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Research Article

"AN INVESTIGATION OF ANALGESIC PROPERTIES OF CLEFMA IN TRPA1 RECEPTOR AGONIST-MEDIATED ANIMAL MODELS OF NOCIFENSIVE BEHAVIOR" AITC-INDUCED EYE WIPE TEST

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A B S T R A C T

Back Ground: Pain can be defined simply as the subjective experience of harm in a part of one's body. In reality, however, there are multiple forms of pain, which involve a variety of distinct biological processes. Exposure to extreme heat, cold, or pressure can be noxious, triggering nociceptive pain. Inflammatory pain, involving the release of cytokines and the infiltration of immune cells, also occurs after injury but can be triggered independently by bacterial infections[1, 2]. Peripheral nociceptive neurons express a multitude of ion channels that have a key role in adaptive and maladaptive pain. These channels participate in the transduction of noxious stimuli into electrical activity in the periphery, enable the propagation of electrical signals along peripheral nerves, and gate the synaptic transmission of information in the spinal cord[1]. Like nociceptive pain, normal inflammatory pain signals that certain body parts have suffered damage and should therefore be protected from subsequent harm. However, inflammatory pain is triggered by the cross-talk between the immune and nervous systems. Interestingly, the recruitment of the immune system after tissue damage initiates a cascade of molecular and cellular processes that mediate the progressive repair of the injured tissues, thereby actively promoting healing. Many of the molecules involved in these processes are lipids and can be either proalgesic or analgesia [3, 4].

Aim & Objective: The present research program aimed to evaluate the analgesic properties of CLEFMA in TRPA1 receptor agonist-mediated animal models of nocifensive behavior by Evaluation of analgesic properties of CLEFMA in AITC-induced eye wipe test.

Materials and Methods: AITC-induced eye wipe test: Protocol: Acclimatize rats for 2-3 minutes in a 30 X 30 X 30 cm plexiglass chamber. Prepare AITC 1000µM stock solution by dissolving it in 90% Ethanol. Dilute the stock solution to prepare 100 µM stock. Administer the drug before AITC application onto the eye. Administer 30µl of AITC (100 µM stock) to the left eye of the rat.

Groups: Graded concentration of 3, 10, 30, 100, 300, 900 mg/kg/ml of CLEFMA samples, Positive Control (Tramadol 60mg/kg/10ml), Negative Control, Vehicle, 1-8 cineole (TRPA1 antagonist), 900mg/kg/ml + 1-8 cineole (TRPA1 antagonist)
Observations: Keep the rat in the plexiglass chamber immediately after the application of AITC (100 µM stock) to the left eye of the rat. Start counting the number of eye wipes per minute. Count the number of eye wipes for 5 min.

Results: The above results clearly show that CLEFMA 100 mg/kg, 300mg/kg, and 900 mg/kg significantly reduce the number of AITC-mediated eye wipes. The above results for CLEFMA validate the results of our previous experiment where CLEFMA acts only on phase one of formalin response. Moreover, in the presence of the TRPA1 antagonist CLEFMA 900mg/kg failed to contain the AITC-induced eye wipes indicating that CLEFMA mediated through TRPA1 receptors in AITC-induced nocifensive behavior. Both AITC and Formalin mediate through TRPA1 receptors.

The antagonists acting on the TRPA1 receptors act mainly by desensitizing the receptors. The minimal drop in eye wipes might be due to the effect of CLEFMA on TRPV1 receptors. CLEFMA is known to interact with TRPA1 and TRPV1 receptors, however, the presence of an antagonist may mediate through the other available receptors.
Conclusions: The current study was planned to elucidate the mechanism of CLEFMA-mediated suppression of TRPA1 agonist-mediated nocifensive behavior. From the above

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study, we observed that apart from TRPV1 receptors, CLEFMA mediates through TRPA1 receptors. The function of TRPA1 in those cells is still poorly understood. Given the widespread potential targets for TRPA1 modulators, it is essential to understand the genetics, biophysics, and physiological role of this fascinating TRPA1 channel.

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INTRODUCTION

Pain can be defined simply as the subjective experience of harm in a part of one's body. In reality, however, there are multiple forms of pain, which involve a variety of distinct biological processes. Exposure to extreme heat, cold, or pressure can be noxious, triggering nociceptive pain. Inflammatory pain, involving the release of cytokines and the infiltration of immune cells, also occurs after injury but can be triggered independently by bacterial infections[1, 2]. Peripheral nociceptive neurons express a multitude of ion channels that have a key role in adaptive and maladaptive pain. These channels participate in the transduction of noxious stimuli into electrical activity in the periphery, enable the propagation of electrical signals along peripheral nerves, and gate the synaptic transmission of information in the spinal cord[1].

Like nociceptive pain, normal inflammatory pain signals that certain body parts have suffered damage and should therefore be protected from subsequent harm. However, inflammatory pain is triggered by the cross-talk between the immune and nervous systems. Interestingly, the recruitment of the immune system after tissue damage initiates a cascade of molecular and cellular processes that mediate the progressive repair of the injured tissues, thereby actively promoting healing. Many of the molecules involved in these processes are lipids and can be either proalgesic or analgesia [3, 4].

Noxious information is processed centrally and unlike the cortex, little is known about the cellular repertoire and the wiring rules of the dorsal horn of the spinal cord. Our incomplete understanding of circuit architecture in the spinal cord and of how it connects with primary afferents may explain the lack of consensus about how noxious information is processed in the CNS. The growing evidence for cross-talk between somatosensory labeled lines lends support to a theoretical framework according to which the nociceptive system operates under combinatorial encoding rules [3, 4]. Fundamentally, pain is a subjective experience that can vary greatly among individuals, even for identical noxious stimuli, and there is great variability in how long patients experience pain when healing from injuries. What explains these differences? What makes certain individuals more susceptible or more resilient to developing chronic pain? Early life stress, mood disorders, existing pain conditions, and genetic mutations are some of the factors that can modulate the risk of triggering the transition from acute to chronic pain[2].

Although itch is not pain, these two sensations share many features. Like removing our hand from a burning flame, scratching an insect bite is a response to a potentially harmful stimulus. The functions of the perceptive and nociceptive systems also appear to be tightly linked. Some analgesics can induce itch and some pruritogens can cause pain. Moreover, suppressing itch can cause pain and, reciprocally, inducing pain can relieve itch. As a consequence of the functional and anatomical proximity of these two systems, the study of itch has often had implications for our understanding of pain.

Sensory information from the periphery is carried to the brain in the form of action potentials. Therefore, the energy induced by sensory stimulation needs to be translated into electrical signals in a process called transduction. In the sense of pain, the transduction of noxious (tissue-damaging or pain-inducing) stimuli takes place at peripheral terminals of nociceptive (pain-mediating) nerve fibers. In light microscopy, the peripheral endings of nociceptive nerve fibers look like free nerve endings. The cell membrane of the free nerve ends expresses various types of receptors that are specialized for transducing mechanical, thermal, and/or chemical stimuli into electrical signals. The sensitivity of these membrane receptors varies with the receptor type providing a molecular basis for the submodality selectivity of pain-related signaling [4]. Among the membrane receptors contributing to the transduction of noxious signals on free nerve endings are those belonging to the transient receptor potential (TRP) family of ion channels. The most widely studied member in the TRP family is the transient receptor vanilloid 1 (TRPV1), a channel activated by noxious heat, low pH, and capsaicin, the pungent component of chili peppers [5, 6]. Another widely studied member of the TRP family expressed on nociceptive nerve terminals is transient receptor potential ankyrin 1 [5, 6]. TRPA1 is the only TRPA subfamily member of TRP channels in mammals, and it is expressed in a subpopulation of TRPV1-expressing nociceptive nerve fibers. It has been estimated that about 30% of the TRPV1-expressing neurons co-express TRPA1 [7-10]]. TRPA1 was originally considered to detect noxious cold (cold pain), while later studies indicate that it is involved in the detection of cold hypersensitivity rather than physiological cold pain. Additionally, TRPA1 is activated by various irritant chemicals and endogenous products of tissue injury (see for further details section Peripheral Drivers of Central TRPA1-Mediated Actions). Activation of TRPA1 and TRPV1 channels leads to an influx of sodium and calcium ions, which induces depolarization of the nociceptive nerve ending needed to generate the centrally propagating nociceptive signal. TRPA1 is expressed not only on peripheral but also on central terminals of nociceptive primary afferent nerve fibers. On central terminals located within the spinal dorsal horn, TRPA1 amplifies glutamate release and thereby transmits nociceptive signals to spinal interneurons and projection neurons [7-10]. Among non-neuronal cells expressing TRPA1 are epidermal keratinocytes, endothelium, vascular smooth muscle, and astrocytes [7-10].

TRPA1 is a nociceptor-specific ion channel expressed in a subset of TRPV1-expressing neurons. As TRPA1 is specific to certain nociceptors, it is an attractive target for various therapeutic conditions such as pain, asthma, and cough [7-10]. So far, several classes of chemicals have been demonstrated to activate TRPA1, including multiple endogenously produced reactive electrophilic mediators such as nitrated fatty acids, 15-deoxy-12,14-prostaglandin J2 (15d-PGJ2), 4-HNE, and 4-

ONE [7-10]. Natural products (NPs) form the basis for many widely used drugs. Turmeric is a member of the ginger family (Zingiberaceae) and is prescribed abundantly for ailments in both traditional Chinese and Indian medicine. Curcuma longa has been traditionally used in Asian countries as a medical herb due to its antioxidant, analgesic, anti-inflammatory, antimutagenic, antimicrobial, and anticancer properties. Major phytoconstituents of turmeric are diarylheptanoids, which occur in a mixture termed curcuminoids that generally makeup approximately 1−6% of turmeric by dry weight. Most crude extracts prepared from turmeric, and even some refined "curcumin" materials, contain three major compounds: curcumin [1, (1 E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptane-3,5-dione, typically 60−70% of a crude extract], demethoxycurcumin (3,20 −27%), and bisdemethoxycurcumin (4, 10 −15%), along with numerous and less abundant secondary metabolites[11-29]. Curcumin, feruloyl methane ((E, E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptane-3,5-dione), has demonstrated benefit in multiple inflammatory conditions in animals and humans. In-vitro studies show Curcumin mediates through TRPA1 receptors, however, supportive in-vivo studies are not available [11-29].

Various curcumin derivatives have been developed that may turn out to be potential candidates for inflammation and nocifensive behavior. Although our understanding of the chronic effects of curcumin is growing, we have limited information about the acute effects on ion channels. It is well documented that many TRPA1 agonists like alkenyl aldehydes (4-hydroxynonenal and 4-oxo-nominal) and 15d-PGJ2 activate TRPA1 by forming Michael adducts with intracellular N-terminal cysteine residues of the channel. Similar to pungent plant-derived TRPA1 agonists such as allyl isothiocyanate (AITC) and diallyl disulfide (DADS), curcumin is also a reactive electrophilic molecule capable of forming adducts with free thiols in molecules such as reduced glutathione (GSH) [5-10].

Although pain has an important physiological role in preserving the integrity of the body, pathological pain also exists. Nerve damage, in surgery patients for instance, sometimes leads to chronic pain conditions that can last years or even decades. Unfortunately, many pathological pain conditions remain poorly understood and resist currently available treatments.

Developing new therapeutic approaches to managing pain will undoubtedly depend on a better understanding of the molecular, cellular, and circuit mechanisms underlying acute and chronic pain states. In the current research, we evaluate the analgesic properties of curcumin in TRPA1 receptor agonist-mediated animal models of nocifensive behavior.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (150–200 g) purchased from Sainath Agencies, Hyderabad were used in all of the experiments. The animals were housed in groups of 3 in a cage in a controlled temperature environment maintained at 22 ± 1^0C with a 12-h light/dark cycle (lights on from 6:00 a.m. to 6 p.m.) and fed standard food pellets and tap water ad libitum. The animals were acclimated to the experimental room for at least 2 h before the experiments. All of the experiments were carried out according to the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals. The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatments. All of the protocols were approved by the Animal Ethics Committee of A. M. Reddy Memorial College of Pharmacy.

Chemicals

CLEFMA, 1-8 cineole, AITC, Formaldehyde, PEG 400, and Cremophor-EL were purchased from Sigma Aldrich. CLEFMA was dissolved in a solution of PEG-400 (66.7%) and Cremophor-EL (33.33%). The dilutions from the stock concentrations were prepared by diluting with Phosphate Buffered Saline (pH 6.1). 1-8 cineole was emulsified with 2% Tween 80 to achieve the desired concentration. Formaldehyde (37-41%) was diluted with normal saline to get 2.5% Formalin.

METHODS

AITC-induced eye wipe test

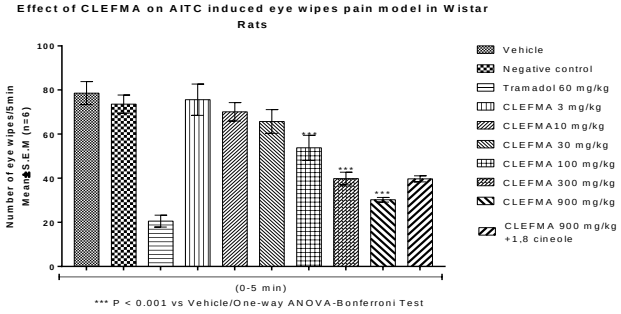
Protocol: Acclimatize rats for 2-3 minutes in a 30 X 30 X 30 cm plexiglass chamber. Prepare AITC 1000µM stock solution by dissolving it in 90% Ethanol. Dilute the stock solution to prepare 100 µM stock. Administer the drug before AITC application onto the eye. Administer 30µl of AITC (100 µM stock) to the left eye of the rat.

Groups: Graded concentration of 3, 10, 30, 100, 300, 900 mg/kg/ml of CLEFMA samples, Positive Control (Tramadol 60mg/kg/10ml), Negative Control, Vehicle, 1-8 cineole (TRPA1 antagonist), 900mg/kg/ml + 1-8 cineole (TRPA1 antagonist)

Observations: Keep the rat in the plexiglass chamber immediately after the application of AITC (100 µM stock) to the left eye of the rat. Start counting the number of eye wipes per minute. Count the number of eye wipes for 5 min.

Data Analysis: Results will be shown as % inhibition of Eye wipes per 5 min (Mean ± S.E.M) for each group. One way of ANOVA will compare the significant difference with vehicle control rats.

RESULTS AND DISCUSSION



The above results clearly show that CLEFMA 100 mg/kg, 300mg/kg, and 900 mg/kg significantly reduce the number of AITC-mediated eye wipes. Moreover, in the presence of the TRPA1 antagonist CLEFMA 900mg/kg failed to contain the AITC-induced eye wipes indicating that CLEFMA mediated

through TRPA1 receptors in AITC-induced nocifensive behavior. AITC mediates through TRPA1 receptors.
The antagonists acting on the TRPA1 receptors act mainly by desensitizing the receptors. The minimal drop in eye wipes might be due to the effect of CLEFMA on TRPV1 receptors. CLEFMA is known to interact with TRPA1 and TRPV1 receptors, however, the presence of an antagonist may mediate through the other available receptors.

CONCLUSION

The current study was planned to elucidate the mechanism of CLEFMA-mediated suppression of TRPA1 agonist-mediated nocifensive behavior. From the above study, we observed that apart from TRPV1 receptors, CLEFMA mediates through TRPA1 receptors.

TRPA1 is a chemosensory channel that constitutes both an attractive drug target and a model channel for studying structural and biophysical properties. The TRPA1 gating mechanisms are still poorly understood. Although it is well accepted that reactive compounds activate the channel via covalent modification of nucleophilic sites in the N terminus, the chemical reactions are not well understood. It is still unclear how channel activation is switched off, and second, how and which biochemical processes control the viability of the channels for subsequent activation after inactivation (desensitization). Since activation of TRPA1 can also occur via a more classical lock-and-key activation, binding sites for these non-electrophilic activators and their role as possible open pore blockers need to be clarified. A better understanding of these mechanisms is critical for screening novel TRPA1 modulators that might be used as possible therapeutic agents. In this regard, it is also important to differentiate between the beneficial effects of the TRPA1 block versus the effects of TRPA1 activation via slow depolarization and subsequent inactivation of voltage-dependent Na^+ or Ca^{2+} channels.

A unique feature of TRPA1 is its huge potential for regulation by dietary intake of channel modulators. Surprisingly, we still do not know which concentrations of these compounds are reached in target organs and what downstream targets of such TRPA1 activation are. It is well established that TRPA1 mediates acute and chronic pain and plays an important role in the initiation but also progression and maintenance of chronic inflammatory diseases and tissue injuries, including asthma, diabetes, arthritis, and skin diseases. In addition to the many effects of neuronal cell types, TRPA1 is an important channel in non-sensory tissues, such as epithelium, smooth muscle cells, and fibroblasts. The function of TRPA1 in those cells is still poorly understood. Given the widespread potential targets for TRPA1 modulators, it is essential to understand the genetics, biophysics, and physiological role of this fascinating TRPA1 channel.

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