



## Experimental Evaluation of Analgesic and Anti-inflammatory Activity of Beta-2 agonists: Salbutamol and Salmeterol

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### ABSTRACT

Role of  $\beta$ -2 adrenergic agonists as analgesics and anti-inflammatory agents can be beneficial due to possible role of  $\beta$ -2 adrenergic receptors in modulation of inflammatory and nociceptive conditions. Therefore, the current study was planned to evaluate analgesic and anti-inflammatory activities of salbutamol and salmeterol in different experimental models in rats. Analgesic effects of test drugs were comparable to aspirin in the tail-flick method. Effect of tramadol was significantly more than the other drugs at all observation times. In carrageenan induced paw edema in rats, all test drugs showed anti-inflammatory activity which was comparable to aspirin at 30 minutes. At 60 and 120 minutes, anti-inflammatory activity of salbutamol (both doses) was significantly less than aspirin ( $p < 0.05$ ) while that of salmeterol was comparable to aspirin. In formalin induced arthritis model, all test drugs showed anti-inflammatory activity which was statistically significantly less than aspirin ( $p < 0.05$ ). Anti-inflammatory activity of salbutamol 2mg/kg was significantly less compared to salbutamol 1mg/kg and salmeterol ( $p < 0.001$ ). Salbutamol and salmeterol, both possess analgesic and anti-inflammatory activity in various experimental models in rats. In acute inflammation activity of salmeterol is comparable to aspirin.

**Keywords:** Tail flick model in rats, carrageenan, rat-paw edema, formalin-induced arthritis.

### INTRODUCTION

Pain has been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. – International Association for the Study of Pain (IASP).<sup>1</sup> Pain and pyrexia are the most common complaints in patients suffering from inflammatory diseases. Inflammation is defined as the reaction of living tissue to injury. It is a defense reaction, the ultimate goal of which is to help the organism get rid of both, the initial cause of injury (e.g. microbes, toxins) and the consequences of such injury (e.g. necrotic cells and tissues).<sup>2</sup> Inflammatory diseases such as rheumatoid arthritis, hepatitis, and asthma are major causes of morbidity in humans. Inflammation plays a major role in the pathogenesis of bronchial asthma. Anti-inflammatory drugs like corticosteroids form the mainstay of treatment in this disease along with bronchodilators like  $\beta$ -2 agonists.

Recent studies suggest that  $\beta$ -2 adrenergic receptors may be involved in increased nociceptive sensitivity<sup>3</sup> and can inhibit nociception and inflammation.<sup>4-6</sup>  $\beta$ 2 adrenergic agonists are claimed to achieve this effect by increasing intracellular cyclic AMP levels. Raised cyclic AMP levels have been suggested to reduce secretion of various cytokines produced by monocyte/macrophages or T cells. Also, it has been shown to inhibit the release of hydrolytic enzymes and other pro-inflammatory mediators.<sup>7</sup> The

anti-inflammatory and analgesic effects of NSAIDs have also been shown to be related to  $\beta$ 2 adrenergic receptors.<sup>8</sup>

Analgesic and anti-inflammatory activity of  $\beta$ 2 adrenergic agonists has been evaluated in a few studies. Anti-inflammatory activity of salbutamol has been reported in experimental models of acute and chronic inflammation in rats.<sup>9</sup> The inhibitory effect on inflammatory processes is seen primarily for CD4 cells as well as for leucocytes with a high density of  $\beta$ -2 receptors present on monocytes, macrophages, and Langerhans cells.<sup>10</sup> An extensive literature search did not reveal any study reporting anti-inflammatory and analgesic activity of salmeterol in animal models. As there is limited published data about analgesic and anti-inflammatory activity of these two  $\beta$ 2 adrenergic receptor agonists, these drugs need to be further explored for these activities. Since corticosteroids form an integral part of treatment in asthma, anti-inflammatory activity of  $\beta$ 2 agonists, if established, will provide an additional mechanism for their efficacy in bronchial asthma. Hence, this study was planned with the objectives of evaluating the analgesic and anti-inflammatory activities of salbutamol and salmeterol in various animal models.

### MATERIALS AND METHODS

Male Albino Wistar rats of either sex weighing 180-250 gm were selected. Study was conducted after approval from the Institutional Animal Ethics Committee (IAEC approval



Number 2092-94), which is an approved body by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi. All procedures were performed in compliance with relevant international, national, local and institutional laws and requirements.

The rats were grouped in separate polypropylene cages on husk bedding with six animals in each cage. Animals were fed with standard pellet diet and water ad libitum. Animals were allowed to adjust to the laboratory conditions such as light, temperature and noise before being subjected to the experiment (acclimatization). They were maintained at ambient temperature of  $23\pm 1^\circ\text{C}$  with help of air coolers and enough humidity on a 12-hour light – dark cycle. They had free access to food and water. Care was taken to avoid coprophagy among animals by the use of net. Similar conditions were provided in laboratory while performing experiments. Study was conducted during the day time (between 10.00 to 18.00 hours).

### Chemicals

Drugs: Salbutamol and Salmeterol in pure powdered were obtained from Cipla Pharmaceuticals Ltd Mumbai, India. Aspirin and chemicals like Sodium Carboxymethylcellulose (CMC) were obtained from Medley Pharmaceuticals Ltd., Mumbai, India, in pure powder form. Carrageenan and Tramadol were obtained from commercial sources. Chemicals were of analytical grade. All the drugs were dissolved in 0.9% normal saline while aspirin was suspended with CMC in normal saline. Fresh solutions were prepared half an hour before experiment.

### Evaluation of Analgesic activity: Tail-flick method<sup>11</sup>

For evaluation of analgesic activity, animals were divided into the following six groups:

Group 1- Control group- normal saline (2ml/kg orally); Group 2- tramadol group: tramadol (10mg/kg intraperitoneal); Group 3- aspirin group: aspirin (300mg/kg orally); Group 4- salbutamol group: salbutamol (1mg/kg orally); Group 5- salbutamol group: salbutamol (2mg/kg orally); Group 6- salmeterol group: salmeterol (10mg/kg orally).

In this study, Tail flick method was used to evaluate analgesic activity of Salbutamol and Salmeterol.

Analgesic activity was assessed using modified method of D Amour and Smith<sup>12</sup> called as Tail Flick method using analgesiometer. Reaction time in seconds was used as unit for measurement of pain and an increase in reaction time was indicative of analgesia. Time between placing the tail of the rat on the radiant heat source and sharp withdrawal of the tail was recorded as 'reaction time'. Cut off time of ten seconds was imposed in all sets of experiments taken as maximum latency so as to rule out thermal injury while noting down the reaction time.

Animals whose mean reaction time was outside the range of five-six seconds were discarded. In all the groups, tail-flick test was performed prior to drug administration and

at 30, 60, 90 and 120 minutes after drug administration and the reaction time at each time interval (test latency) was calculated.

Average reaction times were then calculated and the percentage analgesia was calculated using the following formula:

$$\text{Percentage analgesia} = (\text{TL-BL} / \text{ML-BL}) \times 100$$

Where M.L.= Maximum latency, T.L.= Test latency, B.L.= Basal latency or control latency

### Anti-inflammatory activity

For anti-inflammatory activity animals were grouped into the following five groups:

Group 1- Control: Normal saline 2 ml/kg (p.o.); Group 2- Standard drug: aspirin 300 mg/kg (p.o.); Group 3- salbutamol group: salbutamol (1mg/kg orally); Group 4- salbutamol group: salbutamol (2mg/kg orally); Group 5- salmeterol group: salmeterol (10mg/kg orally).

### Evaluation of Acute anti-inflammatory activity: Carrageenan induced-rat paw edema<sup>13</sup>

The instrument used in this study for recording paw edema was Mercury Plethysmometer (modified by Hardayal Singh and Ghosh).<sup>14</sup> Left hind paw of rats was marked with ink at the level of lateral malleolus; basal paw volume was measured by volume displacement method using mercury Plethysmometer by immersing the paw till the level of lateral malleolus.<sup>15</sup> After one hour of drug administration 0.1 ml of 1% Carrageenan (1% in 0.9% normal saline solution) was injected into sub plantar region of the left hind paw of rats. Paw volume was measured by Plethysmometer just before 1% carrageenan injection, that is, at '0' hours and then at 30 minutes, 1<sup>st</sup> and 2<sup>nd</sup> hour after carrageenan injection.<sup>16</sup>

Same procedure was adopted for rats of all the groups. The percentage inhibition of oedema in animals of all groups was calculated by using the formula:

$$\text{Percentage inhibition} = (\text{Vc-Vt})/\text{Vc} \times 100$$

Where, Vc = Paw volume in control group, Vt = Paw volume in test group

### Evaluation of chronic anti-inflammatory activity: Formalin induced arthritis in rats<sup>17</sup>

Chronic inflammation was induced 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 drug to be tested was given daily for 10 days. Linear cross section (LCS) immediately below the ankle joint of right hind paw was measured daily with Vernier Callipers. The difference in LCS on day one and day 10 was calculated for all groups. Percentage anti-inflammatory effect of particular drug was calculated by the following formula:



$$\text{Percentage anti-inflammatory effect} = \frac{\text{Mean difference in LCS in control group} - \text{Mean difference in LCS in test group}}{\text{Mean difference in LCS in control group}} \times 100$$

### Statistical Analysis

Results are expressed as mean  $\pm$  Standard Error of Mean (SEM). Data was analysed by using Graph Pad Prism software version 8.0.2. Comparison between different groups was done by one-way ANOVA followed by Tukey's test. P value less than 0.05 (p value <0.05) was considered as statistically significant.

## RESULTS

### Analgesic activity: tail flick method

Table 1 shows that the mean reaction time of tramadol was statistically significantly higher when compared to all other groups at all observation times. At 30 minutes, the mean reaction time of aspirin was statistically significantly higher than the test drugs. At 120 minutes, mean reaction time of salmeterol was statistically significantly higher than salbutamol 2mg/kg. Mean reaction time of Aspirin was comparable with salbutamol(1mg/kg), salbutamol (2mg/kg) and salmeterol at 60, 90 and 120 minutes.

### Acute anti-inflammatory activity: carrageenan induced rat paw edema

Table 2 shows that the percentage analgesia of tramadol was greater than all the other groups at all observation times. At 60 minutes, salbutamol 2mg/kg showed higher percentage analgesia than aspirin and salbutamol 1mg/kg. At 90 minutes, percentage analgesia of all test drugs was higher than aspirin. At 120 minutes, salmeterol showed higher percentage analgesia than aspirin.

Table 3 shows that the mean paw volume in all the drug treated groups was statistically significantly lower when

compared to control group ( $P < 0.001$ ) at all observation times. Anti-inflammatory activity is inversely proportional to paw volume. At 60 and 120 minutes mean paw volume in salmeterol group was comparable to aspirin while that of salbutamol (both doses) was significantly more compared to aspirin.

### Chronic anti-inflammatory activity: Formalin induced arthritis in rats

Table 4 shows that the percentage inhibition of carrageenan induced paw edema was maximum at 120 minutes in all the three study groups. Aspirin showed higher anti-inflammatory activity than both the doses of salbutamol and salmeterol at all time intervals in the experiment.

### Chronic anti-inflammatory activity: Formalin induced arthritis

The mean difference between LCS on tenth day and first day was calculated for each group. Lower the difference in LCS, higher is the anti-inflammatory activity. In table 5, the mean differences in LCS in all the three drug treated groups were statistically significantly less when compared to control ( $P < 0.0001$ ). Inhibition of arthritis started from the third day in aspirin group, fifth day in salmeterol group, sixth day in salbutamol (1mg/ kg) and seventh day in the salbutamol (2mg/ kg) group, and maximum effect was seen on the tenth day in all the groups. The least difference in mean LCS was found in the aspirin group.

Aspirin (87.63%) showed maximum percentage anti-inflammatory effect followed by salmeterol (57.59%), salbutamol (1mg/ kg) (48.76%) and salbutamol (2mg/kg) (34.62%).

**Table 1:** Mean reaction time by tail flick model of analgesia in rats (n=6)

Drugs and Doses (mg/kg)	Mean reaction time in seconds				
	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes
Control (2ml/kg p.o.)	3.45 $\pm$ 0.10	3.51 $\pm$ 0.12*	3.53 $\pm$ 0.13*	3.45 $\pm$ 0.11*	3.45 $\pm$ 0.09*
Tramadol (10mg/kg i.p.)	3.78 $\pm$ 0.2	8.38 $\pm$ 0.06	8.96 $\pm$ 0.06**	9.78 $\pm$ 0.07**	9.36 $\pm$ 0.09**
Aspirin (300mg/kg p.o.)	3.61 $\pm$ 0.13	7.96 $\pm$ 0.11	7.28 $\pm$ 0.07	8.12 $\pm$ 0.06	6.90 $\pm$ 0.04
Salbutamol (1mg/ kg p.o.)	3.53 $\pm$ 0.33	6.05 $\pm$ 0.48**	7.20 $\pm$ 0.60	7.93 $\pm$ 0.50	6.70 $\pm$ 0.63
Salbutamol (2mg/ kg p.o.)	3.33 $\pm$ 0.10	6.65 $\pm$ 0.17 <sup>#</sup>	7.60 $\pm$ 0.22	7.05 $\pm$ 0.36	6.00 $\pm$ 0.31
Salmeterol (10 mg/kg p.o.)	3.55 $\pm$ 0.2	6.95 $\pm$ 0.28 <sup>#</sup>	7.20 $\pm$ 0.28	7.85 $\pm$ 0.20	7.30 $\pm$ 0.18 <sup>@</sup>

p.o. = per os (by mouth); i.p. = intraperitoneal. Data analysed using one-way analysis of variance (ANOVA) followed by Tukey's test. Values are expressed as mean  $\pm$  S.E.M. (n = 6 in each group); \* $P < 0.0001$  when compared to all other groups in all columns. Column 2: \*\* $P < 0.0001$  when compared to tramadol and aspirin, # $P < 0.05$  when compared to tramadol and aspirin. Column 3: \*\* $P < 0.05$  when compared to all other groups. Column 4: \*\* $P < 0.05$  when compared to all other groups. Column 5: \*\* $P < 0.0001$  when compared to all other groups, @ $P < 0.05$  when compared to salbutamol 2mg.

**Table 2:** Percentage analgesia in tail flick model of analgesia in rats (n=6)

Drugs and doses (mg/kg)	Percentage analgesia (%)			
	At 30 minutes	At 60 minutes	At 90 minutes	At 120 minutes
Tramadol (10mg/kg i.p.)	73.50	82.00	80.00	67.00
Aspirin (300 mg/kg p.o.)	67.66	57.26	49.22	47.63
Salbutamol (1mg/ kg p.o.)	39.50	56.23	69.90	44.41
Salbutamol (2mg/ kg p.o.)	49.80	64.53	56.00	42.67
Salmeterol (10 mg/kg p.o.)	52.16	56.18	66.35	57.85

**Table 3:** Effects of different drugs on carrageenan induced paw oedema

Drugs and doses (mg/kg)	Paw volume (ml)			
	At 0 minutes	At 30 minutes	At 60 minutes	At 120 minutes
Control (2ml/kg p.o.)	0.36± 0.03	0.8± 0.02	1.08± 0.04	1.21± 0.05
Aspirin (300 mg/kg p.o.)	0.31± 0.03	0.41± 0.03*	0.50± 0.03* <sup>@</sup>	0.31± 0.03* <sup>@</sup>
Salbutamol (1mg/ kg p.o.)	0.33± 0.04	0.58± 0.06*	0.90± 0.06*	0.73± 0.04*
Salbutamol (2mg/ kg p.o.)	0.34± 0.08	0.56 ± 0.08*	0.76± 0.09*	0.56± 0.04*
Salmeterol (10 mg/kg p.o.)	0.31± 0.04	0.51± 0.04*	0.61± 0.04* <sup>#</sup>	0.45± 0.04* <sup>#</sup>

Data analysed using one-way analysis of variance (ANOVA) followed by Tukey's test.; Values are expressed as mean ± S.E.M. (n = 6 in each group); Where p.o. = per os (by mouth); Column 2: \*P< 0.05 when compared to control, Column 3 and column 4: \*P< 0.0001 as compared to control, <sup>@</sup>P<0.05 when compared to salbutamol 1mg, salbutamol 2mg, <sup>#</sup>P<0.05 when compared to salbutamol 1mg

**Table 4:** Percentage inhibition of carrageenan induced rat paw edema by different drugs

Drugs and doses (mg/kg)	Percentage inhibition (%)		
	At 30 minutes	At 60 minutes	At 120 minutes
Aspirin (300 mg/kg p.o.)	47.91	53.40	72.77
Salbutamol (1mg/ kg p.o.)	27.08	16.64	37.22
Salbutamol (2mg/ kg p.o.)	29.16	29.67	51.11
Salmeterol (10 mg/kg p.o.)	35.41	42.14	62.80

Where; p.o. = per os (by mouth)

**Table 5:** Effects of different drugs on linear cross section below the ankle joint in formalin induced arthritis in rats (n =6)

Drugs and doses (mg/kg)	Mean initial LCS	Mean Day 10 LCS	Mean difference in LCS	Percentage anti-inflammatory effect
Control (2ml/kg p.o.)	4.71±0.20	7.55±0.27	2.83±0.22	-----
Aspirin (300 mg/kg p.o.)	4.4±0.26	4.75±0.16	0.35±0.17* <sup>@</sup>	87.63
Salbutamol (1mg/ kg p.o.)	4.31±0.23	5.76±0.21	1.45±0.18*	48.76
Salbutamol (2mg/ kg p.o.)	4±0.19	5.85±0.25	1.85±0.10*	34.62
Salmeterol (10 mg/kg p.o.)	3.85±0.12	5.05±0.12	1.20±0.04* <sup>\$</sup>	57.59

Data analysed using one-way analysis of variance (ANOVA) followed by Tukey's test. Values are expressed as mean ± S.E.M. (n = 6 in each group) Where, LCS = Linear Cross Section, p.o. = per os (by mouth); \*P< 0.0001 when compared to control, <sup>@</sup>P<0.05 when compared to salbutamol 1mg/kg, salbutamol 2mg/kg and salmeterol, <sup>\$</sup>P< 0.001 when compared to salbutamol 2mg/kg.

## DISCUSSION

This study has investigated the protective effect of  $\beta$ -2 adrenergic receptor agonist drugs on nociception model, acute and chronic inflammatory models. Analgesics act on central or peripheral nervous system to relieve pain selectively without altering consciousness.<sup>18</sup> Centrally acting analgesics alter the physiological response to pain and increase the pain threshold. However, generation of

pain impulses at the chemoreceptor level is inhibited by peripherally acting drugs.<sup>19</sup> Tail flick method is generally used for centrally acting analgesics through opioid receptors.<sup>20</sup> This was evident in this study as in all the four drug treated groups, the pain threshold increased significantly during the experiment with tramadol group showing maximum effect. In this method, test animals treated with both doses of salbutamol (1mg/kg and 2 mg/kg) and salmeterol (10mg/kg) have shown an increase



in the reaction time at 30, 60, 90 and 120 minutes post administration compared to pre-treatment levels. Analgesic activity of salbutamol and salmeterol came out to be comparable with aspirin at specific time intervals in the tail-flick model of analgesia. Still, clinical usefulness of these drugs as analgesics in the absence of inflammation is less likely as the inflammatory nociceptive mediators are distinct. Extensive literature search suggests that  $\beta_2$  adrenergic receptor agonist drugs may be more effective in suppressing the release of these distinct inflammatory nociceptive mediators<sup>4-7</sup> and hence, their clinical utility would be better in these clinical scenarios.

Though aspirin has a central component of action, it predominantly produces analgesia through a peripheral action. Hence, maximum analgesic action of aspirin cannot be evident in this method.

The underlying mechanism by which  $\beta_2$  adrenergic agonists inhibit nociception is not investigated in our study; however, it has been reported that they increase the intracellular cyclic AMP levels which further reduce secretion of various cytokines produced by monocyte/macrophages or T cells.<sup>21</sup>

Carrageenan-induced paw oedema is a standard experimental model of acute inflammation. Carrageenan is non-antigenic and devoid of apparent systemic effects; hence it is considered to be the phlogistic agent of choice for testing anti-inflammatory drugs. This model of acute inflammation exhibits a high degree of reproducibility. Therefore, it has proven significant predictive value for clinically useful anti-inflammatory drugs.<sup>12</sup>

Our study demonstrated that both doses of salbutamol (1mg/kg and 2mg/kg) and salmeterol (10mg/kg) effectively reduced the acute inflammation associated with carrageenan injection which is in consensus with the findings of the study conducted by Asha et al and Uzkeser et al.<sup>9,10</sup> Various investigators have tried to explore the mechanisms underlying the anti-inflammatory activity of  $\beta_2$ -adrenergic receptor agonists.  $\beta_2$ -receptors are expressed in neutrophils, monocytes, and macrophages which are involved in the regulation of inflammation.<sup>22-24</sup> It has been suggested that activation of  $\beta_2$  receptors causes intracellular increase of cAMP generated by the activation of adenylyl cyclase in CD4+ lymphocytes, eosinophils and neutrophils, reducing the release of inflammatory mediators from these cells.<sup>21,25</sup>

Anti-inflammatory role of both doses of salbutamol (1mg/kg and 2 mg/kg) and salmeterol (10mg/kg) in chronic inflammation can be suggested by our study findings in formalin-induced arthritis model of chronic inflammation. The result of our study was in accordance with the study conducted by Asha et al and Malfait et al.<sup>9,26</sup>

Collagen type II specific Interferon- $\gamma$  proliferation and mast cell degranulation in joint tissues is diminished by salbutamol in arthritis model. This could be the probable reason for significant anti-inflammatory action of these test drugs by enhancing vascular permeability.<sup>27</sup> Moreover,

Interleukin-12 release by peritoneal macrophages is blocked by salbutamol in a dose dependent manner. It also directly stimulates  $\beta$ -adrenergic receptors on inflammatory immune cells like monocytes, macrophages, Langerhans cells and blocks TNF release.<sup>28-30</sup> This also could be the probable reason for anti-inflammatory action of salbutamol in chronic inflammatory model.

These results are in accordance with already published reports in literature which indicate that  $\beta_2$  adrenergic receptor agonists do have an important role to play as analgesics and anti-inflammatory agents.<sup>9,10,25,31</sup> However, considering the availability of many other drugs having superior analgesic and anti-inflammatory activities, the routine use of  $\beta_2$  adrenergic receptor agonists for these effects cannot be justified. Since anti-inflammatory drugs like corticosteroids form the mainstay of treatment in bronchial asthma along with  $\beta_2$  agonists, anti-inflammatory action of these drugs could be considered an additional mechanism for their usefulness in asthma. Further, it is suggestive that they may be particularly effective in the clinical scenario of inflammatory nociception.

## CONCLUSION

Our study suggests that salbutamol and salmeterol possess significant analgesic activity in rats in tail flick model. Their analgesic activity may be beneficial particularly in conditions characterised by inflammatory nociception. They also have significant anti-inflammatory activity in both, acute and chronic inflammation in carrageenan induced paw edema and formalin induced arthritis in rats, respectively, which could be an additional mechanism for their usefulness in bronchial asthma.

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