, TRPV1 may be a useful target in the treatment of OA pain.

Preclinical modeling of OA pain

The recent development of animal models of OA pain has contributed greatly to increasing our mechanistic understanding and to the testing of novel analgesic strategies [59–61]. The model most frequently used to investigate OA pain mechanisms and used to exam-ine the role of TRPV1 is the monosodium iodoacetate (MIA) model in the rat [7–10,15]. MIA is a chemical inhibitor of glyceraldehyde-3-phosphate dehydroge-nase, and when injected into the knee joint of rats (and mice) it prevents chondrocyte glycolysis resulting in chondrocyte cell death, and as a consequence patho-logical articular changes that resemble human OA [62].

These MIA-induced pathological changes in joint structure are associated with the development of pain behavior that has similarities to the pain symp-toms experienced by OA sufferers including reduc-tions in weight bearing on the affected limb, pain in response to movement of the joint and hypersensitiv-ity referred to uninjured tissues (i.e., secondary tactile allodynia of the hindpaw). Pain behavior appears as early as 1 day post-injection and persists for at least 63 days [59,60,63] and is thought to be driven by both peripheral and central sensitization. Nociceptive C- and Aδ-fibers are sensitized at 14 days following MIA model induction [10,63,64] indicating peripheral

sensitization of joint afferent nociceptors. 28 days following model induction wide dynamic range spinal dorsal horn neuronal responses [65] and himb-limb spinal nociceptive withdrawal reflexes [66] are sensitized to hind paw mechanical stimulation indicating that central nociceptive pathways are also sensitized (i.e., central sensitization). The MIA model is responsive to analgesics or anti-inflammatory agents used in the treatment of human OA, such as diclofenac [67] and morphine [59,61]. Drugs used to treat neuropathic pain, such as gabapentin and amitriptyline, are also efficacious in the MIA model and ATF-3, a marker of nerve injury, is upregulated in the cell bodies of knee innervating afferents [68]. Due to its ease of induction, rapid development and robust and reproducible pain behavior, the MIA model has been the model of choice for the pharmaceutical industry and has been intensively used to broaden our understanding of the role of TRPV1 in OA pain mechanisms as well as in the screening of analgesic compounds including novel TRPV1 antagonists [8,9].

Investigations of the contribution of TRPV1 to OA pain

Over the last decade a significant body of preclinical data has accumulated implicating TRPV1 in the maintenance of established OA pain [7–10,15]. Numerous anatomical studies have demonstrated that the development of pain behavior and OA-like changes in joint structure are associated with enhanced expression of TRPV1 [7,10]. During established pain behavior in the MIA (2 mg) model at 28 days, the percentage of retrograde labeled knee afferents expressing TRPV1 increased from 54.3 to 71.7% [7]. Whether this increased expression within knee afferent cell somas is reflected at the peripheral terminals within the diseased joint is not yet clear. Occasional TRPV1 expressing afferent fibers have been demonstrated in human synovium from OA joint replacement tissue [2].

Clinical findings support an upregulation of TRPV1 at the level of the joint in symptomatic OA. TRPV1 immunoreactivity is increased in synovial tissue taken from people undergoing total knee replacement for OA, compared with postmortem control tissue and is localized to infiltrating macrophages within the synovial lining region as well as deeper layers of the synovium [10]. TRPV1 has also been demonstrated in synovial fibroblasts from patients with symptomatic OA [30]. These findings suggest that the majority of synovial TRPV1 may in fact be localized to non-neuronal cells [10]. It is possible that TRPV1 expressed in these cells has a pro-inflammatory role contributing to peripheral sensitization via the release of inflammatory mediators such as IL-6 [30] a known sensitizer of

joint nociceptors [54] (Figure 2). However, the role of non-neuronal TRPV1 and its contribution to OA pain is not yet fully understood. Increased synovial expression of TRPV1 in OA pain back-translates to the MIA model [10] providing an opportunity to further investigate these mechanisms.

With as yet unknown relevance to pain in OA, the expression of TRPV1 has also been detected in osteoarthritic articular chondrocytes harvested at the time of total knee arthroplasty for symptomatic OA [50]. Although unconfirmed, expression of TRPV1 in these cells may relate to pathological changes within the cartilage. In cartilage explants taken from patients with end-stage knee OA undergoing joint replacement, the pro-inflammatory and cartilage destructive cytokines IL-1 and TNF-α upregulated TRPV1 expression, an effect not seen in normal human synovial fibroblasts [11]. These findings suggest that cytokine-induced stress to the cartilage can regulate TRPV1 expression. Since TNF-α also regulates the expression of TRPV1 in sensory neurones, TRPV1 may influence pain processing at the level of the joint as well as structural pathology in knee OA.

As well as synovial and cartilage pathology, OA is also associated with excessive levels of bone remodeling and the subchondral bone has recently begun to be considered as a potential source of pain in OA [69]. Excessive osteoclast activity during bone resorption is thought to lead to acidification of the extracellular matrix which would be expected to activate TRPV1 expressed on sensory neurones innervating the subchondral bone [70] (Figure 3). In addition, TRPV1 is a known regulator of osteoclast function [51]. Whether TRPV1 contributes to the mechanisms of bone pain in OA has not been yet been proven. However, an important contribution to pain mechanisms in bone cancer, another condition associated with increased osteoclast activity, has been demonstrated [58,71]. TRPV1 expression is increased in mouse models of bone cancer pain and selective blockade of TRPV1 or *TRPV1* genetic deletion attenuates both ongoing and movement evoked pain as well as thermal hyperalgesia [55].

Functional evidence from the MIA model also suggests an enhanced contribution of TRPV1 to nociceptive drive in OA. Capsaicin evoked CGRP release from isolated rat spinal cord is increased in cords taken from OA rats [8] indicating that TRPV1 activity is facilitated in afferent nociceptors during OA pain. The baseline (unstimulated) spinal release of CGRP also increases, possibly reflecting facilitated endogenous activity at TRPV1. This theory is supported by the fact that pain behavior in the MIA model is associated with increased levels of at
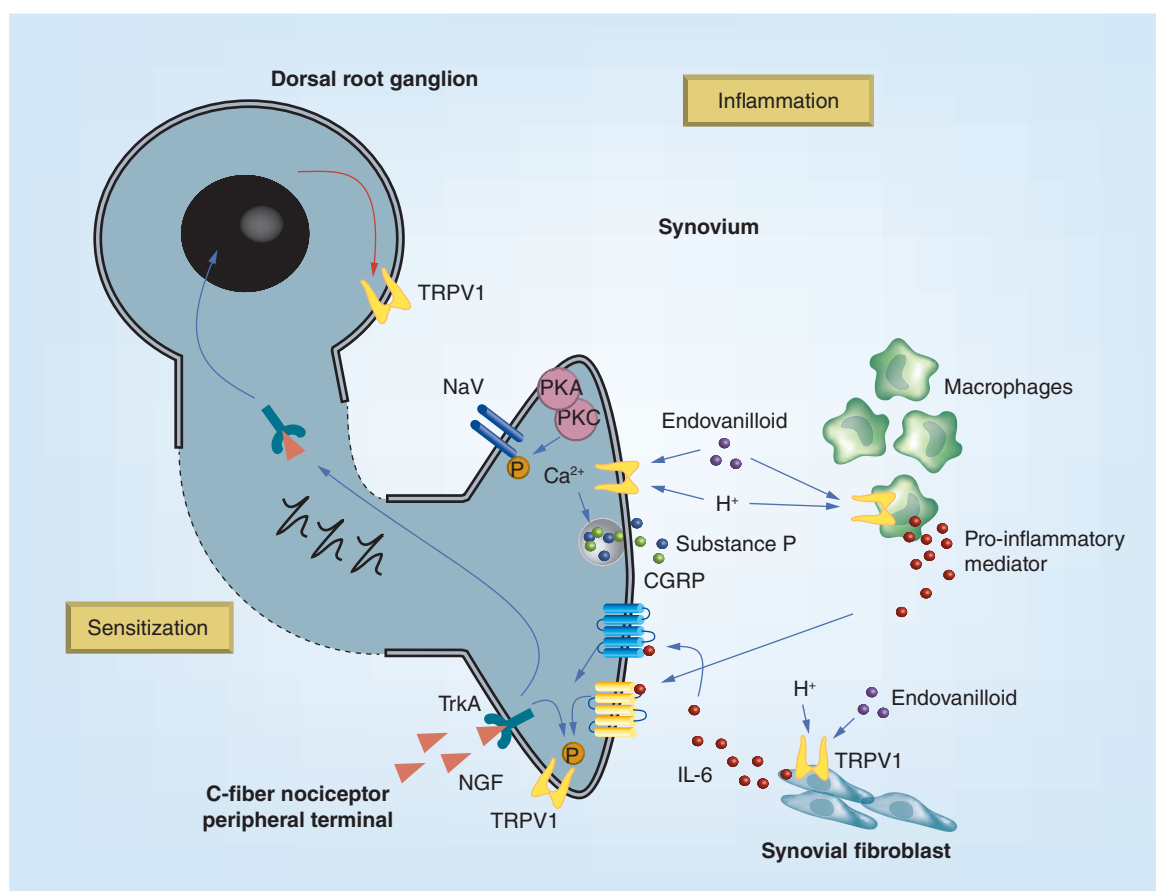
Figure 2. Putative TRPV1 mediated mechanisms contributing to enhanced nociceptive drive from the joint during osteoarthritis. TRPV1 is expressed in joint afferent nociceptors; activation by protons released during tissue injury and endogenously released endovaniloids increased during OA activates TRPV1 causing the peripheral release of CGRP. Subsequent activation of the CGRP receptor (CGRPR) contributes to peripheral sensitization via PKA/PKC mediated phosphorylation of tetrodotoxin-resistant voltage-gated sodium channels. NGF release associated with synovitis phosphorylates and sensitizes TRPV1 via the receptor TrKA and increases TRPV1 expression in joint afferent nociceptors. Finally, TRPV1 is expressed in synovial macrophages and synovial fibroblasts in symptomatic OA and is thought to have pro-inflammatory effect in these cells. TRPV1-mediated release of inflammatory mediators such as IL-6 a known sensitizer of joint nociceptors further contributes to peripheral sensitization and pain in OA.
OA: Osteoarthritis.

least one of its endogenous ligands, 12-HETE [10] (Figure 2). Levels of 12-HETE are increased in joint tissue of MIA-injected rats compared with saline control rats. However, this increase is restricted to the joint, and is not seen in either DRG or lumbar spinal cord [10]. Enhanced release of 12 HETE could have direct relevance to pain since 12-HETE is a known modulator of afferent function [72] and local blockade of endogenous TRPV1 activation by the antagonist JNJ-17203212 reverses the MIA-induced mechanical sensitization of joint afferent nociceptors without any effect in control rats [10]. It is possible that this TRPV1-mediated mechanical sensitization of joint afferent neurones involves CGRP release [64]. The therapeutic potential of blocking TRPV1 expressing afferent inputs is further emphasized by the abolish-

ment of mechanical hypersensitivity and reduction in bone pathology following their functional ablation prior to MIA model induction [73].

Analgesic effects of TRPV1 antagonists in OA pain models

The enhanced expression of TRPV1 in OA provided the impetus for investigations of the effects of TRPV1 antagonists on established OA pain behavior [8,9]. Abbott Laboratories have developed a number of TRPV1 antagonists assessing their analgesic potential in several pathophysiological pain states [40]. Intraperitoneal administration of A-425619 acutely and dose dependently reduced weight bearing asymmetry by up to 47% during the acute inflammatory phase of the MIA (3 mg) model, 4 days postinduction [40]. Its

Figure 3. Putative contribution of TRPV1 to bone pain mechanisms during osteoarthritis. TRPV1 is a known modulator of osteoclast function. Excessive osteoclast activity during bone resorption leads to acidification of the extracellular matrix which activates TRPV1 expressed on nociceptive neurones innervating the subchondral bone. TRPV1 is known to be expressed in human osteoclasts and TRPV1 activation increases the expression of cathepsin K and TRAP two enzymes that maintain and amplify osteoclastic bone resorption activity. TRAP: Tartrate-resistant acid phosphatase.

effects were rapid in onset and prolonged in duration and its low CNS penetration indicated that the anti-hyperalgesic effects were mediated at the level of the diseased joint. Analgesic efficacy of TRPV1 antagonists has also been demonstrated at more chronic and arguably more clinically relevant stages of the MIA model when there is extensive cartilage degradation, subchondral bone thickening and osteophytes. The Abbott compounds A-889425 [15] and A-995662 [8] attenuated the MIA-induced reduction in grip strength when administered orally, 20–21 days following model induction. A-995662 completely reversed grip force 1 h post-administration and remained efficacious for up to 8 h [8]. A-995662 was found to be more efficacious than the NSAID celecoxib [8]. Importantly, the *in vivo* analgesic efficacy of certain TRPV1 antagonists (e.g., A-995662, ABT-102 and A-993610) appears to be enhanced and prolonged following repeated dosing [9]. With A-995662, enhanced efficacy was observed as early as day 5 following a repeated low dose [8]. The MIA-induced reduction in grip force was reversed by 76% at 8 h compared with only 30% following a single administration. Twice daily administration for 12 days resulted in full efficacy at 48 h a time point

not associated with analgesic effects following a single dose. This enhanced efficacy and prolonged action of TRPV1 antagonists following repeated administration is not thought to be due to compound accumulation but due to inhibition of TRPV1 sensitization following sustained inhibition of CGRP release [8]. Furthermore, these findings indicate that tolerance to the analgesic effects of TRPV1 antagonists does not develop following prolonged treatment.

Several studies have aimed to advance the understanding of the role of peripheral versus central TRPV1 receptors in the analgesic effects of TRPV1 antagonists in OA [41]. To this end, the analgesic effects of a centrally penetrant (A-784168) and a peripherally restricted TRPV1 antagonist (A-795614) were compared in MIA rats [41]. The analgesic effects of A-784168 and A-795614 with high versus low CNS penetration were assessed 4 days following MIA (3 mg) using the weight bearing incapacitance test. Oral administration of A-784168 (good CNS penetration) dose dependently attenuated weight bearing asymmetry by up to 85% whereas A-795614 (low CNS penetration) was much less potent (53% attenuation). When the two compounds were injected intrathe-

cally, they demonstrated similar analgesic potency and intracerebroventricular injection of A-784168 also demonstrated analgesic effects following MIA [41]. The authors of this work concluded that TRPV1 receptors expressed centrally play an important role in driving OA pain and is an important site mediating the analgesic effects of systemic TRPV1 antagonists. However, a later study demonstrating that peripheral (intra-articular) administration of the TRPV1 antagonist JNJ-17203212 was equally as effective as systemic (ip.) administration in attenuating weight bearing asymmetry suggests that TRPV1 at the level of the diseased joint makes a greater contribution than central TRPV1 to OA pain [10]. Discordance in these findings may relate to the antagonist used, MIA dose (3 vs 1 mg) and time point post-induction studied (4 vs 14 days). Despite this, both peripheral and central sensitization are key features of the MIA model [63,64] as well as human OA [74], and it is likely that their inhibition by TRPV1 antagonists make a major contribution to the analgesic effects observed [10].

Recent studies have attempted to elucidate the neurophysiological mechanisms underlying TRPV1 antagonist-mediated analgesia in OA. The analgesic effects of A-995662 are closely associated with a reduction in the spinal release of CGRP and glutamate in OA rats [8]. This reduction in afferent neurotransmitter release would be expected to inhibit spinal nociceptive transmission. Indeed this theory is supported by electrophysiological evidence of an inhibition of knee joint mechanical evoked spinal neuronal responses by systemic (iv.) administration of A-889425 in OA rats but not control rats [15] providing a neurophysiological basis for the behavioral analgesia observed. Since A-889425 was administered systemically, it is not clear whether this effect was mediated at the level of the diseased joint or at a central site within the pain pathway. However, electrophysiological data with intrathecal (i.t) JNJ-17203212 exhibited similar inhibitory effects on mechanical evoked responses of spinal neurones in MIA and saline control rats, while inhibition of joint nociceptor mechanosensitivity by local JNJ-17203212 was only observed in MIA rats [10]. It is possible that these studies highlight differences in the role of TRPV1 in the control of nociceptive inputs from the joint [15] versus the hind paw [10] in OA or alternatively the greater relative importance of peripheral versus central TRPV1 in driving nociceptive inputs during OA.

Adverse effects of TRPV1 antagonism

Despite the encouraging analgesic efficacy of TRPV1 antagonists against experimental OA pain, their use is associated with unavoidable on-target-mediated side effects. A loss of noxious heat sensation has raised the concern of burn risk [46]. It is also now well reported that systemic TRPV1 antagonist administration is associated with hyperthermia in rodents and humans [18]. In line with other TRPV1 antagonists (e.g., AMG-517 [18] and ABT-102 [9]) A-995662 causes a rise in core body temperature of around 1°C within 30 min that lasts for up to 7 h [8]. Encouragingly, the hyperthermic effects of A-995662 are attenuated following only the second day of repeated dosing. However, loss of effect of ABT-102 on thermal thresholds following repeated dosing is not observed [75]. Despite attenuation of the hyperthermic effects following repeated antagonist administration, hyperthermia remains a serious concern and the contribution of different modes of TRPV1 activation (heat, protons, chemical ligands) to TRPV1 antagonist-induced hyperthermia has been examined in both rodents and guinea pigs [19]. The blockade of heat or capsaicin activation of TRPV1 does not make a significant contribution, whereas the potency of TRPV1 antagonists to induce hyperthermia relates most closely to their potency to block proton-induced activation of TRPV1. This information has been exploited in the development of a second-generation TRPV1 antagonists without the side effects of burn risk and hyperthermia [76,77]. For instance, AMG8562 an antagonist that potentiates TRPV1 activation by protons through positive allosteric modulation does not cause hyperthermia in rodents [78], however there is a requirement for a 46-fold higher plasma concentration for comparable efficacy when compared with AM8163 a compound that blocks all modalities of activation [78]. The Abbott compound A-1165442 an acid sparing TRPV1 antagonist that potently blocks capsaicin and NADA evoked activation of TRPV1 demonstrated a marked analgesic effect in the MIA model without significantly increasing core body temperature [79]. An additional therapeutic strategy that could be exploited in order to avoid these potentially serious side effects is local administration to the diseased joint. Intra-articular administration of JNJ-17203212 in the MIA model maintained its analgesic efficacy (compared with systemic administration) without altering core body temperature [10]. These data provide scientific rationale for the clinical investigation of intra-articular TRPV1 antagonists in the treatment of human OA pain.

Clinical trials of TRPV1 antagonists in osteoarthritis pain

The analgesic efficacy of TRPV1 antagonists against OA pain in clinical trials remains to be proven. A recent Phase II clinical trial demonstrated analgesic efficacy of topical administration of civamide (cis isomer of capsaicin) in patients with knee OA pain [14] highlighting the therapeutic potential of the blockade

of TRPV1 expressing nociceptors. Owing to a lack of selectivity and hyperthermic side effects, only a small number of novel TRPV1 antagonists have been translated to the clinic. Astra Zeneca have submitted patent applications for benzimidazole derivative TRPV1 antagonists in the treatments of OA pain [80]. A Phase II clinical trial of GRC-6211 an orally active TRPV1 antagonist developed by Glenmark-Eli Lilly was suspended for undisclosed reasons. In a Phase II trial conducted in knee OA pain patients that were insensitive to NSAIDs, the AstraZeneca compound AZD1386 failed to cause clinically relevant changes in the Western Ontario and McMaster Universities (WOMAC) pain scale and the trial was halted [81]. Despite this, pain intensity was significantly decreased in the AZD1386 group versus placebo, however treatment differences were slightly below the level considered clinically relevant and no correction for baseline differences was included. The exclusion of patients that were NSAID sensitive from this trial, that presumably would have an inflammatory component to their OA pain, may have precluded the detection of a clinically relevant improvement in pain outcomes following AZD1386 treatment. NEOMED has received approval to initiate the first-in-human study of its product candidate NEO6860 a TRPV1 antagonist intended for OA-associated pain [82]. The study is expected to commence during the first quarter of 2015 with data delivered by the end of 2015 with a proof-of-concept trial planned for early 2016.

Clinical trials of TRPV1 antagonists carried out in veterinary species may shed light on the analgesic potential of TRPV1 antagonism for human OA pain. A recent randomized controlled trial was conducted in client owned dogs with naturally occurring hip OA [83]. The trial demonstrated that three-times daily oral dosing with the TRPV1 antagonist ABT-116 for 2 weeks did not improve the total pain score, pain severity score or pain interference score as assessed by the canine brief pain inventory questionnaire. Whereas, improvements in all these scores were demonstrated following treatment with the NSAID carprofen and the opioid tramadol [83]. However, night time activity levels during the second week of treatment with ABT-116 were higher than baseline activity levels. In addition, there was a reduction in the number of days that rescue mediation was used during ABT-116 treatment. As with human studies with TRPV1 antagonists, an acute hyperthermic effect was seen following ABT-116 treatment.

Conclusion

It is as yet too early to know whether TRPV1 antagonists have the potential to be effective treatments for pain in OA. With a second generation of TRPV1 antagonists under development that demonstrate analgesic effects in the MIA model and are devoid of the on-target-mediated side effects of hyperthermia [77] and burn risk, the clinical evaluation of TRPV1 antagonists can now progress unhampered.

Future perspective

Pain in OA of the knee is heterogeneous, and influenced by multiple interacting factors. A model of knee OA pain has been proposed [84] that includes a contribution of changes in joint structure, altered central pain processing and patient psychological factors. The extent to which these multiple factors contribute to the pain of knee OA may not be identical in all patients at all stages of the disease. This heterogeneity likely explains why currently available treatments prescribed in a 'one size-fits-all approach' are often ineffective. Evidence from pain questionnaire studies in patients with symptomatic knee OA indicates that distinct phenotypes exist in knee OA [85]. The subgrouping of patients based on their 'pain phenotype' may enable the targeting of treatments to the right individual patient at the right time with an increased likelihood of therapeutic benefit. However, the phenotyping of pain in OA is in its infancy. In the future, defining the contribution of knee pathology, augmented central pain processing and psychological distress to the experience of pain in OA may increase our understanding of specific OA pain phenotypes and their underlying contributing mechanisms and facilitate the development of individualized treatments. The subgrouping of patients based on their pain phenotype, as is being done in low back pain, may reduce heterogeneity and enable the targeting of treatments to patients with a specific pain experience [86]. TRPV1 antagonists may be more likely to benefit some OA knee pain subgroups over others. The known role of TRPV1 in inflammatory hyperalgesia [87] and evidence of increased synovial expression of TRPV1, in infiltrating macrophages in symptomatic OA [10] suggests that TRPV1 antagonists may be more likely to benefit those individuals with a pain phenotype reflective of a predominant contribution of peripheral factors such as inflammation. Although, OA has traditionally been thought of as a noninflammatory disease, histological [10] and ultrasound [88] evidence suggests the presence of inflammation at the level of the joint, with inflammatory mediators detected in serum and synovial fluid [89]. In the future, clinical trials of TRPV1 antagonists run specifically in a subgroup of patients that present with moderate joint damage and low levels of psychological distress could help to reduce heterogeneity and maximize treatment efficacy. Preclinical data suggest

that intra-articular administration of TRPV1 antagonists may be future therapeutic strategy that would maximize analgesic efficacy and at the same time avoid undesirable hyperthermic side effects [10]. However, this remains to be proven in clinical trials that should include OA patients with synovitis or with early OA. Future studies of TRPV1 antagonist-mediated analgesia in preclinical models other than the MIA model, for instance in spontaneous or surgically induced OA, and in TRPV1 knockout mice with OA, should be conducted to bring new insights into the generalizability of the role of TRPV1 in OA pain mechanisms. Targeting TRPV1 mechanisms downstream of channel activation, for instance in the neutralization of CGRP,

may have the potential to modulate pain in OA without producing on-target-mediated hyperthermia. In fact, Eli Lilly have commenced Phase II clinical trials of a monoclonal CGRP antibody in OA patients [90].

Financial & competing interests disclosure

Executive summary

Targeting TRPV1 in the treatment of OA pain; topical & intra-articular capsaicin
- Topical and intra-articular TRPV1 agonists are analgesic in osteoarthritis (OA). However, the relatively low effectiveness of capsaicin cream and the need to reapply, along with the associated skin irritation has limited its use.

TRPV1 background
- TRPV1 is expressed in nociceptive sensory neurones and has a role in inflammatory hyperalgesia. Blockade of TRPV1 is analgesic in animal models of chronic pain.

Development of TRPV1 antagonists
- The pharmaceutical industry has invested major efforts into the development of TRPV1 antagonists and clinical trials for chronic pain are in progress.

TRPV1 & synovial joints
- At the level of the knee joint, TRPV1 is expressed in multiple cell types including sensory afferent neurones, synovial fibroblasts, chondrocytes and osteoclasts. TRPV1 has been demonstrated to be involved in the pathogenesis of inflammatory arthritis.

Preclinical modeling of osteoarthritis pain
- The MIA model of osteoarthritis (OA) exhibits joint pathology and pain symptoms that share similarities to human OA. This model has been used by industry to investigate the role of TRPV1 in OA pain and in the testing of the analgesic efficacy of TRPV1 antagonists.

Investigations of the contribution of TRPV1 to OA pain
- Studies suggest that the contribution of TRPV1 to nociceptive drive increases during established OA pain. Targeting of TRPV1 has the potential to modify structural change as well as peripheral and central sensitization in OA.

Analgesic effects of TRPV1 antagonists in OA pain models
- TRPV1 antagonists attenuate weight bearing asymmetry and reduced grip strength in the MIA model. Studies aimed at investigating the sites and mechanisms underlying TRPV1 antagonist-mediated analgesia indicate the importance of both peripheral and central sites and demonstrate an inhibition of joint nociceptor sensitization and of central nociceptive transmission.

Adverse effects of TRPV1 antagonism
- TRPV1 antagonist-mediated analgesia is associated with serious on-target-mediated side effects including risk of burn injury and hyperthermia in both animal models and humans. Second-generation antagonists have been development devoid of these side effects and clinical trials can now proceed unhampered.

Clinical trials of TRPV1 antagonists in OA pain
- Clinical trials of TRPV1 antagonists in OA are at an early stage and analgesic efficacy is still to be proven.

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