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# TRP Receptors in Arthritis, Gaining Knowledge for Translation from Experimental Models

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**Abstract:** Arthritis is a condition characterised by mainly pain, reduced joint movement and signs of inflammation, such as swelling. The disorder has many different types, of which osteoarthritis (a degenerative joint disease) and rheumatoid arthritis (a chronic autoimmune disease) are the two most common forms. There are >6 million sufferers in the UK and both conditions have a huge potential to impair capabilities and contribute to social and economic burdens. Whilst there are a wide range of arthritic therapies available, many patients under treatment complain of poor pain relief. Thus there is a need for novel therapeutic approaches, and the transient receptor potential (TRP) family of receptor channels has been investigated. One particular area of recent research has been the ligand-gated transient receptor potential vanilloid 1 (TRPV1) channel. Findings from numerous pre-clinical models and scientific studies have shown that TRPV1 desensitisation, or the use of TRPV1 antagonists alleviates pain and some inflammatory aspects. New findings have started to unveil the potential of other TRP channels in mediating arthritic pain and inflammation. With the understanding that the currently available treatments for arthritis are limited, researchers have looked into the exciting prospect that TRP receptor antagonists may be developed into effective, specific drugs, which would potentially protect against the complications of arthritis. These antagonists are still under development, although only data from studies from pre-clinical models are currently available. This review acts to summarize knowledge of the potential influence of TRP receptors in arthritis to date.

**Keywords:** TRP receptors, arthritis, knowledge.

## 1. INTRODUCTION

### 1.1. Arthritis

Arthritis is defined as the inflammation of one (or more) joint(s), and characterised by pain, swelling, warmth, redness (of the overlying skin) and a diminished range of joint movements. Considering the widespread occurrence of arthritis, it is not surprising that there is much research interest. There are many different types of arthritis, but the most common are rheumatoid arthritis (RA) and osteoarthritis (OA), of which osteo-arthritis affects most, especially the elderly.

RA affects people of different ages; it is most common in women and is characterized by a marked inflammation of the synovium. Although the inflammatory changes associated with RA have been extensively investigated, their actual trigger remains unclear. RA is classified based on the presence of joint stiffness following a prolonged rest, rheumatoid factors (RF or RhF), auto-antibodies [1] and increased synovium/synovial fluid buildup in the joint, along with bone erosion [2] Fig. (**1**). OA is a highly common degenerative joint disease that isoften painful and directly associated with ageing and associated previous injuries, such as from certain sports and occupations [3]. By comparison to RA it is considered to have a less overt inflammatory component, although a relatively mild synovial inflammation is present in OA. Its diagnosis relies on damaged bone surface with the formation of osteophytes, reduced mobility of the joint and pain associated to movement [4].

With the advances and efforts to comprehend the mechanisms underlying both RA and OA, there are a range of local and systemic treatments available most targeting RA, especially its related pain and the progression of the immune response. The most common treatments are the non-steroidal anti-inflammatory drugs (NSAIDs) that relieve pain and in some cases inflammation, but do not halt the ongoing disease process. There are side-effects associated with many of the treatments and this is one of the reasons that combination drug treatments have been developed; in the hope that the use of lower doses of each drug, when taken in combination, lessens side effects. Recent clinical research and therapeutic approaches have concentrated mainly on targeting rheumatoid arthritis in an aggressive manner at an early time point, through use of disease modifying antirheumatic drugs (DMARDS), often in combination with each other or with NSAIDs [5]. Furthermore, the 'biologics' are being increasingly used that provide treatment in the form of antibodies against cytokines or other pro-inflammatory component. These are good when effective, with relatively few side effects, but are associated with large expense which limits access. The use of biologics such as anti-TNF drugs to treat RA has improved the life quality of patients, especially when DMARDs and classic anti-

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inflammatory drugs are ineffective. Their use in combination with established DMARDS is also being developed. On the other hand, there are relatively few treatments available for the treatment of OA [6].

Overall there is an unmet need and it is understood that research into new solutions for the management of RA and OA are essential. This review will discuss the relevance of transient receptor potential (TRP) channel-dependent mechanisms in both the establishment and the maintenance of RA and OA and will also address the potential of these channels as pharmacological targets for the treatment of arthritis. We will first examine the fundamental studies into the role of capsaicin that act via the TRP vanilloid 1 (TRPV1) receptor to deplete and desensitise the sensory nerves, followed by pre-clinical studies involving TRPV1 knockout mice, and more latterly selective TRPV1 antagonists. Finally we will review the fragmented evidence that other TRP receptors, especially TRPA1, may have an important role in arthritis. The major studies involving these two TRP channels in arthritis are summarized in the Table **1**.

## 2. CAPSAICIN

### 2.1. The History of Capsaicin and Evidence from Rodent Models of Arthritis

The pungent substance of the *capsicum* was initially extracted and named as capsaicin in 1846 by Thresh [31] with its chemical structure determined nearly a century later [32]. Capsaicin administration evokes the classical signs of inflammation which are associated to neuronal sensitization and thus pain; known as neurogenic inflammation. The existence of a capsaicin receptor was initially postulated by Szolcsànyi and Jancsó-Gábor in 1975 [33]. Early researchers in 1974-1977 gathered evidence from electrophysiological recordings that capsaicin sensitive afferents were in fact shown to be c-polymodal nociceptors [34]. However, a more comprehensive analysis of the interaction of capsaicin and its receptor was conveyed following the molecular identification of the structure of the receptor [7, 35].

It was later established that capsaicin exerts its effects by activating TRPV1 and in turn, causes the release of neuropeptides such as substance P (SP) and calcitonin-related peptide (CGRP) from sensory neurons which accounts for the many of the vasoactive and inflammatory effects of this compound (see [36]; Fig. (**1**).

Indeed, reports in the 1980s showed that SP levels were elevated in the sciatic nerve of rats with severe arthritis [37]. This was later confirmed with increased levels of SP observed in the sciatic nerve and in the knee joint capsule [38]. These findings suggest that SP released from the peripheral nerve endings and the primary afferents stimulating the area is associated with joint dysfunction in animal models of arthritis [37]. SP was soon shown to contribute to the initiation and the maintenance of joint inflammation [39]. Indeed, over the last several decades there has been an understanding that substance P plays a role in arthritis. $NK_1$ receptors for substance P are located within components of the joints, i.e. on primary afferents and also on immune cells [40]. Moreover, the effect of $NK_1$ receptor antagonists was shown to be effective in alleviating arthritis in animal models [41, 42]. However, several attempts have been made to create clinically effective tachykinin $NK_1$ receptor antagonists to attenuate arthritic symptoms. Unfortunately, these studies did not translate into new treatments for arthritis (e.g. [43]). Indeed the clinical trials with $NK_1$ antagonists did not show any benefit in arthritis or in other types of inflammatory pain conditions. The lack of improvement of human arthritis by $NK_1$ antagonists is still of debate with respect to the specific roles of central and peripheral substance P release in arthritis and also in relation to the potency of the drugs tested in the clinical trials [44, 45]. Similarly, CGRP has been implicated in the inflammatory response and pain associated to arthritis, contributing to the angiogenesis and increased neuronal growth in the arthritic joint [46-49]. The involvement of these neuropeptides in arthritis remains under investigation. For example, recent research has shown that biologics such as etanercept, which is a dimer formation of the soluble TNFα receptor, have the ability to reduce levels of substance P and CGRP as the arthritis improves [50].

An important property of capsaicin is that in addition to causing pain, its repeated administration can cause analgesia [51-53]. This effect, called neuronal desensitisation, also accounts for the reduction of the inflammatory pathways mediated by sensory neuron activation. Indeed, capsaicin is suggested to be unique in that once it activates a channel; it results in the TRPV1 channel undergoing a refractory period of desensitisation, where it becomes unresponsive to many stimuli, including capsaicin [54]. More recently it has been suggested that this may be more appropriately called 'defunctionalisation' as the animal or the area of the human treated becomes refractory to general sensory nerve stimulation and this is now acknowledged to be important in the ability of capsaicin treatment to cause pain relief [55, 56].

Despite the historical knowledge that capsaicin administration can alleviate pain and reduce inflammation, it was not too long ago that this compound was first used to treat the symptoms of arthritic pain as discussed in the next section. Following neuronal desensitisation by capsaicin, the activation of the neuronal pathways, including SP and CGRP release, becomes impaired as also does the activation of pathways depending on these neuropeptides. In 1983, Colpaert and collaborators [37] showed that the inflammation observed in adjuvant-induced arthritis was alleviated in rats pre-treated repeatedly with capsaicin. In addition, neonatal treatment with capsaicin was shown to reduce both thermal and mechanical hyperalgesia in rats with adjuvant-induced arthritis [57]. The same study also showed that capsaicin pre-treatment attenuated the weight loss associated with arthritis and improved locomotion. Possibly, low doses (0.075%) of capsaicin induce only a slow release and consequent depletion of pro-inflammatory neuropeptides (SP and CGRP). This may allow the release of anti-inflammatory peptides such as somatostatin or opioid peptides in a functional, relevant manner. Indeed, in a Freund's adjuvant-induced arthritis model of arthritis, somatostatin was shown to be released from the capsaicin-sensitive nerve endings and exert a systemic anti-inflammatory action [58].

**Table 1. Major Studies Involving TRPV1 and TRPA1 in Arthritis**

| **TRPV1** | | **TRPA1** | |
|---|---|---|---|
| | **Cloning of receptor and KO generation** | | **Cloning of receptor and KO generation** |
| 1997 | First cloned from mammalians (rodents) [7] | 1999 | First cloned from mammalians (human fibroblasts) [24] |
| 2000 | KO generated [8] | 2006 | KO generated [25,26] |
| | **Studies with KOs in RA models** | | **Studies with KOs in RA models** |
| 2005 | Pain and inflammation were shown to be reduced in TRPV1KO animals treated i.pl. [9] and i.art.[10] with CFA | 2007 | TRPA1KO mice exhibited normal mechanical hyperalgesia 24h following CFA injection [27] |
| 2006 | TRPV1KO mice injected with CFA (i.art.) presented reduced pain and joint damage [11] | 2011 | TRPA1KO mice presented reduced TNFα-induced bilateral mechanical hyperalgesia over a 7-day time course [17]. The same study showed that CFA-induced mechanical hyperalgesia is abolished from week 1 in TRPA1KO mice, suggesting a role for this receptor in the maintenance of nociception |
| 2008 | TRPV1KO mice exhibited increased CGRP expression in DRG samples following CFA injection, suggesting compensatory mechanisms in these animals [12] | | |
| 2009 | TRPV1 deletion reduced p-ERK activation in DRG samples [13] and increased spinal glial cell and astrocyte activation [14] following CFA injection in the ankle. | | |
| 2010 | TRPV1KO mice showed increased SP in DRG samples following CFA injection in the ankle [16] | | |
| 2011 | TRPV1KO mice showed increased SP in DRG samples following CFA injection in the ankle [16] | | |
| | **Studies with antagonists in RA models** | | **Studies with antagonists in RA models** |
| 2005 | A-425619 oral, i.t. and i.pl. treatments reduced CFA-induced thermal hyperalgesia in rats [18], suggesting both central and peripheral roles for TRPV1 in mediating nociception | 2007 | AP-18 i.pl. treatment reduced the mechanical inflammatory pain and cold-induced hyperalgesia in animals injected i.pl. with CFA [27] |
| 2006 | In vitro incubation of spinal cord slices obtained from rats with CFA- induced paw inflammation, with SB366791 inhibited glutamatergic neuro-transmission [19] | 2008 | Oral treatment with HC-030031 reduced CFA-induced mechanical hyperalgesia in the rat paw [28] |
| 2007 | The TRPV1 antagonists (SB366791 and BCTC) inhibited CFA-induced arthritis in rats. Co-injection with either SB366791 or BCTC in the paw reduced thermal hyperalgesia whilst their i.t. injection reduced mechanical hyperalgesia [20] | 2010 | Systemic treatment with A-967079 in rats injected i.pl. with CFA, reduced the activity of spinal wide dynamic range neurons after noxious mechanical stimuli such as 10 g von Frey hairs and noxious pinch stimulation [29] |
| 2009 | I.pl. CFA-induced inflammatory pain in rats was reduced by repeated treatment with oral ABT-102 [21] | 2010 | Treatment with HC-030031 inhibited the noxious cold hyperalgesia and the mechanical hyperalgesia in mice injected i.p. with CFA [30] |
| 2011 | Both systemic and i.t. SB366791 but not i.pl. injection reduced TNFα-induced bilateral mechanical hyperalgesia in mice, suggesting a central role for this receptor in mediating nociception [17] | 2011 | AP-18 i.t. and i.pl. administration reduced TNFα-induced mechanical hyperalgesia in mice. AP-18 effect was more pronounced when injected i.pl., suggesting a important role for this receptor in mediating nociception via the peripheral nervous system [17] |
| | Studies with antagonists in OA models | | Studies with antagonists in OA models |
| 2005 | I.p. A-425619 treatment decreased MIA-induced increase in weight- bearing differences in rats [18] | 2010 | A reduced neuronal activity was observed in rats systemically treated with A-967079 when tested for high-intensity von Frey hair stimulation [29] |
| 2009 | Oral ABT-102 treatment reduced MIA-induced nociception and increased grip force in rats [21] | 2012 | Neither systemic nor i.pl. treatment with HC-030031 affected the weight bearing responses induced by MIA [23] |
| 2011 | Oral A-889425 ameliorated MIA-induced nociception and loss of gripforce [22] | | |
| 2012 | I.p. AMG9810 reduced MIA-induced thermal hyperalgesia but not ongoing pain [23] | | |

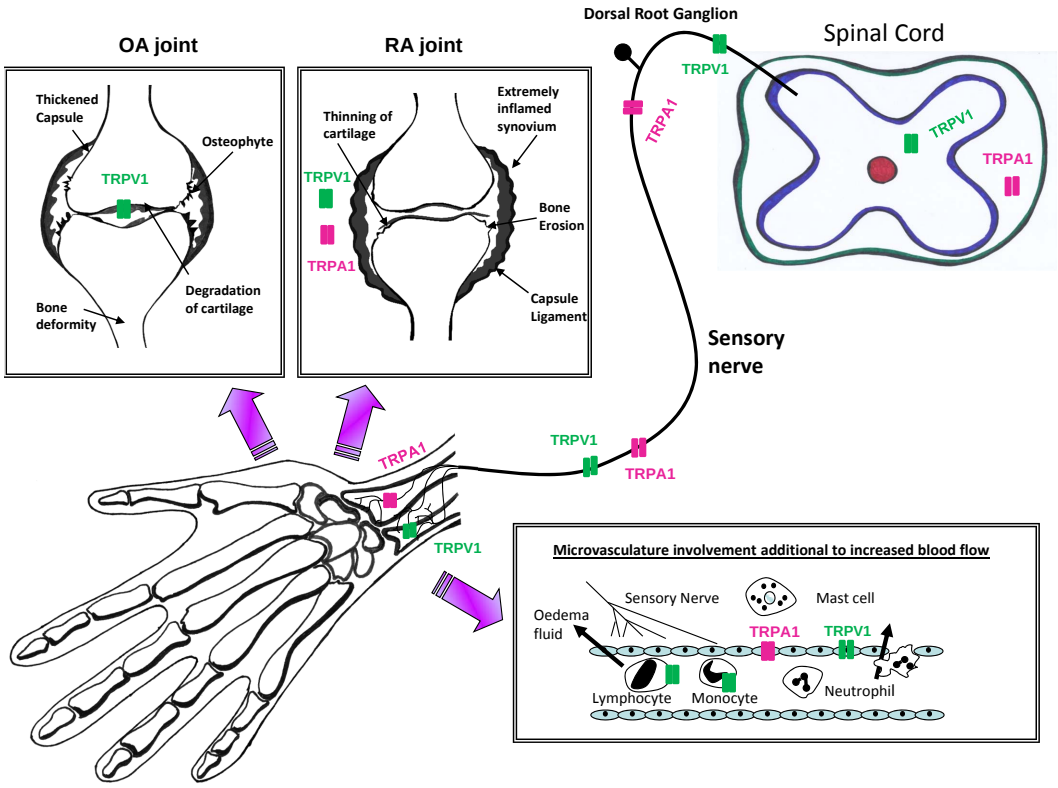


**Fig. (1).** TRPV1 and TRPA1 expression in arthritis. TRPV1 and TRPA1 play an important role on pain sensation in arthritis, with TRPV1 also contributing to the inflammation observed in RA. At present, TRPV1 (green) is known to be localized in OA chondrocytes, synoviocytes, DRGs, spinal cord, peripheral sensory nerves, endothelial cells and mononuclear cells. Evidence to date suggests that TRPA1 (pink) is expressed in synoviocytes, DRGs, spinal cord, peripheral sensory neurons and endothelial cells. TRPC5 is found in RA synoviocytes whilst TRPV4 is found in OA osteoblasts. Other TRP channels such as TRPC6, TRPC1 and TRPC3, TRPM2, TRPM3, TRPM7 and TRPV2 have also been found in the these cells although their roles in RA and OA remain to be elucidated.

### 2.2. Clinical Relevance of Capsaicin

The use of topical creams, lotions and patches to treat inflammatory and arthritic conditions has become widespread since the early 1980s. Currently, there are a range of creams available that contain various percentages of capsaicin. It has been difficult to directly associate the use of such creams with clinical effectiveness, thus clinical studies in this area are important. This is especially so when application of capsaicin-containing creams is associated with initial burning as a result of the stimulation of neurogenic inflammation and pain as discussed above [59, 60]. The benefits of capsaicin-induced neuronal desensitization for chronic neuropathic pain have been recently reviewed [53, 61]. Thus, this section of the review will focus on discussing the use of capsaicin-based creams for the treatment of arthritis.

McCarthy and McCarty [59] were one of the primary investigators of the effects of capsaicin in human arthritis. In their work, patients received topical capsaicin at 0.075% for 4 weeks (4 times a day), which was shown to alleviate the pain (tenderness) in the hand of patients with OA but not RA. These findings showed that although topical capsaicin caused initial pain; its chronic use could be beneficial for the treatment of OA-related pain in humans. As the initial pain caused by capsaicin creams was demonstrated to be a negative aspect of its usage by patients, a study by Schnitzer and collaborators [62] investigated the possibility of developing a maintenance regime for capsaicin treatment with reduced frequency of applications. Twice a day topical application of capsaicin cream (0.25%) was as effective as a 4 times a day in alleviating the pain in the hands of OA patients. Importantly, capsaicin-treated patients showed reduced joint swelling, greater movement and grip strength elevated by 30% in week three just under double compared to placebo-treated patients. Thus, a less frequent treatment with capsaicin cream resulted in an effective reduction of OA-related pain and inflammation whilst causing less discomfort for the patient [62]. Furthermore, topical application of capsaicin cream (0.075%) on the knee of RA patients for 4 weeks reduced the synovial levels of SP, in addition to prostaglandin $E_2$ ($PGE_2$) and interleukin-6 (IL-6) which are well established mediators of RA [63].

These studies show that capsaicin creams, at different concentrations, are effective at relieving arthritic pain. More recently, a 8% capsaicin patch has been developed for the treatment of neuropathic pain and it has been licensed for use in Europe and United States of America (Qutenza; [64]). Indeed, clinical trials demonstrated that a single 60-minute of the 8% patch attenuated neuropathic pain for up to 12 weeks [53]. It is thought that this high concentration of capsaicin seems to cause a quicker neuronal desensitization and to have longer term analgesic effects [53]. It remains to

**Table 2. Endogenous Activators of TRPV1 and Their Role in Arthritis**

| ENDOGENOUS ACTIVATORS | ROLE IN ARTHRITIS |
|---|---|
| Anandamide [65,66] | Analgesic [67] and pro-algesic [68] effects in experimental arthritis in rats and inhibition of cartilage degradation by reducing glycosaminoglycan release [69]. |
| Lipoxygenase products (e.g. $LTB_4$) [70,71] | Produced by normal and OA osteoblasts [72]. Also, there is increased $LTB_4$ release from synovial tissue of OA patients [73]. |
| ROS (e.g. $H_2O_2$) [74] | Increased levels of $H_2O_2$ released from synovial T lymphocytes were found in RA patients [75]. In animal models, reduced $H_2O_2$ production was associated to increased cartilage degradation [76]. $H_2O_2$ can also cause nociception in mice [74], although its role in mediating arthritic pain has not yet been addressed. |
| **ENDOGENOUS MODULATORS (via activation of intracellular pathways)** | |
| Bradykinin [77] | Released in RA and promotes bone resorption [78,79]. Its receptors can be up-regulated by TNFα and IL-1β [80]. |
| PAR-2 agonists [81] | PAR-2 expression in RA- and OA-derived synoviocytes are implicated in disease progression [82,83] and arthritic pain [84]. |
| NGF [85] | Mediates arthritic pain in animal models [85,86]. It is secreted by RA synoviocytes and promotes synoviocyte and T lymphocyte proliferation [87]; synovial nerve sprouting [49]; and increased osteochondral angiogenesis in RA and OA [88]. |

be investigated if the same formulation will be an effective therapy for arthritic pain.

### 2.3. TRPV1

Capsaicin selectively stimulates the primary afferents by activating and binding to TRPV1 that was cloned in 1997 [7]. TRPV1 is a ligand-gated, non-selective ion channel that functions as an integrator of multiple nociceptive stimuli including heat (>43$^{o}$C), low pH, and a variety of putative endogenous lipid ligands, in addition to capsaicin. Indeed, endogenous TRPV1 agonists and modulators are known to play an active role in arthritis and are summarized in Table **2**. Thus, the fact that capsaicin treatment alleviates pain and inflammation in arthritis; in addition to TRPV1 being activated by a variety of inflammatory stimuli and the wide expression of this channel in different tissues and cells, makes it an attractive drug development target for the treatment of arthritic conditions. Here, we discuss the importance of TRPV1 expression in the context of arthritis.

TRPV1 positive sensory nerves are present in the mouse knee and ankle joints [89]. Combined studies of tracing and immunohistochemistry revealed that approximately 40% of articular afferent nerves are TRPV1 positive and most of them also contain classic neuropeptides. The release of neuropeptides within the joint would be expected to cause dilatation. Perhaps surprisingly though, in the mouse knee joint, the TRPV1 agonist capsaicin mediates vasoconstriction [90]. This response has also been observed in certain vascular tissues and is not abolished by denervation [91]. This suggests that the ability of capsaicin to mediate vasoconstriction may be due to the presence of non-neuronal TRPV1 receptors. Besides being expressed on sensory neurons, there is evidence for functional TRPV1 receptor channels on synoviocytes [92, 93] and glial cells [94] in addition to blood vessels. Recently, TRPV1 was found to mediate cell death in rat synovial fibroblasts through calcium entry-dependent ROS production and mitochondrial depolarization [95]. Moreover, it has been proposed that the microRNA (miR-146a) plays an important role in OA by modulating inflammatory mediators such as TNFα, IL-6 and IL-8 as well as pain-related molecules such as TRPV1 in human glial cells, perhaps playing a pivotal role in influencing knee joint homeostasis and arthritic pain through effects on glial cells [96]. Thus, the potential exists for both neuronal and non-neuronal localisation of TRPV1 contributing to pain and inflammation [for review see: 97, 98].

Similarly to capsaicin, TRPV1 activation by other agonists triggers the release of CGRP and SP. Whilst it is now realised that substance P is unlikely to influence the arthritic disease process in humans, the role of CGRP is less well understood. CGRP acts through vasodilation of vessels, which causes redness and warmth at the surface [99, 100] in different tissues including in the joint [101]. CGRP, like substance P, may also influence endothelial cell proliferation in the joint [102] although the relevance of this is currently unknown. It has been suggested that blocking CGRP may alleviate pain in temporomandibular joint disorder [103]. This is of interest as it is known that the trigeminal ganglia is involved in CGRP-mediated pain processing in migraine, where CGRP antagonists have been shown to be of benefit in clinical trials [104].

### 2.4. TRPV1 Knockout Mice (TRPV1KO) and Investigations in Inflammatory Joint Inflammation/Pain

TRPV1KO mice were established shortly after the cloning of the TRPV1 receptor. The TRPV1 gene was disrupted by deleting an exon encoding part of the fifth and all of the sixth putative transmembrane domains of the channel, together with the intervening pore-loop region, meaning the TRPV1 receptor is expressed on the cell

membrane, but it is not active [8]. They grow and reproduce normally. Importantly, a selective loss of thermal hypersensitivity during inflammatory responses was shown [8, 105] and diminished joint inflammation and pain in models of CFA-induced arthritis in the TRPV1 KO mouse, when compared with WT mouse [9]. This study was complemented by that of Keeble and collaborators [10] who showed that CFA-induced mono-arthritis was associated with less thermal hyperalgesia and knee swelling, despite TNFα levels remaining similarly high in WT and TRPV1 KO mice. In addition, another group showed by using a similar model of inflammatory pain that TRPV1KO mice experience less joint damage and altered weight distribution [11].

Mechanistic studies indicated that TNFα, present in high levels in the WT and TRPV1 KO mouse, induces thermal hyperalgesia via a TRPV1-dependent mechanism that also involved cyclooxygenase products and other cytokines such as IL-1β [15]. It was suggested that there are a series of peripheral events through which TNFα triggers and mediates inflammatory pain in diseases with a symmetrical aspect such as arthritis. A study carried out by Chen and collaborators in 2009 [14], aimed to elucidate if TRPV1 receptors were able to activate spinal cord glial cells, and therefore allowing the assumption that this channel was involved in pain stimulation. They showed TRPV1 KO mice did not develop hypersensitivity in adjuvant-induced arthritis as expected when compared to WT littermates. However, immunohistochemical analysis revealed that TRPV1 receptors are able to activate spinal glia, in the WT but not TRPV1 KO mice, suggesting a direct relationship with the observed pathological pain and inflammation [14]. Moreover, it was later demonstrated that this was associated with ERK signalling [13]. Similar findings were described by Honore and collaborators also using TRPV1 KO mice [21]. In their study, motor function was measured using photo-beam activity monitors and movement apparatus in addition to thermal sensitivity and calcium concentrations *in vitro*. Recently, it was established that central, but not peripherally expressed TRPV1, plays a role in the bilateral mechanical hyperalgesia induced by TNFα [17].

The studies, involving TRPV1 KO mice, are under a situation where the receptor deletion was life-long. This is of course different to the normal situation in animal and humans that develop arthritis. However, they provide evidence towards the hypothesis that TRPV1 antagonists may be beneficial in the treatment of arthritis. Moreover, this concept is supported by the knowledge that capsaicin creams that act, initially at least, via the TRPV1 receptor are beneficial in the human.

### 2.5. TRPV1 Antagonists and Inflammatory Inflammation/Pain, Relevant to Joint Inflammation and RA

The studies above demonstrate the involvement of the TRPV1 receptor in pain and inflammation associated with arthritis. Without doubt, these results heighten interest in the probability that TRPV1 antagonists would also be beneficial. Indeed, there have been key demonstrations that suggest TRPV1 antagonists have beneficial effects in relieving the symptoms associated with arthritis [106].

The TRPV1 antagonist, A-425619 was shown to reduce complete Freund's adjuvant-induced pain. It also alleviated pain after either i.t. administration or local injection into the inflamed paw [18], demonstrating the potential of TRPV1 antagonists. Furthermore Lappin and co-workers [19] showed that the TRPV1 antagonist SB366791 additionally inhibited glutamatergic neuro-transmission following CFA-induced peripheral inflammation. A study carried out by Kanai *et al.,* [20] explored two TRPV1 antagonists (SB366791 and BCTC) in a CFA model of adjuvant-induced arthritis in the rat. They suggested that the antagonists inhibited thermal, but not mechanical hyperalgesia when given locally. However, the antagonists inhibited the mechanical hyperalgesia when given intrathecally, thus supporting the concept that central, in addition to peripheral TRPV1 mechanisms are important for pain sensation [17, 20].

Honore and collaborators [21] showed that the potent and selective TRPV1 antagonist, ABT-102 (Abbott), is an effective agent in blocking thermal and mechanical pain in a range of models. Importantly, there was an increase in analgesic efficacy with repeated administration of ABT-102 for 5-12 days. These beneficial effects were seen also with a structurally distinct TRPV1 antagonist, A-993610 [21]. By this time it was realised that TRPV1 antagonists induced an adverse hyperthermia. However, ABT-102 had a mild effect on core body temperature that appeared tolerated [21]. The authors concluded that the analgesic efficacy of ABT-102 supported its testing in clinical studies.

### 2.6. Research using Models for OA and other Arthritic Conditions

There is evidence for TRPV1 up-regulation in models of OA [107] and increased joint innervations in rats treated intra-articularly with monoiodoacetate (MIA) involving CGRP positive sensory neurons [108]. The TRPV1 receptor antagonist, A-889425, has been investigated in this model of OA. Effects on grip behaviour and firing of neurons were studied. A-889425 (10-300 μmol/kg, p.o.) attenuated loss of grip force and this was associated with reduced mechanical sensitivity. In addition, the increase in spontaneous firing following OA induction was reduced by A-889425 treatment [22]. Repeated treatment with another TRPV1 antagonist, A-995662, was also effective in attenuating the MIA-induced osteoarthritic pain in rats. This antagonist was suggested to inhibit glutamate and CGRP release from the spinal cord [109]. Similarly, the TRPV1 antagonist ABT-102 attenuated pain in a model of osteoarthritic pain induced by MIA [21]. These results provide pre-clinical evidence of a potential role for TRPV1 antagonists in treating OA.

There are, as discussed in the introduction, many types of arthritis and the intense pain associated with episodes of gout are well known. Gout is induced by deposition of monosodium urate crystals in joints and this can be modelled in rats. Recently, it was shown that treatment with the selective TRPV1 receptor antagonists SB366791 or AMG9810 substantially inhibited the pain sensitivity and the swelling (oedema formation) observed in response to monosodium urate injection. Whilst a range of other mediators are involved, this work cited the importance of mast cell-dependent mechanisms with the clear involvement

of TRPV1 [110]. Thus there may be a use for TRPV1 antagonists alleviating the sharp pain associated with gout.

### 2.7. Clinical Trials

The efficacy of TRPV1 antagonists in pre-clinical models is supported by the fact that there have been at least 7 antagonists that have progressed to clinical trials, where one of the first, SB-705498, progressed from phase 1 trials a number of years ago [106]. However, to our knowledge, the efficacy of a TRPV1 antagonist in human arthritis has yet to be published. It is difficult to know at this stage, whether this is due to the delay in obtaining suitable drugs for humans, or whether clinical trials have been performed, but the data is at present unpublished.

## 5. EVIDENCE FOR THE INVOLVEMENT OF OTHER TRP CHANNELS IN ARTHRITIS

In recent years there has been exciting basic research into the pathophysiological relevance of other TRP channels that have been shown to be expressed in different cells and possess biological functions. The new research aims to understand the importance of these channels for pain regulation and inflammation, including arthritis.

### 5.1. TRPA1

The transient receptor potential ankyrin 1 (TRPA1), in addition to TRPV1, has been shown to play a role in mediating inflammatory pain. TRPA1 was first cloned in 1999 [24] and it is known to be expressed in the same sensory neurons as TRPV1, and activated by a range of endogenous and exogenous agonists (see: [98]). This review will highlight the importance of TRPA1 activation by cold [111] and by endogenous agonists for arthritis. TRPA1 endogenous activators are often electrophilic compounds such as reactive oxygen species [112, 113] and lipid mediators such as 15d-PGJ2 [114]100; which are generated in arthritis [115-118].

Research using rodent models has revealed the role of TRPA1 role in different pain models which have been recently reviewed [119-121]. However, little is known of the specific roles of TRPA1 in arthritis. In addition to TRPA1 expression in sensory neurons, TRPA1 alongside TRPV1 is found expressed in non-neuronal cells such as rat endothelial cells [122] and human synoviocytes [92]. TRPA1 expression in synoviocytes and endothelial cells is functional, i.e.; elicits calcium influx when stimulated [92, 122]; suggesting this receptor could be an important mediator of joint inflammation and pain. It is only recently, with the generation of TRPA1KO mice and selective TRPA1 antagonists, that the role of this channel in arthritic pain has been explored. These studies have used models of inflammatory pain which resemble RA and models of OA.

In 2007, Petrus and collaborators [27] published the first evidence of a selective TRPA1 antagonist being effective in reducing arthritic pain. By using a model of i.pl. CFA-induced inflammatory pain, co-injection with the selective TRPA1 antagonist AP-18 abolished mechanical hyperalgesia in mice. The same study showed TRPA1 KO mice exhibited normal mechanical hyperalgesia 24h following CFA injection. Recently, our group developed a model of bilateral mechanical hyperalgesia induced by i.pl. TNFα, a model of pain relevant for the study of arthritis [17]. We found that both intrathecal (i.t.) and intraplantar (i.pl.) administration of a selective TRPA1 antagonist, AP-18, caused analgesia. This analgesic effect was more pronounced in animals treated with i.pl. AP-18, suggesting an important role for these receptors at a peripheral level, in contrast with a central role for TRPV1 receptors observed in the same model. This research showed for the first time that although these receptors are expressed in the same sensory neurons, they play specific and additional roles in the mediation of pain. In addition, the same study showed that TRPA1 KO mice exhibited analgesia when treated either with i.pl. TNFα or intrarticular CFA, in comparison with WT littermates. We highlight here that CFA-induced pain was decreased in TRPA1 KO mice only from 1 week after CFA injection. These results together with those described by Petrus *et al*. [27] suggest a primary role for TRPA1 in the maintenance of mechanical hyperalgesia in inflammatory states. Similarly, a study performed in rats [28] showed the oral effects of another selective TRPA1 antagonist HC-030031 in reducing the mechanical hyperalgesia caused by CFA in the paw. In addition, systemic administration of the selective TRPA1 antagonist A-967079 in rats injected i.pl. with CFA, reduced the activity of spinal wide dynamic range neurons after noxious mechanical stimuli such as 10 g von Frey hairs and noxious pinch stimulation [29]. Interestingly, TRPA1 expression is increased in small and medium size DRG neurons following i.pl. CFA [123].

Although the participation of TRPA1 in mediating pain in models of RA is clear, its role in OA is still of debate. To date, there are only 2 reports of studies aimed at elucidating TRPA1 involvement in MIA-induced OA-related pain. The first performed in 2010 by McGaraughty and collaborators [29], showed a reduced neuronal activity in rats systemically treated with A-967079 when tested for high-intensity von Frey hair stimulation (300 g) and compared to vehicle-controls. On the other hand, the study of Okun and collaborators [23] which investigated the participation of TRPA1 in ongoing pain, showed that neither systemic nor i.pl. treatment with the selective TRPA1 antagonist HC030031 was able to interfere with weight bearing responses caused by intra-articular MIA. These differences may be due to experimental models suggesting that TRPA1 may play a role in OA-related pain when a mechanical stimulus is involved but not continuous pain.

As mentioned before, TRPA1 can be activated by cold (10-17$^{o}$C). The relationship between cold exposure and increased pain in arthritic joints has been suggested [27, 124, 125] and thus, it is sensible to speculate if TRPA1 plays a role in this increased sensitivity. Few studies have addressed this field but it has now been described that although TRPA1's role in sensing cold in physiological conditions is conflicting (see: [121, 126]), that a role for TRPA1 in cold-induced responses in animals with pre-established inflammation is evident. Indeed, Petrus and collaborators [27] showed that co-injection with AP-18 reduces CFA-induced cold hyperalgesia (5$^{o}$C) in the rat paw. In a different experimental setting, cold hyperalgesia was tested in mice with their CFA-treated paws (i.pl.) sprayed with tetrafluorethane that is known to cause licking behaviour. This response was reduced by systemic treatment with HC-030031 [30]. A study performed by Obata and collaborators

[123] showed that TRPA1 anti-sense knockdown causes a reduction in cold hyperalgesia induced by CFA i.pl. injection in rats, although the same treatment failed to prevent the thermal and mechanical hyperalgesia triggered by CFA. Overall, these results suggest in a broad sense, a link between cold sensation and inflammation in RA. On the other hand, the mechanisms underlying this relationship remain unclear.

Although there is clear evidence from animal models that TRPA1 plays a role in the mechanical and cold responses related to arthritis, the potential use of TRPA1 antagonists to treat human pain remains to be addressed. In addition, research into the role of TRPA1 channels in arthritis is still novel and very little is understood of TRPA1 participation in arthritis. Plus, the identification of the specific endogenous agonists playing a role at different stages of RA and OA would be extremely important. Thus, there is still a great need of fundamental mechanistic studies in order to fully understand the role of TRPA1 channels in both RA and OA. Studies from our laboratory have revealed the distinct roles of TRPV1 and TRPA1 in a single study. This highlights the potential that a combined treatment of TRPV1 and TRPA1 antagonists may be a more beneficial therapeutic approach than inhibiting with of these TRP receptors alone.

### 5.2. Other TRP Channels

A potential role for other TRP channels, in addition to TRPV1 and TRPA1, in arthritis has been speculated. Perhaps, the most compelling evidence is in regards to TRPV4 and TRPC5 (transient receptor potential canonical 5). In addition to being neuronally expressed [127, 128], both receptors are functionally expressed in human synoviocytes either derived from commercial cell lines or obtained from RA patients [92, 129, 130].

Indeed, the suggested role of TRPC5 in pain and RA is very new. This channel was first described as an important regulator of brain development [131]. Very recently, this receptor channel was revealed as a peripheral cold sensor in *naive* mice [132]. Interestingly, treatment of RA-derived fibroblast-like synoviocytes (FLS) with either an anti-TRPC5 antibody or the knockdown of TRPC1/5 expression, increases metalloproteinase expression (MMP-2, -9 and -1) [129]; suggesting TRPC5 plays a possible protective role in RA. Thioredoxin is one of the endogenous activators of TRPC5 and has been suggested to play a role in arthritis. The reduced thioredoxin form is able to break disulphide bridges between cysteine residues and activate TRPC5 [129]. Systemic thioredoxin treatment was shown to reduce arthritis severity in a murine model of RA induced by mAb/LPS injection. The same study described a protective response to arthritis in thioredoxin overexpressing transgenic (TG) mice [133]. In addition, *in vitro* studies showed that thioredoxin inhibits MMP release from FLS, an effect that was reduced in the presence of an anti-TRPC5 antibody [129]. Thioredoxin has been also linked to innate immune cell regulation (neutrophils and macrophages) and inhibition of neovascularisation [134], as well acting as a co-stimulatory molecule for IL-6 and IL-8 production in FLS treated with TNFα [135].

TRPV4, on the other hand, has been primarily implicated in OA. TRPV4 is a sensor for warmer temperatures (30-34$^{o}$C; [136]) and in addition to its expression in neurons and synoviocytes, TRPV4 protein has been localised to in mouse chondrocytes [137]. The same study showed that TRPV4 deletion in mice is associated to a severe, progressive OA at a young age, particularly in male mice, accompanied by significant increases in ossification of joint tissues. Also, TRPV4 deletion results in a reduction of osmotic sensitivity by chondrocytes, which in turn could result in decreased adaptative changes in response to cartilage overloading and degeneration. The collected evidence suggests a chondroprotective role for this channel in OA.

Other TRP channels such as TRPC6, TRPC1 and TRPC3, TRPM2 (transient receptor potential melastatin 2), TRPM3, TRPM7 and TRPV2 are also expressed in synoviocytes, chondrocytes, mononuclear cells and neutrophils [92, 138-140]; although their roles in RA and OA remain to be elucidated.

## 6. CONCLUSION

An American investigation into the mortality rates of arthritis, involving over 5000 individuals with RA, found that those with the condition had a mortality rate at least twice as high (if not more) than those without it [141-143]. These statistics allow us to further acknowledge the severity of arthritis. Moreover, it is established that arthritic patients are often unhappy with the level of pain relief that is achieved with currently available drugs [144]. The established drugs currently used can be divided into the main groups of those that relieve pain and those that alter the disease progression [145]. Those involved in pain relief with a potential for little anti-inflammatory relief are the analgesics or painkillers like dihyrocodeine and paracetamol. The non-steroidal anti-inflammatory drugs (NSAIDs), inhibitors of cyclo-oxygenase, (e.g. proxens) work to combat the pain and are also classed as anti-inflammatory. Within these groups we would also place the capsaicin creams and patches. The NSAIDs are extremely widely used, but do not halt disease progression in rheumatoid arthritis. Also there are problems with dependency (opioids) liver adverse effects (paracetamol) and internal bleeding (systemic cyclo-oxygenase inhibitors). Thus, potentially TRPV1 and TRPA1 antagonists may be as effective, whilst also having less serious side-effects. Other drugs such as the corticosteroids (e.g. prednisone, the most commonly prescribed corticosteroid) work to control the inflammation and can be injected locally, but they have to be used with care. Finally, there is a wealth of disease modifying anti-rheumatic drugs available, of which methotrexate is the best known and here we also include the biologics (antibody therapy), that are used alone or in combination to attenuate or halt the progression of arthritis. Like many drugs, these medicines currently used for arthritis can have undesirable side effects. However, it is highly unlikely, from present knowledge, that TRP-related drugs can influence the immune aspect of these drugs in a similar manner. On the other hand, without doubt there is an unmet need for effective pain killers and it is here that the TRP-related drugs fit best when using the current knowledge, discussed in this review. They may be suitable for several applications including topical, in addition to

systemic with a potential for use in combination therapies, in addition to their use alone.

Without doubt, the TRPV1 cation channels have been clearly demonstrated to play a key role in pain perception. This understanding has given new optimism in finding novel possible treatments for chronic pain diseases like arthritis, especially OA that has currently fewer treatment options. This has been particularly so with respect to TRPV1 antagonists and we believe this optimism should be extended to other receptors, specifically TRPA1.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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