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Potential Therapeutic Effects of Snake Venom Components on Pain Management in Rheumatoid Arthritis Patients

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Abstract

This paper reviews the existing literature on the uses of snake venom in the treatment of rheumatoid arthritis and pain management. Venom from the families *Elapidae* and *Viperidae* have been shown to have anti-inflammatory and analgesic effects. The analgesic findings of research on arthritis murine models are discussed, and the existing uses of snake venom in medicine on the therapeutic potential of venom in the pain management of rheumatoid arthritis are taken into account. Snake venom has anti-inflammatory effects by reducing levels of pro-inflammatory cytokines and increasing levels of anti-inflammatory cytokines. Additionally, snake venom can reduce structural damage from prolonged inflammation by acting as a TNF-alpha blocker, and by inhibiting the proliferation of fibroblast-like synoviocytes. The mechanisms of snake venom pain modulation seen in murine pain models follow the cholinergic and opioidergic systems. Analgesic findings involving the cholinergic system concluded not only that the effects of snake venom have similar effects to morphine, but also that no withdrawal symptoms were observed after administration of venom stopped. Notably, the studies that determined opioidergic mechanisms to pain modulation observed that snake venom targets kappa and delta opioid receptors instead of the mu receptors, which are more involved in addictive behavior. Tolerance was not observed with intermittent administration of venom. These results show incredible promise for a non-addictive analgesic that could be used for pain management in rheumatoid arthritis patients.

Introduction

Venomous snakes such as those in the viper and elapid families are perceived as dangerous animals on a global scale. While this is not without good reason and snake bites do contribute to morbidity in human populations (between 81,000 and 138,000 deaths and around three times as many amputations and other permanent disabilities each year (World Health Organization [WHO], 2021)), snake venom components have beneficial application in the medical world as well. Ayurveda, a holistic healing system in India that dates back more than 5,000 years, exemplifies these contrasting properties (Lad, 2006). Snake venom is mentioned not only in Visha Chikitsha (the section of Ayurveda that focuses on treatment of poisoning) in regard to antivenom and treating bites, but also as a therapeutic agent for various diseases due to its physiologically active components (Sudhakar et al., 2017). More specifically, cobra venom has been used historically in Ayurveda in the treatment of arthritis and other chronic diseases (Gomes, 2010).

Not only does the use of snake venom in medicine have historical context, but it has more current applications as well. Chinese physicians are implementing the use of snake venom products to treat stroke patients, and research has been conducted surrounding its analgesic, anti-cancerous, and anti-inflammatory effects (Sudhakar et al., 2017). Additionally, six venom-based drugs have been FDA approved and are utilized for procedures and pathologies such as plastic and abdominal surgery, hypertension, heart attacks, and stroke (Abd El-Aziz, 2019). Ancord, a drug that was developed from Malayan pit viper venom and used to treat ischemic attacks due to its anti-coagulating and defibrinogenating properties is paving the road for the introduction of venom in the pharmaceutical world (Sherman, 2002).

From an adaptive perspective, snake venom primarily functions as a mechanism with which the organism can immobilize/pre-digest prey and defend itself. There are four families that are entirely or at least partly composed of venomous snake species: Elapidae, Viperidae, Hydrophiidae, and Colubridae (Dodd-Butera, 2014). This review will specifically focus on families Elapidae and Viperidae, as a large majority of the research done surrounding the medical application of snake venom involves species within these groups. Both elapids and vipers are front fanged snakes that belong to the superfamily Colubroidea. Notable species of the elapid family are cobras of the genus *Naja*, and a well researched species in the viper family is *Crotalus durissus terrificus.* Venom from each of these families varies in composition, yet shares certain features that make them candidates for successful therapeutics in a variety of pathological conditions.

There are three primary categories of venoms based on their physiological and pharmacological effects: hemotoxins, neurotoxins, and cytotoxins. Hemotoxins act on the cardiovascular system, neurotoxins affect the nervous system, and cytotoxins cause damage to cells. Snake venom is often described as a complex cocktail of biologically active components including enzymes and other proteins, as well as amines, lipids, nucleosides, carbohydrates, and metal ions. The composition of snake venom varies drastically among species and even across geographical areas within the same species (Powell, 2005). Previous studies have found that some individual venoms have around 100 components, and it is likely that around 90-95% of the dry weight is made up of proteins and peptides (Abd El-Aziz, 2019).

The unique composition of snake venom makes it an abundant source of possibilities and blueprints for human therapeutics. One particular region where there is potential is in the treatment and symptom management of inflammatory diseases. Specifically, venom derived

medications could be an avenue with great potential in the treatment of rheumatoid arthritis (RA), due to the anti-inflammatory and analgesic properties that snake venoms have been found to possess.

The purpose of this paper is to review existing literature on the therapeutic potential of snake venom components for pain management in rheumatoid arthritis patients. While there are many current therapies targeting inflammation in rheumatoid arthritis patients, very few specifically target the alleviation of pain, which is the top priority symptom for improvement in most RA patients. RA pain is multifaceted, and therefore the approach to pain management must be as well. Sources and mechanisms of pain and hence its treatment in RA include inflammation, structural damage, pain sensitization, and central pathways like the cholinergic and opioidergic systems. Existing research on snake venom in murine models has offered promising outcomes for the alleviation of inflammation and associated pain in arthritis, and other research has observed analgesic actions of snake venom in other diseases and areas of human health. The combination of this research provides the framework on which this paper will summarize current knowledge and potential avenues of study in the development of pain-targeting medications for RA patients.

Background Information: Rheumatoid Arthritis Research and Therapeutics

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease in which the immune system primarily attacks healthy tissue of synovial joints (NIH). The disease affects between 0.5-1.0% of the developed world population, and is a significant cause of disability in the United States as well as worldwide (Boonen, 2011). The primary characteristic of RA is the progressive destruction and inflammation of synovial joints, most commonly in metacarpophalangeal, proximal interphalangeal, metatarsophalangeal, wrist, and knee joints

(Grassi et al., 1998). Articular manifestations include symmetric joint swelling, tenderness, stiffness, and motion impairment, and general symptoms such as fevers, fatigue, weight loss, and discomfort are also common (Grassi et al., 1998).

Of the entire rheumatoid arthritis symptom picture, pain has consistently been the primary reason why patients visit a rheumatologist. A 2005 study found that while the general health status of RA patients in Norway improved between the years of 1994 and 2001, alleviation of pain remained the highest priority in both cohorts (Heiberg et al., 2005). In another study, 88% of participants selected pain as their top priority for improvement during a year of treatment (ten Klooster et al., 2007). Pain scores are also disproportionately greater in women, minorities, and those with lesser levels of education, and pain is a top contributor to emotional health in RA patients (Wolfe, 2007; Lee, 2013).

One of the main treatments for pain in RA patients is the administration of disease modifying antirheumatic drugs (DMARDs), which act peripherally to reduce the inflammatory response and the pain associated with it. Additionally, non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen are often suggested to patients to manage their pain. These medications can be coupled with over the counter medications such as acetaminophen to further alleviate pain. When the combination of NSAID and acetaminophen administration has failed to provide relief, weak opioids are considered (Lee, 2013). Despite the analgesic effects that oral opioids provide, adverse reactions such as nausea, vomiting, dizziness, and constipation are frequent and may offset the benefits (Whittle et al., 2011). Additionally, the use of opioids poses the risk of dependence on the medication, as well as the development of opioid induced hyperalgesia, or enhanced sensitivity to pain (Crofford, 2010).

Therapies for RA have generally shifted focus from symptom management to the treatment of underlying inflammation that causes the symptoms (Colmenga et al., 2012). Biologic disease modifying drugs are another ever expanding category of current medications available for treatment of RA. These also act to reduce immune responses in the body. For example, TNF inhibitors are used to block tumor necrosis factor (a proinflammatory cytokine) activity. Similarly, Abatacept prevents the overactivity of T cells, and Tocilluzumab inhibits the activity of another proinflammatory protein, IL-6 (Johns Hopkins Arthritis Center, 2018).

The development of new biologic disease modifying drugs and other therapies that target inflammation offers promise for slowing disease progression and joint damage in RA patients. However, even when inflammation has been controlled, patients will often experience flare ups, or periods of symptom exacerbation and increased pain. Additionally, pain flare ups can occur without the swelling or warmth that is associated with inflammation (Hewlett et al., 2012). The alleviation of inflammation has not been exactly correlated with the alleviation of pain, suggesting the presence of other non-inflammatory contributions to pain in RA patients as well. Although the use of DMARDs is effective in reducing some inflammatory pain, they are not made to provide immediate pain relief and many patients will still suffer from at least moderate pain alongside adverse effects (which may be amplified by certain analgesic medications) while taking these medications (Wolfe, 2007).

While the pathways are complex and not entirely understood, it is agreed that different types of pain mechanisms all contribute to chronic pain experienced by rheumatoid arthritis patients, including peripheral and central mechanisms of pain. There have been few clinical trials examining the efficacy of treatments specifically targeting pain mechanisms, and further

research in this area is needed to discover effective methods of managing pain on both the peripheral and central level (Lee, 2013).

Pain Mechanisms and Venom Effects

The current understanding of rheumatoid arthritis pain divides pathways into inflammatory and non-inflammatory pain, and these categories can further be divided into varying peripheral and central mechanisms. The majority of current therapies target peripheral inflammatory pain, and further research is needed on the CNS pain processing abnormalities in rheumatoid arthritis. This paper will outline these pain mechanisms and describe the existing evidence of, and potential future directions for, therapeutic application of venom on each category of pain.

Inflammation is an important contributor to pain in RA, and has acute, immediate effects as well as long term effects that lead to pain even in the absence of the inflammation. Chemical mediators of inflammation including cytokines contribute to pain, and venom components have been found to reduce inflammatory cytokine levels and increase anti-inflammatory cytokine levels. Persistent inflammation can lead to structural changes of the joint and bone, further contributing to pain. Reductions in certain pro-inflammatory cytokines have been shown to decrease not only the inflammatory response but also the structural damage associated with it. Additionally, pathological activity of fibroblast-like synoviocytes which lead to bone erosion and pain could be reduced by snake venom.

Sensitization is also a key component of pain in RA. Sensitization can occur peripherally leading to primary hyperalgesia, or centrally causing secondary hyperalgesia, both of which refer to an increased sensitivity to stimuli and greater transmission of pain signals (Vardeh & Naranjo, 2017). Mechanical and thermal hyperalgesia have been found to be suppressed in several

murine models with the administration of snake venom. Inflammation can also affect central pain processing, so a decrease in inflammation with snake venom could positively affect central pain and sensitization as well.

The effect of snake venom from elapids and vipers on cholinergic and opioidergic mechanisms of pain are arguably the most promising relevant to treating non-inflammatory pain in diseases such as rheumatoid arthritis. In one study, snake venom acting on cholinergic receptors to produce analgesia was found to be just as effective as morphine, with a longer lasting effect (Cheng et al., 2009). Additionally, several studies have found that snake venom targets opioid receptors, but not necessarily the subtype that is most involved in addictive behavior and response. This finding could warrant further research to establish whether snake venom could be as effective as opioid analgesics without the negative effects of tolerance and addiction.

The effects of snake venom will now be highlighted and explained in further detail as they apply to specific pain pathways relevant to rheumatoid arthritis. The majority of the papers discussed describe studies on murine models, though human trials with snake venom have been conducted and are introduced later in the paper.

Peripheral Inflammation and Pain

Peripheral inflammation has generally been described as the main cause of pain in RA patients, and pain is considered to be one of the four cardinal signs of inflammation. During inflammation, chemical mediators such as bradykinin and prostaglandins are released to heal tissue damage, and subsequently cause pain and hyperalgesia by opening TRP channels and reducing the threshold at which they open. Additionally, swelling and tissue buildup associated

with inflammation can compress nerve endings, sending pain signals to the brain. Reducing inflammation is undoubtedly associated with the reduction of peripheral pain caused by it.

Proinflammatory cytokines, chemical messengers produced by activated macrophages, play a significant role in both the initiation and persistence of pain in autoimmune diseases such as RA by upregulating inflammatory responses. These include several interleukins (IL), including IL-1β, IL-6, IL-8, and IL-17, as well as tumor necrosis factor alpha (TNF-α). IL-1β has been found to produce hyperalgesia by increasing the production of substance P (a neurotransmitter involved in nociception) and prostaglandin E2, a mediator in RA inflammation and pain (Zhang, 2007). This pronociceptive action is likely mediated by signaling cascades that lead to bradykinin, prostaglandin, and nitric oxide production (Sommer & Kress, 2004). TNF-α stimulates the production of other proinflammatory cytokines in a positive feedback manner, creating a cytokine cascade which activates cyclooxygenase enzyme conversion of arachidonic acid to prostaglandin, decreasing pain thresholds.

Anti-inflammatory cytokines such as IL-4, IL-10, IL-11, and IL-13 are molecules that mediate the pro-inflammatory cytokine response. For example, IL-10 decreases the expression of the pro-inflammatory cytokines mentioned above, and inhibits macrophage activity and activation, which are processes that promote inflammation (Zhang, 2007; Gomes et al., 2010). Shifting the balance from greater levels of pro-inflammatory cytokines to increased anti-inflammatory cytokines in RA patients could reduce inflammation and the pain associated with it. The following studies have observed the anti-inflammatory effects of snake venom components on murine arthritis models, and many have produced results suggesting that venoms can produce this positive shift from proinflammatory to anti-inflammatory cytokines.

A handful of studies have utilized venom from elapids, particularly the species *Naja kaouthia* and *Naja naja,* in murine arthritis models to study the anti-inflammatory and anti-arthritic properties of the venom or its specific components. Gomes et al. (2014) observed the effects of NN-32, a cytotoxic protein from *Naja naja* venom, on arthritic rats. It was found that while arthritic rats showed significantly increased levels of inflammatory cytokines TNF-α, IL-17, and cytokine-induced neutrophil chemoattractant 1 (CINC-1, a rat cytokine (homolog of IL-8) with hyperalgesic properties) compared to non-arthritic control rats, NN-32 treatment significantly decreased levels of these cytokines. Another study by the same researchers found that IL-10 levels were decreased in adjuvant induced arthritic rats, but the levels were significantly restored when treated by *Naja kaouthia* venom (Gomes et al., 2010).

Liu et al. (2009) produced similar results using cobratoxin, a neurotoxin from a *Naja* cobra, on complete Freund's adjuvant (CFA) induced arthritis rats. The arthritic rats showed increased serum levels of TNF-α, IL-1, and IL-2, and decreased levels of IL-10. With the cobratoxin treatment, the rats exhibited lower proinflammatory cytokine levels, and a reversal of the CFA induced IL-10 decrease. Ruan et al. (2013) found similar results with neurotoxin-NNA, another peptide from *Naja naja atra*: Treatment with the peptide exhibited a dose dependent decrease in TNF-α and IL-1β levels in rat models of inflammation. These studies add to the evidence that cobra venom could modulate the production of inflammatory cytokines in RA and subsequently reduce inflammatory pain.

A study by Zhu et al. (2016) compared the effects of cobratoxin from *Naja naja atra* to dexamethasone, a corticosteroid that relieves inflammation. Dexamethasone administered to arthritic rats showed greater effects on acute inflammation than the cobrotoxin, but inhibition of

the long-term inflammatory process (observed by a decrease of cytokines IL-6, TNF-α, and IL-1β) was strong in both dexamethasone and cobratoxin treated rats.

Another experiment that orally administered cardiotoxin (CTX) from *Naja naja atra* to adjuvant-induced arthritic rats showed that CTX treatment significantly lowered serum IL-6 and IL-17 levels compared to those without treatment (Chen et al., 2015). The study found no difference in IL-10 levels between control and arthritic rats, regardless of whether they were treated with CTX. The maintenance of the levels suggests that orally administered CTX has anti-inflammatory properties by decreasing pro-inflammatory cytokine levels and maintaining pro-inflammatory cytokine levels. This study specifically assessed the analgesic effects of CTX by using the formalin and acetic acid writhing tests, and compared the effects of CTX to that of aspirin (used as a positive control in the model). Rats treated with CTX showed slightly greater anti-inflammatory and analgesic effects, suggesting the potential for components of venom to function as NSAIDs (Chen et al., 2015).

Joint Destruction

Prolonged inflammation of the joint in result of an autoimmune response causes synovial membrane alteration and bone destruction, both of which subsequently lead to pain. Several studies have confirmed that arthroplasty significantly reduces pain in patients with arthritis, indicating that structural changes due to inflammatory damage persist beyond the inflammatory pain caused from the initial autoimmune attack (McWilliams & Walsh, 2017).

The use of tumor necrosis factor (TNF) blockers, a more recent therapeutic option for RA, provides a correlation between the cytokine TNF-α and bone erosion. Several studies have found that the five TNF blockers that are currently in use have all been correlated with continued inhibition of bone erosion (Schett, 2011). The positive effect of TNF inhibitors provides

evidence that a decrease in the cytokine TNF-α could have beneficial effects on reducing not only initial inflammatory pain but also pain induced by bone erosion and other structural changes. Therefore, snake venom components that have been found to decrease inflammatory cytokine levels, including TNF-α, could play a role in decreasing pain in RA patients.

The previously mentioned papers from Gomes et al., Zhu et al., and Ruan et al. all found a decreased level of TNF-α with snake venom treatment on rat models. Additionally, Zhu et al. (2016) found that cobrotoxin from *Naja naja atra* venom inhibited the activation of nuclear factor kappa B (NF-κB). NF-κB is a transcriptional factor that plays a role in inflammation by expressing pro-inflammatory cytokines, including TNF-α, and inhibition of NF-κB has been shown to delay progression of joint destruction in animal arthritis models. Another study also found that cobrotoxin has an inhibitory effect on NF-κB activation, which led to decreased levels of TNF-α (Park et al., 2005). These studies indicate that cobra venom can decrease proinflammatory cytokine levels, affecting not only inflammatory pain but also pain associated with physical destruction of the joint.

Fibroblast-like synoviocytes (FLS) significantly contribute to the pathogenesis of RA. They reside in the intimal lining of the synovium, producing and recruiting inflammatory cytokines and proteolytic enzymes that cause destruction to the extracellular matrix. FLS activity causes further joint damage by expanding the intimal lining from the normal 1-2 cells to 10-20 cells deep and forming a pannus, which subsequently erodes the bone and causes pain (Bartok & Firestein, 2011). Bartok and Firestein (2011) propose that targeting FLS could produce positive clinical outcomes in RA without compromising systemic immunity. Zhu et al. (2016) assessed the effects of cobrotoxin extracted from Naja naja atra venom on the proliferation of FLS. It was found that cobrotoxin had an inhibitory effect on the proliferation of

FLS through the inhibition of the NF-kb signaling pathway. Pannus formation was also significantly alleviated by cobratoxin, a neurotoxin from the Thailand cobra (Liu et al., 2009).

Peripheral Pain Sensitization

In addition to structural changes seen in RA, long term inflammation alters nociceptive signaling and these long lasting effects result in peripheral pain sensitization, which refers to an increased sensitivity of nerve fiber endings residing outside of the central nervous system. (Biddle & Sofat, 2020). Peripheral sensitization plays a significant role in chronic RA pain, and results in primary hyperalgesia (Prescott, 2017). In addition to prostaglandins and bradykinin, other inflammatory mediators and factors such as cytokines, serotonin, and histamine are released during an inflammatory response. These components affect primary afferent neurons by binding to their corresponding receptors at nociceptive terminals. Sustained inflammation upregulates ion channels that leads those neurons to become more sensitive to the inflammatory mediators (Schaible, 2002). Consequently, these neurons exhibit an increased rate of action potential firing, thus resulting in local pain hypersensitivity (Prescott, 2017).

Zhu et al. (2013) studied murine rheumatoid arthritis models, finding that *Naja naja atra* venom exhibited dose-dependent analgesic effects and inhibited mechanical hyperalgesia. Ruan et al. (2013) found similar results, showing that the *Naja naja atra* venom peptide Neurotoxin-Nna reduced CFA-induced tactile hyperalgesia. A 2009 study observing the effects of cobratoxin on Freund's adjuvant-induced arthritis in rats found that arthritis symptoms were suppressed, and CFA-induced mechanical and thermal hyperalgesia were inhibited with daily administration of cobratoxin 11-19 days after CFA administration (Liu et al., 2009).

Central Inflammatory Pain and Central Sensitization

A central inflammatory component to pain in RA also accompanies the peripheral causes. Synovitis generates bioactive substances that sensitize peripheral nerves. Additionally, the generation of pro-inflammatory cytokines contributes to central sensitization by elevating levels in the central nervous system. Central sensitization causes pain hypersensitivity by increasing the excitability of afferent sensory neurons and hence transmission of pain signals. This often occurs at the level of the spinal cord, where an enhanced release of glutamate, an excitatory neurotransmitter, and substance P (a neuropeptide involved in pain) causes hyperalgesia and allodynia (Lee, 2011).

Cytokines circulating in the body can enter the central nervous system likely due to a compromised blood brain barrier during chronic inflammation, and have central effects on pain processing (McWilliams, 2017). One study found that in the early phase of the collagen induced arthritis model in rats, mechanical allodynia (the pathological experience of pain with non-noxious stimuli that normally do not produce a pain response) and hyperexcitability in the spinal cord was associated with an increase in CSF IL-1β levels, prior to the onset of clinical signs of arthritis (Nieto et al., 2016). This suggests a causal relationship between inflammatory arthritis and central sensitization. Similarly, inflammatory cytokines such as tumor necrosis factor-a, IL-1β, and IL-6 act directly on nociceptive nerve cells in the dorsal root ganglion of the spinal cord and induce allodynia and diffuse hyperalgesia (Schaible et al., 2010).

As discussed earlier, snake venom and its components have been shown to produce anti-inflammatory effects by decreasing the levels of pro-inflammatory cytokines, which could be promising in relieving inflammatory pain not only peripherally but also pain on a central level.

Suppression of joint inflammation is often not correlated to a remission in pain, however, suggesting that central sensitization cannot be entirely reversed by the treatment of inflammation

(McWilliams, 2017). Inflammation is undoubtedly a contributor to RA pain, but as a 2015 study found, changes in inflammation explained less than half of changes in pain (Druce, 2015). The bilateral characteristics and autonomic pathologies associated with RA indicate that there is a central component to pain in chronic diseases such as rheumatoid arthritis in which changes in the central nervous system lead to an alteration in the processing of signals coming from damaged and inflamed joints, even when inflammation has subsided (Meeus et al., 2012). Zhang et al. (2006) assessed the analgesic effects of crotoxin (a neurotoxin isolated from *Crotalus durissus terrificus* venom) in mice models, finding that it exhibited dose-dependent analgesic action, likely mediated both peripherally and centrally. The study concluded that the action of the toxin is mediated by the central nervous system because both intracerebral ventricular and periaqueductal gray area administration of the venom produced significant analgesic effects. The study confirmed this by drawing from previous evidence that analgesia in response to hotplate and tail-flick tests in mice has a central action, which was also seen in the Zhang et al. study. Notably, no neuronal damage was seen in the murine models receiving central crotoxin injections (Zhang et al., 2006).

Cholinergic Pain Modulation

Neurotoxins in cobra venom have been found to target nicotinic and muscarinic acetylcholine receptors, which are expressed readily throughout the peripheral and central nervous system and are involved in the regulation of pain (Liu et al., 2009). Acetylcholine receptor agonists are emerging as promising agents in treating chronic and inflammatory pain (Bagdas, 2019).

Several studies have identified that the analgesic effects of snake venom could be mediated by blocking the transmission of nerve impulses before they have reached the central

nervous system. Postsynaptic alpha neurotoxins inhibit ion channel activity and block synaptic transmission, and postsynaptic muscarinic receptors have been found to be involved in antinociception (Cheng et al., 2009; Bartolini, 1992). Cheng et al. (2009) found that cobratoxin from *Naja kaouthia* inhibited pain-evoked discharge of neurons in the central nervous system in rats. The effect was attributed to the involvement of muscarinic cholinergic receptors, because the pre-administration of atropine (a muscarinic cholinergic antagonist) blocked the anti-nociceptive response (Cheng et al., 2009). Additionally, the authors concluded that nicotinic cholinergic receptors may also be involved in nociception, because peripheral antinociceptive and anti-inflammatory effects were antagonized by methyllycaconitine, an alpha-7 nicotinic receptor antagonist (Liu et al., 2009). Interestingly, the inhibition of pain responses with cobratoxin persisted for at least 2 hours, while morphine administration only produced anti-nociceptive effects for less than thirty minutes (Cheng et al., 2009).

Ruan et al. (2013) studied the anti-inflammatory effects of neurotoxin-Nna, a peptide from *Naja naja atra* venom, finding that it displayed analgesic properties by binding to the alpha subunit of the nicotinic acetylcholine receptor, blocking transmission of the nerve impulse. The study also concluded that treatment with neurotoxin-Nna reduced tactile hyperalgesia induced by complete Freund′s adjuvant in the murine inflammatory model. Najanalgesin, another component of *Naja naja atra* venom, elicited an antinociceptive effect in a rodent model that lasted for 6 hours after the intraperitoneal injection (Jiang et al., 2008). Similar to the Cheng et al. findings, pre-treatment with atropine blocked the antinociceptive effect, suggesting cholinergic mechanisms responsible for the pain relief. Postmortem exams of the rodents revealed no internal damage, nor was locomotion impaired during the study, suggesting that there were minimal adverse effects to the najanalgesin administration (Jiang et al., 2008).

Opioidergic Pain Pathways

Pain is perceived via ascending pain pathways that are mediated at several varying levels. The ascending pain pathway involves a first order sensory neuron (in the peripheral nervous system) that fires action potentials in response to a noxious stimulus and synapses on a second order neuron in the dorsal horn of the spinal cord. This neuron decussates and travels up the spinal cord contralaterally in the spinothalamic tract until it synapses in the thalamus with the third order neuron that will send the signal to the somatosensory cortex of the cerebrum.

Pain perception can be modulated by several mechanisms, arguably the most relevant to venom analgesia findings being the opioid peptidergic system. Peripheral sensory neurons express opioid receptors on their terminals that, when activated by endogenous or exogenous opioids, inhibit the pain signal from being transmitted to the second order neuron (Stein et al., 2009). There are three subtypes of opioid receptors in the body - delta, kappa, and mu - and different ligands show varying affinities for the three types. It is well understood that the mu receptor is responsible for analgesic effects but also plays a large role in the reward system and addictive opioid-related behaviors (Le Merrer et al., 2009). Mu receptor agonists are widely used in pain management but also have the greatest potential for abuse and addiction (Albert-Vartanian, 2016). In fact, in mice lacking the mu opioid receptor gene, analgesia, reward effect, and withdrawal symptoms were abolished (Matthes et al., 1996). Kappa opioid receptors are also involved in pain regulation, and peripherally restricted kappa opioid receptor agonists have been found to relieve inflammatory, visceral, and chronic pain (Vanderah, 2010). Delta opioid receptors also mediate inhibition of pain, and delta receptor agonists are effective in relieving chronic pain, inflammatory pain, and malignant bone pain (Pradhan et al., 2011; Vanderah, 2010). Kappa and delta opioid agonists have been shown to produce impressive analgesic

effects with a potentially lower risk of abuse than those analgesic targeting mu opioid receptors (Vanderah, 2010).

It is for this reason that the use of snake venom for pain management in diseases such as rheumatoid arthritis is so promising. Multiple studies have attributed the antinociceptive effects of snake venom with targeting opioid receptors, and while the findings regarding tolerance build up with continued administration have varied, there is still promising evidence for the use of venom for pain management via opioidergic pathway mechanisms.

Najanalgesin isolated from Naja naja atra venom was found to induce an antinociceptive effect lasting up to 6 hours after injection in a murine model, and pretreatment with atropine, a muscarinic antagonist, and naloxone, an opioid receptor antagonist, blocked the pain-relieving effect. This suggests that the mechanisms for pain relief include both cholinergic and opioidergic pathways (Jiang et al., 2008). A study by Mancin et al. (1998) administered crotamine, a neurotoxin derived from *Crotalus durissus terrificus* venom (Cdtv), via intraperitoneal injection to murine models and found that the dose-dependent analgesic activity of the neurotoxin was also inhibited by naloxone, suggesting that the mechanism of action was opioidergic. This study determined that the antinociceptive effect of crotamine is 30-fold greater than that of morphine, respective to their dosage, and did not have any negative effects on internal organs. It was also concluded that the analgesic activity involves both central and peripheral mechanisms.

Several studies have gone further and have identified the specific opioid receptors on which venom components are acting. Konno et al. (2008) determined that crotalphine, another analgesic peptide from *Crotalus durissus terrificus,* suppressed hyperalgesia and induced antinociception mediated by kappa opioid receptors. Notably, the long-lasting antinociceptive

effects did not cause development of peripheral tolerance or withdrawal symptoms. The study also assessed the efficacy of synthetic crotalphine as an analgesic, and found that it had dose dependent antinociceptive effects similar to the crude crotalphine.

Another study found similar results that Ctdv exerts its antinociceptive effects by acting upon kappa opioid receptors. While tolerance to the effect was observed when administering the venom for 14 days, the effect was reestablished 7 days after the administration was stopped. The administration of the venom every 5 days for 65 days did not lead to tolerance build up. Additionally, symptoms of abstinence syndrome or withdrawal were not observed. It is possible that this finding is due to the venom exerting its effects on kappa opioid receptors, rather than the mu receptors that are more involved in addiction. No histopathological or locomotive changes were observed in the mice (Brigatte et al., 2001).

Conversely, another study by Picolo et al. (2000) concluded that delta opioid receptors, rather than kappa opioid receptors, act to mediate the analgesic effect of Cdtv, as antagonists of delta opioid receptors stopped the antinociceptive effects. Similar to the findings of Konno et al, prolonged administration of the venom did not lead to tolerance, and locomotion in the rat models was not affected, suggesting minimal neurological side effects (Picolo et al., 2000).

Discussion

Snake venom could provide a multi-faceted approach to pain management in rheumatoid arthritis patients due to the scope of mechanisms on which it has been found to act regarding inflammation and pain. While inflammation does not comprise the entire picture of pain in RA, administration of drugs targeting the underlying inflammation still leads to reductions in pain in many patients. Snake venom, particularly from cobras and elapids, has anti-inflammatory properties that could serve as a mechanism by which to reduce the underlying inflammatory

properties of the disease. These properties could reduce both peripheral and central inflammation, and potentially prevent further joint damage and sensitization of nerves.

The discovery that the analgesic effect of snake venom is mediated by cholinergic mechanisms is incredibly promising. Studies have compared the efficacy of venom from species such as *Naja naja atra* to morphine in the treatment of pain, with several cases finding that the venom is just as effective as the opioid analgesic. Most notably, the receptors on which the venom is acting do not belong to the opioidergic system, and the analgesic action was not accompanied by addiction or withdrawal in murine models. Findings that associate the analgesic effects of snake venom with opioidergic systems are just as promising. Many of the components of venom do not act on the mu opioid receptor, which is the most involved with the addictive properties and withdrawal associated with opioid analgesics. The peripheral kappa and delta receptors may be downregulated with prolonged exposure, without the withdrawal syndrome of central downregulation. Further research is needed to determine the exact mechanisms by which venom acts in the opioidergic system that may cause tolerance but not withdrawal. Targeting kappa opioid receptors for the treatment of pain without the side effect of addiction could be a safer method of administering potent analgesics (Beck & Dix, 2019).

While the majority of this paper has described findings of snake venom effects on pain in murine models, there have been multiple phase I and phase II human trials utilizing venom for the treatment of various pathologies and post-operative care, all showing varying levels of response (Reid, 2011). A 2002 phase I trial assessed the effects of crotoxin in patients with advanced cancer, and while managing pain was not the primary goal of the study, eighteen of the twenty-three participants in the study reported a significant decrease, and even disappearance, of pain after several weeks of treatment. In fact, one patient suspended their regular administration

of morphine after three weeks. Several intermittent neurological side effects, including diplopia, palpebral ptosis, and strabismus were observed, but disappeared between 15-21 days of administration (Cura et al., 2002). Pure cobrotoxin was also found to be useful in postoperative pain management, and its effects lasted twice as long as morphine, despite needing only 150th of the amount of drug (per kg) (Wang et al., 1999).

Conclusion

Pain has persistently been shown to be the primary concern in individuals with rheumatoid arthritis. Pain impacts quality of life through a myriad of effects including greater physiological and psychological distress, hindered social and physical functioning, and greater healthcare costs (Biddle & Sofat, 2020). A 2012 study collected transcripts from patients experiencing RA flares, one participant describing how their pain makes them feel: "I'm hurting. Help me, I don't want to feel like this. When I go into my doctor and he says 'Why are you in here today?' I say 'Because I can't function like this. I'm hurting, I want to kill myself' (Hewlett et al., 2012). Chronic pain has detrimental effects on the individuals experiencing it, their families, and their social circles.

While targeting the underlying inflammatory causes of RA has been shown to provide some pain relief, treatments focusing on central mechanisms of pain are yet to be explored in depth. Often, the allopathic approach of treatment with DMARDs, NSAIDs, and other over the counter medications reduces pain but does not provide the relief desired in RA patients. However, the use of more effective analgesics such as opioids are usually avoided due to their undesirable side effects and risk of addiction. Components of snake venom could reduce pain via both inflammatory and central pain mechanisms, while reducing the risks associated with current pain management therapies.

The efficacy of snake venom in reducing pain, comparable to that of morphine, could offer an alternative solution to the use of opioid analgesics. With such a high abuse potential, prescribed opioids have significantly contributed to the growing concern of opioid related disability and mortality in the United States (Brown & Sloan, 2017). In 2019, 14,139 prescription opioid related deaths were reported in the US, and addiction to prescribed opioid agonists that specifically target mu receptors poses a significant public health threat (National Institute on Drug Abuse; Beck & Dix, 2019). When primary care and chronic pain physicians rightfully prioritize mitigating discomfort and pain in patients, prescribing opioids offers an effective but dangerous solution. If snake venom components could produce similar, if not more effective, analgesic results without the potential for abuse, tolerance and withdrawal, the management of pain in rheumatoid arthritis and other chronic diseases could be drastically altered for the better.

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