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RESEARCH ARTICLE

NEURAL MODULATION OF INFLAMMATION

*Mehnaaz Sameera Arifuddin[1], K. Sai Krishna[2], Mohammed Abdul Hannan Hazari[3], B. Dinesh Kumar[4]

[1] M.D., Deccan College of Medical Sciences, Hyderabad

[2](M.B.B.S), Osmania Medical College, Hyderabad

[3]M.D., Deccan College of Medical Sciences, Hyderabad

[4]Ph.D., National Institute of Nutrition, Hyderabad

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ABSTRACT

Background: In the present situation of emerging cases of drug allergies, auto-immune disorders and adverse reactions to anti-inflammatory drugs, there is a need to find an alternative for handling abnormal immune status which causes inflammatory storm. There is a growing need to exploit the nervous system for understanding the role played by neurons in the inflammatory process.

Aims and objectives: The purpose of this study was to understand the anti-inflammatory role of parasympathetic nervous system using Bethanechol chloride and to study the contribution of neurons in modulating inflammation using 2% Carrageenan.

Materials and methods: Thirty female Wistar (WNIN) rats were selected for this study. Twenty five WNIN rats, divided into five groups were selected for studying the anti-inflammatory activity of Bethanechol chloride. The remaining five rats were selected to elicit the inter-dependent natures of immune and nervous systems. 2% Carrageenan was injected in both the hind paws. In both the study protocols, increase in paw volume was measured using plethysmometer and subjected to calculations.

Results: A significant anti-inflammatory activity of Bethanechol chloride compared to control group was observed. In the other part of study, the right paw, in which Carrageenan re-constituted in Lidocaine was injected, showed nearly no signs of inflammation in the initial hours, while left paw injected with Carrageenan dissolved in distilled water presented with a typical Carrageenan-induced paw oedema. However, one to two hours later (which corresponds to the half-life of Lidocaine), the right paw also showed the same degree of oedema.

Conclusion: We conclude that Bethanechol chloride has anti-inflammatory activity at small doses, and therefore can be used as an adjuvant in the treatment of chronic inflammatory disorders. This study also shows that immune system works in conjunction with the nervous system in modulating inflammation.

KEYWORDS: immune system, nervous system, inflammation, Bethanechol chloride, Carrageenan, Lidocaine

INTRODUCTION:

The impact of an immune response on the nervous system has long been apparent; however, the influence of the nervous system on immune responses is not well understood. The complex characters of a coordinated immune response of the body complicate research in this area. Any entity would be compromised if its defence and communication systems did not interact-humans are no exception.

This study aims to highlight the role of muscarinic receptors (M1 and M2) in modulating inflammation via the use of Bethanechol chloride in rat model. It is a directly acting cholinergic receptor stimulant with predominant muscarinic agonistic activity. By increasing the tone of parasympathetic nervous system and stimulating receptors on macrophages, Bethanechol, like other choline esters, is expected to have an anti-inflammatory response.[1]

A dual relationship exists between inflammatory process and nervous system and this has been studied using different animal models.[2] The signs of inflammation like vasodilation and plasma extravasation were elicited by stimulation of nerves. However, in our study, we attempt to understand the extent of autologous nature of the immune system. The inflammatory stimulus is introduced and neuronal activity is totally blocked with local anaesthetic and compared with controls for signs of inflammation, thus eliciting the extent of autologous nature of immune system.

MATERIALS AND METHODS:

*Corresponding author: Dr. Mehnaaz Sameera Arifuddin | Email: mehnaaz@deccancollegeofmedicalsciences.com

Thirty adult female Wistar NIN (WNIN) rats developed by National Institute of Nutrition (NIN), Hyderabad and maintained at the National Centre for Laboratory Animal Sciences (NCLAS), Hyderabad were included in this study. Their age ranged from 4-6 weeks; and body weight from 100-150 gm. The study was conducted according to Institutional Animal Ethics Committee (IAEC) [ref: (1/2011) (P14/7-2011/GBR)] of the NCLAS & Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

The study was conducted in two parts – first part using 25 rats and second part 5 rats. The first part of the study analyses the anti-inflammatory activity of Bethanechol chloride. Twenty five WNIN rats were divided randomly into 5 groups, with 5 rats in each group, and kept for overnight fasting. The 1st group (control group) received 10 ml/kg of distilled water into the plantar side of right hind paw. The 2nd, 3rd and 4th group rats received the test drug, Bethanechol chloride in an increasing dose as described by Paget et al (1974).[3] The 5th group was administered 60mg/kg Aspirin orally. The dose administered to all the 5 groups is given in Table 1. The right hind paw was marked with ink at the level of lateral malleolus. 100 µL of 2% Carrageenan solution was later injected into the plantar side of the right hind paw of rats to induce oedema. The method given by Winter CA et al. (1962)[4] was followed with slight modifications while administering Carrageenan solution. The paw volume was measured plethysmographically immediately (0 hour) after injecting Carrageenan solution. Subsequent measurements were done at the end of 1hr, 2, 4, 6 and 24 hours. The increase in paw volume was calculated in percentage compared with control as follows:

$$\% \text{ Inhibition} = \frac{(C_t - C_0)\text{Control} - (C_t - C_0)\text{Treated}}{(C_t - C_0)\text{Control}} \times 100$$

Where C_t is the paw volume 1hr, 2hr etc. after Carrageenan injection and C_0 is the paw volume before Carrageenan injection.

The second part of the study analyses the neural role in inflammation wherein the remaining five rats were injected with 2% Carrageenan in both paws of hind limbs. To ensure total loss of neural activity in the right paw, Carrageenan injected in the right paw was re-constituted in 0.5% Lidocaine. The left paw injected with Carrageenan dissolved in distilled water acted as control with normal neural activity. Inflammation in both the paws was compared by measuring increase in paw volume using plethysmometer. The calculations were same as used for the above model.

STATISTICAL ANALYSES:

Randomization ensured that the allocation of treatment to animal groups was independent of their characteristics and was similar in all the groups. It was also ensured that during randomization, base variables were homogenized and were allotted to a different group. Recorded values are reported as Mean ± Standard Deviation. Statistical analyses were performed using GraphPad Prism 5 and SPSS 13.0. Data was analysed using ANOVA. Post-hoc tests were done for statistical significance which was set at $P < 0.05$.

RESULTS:

Anti-inflammatory activity of Bethanechol chloride: 2% Carrageenan induced paw oedema was significantly reduced by Aspirin (standard), test drug (Bethanechol) at different time points. Table 2 and Figure 1 give the change in paw volume and the anti-inflammatory activity of Aspirin and varying doses of Bethanechol chloride.

The anti-inflammatory activity of Bethanechol chloride was observed throughout the study duration and was comparable to that of Aspirin. At a lower dose (1.35mg/kg), Bethanechol chloride was shown to lower the paw volume throughout 24 hours but was not equivalent to that of Aspirin. During the first 2 hours, Aspirin showed greater anti-inflammatory activity compared to Bethanechol chloride as assessed by change in the paw volume. Over the next 4 hours, the anti-oedema effect of Bethanechol chloride at a dose of 6.25 mg/kg and 13.5 mg/kg was much more than that of Aspirin. After 6 hours there was gradual decrease in paw oedema in all the 5 groups, which continued till the end of the study duration. Bethanechol appears to act best at a dose of 6.25 mg/kg.

Neural role in inflammation: The right paw volume was measured after injecting Carrageenan re-constituted with Lidocaine and the left paw volume was measured after injecting Carrageenan re-constituted with distilled water. There was no significant change in the right paw volume until the waning of Lidocaine action (half-life 1-2 hours). The results obtained are shown in Figure 2.

Table 1: Dosage administered

Category	Group	Drug administered	No. of rats	Dosage	Volume of 2% Carrageenan (µL)
Control	1	Distilled water	5	10 ml/kg	100
Test	2	Bethanechol chloride	5	1.35 mg/kg	100
	3		5	6.25 mg/kg	100
	4		5	13.5 mg/kg	100
Standard	5	Aspirin	5	60 mg/kg	100

Table 2: Anti-inflammatory role of Bethanechol chloride on 2% Carrageenan induced rat paw oedema

Time (hrs.)	Control Mean ± SD	Beth 1.35 mg/kg Mean ± SD	Beth 6.25 mg/kg Mean ± SD	Beth 13.5 mg/kg Mean ± SD	Aspirin 60mg/kg Mean ± SD
0	0.12 ± 0.02	0.10 ± 0.03	0.11 ± 0.04	0.20 ± 0.07	0.11 ± 0.04
1	0.47 ± 0.08	0.34 ± 0.13	0.30 ± 0.09	0.39 ± 0.10	0.27 ± 0.10
2	1.00 ± 0.13	0.53 ± 0.14	0.42 ± 0.06	0.52 ± 0.14	0.40 ± 0.09
4	1.38 ± 0.28	1.04 ± 0.27	0.78 ± 0.12	0.74 ± 0.24	1.03 ± 0.20
6	1.77 ± 0.18	1.29 ± 0.23	0.93 ± 0.16	1.06 ± 0.29	1.24 ± 0.21
24	1.06 ± 0.22	0.84 ± 0.20	0.45 ± 0.10	0.84 ± 0.25	0.76 ± 0.09

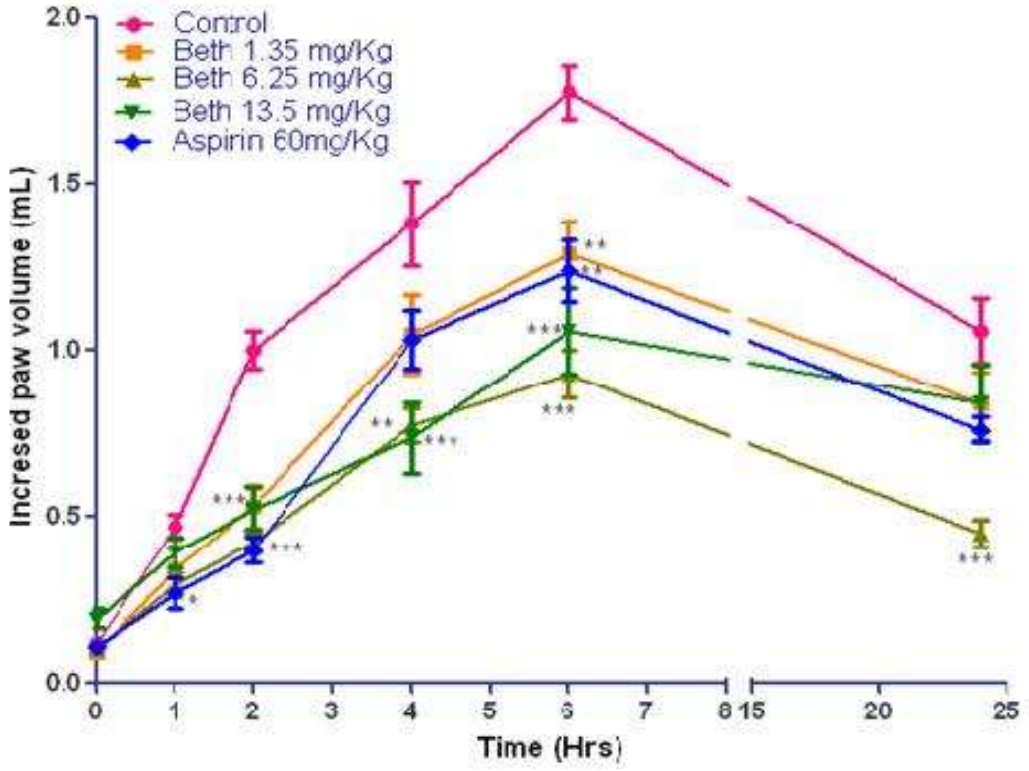

Figure 1: Anti-inflammatory activity of Bethanechol chloride at different doses when compared with Aspirin. (*P<0.05, **P<0.01, ***P<0.001).

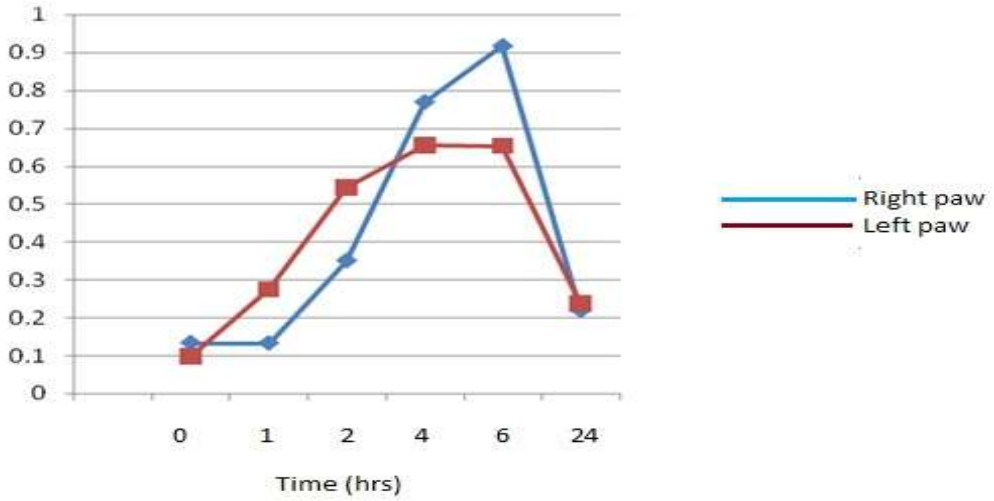

Figure 2: Mean increase in paw volume of both right and left paws.

DISCUSSION:

Many researchers have focused on developing drugs which can block the inflammatory cascade. However, the adverse effects associated with such drugs can limit their use. Hence, it becomes important to find out novel ways of modulating the inflammatory process. Various studies have highlighted the anti-inflammatory role of the vagus nerve; and thus, this feature can be used as an alternate measure to treat inflammatory disorders.

The immune system no longer functions independently; it functions in consort with the nervous system. Humoral mediators of inflammation activate the nervous system by crossing the blood-brain barrier or by causing local production of cytokines. Dr. Tracey and other investigators have discovered a third route by which inflammatory mediators can activate the nervous system: the vagus nerve. They later discovered that the cholinergic anti-inflammatory pathway functions as the motor component of innate immunity.[5] The vagus nerve is the main nerve of the parasympathetic division of the autonomic nervous system. Recent studies have shown that afferent component of the vagus nerve carries signals to the brain about any inflammatory process occurring in the periphery. Vagus nerve, its neurotransmitter Acetylcholine (Ach) and the α7 subunit of the nicotinic Ach receptor constitute the cholinergic anti-inflammatory pathway. Previous experimental data show that Ach decreases the production of cytokines like TNF, IL-1β, IL-6 and IL-18 by macrophages. However, it does not interfere with the release of IL-10 indicating that Ach directly inhibits pro-inflammatory cytokine production.[6] Thus, the release of Ach via activation of its cholinergic receptor can detour the inflammatory cascade of reactions providing an early intervention into the treatment of inflammatory diseases.[7]

Some organs like the spleen and joints are not innervated directly by the vagus. However stimulation of vagus as a result of injury or inflammation causes stimulation of autonomic fibres to release nor-epinephrine which in-turn causes release of Ach. This pathway is known as non-neuronal cholinergic system. Increase in Ach levels in the periphery via activation of α7 nicotinic receptor causes down regulation of release of pro-inflammatory cytokines reducing the inflammatory response. The α7 subtype of nicotinic receptor mediates most of the peripheral anti-inflammatory effects of Ach. However the mechanism of action also depends on the model studied. Stimulation of α7 receptor antagonises the nuclear factor κB pathway in macrophages in vitro; and in a murine model of surgical intestinal inflammation, it causes activation of Janus kinase pathway and transcription factor 3. Cholinergic anti-inflammatory reflex is also influenced by central nervous system (CNS) via muscarinic Ach receptors, M1 and M2. M1 receptor activation or M2 receptor inhibition causes increase in efferent vagal activity and decreases TNF levels in the periphery.[8] In peripheral organs signalling via muscarinic receptors is not required for vagal nerve control of inflammation. However, central muscarinic transmission is important in diminishing inflammatory responses. In a rodent model of endotoxemia, administration of muscarine or the M1 muscarinic receptor agonist McN-A-343 intracerebroventricularly decreased serum TNF levels.[6]

The inflammatory reaction induced by administration of 2% Carrageenan in WNIN rat paw manifest in the form of oedema which commonly peaks at 6 hours while the second phase develops after 24 hours. The second phase of oedema after Carrageenan administration may be due to oxygen-derived free radicals and production of inducible cyclooxygenase besides elevated production of prostaglandins. It has been

reported that this phase is sensitive to both steroidal and non-steroidal anti-inflammatory agents.[9]

In our study, we were able to establish the anti-inflammatory role of Bethanechol chloride; and also that there is a role of muscarinic receptors in controlling inflammation. Bethanechol chloride could significantly interfere with the inflammatory process caused by 2% Carrageenan; and when compared with controls all the three doses of Bethanechol were able to subdue inflammation. Bethanechol has been clinically used for the treatment of atony of bowel [10] and bladder.[11] It can be further evaluated for its anti-inflammatory role and can be used as an adjuvant anti-inflammatory agent. This study revealed that non-steroidal anti-inflammatory agent like Aspirin has better anti-oedema effect in early hours whereas Bethanechol showed better response in the later hours of inflammatory process.

This study also attempted to understand the neural role in inflammation. The neural activity in the right paw was suppressed by Lidocaine (administered along with Carrageenan) whereas in the left paw the neural activity was normal. The increase in mean right paw volume was negligible in the first hour. During the second hour, increase in mean right paw volume was much less when compared to mean left paw volume. But after 2 hours, (which corresponds to the half-life of Lidocaine), the mean increase in right paw volume was greater than left paw, showing an exaggerated response. These results indicate the role of nervous system in inflammation. The exaggerated response may be due to excess stimulation of nerves by the accumulation of potential vasoactive amines, etc. Hence it confirms that immune system is no longer independent; it needs an efficient modulator like nervous system. The results of this study necessitate further exploration of this new model of inflammation with suppressed neural activity to potentiate our findings probably with the use of long-acting local anaesthetics. This animal model can also be standardized for similar experimental set-ups.

CONCLUSION:

This study was able to demonstrate that Bethanechol chloride acts via muscarinic receptors to modify the inflammatory process at comparatively lower doses than Aspirin. Thus its role can be further evaluated for treating chronic inflammatory disorders. For eliciting the role of nervous system in inflammation, we have studied the extent of plasma extravasation (paw oedema) which highlights the importance of an intact nervous system in modulating the inflammatory process.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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