The TRPV3 Receptor as a Pain Target: A Therapeutic Promise or Just Some More New Biology?

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Abstract: Human beings have evolved with a powerful peripheral thermo-sensory capacity to maintain thermal homeostasis and to avoid potentially harmful stimuli. Specialized sensory neurons are believed to mediate temperature sensation. Among the candidate heat-sensitive transient receptor potential (thermoTRP) receptors implicated in this process is the TRPV3 receptor, which is identified as a molecular sensor of "warm to hot" temperatures. This review focuses on recent insights into the therapeutic potential of TRPV3 receptor blockers in pain treatment, efficacy and safety of TRPV3 antagonists during preclinical studies, and their progress into clinical development.

TRPV3 MOLECULAR BIOLOGY

The TRPV3 (TRP cation channel, subfamily V, member 3) receptor belongs to the TRPV family of thermo receptors. It is a close relative of the TRPV1 receptor, which is activated by noxious heat. The TRPV3 receptor gene was simultaneously identified by three independent research groups. Database searches for TRP-related ESTs from human testes library revealed a putative ORF encoding hTRPV3 receptor sequence [1]. In another investigation involving homology searches of Human Celera and public database for TRPV1 like genes, multiple new exons with a great degree of sequence similarity to the ankyrin and trans-membrane domains of TRPV1 were identified. The sequence comprised of a 2370 base pairs ORF encoding the TRPV3 receptor [2]. The product of this gene was initially referred to as vanilloid receptor-like protein 3 (VRL3) and later renamed as TRPV3 conforming to the latest TRP receptor nomenclature [3]. Almost simultaneously, identification of murine TRPV3 from the skin of newborn mouse was reported [4]. Both TRPV1 and TRPV3 genes map to human chromosome 17p13 and mouse chromosome 11B4. They are spaced at a distance of 10 kb and transcribed in the same direction. This suggests the possibility that both are derived from a single gene duplication event. TRPV3 receptor protein is composed of 790 amino acids (791 in the mouse) with three predicted ankyrin domains, six transmembrane (TM) domains and a pore-loop region between last 2 membrane spanning regions. TRPV3 receptor is reported to have ∼ 43%, 42%, 41% and 30% identity to hTRPV1, TRPV4, TRPV2 and TRPV5 and TRPV6 receptors, respectively [2].

TRPV3 TISSUE EXPRESSION

Unlike TRPV1 and TRPV2, which are highly abundant in sensory neurons (though are also present, albeit at much lower levels, in keratinocytes), TRPV3 is predominantly expressed in keratinocytes. TRPV3 expression is well documented in the literature both in human and rodent skin using various detection techniques from northern blots through PCR and immunostaining to in-situ hybridization [4-6]. Of note, the reported absence of TRPV3 in skin cells in a few early studies was later attributed to technical artifacts such as receptor loss during tissue dissociation. With regard to neuronal expression, the presence of TRPV3 in human DRG neurons is firmly established [2, 4]. There are, however, conflicting reports in rodents with some early studies demonstrating absence of TRPV3 receptor in rodent sensory neurons [4, 5, 7]. Subsequently, TRPV3 expression was reported in the rodent neuronal cells, including DRG, trigeminal, hippocampal and superior cervical neurons, peripheral nerve endings and certain brain regions [1, 2, 8-10]. The reason underlying these discrepant reports is unclear but is likely to involve a combination of receptor loss during tissue processing and differences in the detection limit of various methods. The CNS expression of TRPV3 includes ventral motor neurons, superior cervical ganglion neurons, deeper laminae of dorsal horn, and nigral dopaminergic neurons [11]. Its physiological role in these areas remains to be investigated.

The presence of functional TRPV3 receptors in skin cells has been amply demonstrated using electrophysiology assay [12, 13]. TRPV3 was found to be abundantly expressed both in differentiated basal layer cells and undifferentiated suprabasal layers of mouse epidermis [5]. By contrast, absence of TRPV3 expression in cultured mouse primary skin keratinocytes, as well as in established epidermal cell lines was reported [4]. These differences could be due to receptor down regulation during tissue dissociation procedure and/or long-term culture. The high level of expression of this "warm" receptor in skin *vs* DRG neurons may be important for the prevention of constitutive activation of the channel by heat since the skin has a lower resting temperature (34°C) compared to DRG neurons (37°C).

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Apart from skin and neuronal cells, TRPV3 receptor is also expressed in nasal and oral cavity, namely in the tongue and palate [12]. In the mouth, TRPV3-like immunoreactivity is largely restricted to epithelial layers facing oral cavity. Its presence in the tongue and palate implies that TRPV3 may function as a target for flavor actions of plant derived derivatives such as carvacrol from oregano.

TRPV3 AND THERMAL ACTIVATION

The mammalian nervous system detects temperature over a range extending from noxious cold to noxious hot. This capacity involves selective activation of specific neuronal subpopulations that fire over discrete ranges of skin temperatures. ThermoTRPs are candidate mediators of this thermosensory diversity. Each of the thermoTRPs has a specific activation threshold temperature range. For example, TRPV1, TRPV2, TRPV3 and TRPV4 are responsible for mediating thermal sensitivity in warm to hot temperature ranges [14]. Temperature sensitivity for some of the thermoTRPs is believed to be mediated by a specific C terminal domain, the so-called TRP box region [15]. The specific structural domains conferring warm-sensitivity in TRPV3 receptors are yet to be determined. Nonetheless, TRPV3 channel activation by warm to hot temperatures was demonstrated both in cloned and recombinantly expressed human TRPV3 receptors as well as native TRPV3 expressed in skin keratinocytes [4, 13].

POLYMODAL ACTIVATION – ALSO FOR TRPV3 RECEPTORS?

Most thermosensitive TRP channels can be alternatively activated by non-thermal stimuli. For instance, TRPV1 is activated by capsaicin, protons, and endocannabinoids, just to cite a few examples [16-18], whereas TRPV4 responds to hyposmolarity, arachidonic acid metabolites and 4alpha-PDD [19, 20]. This polymodal means of activation makes thermoTRPs extremely versatile receptors to detect noxious stimuli; indeed, TRV1 was referred to as a "molecular gateway to the pain pathway" [17]. A number of thermoTRPs, in specific TRPV1, are also activated by factors released from injured cells to the so-called "inflammatory soup" [18].

A variety of non-selective natural compounds activate TRPV3 receptors, including camphor, menthol, carvacrol, and eugenol [7, 12]. Camphor is a plant-derived monoterpene, known to sensitize human skin to warm temperatures. Camphor activates TRPV3 receptors in mouse primary keratinocytes where it also potentiates responses to warm temperature. Camphor induces currents in keratinocytes derived from wild-type mice and, importantly, this response was absent in keratinocytes obtained from TRPV3 knock out mouse [12]. Thus, camphor sensitivity is considered as a relatively specific functional marker for TRPV3-expressing cells. However, camphor is a weak agonist of TRPV3 receptors, requiring concentrations of 5-10 mM or even higher [21]. Menthol is another monoterpene agonist that activates TRPV3 receptors but only to a minor extent (~ 65% of camphor effect), indicative of a partial agonist [21].

Interestingly, camphor and menthol exhibit the opposite thermal sensation of feeling warm and cool, respectively, yet both activate the TRPV3 receptor. Since TRPV3 has not yet been implicated in cold sensation, this paradox remains to be resolved. When applied to the skin, menthol creates a cooling sensation, followed by a local anesthetic action. This activity is inconsistent with the proposed role of TRPV3 as a nociceptor. Therefore, it is likely that the primary target for the well-known menthol effects is not TRPV3 but the TRPM8 receptor. At higher concentrations menthol may also activate other channels that include TRPA1 and TRPV3, but it is unknown if these concentrations are ever achieved *in vivo*.

Carvacrol, a major ingredient in oregano, evokes sense of warmth when applied to skin and sensitizes the skin. Carvacrol is likely to produce these effects *via* TRPV3 receptor activation since it was shown to activate mouse and human TRPV3 expressed in HEK cells as well as native TRPV3 in rat tongue [12]. Carvacrol mimics the pharmacological properties of other TRPV3 agonists like 2-APB and heat. Furthermore, extracellular Ca^{2+} is required for TRPV3 channel activation by carvacrol and its effect is blocked by ruthenium red. Carvacrol is, however, not a selective activator of TRPV3 since it also activates TRPA1 [12].

Eugenol, the principal active ingredient in clove, is used as a topical analgesic agent in dentistry. Eugenol induces Ca^{2+} influx in HEK cells heterogeneously expressing mouse TRPV3 receptors and in mouse keratinocytes [12]. Mouse keratinocytes release IL-1α in response to eugenol stimulation. However, eugenol is not selective for TRPV3 either. Indeed, it activates the TRPV1 receptor. Additional plant-derived monoterpenes known to activate the TRPV3 receptor include carveol and thymol [21]. Overall, TRPV3 activation by these pungent, plant-derived chemicals implies a central role for TRPV3 in protective, chemosensory mechanisms of skin.

2-APB was the first synthetic, non-terpenoid agonist of the TRPV3 receptor. It is a shared activator of TRPV1, TRPV2 and TRPV3 receptors [13]. It not only evokes currents and Ca^{2+} influx in HEK cells expressing these receptors, but also potentiates effects of heat. Conversely, 2-APB inhibits TRPM8 and TRPC6 channels [22, 23]. Moreover, 2-APB also blocks IP3 receptors, native store-operated channels (SOCs), ER Ca-ATPase pumps, mitochondrial transition pore and few other ion channels. On SOCs, 2-APB exerts a bi-phasic effect: at low concentrations it enhances whereas at higher concentrations it blocks channel activity [24-26]. In summary, although 2-APB is widely used as an agonist in TRPV3 receptor investigations, caution should be exercised when interpreting the results to rule out a simultaneous action on receptors other than TRPV3. Recently, incensol acetate, a constituent of *Boswellia cerrata* resin, was suggested to function as a selective TRPV3 receptor agonist in the CNS [27]. However, the nociceptive properties of incensol acetate remain to be described.

There is substantial experimental evidence that heat opens the channel and various other agonists reduce the heat activation threshold of TRPV3 as exemplified by 2-APB which acts in concert to enhance the effect of heat on TRPV3. Unfortunately, a specific and selective TRPV3 receptor agonist with nociceptive properties is yet to be

identified. In the absence of such a compound, proving TRPV3 target receptor occupancy by the antagonists *in vivo* remains a daunting task.

WHAT ACTIVATES TRPV3 DURING DISEASE? DO ENDOGENOUS ACTIVATORS EXIST?

Polymodal activation of TRPV3 by specific, pathologically relevant agents has been reported. Although TRPV3 was initially recognized for its ability to be activated by warm temperatures, it is now known to serve as a molecular sensor to detect changes in the cellular milieu that occur during tissue injury or inflammation. Several endogenous activators of TRPV3 receptors have been identified. For example, TRPV3 channel activity is positively modulated by action of protein kinase C (PKC), nitric oxide (NO) and unsaturated fatty acids. Treatment of TRPV3/ HEK cells with Phorbol-12-myristate-13-acetatate (PMA), an activator of PKC, was found to potentiate TRPV3 channel function and this effect was blocked by the PKC inhibitor Ro318220 [28]. Activation of PKC in skin cells and sensory neurons is an important event downstream of proinflammatory receptor activation. This study has also shown that TRPV3 channel function gets directly potentiated by arachidonic acid and other unsaturated fatty acids. The ability of arachidonic acid to potentiate 2-APB-induced TRPV3 channel activation was demonstrated in TRPV3-expressing HEK cells and Xenopus oocytes, as well as in mouse primary keratinocytes carrying native TRPV3 receptors [28]. Saturated fatty acids were devoid of this effect. Among unsaturated fatty acids, the potency for channel function enhancement (which ranged from 2 fold to 10 fold) seemed to depend on the position of double bonds in the fatty acid rather than on the number of double bonds. The most potent ones were found to be 20:3 n9, 20:4 n6, 20:5 n3, all of which contain the double bond starting at 5th position from the carboxylated end [28]. Whether arachidoic acid interacts with TRPV3 channel protein or partitions into the lipid bilayer remains to be resolved. Interestingly, arachidonic acid-mediated TRPV3 channel potentiation does not require any metabolism of arachidonic acid. Although some unsaturated fatty acids are known to activate PKC, arachidonic acid-mediated potentiation of TRPV3 channel function appeared to be independent of PKC activation inasmuch it was not blocked by PKC inhibitors [28]. Importantly, this mechanism appears to be highly specific for TRPV3 receptors since the function of other "dermoTRPs" (e.g. TRPV1, TRPV2 and TRPV4) are not affected by arachidonic acid but only by its metabolites. For instance, TRPV1 gets activated by lipoxygenase products of arachdonic acid, whereas the epoxygenase product stimulates TRPV4 [20, 29, 30].

Arachidonic acid is an endogenous mediator of inflammatory response in skin cells. It is released either from infiltrating lymphocytes or produced within sensory fibers or skin cells following membrane PLC activation by other inflammatory mediators. In severe cases of "involved psoriasis," arachidonic acid concentration in the epidermis reaches very high levels, close to the concentration at which it shows robust TRPV3 potentiation under *in vitro* conditions [31, 32]. Even under less severe conditions, the combined concentrations of free unsaturated fatty acids are likely to be sufficient to achieve TRPV3 activation. Moreover, *in vitro*

the potentiating effect of arachidonic acid was found to increase with longer incubation. Combined, these effects render arachidonic acid a pathologically relevant means for TRPV3 receptor activation during skin inflammation.

NO is one of the important and pleiotropic cell-signaling molecules that modulate diverse biological processes [33]. cGMP is considered to be the major mediator of NO signaling. NO is produced in cells during inflammation and injury. NO-mediated signaling could also be independent of cGMP as exemplified by S-nitrosylation of proteins as a post-translational modification. NO gating of TRP channels is conferred by structural motifs at the N terminal site of the putative pore-forming region. This region contains cytoplasmically accessible cysteine residues with free sulfhydryl groups. Channel activation by NO appears to be a shared feature of TRPV1, TRPV3 and TRPV4 as well as TRPC5 [34]. Ca^{2+} influx *via* nitrosylated TRP receptor may exert positive feed back regulation. Alternatively, there may be suppressive effect such that NO suppresses Ca^{2+} entry. The outcome of NO-induced TRP channel gating may thus depend on the crosstalk and balance between NO and Ca^{2+} signals. Thus, the TRPV3 receptor belongs to a new category of cell surface receptors that can integrate NO and Ca^{2+} signals.

Stimulation of phospholipase C (PLC)-coupled receptors co-expressed with TRPV3 in HEK cells was reported to enhance the ability of carvacrol and other TRPV3 agonists to activate TRPV3 [12]. Examples of receptors that potentiate TRPV3 activity include the muscarinic receptor type 1, histamine receptor and bradykinin receptor. In other words, PLC-activated, G-protein coupled receptors may potentiate TRPV3 function under inflammatory conditions when these mediators are produced and released in mass amounts. This implies an important role for TRPV3 receptor during inflammation as an integrator of signals from GPCRs at the level of neuronal input to PNS and CNS. Thus, TRPV3 might be involved in the initiation and maintenance of sensory hypersensitivity during tissue inflammation.

TRPV3 RECEPTOR: ROLE IN CUTANEOUS SIGNALING

The very expression of TRPV3 in skin suggests it's role in thermosensation [35]. Indeed, skin TRPV3 is activated by warm to hot temperatures and is believed to mediate keratinocyte responses to heat. Although heat-sensing neurons are thought to terminate as free nerve endings in the dermis, some processes extend into epidermis where TRPV3 is expressed. Heat-induced TRPV3 signals are believed to get transferred from the keratinocyte to nearby free nerve endings, thereby causing sensation of warmth or heat. Although no synapses have been found between keratinocytes and sensory termini, keratinocytes often surround the nerve fibers and it is possible that they can transduce signals through chemical mediators. Skin keratinocytes are known to release signaling molecules upon specific receptor activation. For example, endothelin receptor agonists were shown to stimulate beta-endorphin release from cultured keratinocytes, which, in turn, acts on local neuronal μ-opioid receptors to inhibit nociception [36]. TRPV3 activation on keratinocytes may lead to similar release of substances that, in turn, activate adjacent dermal or

epidermal sensory nerve terminals. Although no specific factor has been identified in this context, a number of candidate mediators exist. For example, keratinocytes when activated by camphor, 2-APB or heat released PGE_2 [37]. Eugenol was shown to increase intracellular Ca^{2+} and induce release of IL-1α from mouse skin 308 keratinocytes [12]. The TRPV3-dependant IL-1α release may have special importance since IL-1α is an important mediator of cutaneous inflammation.

Keratinocytes cultured from TRPV3 over-expressing animals showed augmented TRPV3 ion channel activity and increased PGE_2 mediator release in a gene-dosage dependent manner [37]. Such keratinocytes showed robust release of PGE_2 in response to 2-APB, camphor or heat (42°C) stimulation. Removal of extracellular Ca^{2+} abolished this TRPV3-dependant PGE_2 release. Peripheral nerve endings are known to express all four receptors for prostaglandins [38-41]. Thus, TRPV3-dependant PGE_2 release from keratinocytes may contribute to sensory functions including acute nociception and hyperalgesia.

In another study [42], transgenic Nh mice over-expressing mutated TRPV3 receptors showed enhanced release of nerve growth factor (NGF), a powerful sensitizer of sensory neurons, from epidermal sheets at 33°C. It would be interesting to know if over-expressed wild-type TRPV3 receptor mimics this effect.

Recently, activation of TRPV3 receptors in DRG neurons in response to ATP released from heat-activated keratinocytes has been described in a co-culture system [43]. This effect by ATP release is absent in keratinocytes from TRPV3 deficient mice. This study provides strong evidence that ATP is a messenger molecule for TRPV3-mediated thermotransduction in skin.

Taken together, these interesting observations indicate an important role for TRPV3 in skin-nerve cross talk. Further studies to demonstrate blockade of mediator release by TRPV3 specific antagonist or siRNA are much awaited to endorse functional role for TRPV3 in cutaneous signaling.

TRPV3 AS A PAIN TARGET: HOW IS IT DIFFERENT FROM OTHER TRP RECEPTORS?

The TRPV3 receptor is unique among thermoTRPs in that it gets continuously sensitized on repeated agonist application [1, 4, 7]. This is in contrast to other structurally and functionally related TRP receptors (such as TRPV1, TRPV4, and TRPA1), which get desensitized under such conditions. This feature has been observed both with recombinant TRPV3 and native TRPV3 on keratinocytes and is stimulus independent [7]. It was recapitulated with all known TRPV3 agonists, including heat, 2-APB, camphor, carvacrol and eugenol [7] Ca^{2+} was shown to play an important role in sensitization of TRPV3 on repetitive stimulation. In fact, somewhat paradoxically, the actual sensitization of TRPV3 channel was shown to involve relief from Ca^{2+} mediated inhibition of channel function. Ca^{2+} inhibits TRPV3 from both extracellular and intracellular sides. Intracellular Ca^{2+}-dependant channel inhibition is mediated by calmodulin. Calmodulin binds to TRPV3 receptor protein at 108-130 N terminal site [44]. Extracellular inhibition of TRPV3 channel function is mediated by interaction of Ca^{2+} with a specific aspartic acid

residue (Asp641) at pore loop region. Indeed, mutation of Asp641 to Asn abolishes sensitization property of the channel. Thus, it appears that inhibition of TRPV3 channel function by intra- and extracellular Ca^{2+} keeps the channel inactive under resting conditions.

Sensitization is accompanied by a decrease in calmodulin-dependant inhibition, as well as a reduction in Ca^{2+} block at the pore loop. Thus, on repeated agonist stimulation these inhibitory actions are sequentially reduced which appears as sensitization on repetitive stimulations. TRPV3 channel is known to get sensitized by PKC, unsaturated fatty acids and it is observed that sensitized TRPV3 channel is less inhibited than naïve channel. It is speculated that Ca^{2+} binds with reduced affinity to calmodulin and to Asp when the channel is in sensitized conformation. This calls for further investigation in this area to enhance our understanding of conformation shifts of sensitized TRPV3 channel in context of pain and inflammation.

Based on this unique property of TRPV3 channel, it is tempting to speculate that in chronic pain continuously sensitized TRPV3 contributes to the aberrant and on-going pain signal transmission.

TRPV3 AS A TARGET IN HEAT PAIN SIGNALING IS VALIDATED BY TRPV3 DEFICIENT MICE

Studies with TRPV3 deficient mice confirmed the role of TRPV3 in thermosensation [7]. TRPV3 knockout mice have normal body weight, normal internal temperature, normal open field responses and motor activity, but exhibit deficits in sensing warm temperature. In a two temperature plate-based thermotaxis assays, the TRPV3 knockout mice spent less time on a warm surface than wild-type animals and exhibited delay in the time to prefer the warm surface, indicating strong deficits in heat response. Moreover, the knockout animals showed increased thermal nociceptive threshold manifested by significant withdrawal latencies at 55°C in a hot plate test and delayed tail flick responses at 50°C or above in a tail immersion assay, indicating strong deficits in response to noxious heat. As expected, they showed no difference compared to wild type in sensation of cold temperature [7].

TRPV3 OVER-EXPRESSING MICE

In vivo studies with transgenic mice over-expressing TRPV3 in keratinocytes also support the role of this receptor in thermal pain sensation [37]. Interestingly, no significant differences were found in thermal sensitivity between transgenic mice and the wild-type. It was suspected that presence of functional TRPV1 on keratinocytes masked the pro-nociceptive effects of TRPV3. To confirm this, pharmacological inhibition of TRPV1 receptor was attempted with the selective antagonist JNJ 7203212. Under these experimental settings, the TRPV3 transgenic mice consistently exhibited greater thermal nociception than wild-type. These data suggest that TRPV3 channels facilitate heat-evoked activation of nociceptive signaling pathways.

TRPV3 IN DISEASE STATES

There is good evidence that TRPV3 receptor levels are altered in certain disease states. For example, TRPV3

expression is up-regulated in the Chung model of neuropathic pain in rats [45] where neuropathic pain is induced by constriction of sciatic nerve during surgical procedure. These animals exhibit typical symptoms of neuropathic pain (such as thermal hyperalgesia, mechanical hyperalgesia and mechanical allodynia) within 1-3 days post surgery and these effects last until almost 50 days. RT-PCR analysis of L4 and L5 DRG neurons from Chung model animals and sham operated controls revealed robust elevation of TRPV3 message.

More important, women experiencing breast pain (mastalgia) showed up regulation of TRPV3 and TRPV4 in basal keratinocytes in the skin [46]. Breast pain and tenderness is common in ~70% of women at some time. The symptoms are attributed to stretching of nerves with increase in breast size. TRPV3-like immunoreactivity is increased in these patients such that increase in TRPV3 protein correlated with disease score, implying a role for this receptor in mastalgia.

Enhanced TRPV3-like immunoreactivity in avulsed human DRG neurons was also reported [2]. Cervical DRG neurons whose roots were avulsed from spinal cord after traumatic injury to the brachial plexus were collected from ten adult patients during surgical repair. Age matched controls were used to compare the difference in receptor protein levels. TRPV3-like immunoreactivity was increased significantly on injured neurons. In another clinical study, skin and nerve preparations from patients with nerve injury, avulsed dorsal root ganglia, injured spinal roots and diabetic neuropathy showed significantly increased expression of TRPV3 receptor in injured brachial plexus nerves [10]. TRPV3 expression increased in peripheral nerve proximal to the site of injury. Increase in injured nerves indicates its export from ganglion or accumulation at site after injury. TRPV3-immunoreactive sensory nerves also increased in DRG following avulsion injury such as central axotomy. After nerve injury, TRPV3-like immunoreactivity appeared to be increased in both number and intensity along with that of TRPV1. In tissue preparations from diabetic neuropathy patients, TRPV3, however, showed a decrease in immunoreactivity in the skin [10]. This could be due to abnormally thin epidermis in diabetics causing loss of TRPV3 receptor. In injured ventral spinal cords, the TRPV3-like immunoreactivity was reported to diminish after chronic injury; again, this effect is probably due to impaired peripheral transport [10].

Keratinocytes are strategically positioned at the interface between external environment and body's internal milieu and have been recognized for their role in thermal and pain sensation [7]. TRPV3 channel function in keratinocytes is potentiated by arachidonic acid. Concentration of arachidonic acid is elevated in inflammatory skin diseases such as psoriasis. Hence, TRPV3 may be involved is producing sensory hypersensitivity in general inflammatory dermatoses such as psoriasis [28]. Keratinocyte participation in thermal pain transduction and dermatitis *via* TRPV3-dependant PGE_2 release was also postulated [37]. PGE_2 is known to promote thermal and mechanical hypersensitivity by interacting at G protein-coupled EP receptors in sensory neurons [47]. TRPV3 receptor over-activity has been implicated in the development of atopic dermatitis [48].

Release of IL-1 from keratinocytes in a TRPV3-dependant manner also supports the involvement of TRPV3 in the development of psoriasis [12]. IL-1 is an important skin-specific pro-inflammatory cytokine and a known mediator of cutaneous inflammation. Some of the anti-psoriatic drugs such as hydrocortisone and 2-OH vitamin D3 work though IL-1 release inhibition [49]. Taken together, these observations support the involvement of TRPV3 in skin inflammation.

In summary, TRPV3 with its demonstrated role in hyperalgesia, pain transduction and inflammatory signaling, its continuous sensitization properties and its expression pattern, appears to be a valid target for treating many pain and inflammatory conditions such as arthritis, some forms of neuropathic pain such as diabetic neuropathy and skin disorders such as itch and dermatitis.

TRPV3 SELECTIVE ANTAGONISTS FOR PAIN RELIEF: PRECLINICAL PROOF OF CONCEPT

Until recently, only two non-selective TRPV3 receptor antagonists were known, namely ruthenium red and diphenyltetrahydrofuran (DPTHF) [50]. At present, two pharmaceutical companies (Glenmark and Hydra Biosciences) have announced their engagement in the TRPV3 target area and published *in vitro* and *in vivo* profiles of proprietary TRPV3 antagonists (Table **1**).

Hydra Biosciences was the first entrant in this area with disclosure of efficacy of their TRPV3 antagonists in animal models. Their compound 82 was effective in the carrageenan model of acute inflammatory pain at a dose of 200 mg/kg dose i.p., although it was much less effective than diclofenac [51]. However, compound 64 was reported to be more effective than diclofenac at equivalent doses in the carrageenan model on i.p. administration [52]. Moreover, it was effective in reversing formalin-induced flinches, thermal injury pain, and inflammatory pain in the complete Freund's adjuvant (CFA) model at the same dose. Importantly, this efficacy was seen in the absence of catalepsy, sedation and other CNS adverse effects that are typically seen with morphine. Hydra Biosciences signed a collaboration agreement with Pfizer Global Research & Development aimed at developing TRPV3 antagonists for treatment of pain in July 2007 [53]. As of today, there is no further development reported for any of their TRPV3 antagonists.

Glenmark is the second pharmaceutical company with public disclosure of a TRPV3 antagonist program. Glenmark reported a series of potent and selective TRPV3 receptor antagonists. GRC 15133 was the first compound to provide evidence of anti-hyperalgesic activity due to TRPV3 blockade in animal pain models [54]. It showed dose-dependant reversal of inflammatory mechanical hyperalgesia in the CFA model with an ED_{50} of 8 mg/kg on i.p. dosing. It also reversed CFA-induced thermal hyperalgesia at a dose of 30 mg/kg with maximum effect comparable to that of diclofenac. It was efficacious in reversing mechanical hyperalgesia in CCI model with an ED_{50} of 4.8 mg/kg on chronic dosing. GRC 17173 was the first potent, selective, and orally available antagonist to show efficacy in all pain models tested [55]. It reversed both CFA-induced thermal hyperalgesia and CCI-induced mechanical hyperalgesia with ED_{50} values of 1.26 mg/kg and 1.46 mg/kg, respectively,

Table 1.   TRPV3 Antagonists Profile and in Developmental Status

Compound	In Vitro		In Vivo Efficacy	Route of Administration	Developmental Stage
	Antagonism IC_{50}	TRP Selectivity (on V1, V4, M8, A1)			
Hydra Biosciences Compound # 64	200-300 nM - EP	> 39 fold	In Carrageenan Model efficacious at 200 mg/kg, less efficacious than Diclofenac 100 mg/kg	i.p.	No development reported
Hydra Biosciences Compound # 82	200-1000 nM - EP	> 40 fold	In Carageenan Model efficaceous at 200 mg/kg, efficacacy better than Diclofenac 100 mg/kg	i.p.	No development reported
Glenmark Pharmaceuticals GRC 15133	254 nM – Ca^{2+} influx	> 39 fold	ED_{50} between 4-8 mg/kg in CFA and CCI model, efficacy superior to Diclifenac and Pregabalin respectively	i.p.	Preclinical
Glenmark Pharmaceuticals GRC 17173	180 nM - Ca^{2+} influx	> 55 fold	ED_{50} between 1-2 mg/kg in CFA and CCI model, efficacy superior to Diclofenac and Pregabalin respectively	Oral	Preclinical
Glenmark Pharmaceuticals GRC 15300	Unpublished	> 100 fold	FCA, CCI, OA	Oral	Clinical Phase I

EP- Electrophysiology, ip - intraperitoneal, CFA - Freunds Coplete Adjuvant, CCI - Chronic Constiction injury, OA- Osteoarthirits.

upon oral administration. In preclinical studies, these antagonists produced sufficient evidence to justify clinical studies to investigate the possibility that TRPV3 blockers may ameliorate both thermal and mechanical hyperalgesia following nerve or inflammatory injury in man. Indeed, GRC 15300 had already entered phase I clinical trials [56]. GRC 15300 is a potent, selective, orally available TRPV3 antagonist whose pharmacokinetic properties are yet to be published. In addition to showing efficacy in inflammatory and neuropathic pain models, it also showed promising therapeutic action in MIA-induced osteoarthritis model where it reversed mechanical hyperalgesia which is typically experienced in the painful joints in human osteoarthritis. GRC 15691 is a second IND qualifying TRPV3 antagonist for clinical development.

TRPV3 ANTAGONISTS: SAFETY CONCERNS

The very existence of TRPV3 in keratinocytes raises the possibility of an undesirable side-effect of TRPV3 blockade on skin barrier integrity. As discussed above, TRPV3 is expressed in the skin, both in keratinocytes and hair follicles, and TRPV3 knock out mice showed subtle and transient hair irregularity in the abdominal area. Based on these findings, it was speculated that TRPV3 antagonists may have undesirable side-effects on skin barrier integrity. Detailed investigation of TRPV3 knock out mice, however, showed that there were no gross anatomical skin defects. Epidermal and dermal layer thickness, keratinocyte specific markers, and skin epidermal barrier integrity were all found to be normal [7]. By contrast, it was shown that in rodents with a constitutively acting mutant TRPV3 channel the channel over-activity results in a hair loss phenotype [57]. Also, in the "hair-less phenotype" of DS-nh animals, which is used as a model of atopic dermatitis, studies have confirmed role of TRPV3 "gain of function" mutation causing hairless phenotype [57, 58]. In summary, it is TRPV3 over-activity, and not blockade, that appears to be deleterious.

COULD TRPV3 ANTAGONISTS ALTER BODY TEMPERATURE?

Both TRPV1 and TRPV3 are thermoreceptors. Knock out mice for each of these receptors are similar in that they have normal core body temperature. However, pharmacological blockade of TRPV1 receptor produces hyperthermia in preclinical and clinical settings. With this knowledge, there seems to be a legitimate concern over possible adverse effect of thermoTRP channel antagonists on homeostatic control of body temperature. Do TRPV3 antagonists increase body temperature?

TRPV1 antagonists are believed to increase body temperature by causing skin vasoconstriction and cold-induced thermogenesis [59]. A TRPV1 receptor located in the GI tract is believed to function as body temperature regulator. TRPV1 and TRPV3 genes lie on the same chromosome and are believed to have arisen due to gene duplication event. TRPV3 senses a wide range of temperatures including the physiological range of 33-39°C and non-physiological range up to 50°C. On the contrary, TRPV1 is activated only at non-physiological temperatures. Considering these important differences, it rather seems a more likely possibility that while TRPV1 functions as a temperature regulator, TRPV3 may just be a temperature sensor.

Another important difference between TRPV1 and TRPV3 receptor pharmacologies is that TRPV1 agonists (capsaicin and resiniferatoxin) cause hypothermia in preclinical species. Thus, TRPV1 antagonist may produce hyperthermia as a consequence of suppression of endogenous agonist-induced hypothermia. TRPV3 receptor agonists are not known to produce hypothermia after receptor activation. This hypothesis is in accord with the important observation that TRPV3 over-expressing transgenic mice do not show subnormal rectal temperature compared to the wild type mice [37].

Most important is the evidence from preclinical studies with TRPV3 selective antagonists. Temperature effects of TRPV3 antagonists of Hydra Biosciences are not described in literature. However, none of the TRPV3 selective antagonists synthesized by Glenmark produce significant body temperature changes in rodents or ferrets (unpublished data). This was observed even at high doses beyond efficacious dose range. Phase I clinical trials with GRC 15300 are on-going.

COULD TRPV3 ANTAGONISTS AFFECT HEAT SENSITIVITY?

Since TRPV3 blockade ameliorates thermal hyperalgesia, it could be speculated that TRPV3 antagonists may interfere with heat sensation, raising concerns about safety under sun exposure. Indeed, this was observed in clinical studies with specific TRPV1 antagonists [60]. Of note, some TRPV1 antagonists synthesized by Merck also interfered with the ability of volunteers to distinguish between safe and noxiously hot food, drinks, and showers. At present, it is unclear if this unwarranted side-effect is specific for MK-2295 or a general problem with TRPV1 antagonists. Total attenuation of thermosensation might be responsible for ablation of normal heat sensation to hot drinks and sunburns. A selective TRPV3 antagonist is expected to be devoid of any such effect since its function is expcted be taken over by compensatory thermoTRPs such as TRPV1 (hot) and TRPV4 (warm) to restore normal warmth and heat sensation. Additional preclinical studies to prove normal thermo-sensation with this class of antagonists may allow a clearer differentiation of TRPV3 receptor blockade from other TRPs in pain.

TRPV3 ANTAGONISTS – WILL THEY FIND A NICHE IN PAIN MANAGEMENT THERAPY?

TRPV3 has a well-defined tissue distribution [8]. At the same time, its presence on peripheral nerves and its abundance in skin keratinocytes implies a pivotal role for TRPV3 in sensory pathways. Peripheral nerve activation has been identified as a causative event behind neurogenic inflammatory responses during acute and chronic pain conditions. Neurogenic inflammation encompasses a series of vascular and non-vascular inflammatory responses, triggered by the activation of and subsequent release of inflammatory neuropeptides. Hence, targeting an upstream-located pain receptor like TRPV3 provides an attractive opportunity for early therapeutic intervention of pain signal transmission, thereby providing better disease control.

Over the past few years, there has been significant progress in human experimental pain research and new techniques have been developed for induction and measurement of mechanistically distinct pain responses. Clearly, availability of a human experimental TRPV3 receptor-dependant pain model could be instrumental in smooth transitioning of TRPV3 antagonists from preclinical to clinical pain studies.

In conclusion, the TRPV3 receptor, being positioned upstream in the pain pathway, provides a logical strategy to start therapeutic intervention at the beginning of pain chain. The TRPV3 antagonist GRC 15300 has entered into clinical trials after demonstration of promising preclinical efficacy

and safety. We are poised to discover soon if TRPV3 antagonists prove superior to or at-least equivalent in, efficacy to existing analgesic agents in the absence of adverse effects of other centrally acting drugs in treatment of pain. We predict that TRPV3 antagonists will remain in the focus of drug discovery and development. Outcome from proof-of-concept clinical trials will determine if they find a specific therapeutic niche in pain management.

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