

RESEARCH ARTICLE

ANTI-INFLAMMATORY PROPERTY OF
SALBUTAMOL ON ACUTE AND
CHRONIC MODELS OF INFLAMMATIONAsha D Jadhav, Sudhir L Padwal, Rakesh R Jadhav, Swapnil S
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Key Words

Salbutamol; Inflammation;
Carageenin**Background:** β 2-Adrenergic receptor agonist agents were able to suppress the immune response by inhibiting release of IL-12 and production of TNF- α by the direct stimulation of β -adrenergic receptors on inflammatory immune cells. In addition to bronchodilator action, salbutamol could exert anti-inflammatory action.**Aims and Objective:** To find the anti-inflammatory effect of salbutamol in acute and chronic models of inflammation.**Materials and Methods:** Wistar albino rats of either sex weighing 200–250 g were used. Anti-inflammatory activity of salbutamol (2 mg/kg) was evaluated and compared with aspirin (300 mg/kg) using acute carrageenan method and formalin-induced arthritis method of inflammation. The result was statistically analyzed by one-way analysis of variance followed by Bonferroni test.**Results:** The tested drug salbutamol at a dose of 2 mg/kg was found to possess significant anti-inflammatory activity in both acute and chronic methods of inflammation as compared to control but lesser than that of aspirin in carrageenan-induced paw edema and comparable with that of standard drug aspirin at a dose 300 mg/kg in formalin-induced arthritis.**Conclusion:** Salbutamol possesses anti-inflammatory activity in acute and chronic methods of inflammation. Stimulation of β 2-adrenergic receptors may have a predominant role in modulation of anti-inflammatory activity.

INTRODUCTION

Common inflammatory diseases include rheumatoid arthritis, osteoarthritis, hepatitis, and asthma. The pathological changes underlying inflammatory conditions are hemodynamic changes, polymorphonuclear leukocyte infiltration, and secretion of inflammatory mediators.^[1] At present, nonsteroidal anti-inflammatory drugs and corticosteroids are useful in treating inflammation. Anti-inflammatory mechanisms of these drugs depend on inhibiting the synthesis of chemical mediators during inflammation.^[2,3] Long-term use of these agents can lead to serious adverse effects such as gastric intolerance, bone marrow depression, and water and salt retention. Hence, there is a need to find and develop new anti-inflammatory drugs with fewer side effects.

The two phases of inflammatory processes are acute and chronic. The predominant features of acute inflammation include fever, pain, and edema, whereas the important characteristic of chronic inflammation is cellular proliferation. In

experimental models, acute inflammation is induced by histamine, carrageenan, serotonin, formalin, dextran, bradykinin, and prostaglandin. Models of chronic inflammation are provoked by subcutaneous implantation of foreign bodies and arthritis model.^[4]

Salbutamol is a selective β 2-adrenergic receptor agonist used as bronchodilator in treating bronchial asthma. Recently it is found that it exerts anti-inflammatory effect by unknown mechanism. β 2-Receptors are also expressed by cells involved in the regulation of inflammation, particularly by neutrophils, monocytes, and macrophages.^[5-7] Stimulation of β 2-receptors on these immunocompetent cells results in anti-inflammatory effects.

Salbutamol exerts anti-inflammatory effect in acute inflammation by inhibiting release of mediators in early phase of carrageenan inflammation in marine model of pleurisy but it was ineffective in suppressing the late response.^[8] Another study conducted by Whelan et al.^[9] reported that only

long-acting β 2-agonist salmeterol had anti-inflammatory activity, but short-acting β 2-agonist salbutamol had little or no effect.^[9]

Hence in view of these inconclusive results, it is not clear whether salbutamol had anti-inflammatory activity in acute pain management. Very few studies were carried out on the anti-inflammatory effect of salbutamol for chronic pain management. This study aims to evaluate the anti-inflammatory activity of salbutamol in acute inflammation method (carrageenan-induced hind paw edema) and chronic inflammation method (formalin-induced arthritis).

MATERIALS AND METHODS

This study was carried out after approval from the institutional animal ethics committee.

Animal: Wistar albino rats of either sex weighing 180–200 g were used. The rats were grouped in separate cages with six rats in each cage. They were maintained in a colony room at ambient temperature of $23 \pm 1^\circ\text{C}$ with the help of air conditioner and enough humidity on a 12-h light/dark cycle. They had free access to food and water. The study was carried out during the daytime (between 1000 and 1800 hours).

Chemicals: Aspirin and carboxymethyl cellulose (CMC) were obtained as kind gift from Medley Pharmaceuticals, Mumbai. Salbutamol was received as gift sample from Cipla, Mumbai. Carrageenan (1% in 0.9% saline) and tramadol injections were obtained from commercial sources.

Acute inflammation—Carrageenan-induced hind paw edema in rats^[10]: Paw edema was induced in the rats by an intradermal injection of 0.1 ml carrageenan (1% in normal saline) into the plantar surface of the right hind paw of the rats. The rats were divided into the following groups ($n = 6$ in each group):

| Group | Drug given | Dose |
|------------|---------------------------------------|------------------|
| Control | Normal saline | 2 ml/kg orally |
| Salbutamol | Salbutamol dissolved in normal saline | 2 mg/kg orally |
| Aspirin | Aspirin dissolved in CMC | 300 mg/kg orally |

The acute phase of inflammatory reaction, that is, edema volume, was determined using

plethysmometer^[11] before and 180 min after carrageenan injection. All the drugs were administered 1 h before injecting carrageenan.

Percentage inhibition of paw edema (i.e., acute inflammation) was calculated as follows:

$$\% \text{ Inhibition given time interval} = (\text{Paw volume in control group} - \text{Paw volume in test group}) \times 100 / (\text{Paw volume in control group})$$

Chronic inflammation—Formalin-induced arthritis in rats^[12]: Chronic phase of inflammation was induced in rats by subcutaneous injection of 0.1 ml of 2% formalin under the plantar aponeurosis of right hind paw of albino rats on first and third day of the experiment.

The rats were divided into three groups ($n = 6$ in each group) as follows:

| Group | Drug given | Dose |
|------------|---------------------------------------|------------------|
| Control | Normal saline | 2 ml/kg orally |
| Salbutamol | Salbutamol dissolved in normal saline | 2 mg/kg orally |
| Aspirin | Aspirin dissolved in CMC | 300 mg/kg orally |

The drug to be tested was given daily for 10 days. The linear cross section (LCS) immediately below the ankle joint of right hind paw was measured daily using Vernier caliper. The difference in LCS on day 1 and day 10 was calculated for all groups.

Percentage anti-inflammatory effect of particular drug group was calculated as follows:

$$\% \text{ anti-inflammatory effect} = (\text{Mean difference in LCS in control group} - \text{Mean difference in LCS in test group}) \times 100 / (\text{Mean difference in LCS in control group})$$

RESULTS

Carrageenan-induced hind paw edema method: Salbutamol and aspirin showed decrease in paw edema at 1, 2, and 3 h intervals. Aspirin and salbutamol had a statistically significantly ($p < 0.05$) less increase in paw volume as compared to control. Similar to aspirin, salbutamol also has less increase in paw volume, but this difference is statistically significant at 3 h in which the decrease in paw edema is less than aspirin.

Table 1: Effect of drugs on paw volume in carrageenan-induced paw edema in rats

| Groups | Paw volume increase (ml) | | | % Inhibition at 3 h |
|-------------------------|--------------------------|----------------|-----------------|---------------------|
| | 1 h | 2 h | 3 h | |
| Control (normal saline) | 0.53 ± 0.12 | 1.33 ± 0.20 | 1.60 ± 0.22 | - |
| Aspirin (300 mg/kg) | 0.14 ± 0.05** | 0.10 ± 0.03*** | 0.24 ± 0.07***# | 84.89 |
| Salbutamol (2 mg/kg) | 0.23 ± 0.03* | 0.50 ± 0.09*** | 0.83 ± 0.03** | 47.91 |

Values are mean ± SEM, n = 6 in each group. * p-value < 0.05 as compared to control, ** p-value < 0.01, *** p-value < 0.001 as compared to control, # p-value < 0.05 as compared to salbutamol.

Table 2: Effect of different drugs on LCS below the ankle joint in formalin-induced arthritis in rats

| Control | Mean LCS | | | % Anti-inflammatory effect |
|----------------------|-------------|----------------|----------------|----------------------------|
| | Initial | Day 10 | Difference | |
| Control | 5.02 ± 0.08 | 6.60 ± 0.15 | 1.57 ± 0.11 | - |
| Aspirin (300 mg/kg) | 4.47 ± 0.80 | 4.78 ± 0.14*** | 0.38 ± 0.11*** | 76.05 |
| Salbutamol (2 mg/kg) | 5.19 ± 0.24 | 5.62 ± 0.39* | 0.42 ± 0.16*** | 73.61 |

Values are mean ± SEM, n = 6 in each group. *** p-value < 0.001 as compared to control.

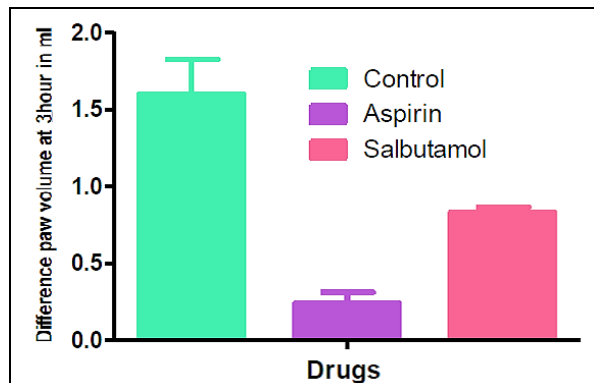


Figure 1: Carrageenan-induced hind paw edema at 3 h

For aspirin group, the percentage inhibition at 3 h is 84.89%, which is greater than that for salbutamol group (47.91%). The results are given in Table 1 and Figure 1.

Formalin-induced arthritis method: The mean difference between the LCS just below the ankle on day 1 and day 10 was calculated for each group. Lower the difference in LCS, higher the anti-inflammatory action. Table 2 shows that the least difference in the mean LCS was found in the aspirin group, which was statistically significantly ($p < 0.05$) lower than that in the control group. The difference in the LCS in salbutamol group was also significantly less as compared to that in control group ($p < 0.05$). The mean difference in the LCS in aspirin group and salbutamol group was comparable. The percentage anti-inflammatory effect was highest with aspirin group but was comparable with salbutamol group ($p > 0.05$).

DISCUSSION

Salbutamol is selective β_2 -receptor agonist used in treatment of bronchial asthma and chronic obstructive pulmonary disease.^[13] Along with the bronchodilator action, it could also have anti-inflammatory activity by stimulating the β_2 -receptor. This study was carried out to investigate the anti-inflammatory activity of salbutamol, a β_2 -adrenergic receptor agonist drug, on acute (carrageenan-induced) and chronic (cotton pellet-induced) inflammation models.

Inflammatory response is multifactorial polyphasic tissue reaction ranging from rapid, short-lived increase in vascular permeability to prolonged cellular infiltration and proliferation. New anti-inflammatory drugs are tested with acute, subacute, and chronic model of inflammation. Subcutaneous injection of carrageenan into rat paw produces plasma extravasation and inflammation characterized by increased tissue water and plasma protein exudation with neutrophils extravasation and metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase pathway.^[14] There are biphasic effects in carrageenan-induced edema. The first phase begins immediately after injection and diminishes in 1 h.

Second phase begins at 1 h and remains through 3 h. The first phase is mediated through the release of histamine, serotonin, and kinin, whereas the second phase is related to the release of prostaglandins and slow-reacting substances.^[14,15]

This study showed that salbutamol (2 mg/kg) has significant anti-inflammatory activity in acute carrageenan-induced paw edema method, which was in accordance with the study conducted by Uzkeser et al.^[16] Possible mechanism exerted by salbutamol could be due to β_2 -adrenergic receptors stimulation, which is important in the suppression of inflammation. β_2 -Receptors are expressed by cells involved in the regulation of inflammation, in particular by neutrophils, monocytes, and macrophages.^[6-8] Salbutamol was able to inhibit the first phase of the inflammatory reaction induced by carrageenan in the pleural cavity, but ineffective in suppressing the late response.^[17]

In formalin-induced arthritis model of chronic inflammation, maximum anti-inflammatory activity

was indicated by percentage anti-inflammatory effect. In our study in formalin-induced arthritis method, salbutamol showed the anti-inflammatory effect suggesting that they exert anti-inflammatory response in chronic pain. The result of our study was in accordance with that of the study conducted by Malfait et al.^[18] The possible mechanism suggested could be that salbutamol reduced IL-12 and TNF- α release by peritoneal macrophages in a dose-dependent manner, as well as TNF release by the direct stimulation of β -adrenergic receptors on inflammatory immune cells, which is not only for CD4 cells and synovial cells but also for other leucocytes with a high density of β 2-receptors such as monocytes, macrophages, and Langerhans cells.^[19-21] In addition, salbutamol helps to diminish CII-specific IFN- γ production and proliferation and block mast cell degranulation in joint tissues in arthritis model. It could exert significant anti-inflammatory action by inhibiting mast cell degranulation that might contribute to the acute inflammatory phase of arthritis by enhancing vascular permeability.^[22]

CONCLUSION

Our study suggests that salbutamol possesses anti-inflammatory activity in acute and chronic method of inflammation. Stimulation of β 2-adrenergic receptors may have a predominant role in modulation of anti-inflammatory activity. However, further studies need to be carried out to know exact possible mechanism of anti-inflammatory activity.

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