Exploring the Anti-Inflammatory Effects of Aprepitant: An Antagonist of the Neurokinin-1 Receptor

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ABSTRACT

Aim: Substance P participates towards pain aetiology by activating on the NK-1R, the neurokinin-1 receptor, which is found on sensory neurons that perceive noxious stimuli. SP is recognised to have biological action through G-protein-coupled neurokinin receptors named Neurokinin 1 Receptor (NK-1R), Neurokinin 2 Receptor (NK-2R) and neurokinin 3 receptors (NK-3R). Among the three, NK-1R has the highest affinity for substance P. The NK-1R receptor is a G-protein-coupled receptor that is involved in inflammatory disorders. Aprepitant is a highly selective non-peptide neurokinin-1 receptor antagonist that is approved to lessen nausea and vomiting brought on by chemotherapy. **Materials and Methods:** The purpose of this study is to see if Aprepitant has any anti-inflammatory activity in the cotton pellet granuloma technique. Pellets with granuloma tissue around them were carefully dissected, cleared of extraneous tissues, and dried at 60°C at the end of the research. Under both moist and dry circumstances, and each particle's surrounding granuloma tissue's mean weight was determined. **Results:** The study's goal is to evaluate the anti-inflammatory impact and Aprepitant prevents granuloma formation (48.68%) at a dose of 80 mg/kg to the conventional medicine indomethacin (2.5 mg/kg), which demonstrated the maximum suppression of granuloma tissue development (55.34%). **Conclusion:** When compared to a control without an ulcerogenic impact, the histology of granuloma in aprepitant treated group tissue indicated considerable prevention of necrosis and exudates.

Keywords: Chronic inflammation, Substance P, Aprepitant, Neurokinin 1 receptor, Cytokines.

INTRODUCTION

Substance P (SP) is a tachykinin peptide family member that is extensively expressed across the animal kingdom. Members of the tachykinin peptide family function in a variety of neuronal signalling pathways, affecting emotions and emotional responses. SP has a net positive charge at physiological pH and is composed of 11 amino acids (undecapeptide) (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2).[1] Tachykinin (Neurokinin: NK) receptors, which have rhodopsin-like membrane structures made up of seven hydrophobic transmembrane domains joined by extracellular and intracellular loops and associated with G-proteins, mediate the biological effects of substance P. Tachykinin receptors come in three varieties, with NK-1, NK-2, and NK-3 showing selectivity for neurokinin A, substance P, and neurokinin B, respectively. The Central Nervous System (CNS) and peripheral nervous system both contain Substance P (SP), an undecapeptide. High-affinity neurokinin-1 receptors are the site of the biological and immunological action of SP released from peripheral neurons (NK-1R). Immune cells also produce SP, which controls immune cell activity in a paracrine or autocrine manner. SP is expected to exert its biological action through the G-protein-coupled neurokinin

receptors known as neurokinin 1 receptor, neurokinin-2 receptor, and neurokinin 3 receptors. The most affinitous receptor for SP is the NK-1 Receptor.[2,3] By binding to the Neurokinin-1 Receptor (NK-1R), Substance P (SP), which is secreted by nerves and inflammatory cells like eosinophils, lymphocytes, macrophages, dendritic cells, which trigger the release of other substances such as histamine, allergens, leukotrienes, and prostaglandins from sensory nerves. Inflammatory disorders have been associated with elevated SP levels and uncontrolled NK-1R expression, which are correlated with disease activity. The cytokine release from mast cells, lymphocytes, monocytes, macrophages, oxygen radicals, and arachidonic acid derivatives is increased by SP, as are lymphocyte proliferation and immunoglobulin synthesis. This amplifies tissue damage and the inflammatory response. Additionally, it causes local vasodilatation and changes vascular permeability, which improves leukocyte distribution and accumulation in tissues for the expression of localized immunological reactions.[4] Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used due to their broad spectrum of activity. However, it has a lot of side effects. The U.S. Food and Drug Administration has authorised a highly specific non-peptide neurokinin-1 receptor (NK-1R) antagonist called Aprepitant for the treatment of chemotherapy-related vomiting and nausea. Aprepitant is currently used in clinical practise and is considered safe and well tolerated. Additionally, it has been noted that Aprepitant has particular anti-inflammatory effects on rheumatoid arthritis,[5] prurigo nodularis,[6] osteoarthritis pain, cancer.[7] And also, we predicted that Aprepitant could be useful for treating chronic inflammatory pain and preventing microglia activation and inflammatory cytokine production in HIV infection.[8] Consequently, this work provides a new understanding of the function of aprepitant in anti-inflammatory responses, and consequently, a promising therapeutic approach for inflammatory pain.

MATERIALS AND METHODS

Ethical considerations and experimental design

This study employed Wistar rats weighing 300-400 g. The rats were taken from the Nandha College of Pharmacy's animal home in Erode, Tamil Nadu, India. All experimental techniques utilised in this work were approved by the Institutional Animal Ethics Committee (688/02/C-CPCSEA), proposal number NCP/IAEC/NO: 2019-2020/07, and were in compliance with the Institute Animal Ethics Committee's norms (IAEC).

Aprepitant pills (80 mg) were delivered by the Erode Cancer Centre in Erode, Tamil Nadu.

Cotton pellet granuloma model an experimental model

During procedure, two 10 mg autoclaved sterile cotton pellets were inserted in the dorsal surface of the rats, one near each groyne, to induce granulomatous lesions following an earlier procedure. Following acclimation, all rats of both sexes were divided into three groups of six animals each. The test drug was administered orally for seven days at a dose of 80 mg/kg. The rats in the standard group were given indomethacin for seven days in a row, whereas the rats in the control group were given a vehicle (5% gum acacia). All of the animals had unlimited access to food and water. After the granuloma tissue-enclosed pellets were carefully removed on the eighth day, they were separated from extraneous tissues, and they were dried at 60ºC. On average, each pellet's surrounding granuloma tissue was weighed. The pellets were weighed both wet and dry. The amount of granulation tissue produced is determined by the difference between the starting and end weight of the cotton pellet. The weights of rats treated with standard and test were compared to the weights of granulation tissue generated in the control group.[9,10]

The following formula was used to compute the percentage suppression of granuloma tissue development in rats

$$\% \, \text{Inhibition} = \left[\frac{\left(\text{Control} - \text{Test} \right)}{\text{Control}} \right] \times 100$$

Statistical Interpretation

The findings were presented as mean±SEM. Graph Pad version 3 was used to do the Dunnett's t test and one-way analysis of variance (ANOVA) were used to the data. The results of a significant ANOVA test do not specify which pairs of means were different. After the ANOVA has been conducted, Dunnett's can be used to determine the pairings that have significant differences, p values between <0.001 and <0.01 were deemed significant.

RESULTS

Effect of Aprepitant on granuloma-associated inflammation in rats

Comparatively to control rats, Aprepitant was able to significantly reduce the granuloma-associated inflammation in rats following the treatment period Table 1. This was demonstrated by the decrease in the

Table 1: The impact of a Aprepitant on rats treated with cotton pellets that develop granulomas.			
Group	Dose (mg/kg)	Granuloma weight (dry) mg	(%) inhibition
Control group	5% gum acacia	46.67±0.612	-
Standard group (Indomethacin)	2.5 mg/kg	22.38±0.566	55.34
Test group (Aprepitant)	80 mg /kg	28.85±1.497	48.68

The results are given as mean±SEM (*n*=6), with *p*<0.01 in comparison to the control group.

dry and wet weights of the cotton pellets. Indomethacin medication was also successful in reducing the granuloma. Pus was observed in the test-treated groups although not in the control groups. When compared to the standard medication Indomethacin (2.5 mg/kg), which exhibited the maximum inhibition (55.34%) of the development of granuloma tissue in this investigation, Aprepitant had a substantial anti-inflammatory action and inhibited granuloma formation (48.68%) at a dose of 80 mg/kg Figure 1, which was further confirmed by histopathological proof mentioned in Figure 2.

DISCUSSION

The inflammatory response is a crucial aspect of tissue reaction to noxious stimuli, involving various inflammatory cells such as lymphocytes, macrophages, and neutrophils. These cells release specific substances,
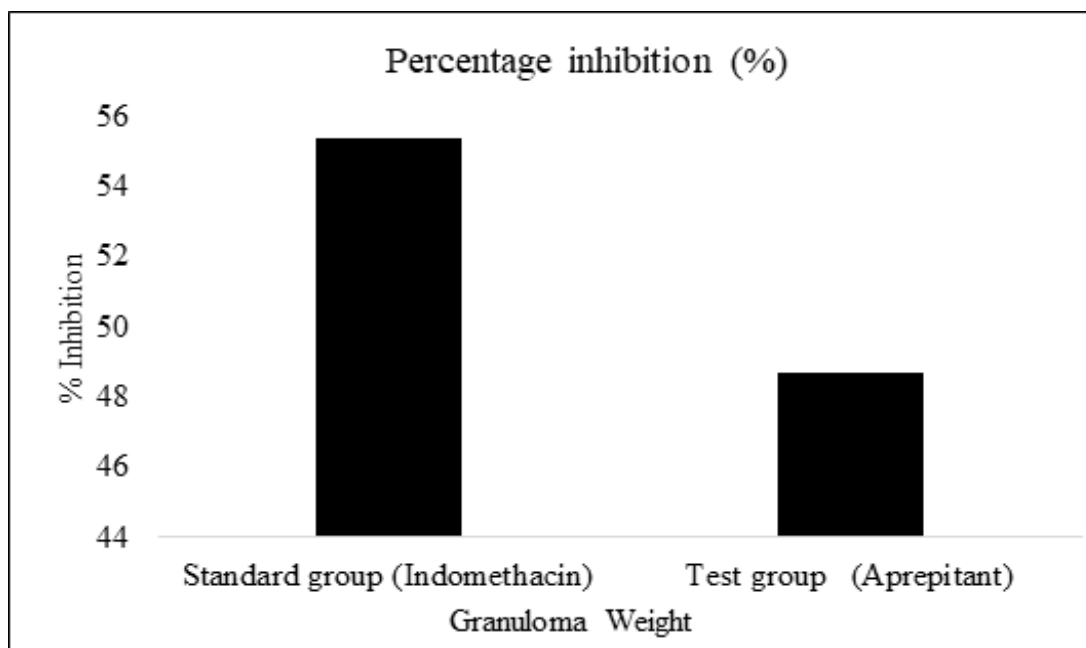

Figure 1: % Inhibition in mean weight of Cotton pellet.

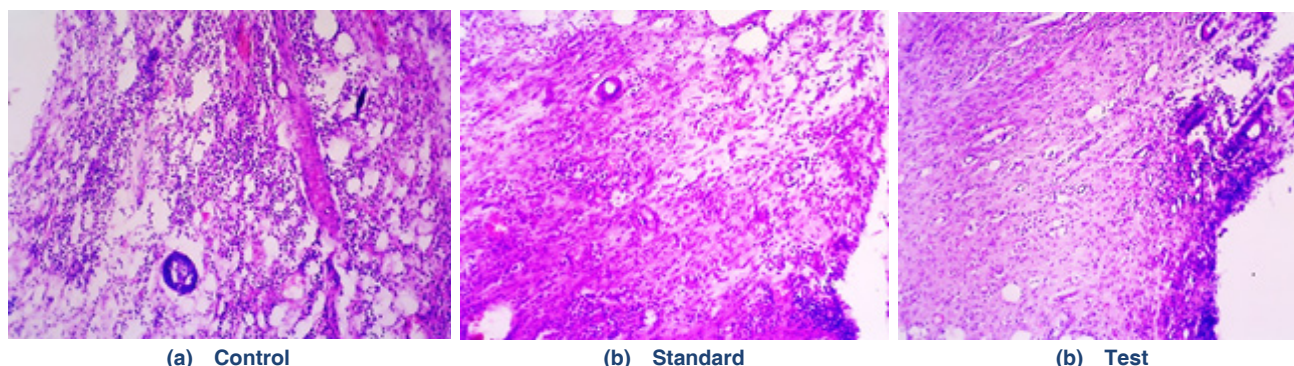
(a) Control   (b) Standard   (b) Test

Figure 2: Histopathological Studies.

including vasoactive amines, peptides, eicosanoids, proinflammatory cytokines, and proteins, in response to inflammation. Substance P (SP) is released by neurons and inflammatory cells, contributing to inflammatory disorders by binding to the Neurokinin-1 Receptor (NK-1R), a nociceptor. This study investigates the anti-inflammatory activity of Aprepitant using a cotton pellet granuloma model, focusing on microglial activation, p38/MAPK, and JNK phosphorylation. The study reveals that Aprepitant acts as an antagonist of the Substance P receptor, down-regulating JNK phosphorylation, p38/MAPK, and microglial activation. This inhibitory effect is crucial in alleviating inflammatory pain by reducing the expression of proinflammatory cytokines like TNFα, IL-6, and IL-1β. Current research suggests that Aprepitant may offer therapeutic benefits extending beyond its FDA-approved application for alleviating vomiting and nausea induced by chemotherapy. Notably, Aprepitant demonstrates anti-inflammatory properties in various conditions, including rheumatoid arthritis, prurigo nodularis, osteoarthritis pain, and cancer.[5-7] In the context of the cotton pellet-induced granuloma model, Aprepitant proves to be a potent suppressor of the proliferative phase of inflammation. The study's controlled experimental design, including randomization and a control group, enhances internal validity. Aprepitant demonstrates substantial efficacy with 48.68% inhibition compared to the standard indomethacin's 55.34% inhibition. Histopathological analysis supports these findings, with Aprepitant showing notable reduction in inflammation, chronic inflammation, granulation tissue, and blood vessels compared to the control. Comparing the study's methods with existing literature, the use of a cotton pellet granuloma model aligns with established approaches for assessing chronic inflammation's proliferative and transudative components. The findings regarding Aprepitant's efficacy in inhibiting inflammation are consistent with its known anti-inflammatory effects in various conditions. However, the slightly lower inhibition compared to indomethacin might be attributed to differences in their mechanisms of action.

To further validate the results, future studies could compare Aprepitant with other anti-inflammatory agents in the different model. Understanding these differences can contribute to the broader context of anti-inflammatory interventions and their specific mechanisms. Additionally, exploring the molecular pathways affected by Aprepitant in comparison to other drugs may provide insights into its unique mode of action. Overall, this study provides valuable information on Aprepitant's potential as a therapeutic option for inflammatory pain and warrants further exploration in diverse clinical scenarios.[13-15]

CONCLUSION

A tachykinin peptide known as substance P is involved in tissue homeostasis, pain perception, wound healing, and neurotransmitter function. Aprepitant, a neurokinin receptor antagonist, has been shown to cure chemotherapy-induced emesis. The goal of the current investigation was to influence Aprepitant as anti-inflammatory drug. Surprisingly, in a cotton pellet granuloma model, Aprepitant has anti-inflammatory effects, which may be brought about by the reduction of pro-inflammatory cytokines.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SP: Substance P; **NK-1R:** Neurokinin-1 receptor; **TNFα:** Tumor necrosis factor; **IL-6:** Interlukin-6; **IL-1β:** Interluekin-1 beta; **p38/MAPK:** Mitogen-Activated Protein Kinase; **JNK:** c-Jun N-terminal kinase; **SEM:** Standard error mean; **ANOVA:** Analysis of variance; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institute Animal Ethics Committee.

SUMMARY

The use of rats as a model might limit the direct translation of findings to humans, as animal responses may not fully represent human physiological responses and specific model of inflammation (cotton pellet granuloma), which may not fully capture the complexity of inflammatory processes in different tissues or conditions. Administering a single dose of Aprepitant without exploring long-term effects or dose-response relationships limits a comprehensive understanding. The quantification of granulation tissue may have inherent variability, and the study does not delve into the molecular mechanisms underlying the observed anti-

inflammatory effects. The study employed a controlled experimental design, using randomization and a control group, which enhances the internal validity of the results. Standard indomethacin shows 55.34% inhibition, while Aprepitant shows 48.68% inhibition of inflammation. Aprepitant demonstrates substantial efficacy but slightly lower inhibition compared to the standard. Whereas in histopathology in control significant acute inflammation, necrosis, exudates, and lipids indicate severe pathological changes when compared to standard presence of granulation tissue, blood vessels, and moderate chronic inflammation suggest a partial mitigation of pathological features. While aprepitant showed mild to severe chronic inflammation with granulation tissue and blood vessels indicates a notable reduction in inflammation, aligning with the quantitative inhibition results. So, the measurement of granulation tissue provides a tangible and measurable outcome, enhancing the objectivity of the results. The observed anti-inflammatory properties of Aprepitant align with its known effects in treating nausea and vomiting induced by chemotherapy. This consistency supports the validity of the findings, indicating that Aprepitant may have broader applications beyond its originally approved indications. The identification of Aprepitant's anti-inflammatory properties opens avenues for further research and potential therapeutic applications. If validated in human studies, it could offer a novel approach to managing inflammatory conditions, potentially reducing the reliance on traditional anti-inflammatory drugs with known side effects. Human trials are essential to ascertain the applicability of Aprepitant's anti-inflammatory effects in clinical settings. The observed decrease in pro-inflammatory cytokines aligns with the known mechanism of action of Aprepitant as a neurokinin receptor antagonist. The differences from other studies may stem from the unique focus on a cotton pellet granuloma model, highlighting the context-dependent nature of anti-inflammatory responses. Further research is needed to explore the specific pathways involved and to compare these results with other models of inflammation and to explore the broader implications for clinical practice and drug development.

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