

## **Relative Influence of adrenergic $\beta$ -agonist and antagonist on the inflammation and their interaction with aspirin**

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### **ABSTRACT**

*Aim of the present study was to evaluate the effects of adrenergic  $\beta$ -agonist and antagonist on acute and subacute inflammation and to study their possible interaction with aspirin, a commonly used NSAID. Propranolol in the dose of 4.5 mg/kg, 9 mg/kg, atenolol in the dose of 1mg/kg and 4.5 mg/kg, terbutaline in the dose of 0.45mg/kg and 0.9mg/kg and aspirin in the dose of 54,200 mg/kg were administered orally in different groups of albino rats, to study their effect on inflammation induced by carrageenan or a foreign body. Aspirin (54 mg/kg) was administered with terbutaline (0.45 mg/kg) propranolol and atenolol (4.5mg/kg and 1mg/kg) to separate groups in order to study their interaction. Terbutaline, propranolol and atenolol produced significant anti-inflammatory activity in acute as well as sub acute inflammation model except atenolol in sub acute model. Sub anti-inflammatory (low dose) dose of terbutaline potentiated the anti-inflammatory activity of aspirin where as propranolol and atenolol failed. In conclusion, adrenergic  $\beta$  agonist and antagonist showed significant anti-inflammatory activity and co-administration with aspirin potentiated anti-inflammatory response of aspirin.*

**Keywords:** Aspirin, anti-inflammatory agent, terbutaline, propranolol and atenolol

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### **INTRODUCTION**

Inflammation is a local response of tissue to injury and body defense reaction in order to eliminate or limit the spread of injurious agent [1]. It is characterized by accumulation of fluid and leukocytes in extravascular tissue and it is closely intertwined with process of repair [2]. The process of inflammation consists of vascular and cellular phase. Vascular events are due to alteration in vascular calibre, blood flow and permeability where as cellular events consists of accumulation of leukocytes, to engulf the inflammogens to protect normal tissue [3, 4]. In recent years increased understanding of the inflammatory mechanism and mediators involved has led to the development of newer anti-inflammatory agents like monoclonal antibodies and antagonists of inflammogens [5]. Interestingly, several other drugs like adrenergic agonists and antagonists have also been reported to possess anti-inflammatory property.

Earlier adrenergic agonists like adrenaline; noradrenaline and isoprenaline have been reported to potentiate thermic edema of rat paw [6]. Surprisingly in another study adrenaline, noradrenaline by virtue of their vasoconstrictive property and isoprenaline by reducing vascular permeability have been reported to possess anti-inflammatory activity [8]. However beta-2-agonist like salmeterol and salbutamol has been reported anti-inflammatory in marine model of pleurisy [8]. Paradoxically drug like propranolol act as anti-inflammatory in carrageenan and catheter induced inflammation [9, 10]. On the other hand propranolol has been reported to potentiate inflammation [11]. In view of above mentioned controversies it is very difficult to conceive that both agonists and antagonist producing the same effect.

Therefore, the present study was planned to investigate the effect of some selective  $\beta$ -2 agonist like terbutaline and specific as well as nonspecific beta blocker like atenolol and propranolol on acute and subacute model of inflammation in albino rats. The other objective was to investigate the interaction of said agonist and antagonist with aspirin.

## MATERIALS AND METHODS

### Animals

Male albino Wistar rats (150-270g) were used. The animals were acclimatized to normal laboratory conditions with 12:12 hr. natural light: dark cycle and were fed with standard laboratory diet with free access to water.

### Acute inflammation

Rats were divided into six groups comprising six animals in each group. They were starved overnight with water *ad libitum* prior to day of experiment. The control group received 0.5 ml of 1% gum acacia suspension orally. While the other groups received different drug treatment as per **Table 1**.

Thirty Minutes after drug administration, acute inflammation was induced by injecting 0.05 ml at 1% carrageenan (sigma Company, St Louis) in normal saline into the sub planter region of the left hind paw, as per the method given in literature [12]. A mark was applied on the leg at the malleolus to facilitate subsequent readings. The paw edema volume was measured by mercury displacement with the help at plethysmograph at 0, 0.5, 1, 3, and 5 h after injecting carrageenan. The difference between 0 h and subsequent readings was considered as edema volume. The percentage inhibition of edema in various groups was calculated using the formula :

$$\% \text{ edema inhibition} = 1 - V_t / V_c \times 100$$

$V_t$  and  $V_c$  were edema volume in drug treated and control groups respectively.

### Subacute inflammation

Subacute inflammation was induced by a slightly modifying the method given by D'Arcy [13]. Four groups of six rats each were used. Under light ether anaesthesia, hair in the axilla and the groin were clipped, and two sterile cotton pellets, weighing 10mg each and two sterile grass piths (25 × 2 mm) were implanted subcutaneously, through a small incision, either in the axilla or the groin, at random. The wounds were then sutured and the animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the experiment. The rat then received treatments as shown in **Table 3**.

The treatment was started on the day after the implantation and was repeated every 24 h regularly for 10 days. On the 11<sup>th</sup> day the rats were sacrificed with an overdose of ether anaesthesia, and the cotton pellets, grass piths were removed. The pellets, freed from extraneous tissues, were dried overnight at 60°C and their dry weight measured. Net granuloma formation was calculated by subtracting the initial weights of cotton pellet from the final weights. Mean granuloma dry weights for the various groups was calculated and expressed as mg/100gm body weight. The glass piths were preserved in 10% formalin for histopathological study after H and E staining.

### Drugs and Doses

The clinical doses for various drugs were converted to rat equivalent doses with the help of converting table devised by Paget and Barnes cited by Gosh [14]. All the drugs were dissolved or suspended in water for oral administration in the volume of 5ml/kg-body weight.

- 1) **Aspirin** (IP grade, Dept. of p'cology J. N. Medical College, Belgaum) in 2% gum acacia suspension was administered at 54 and 200 mg/kg doses and also used as standard anti-inflammatory agent for the comparison.
- 2) **Terbutaline Sulphate** (Astra idl, Hyderabad) was used in dose of 0.45 mg/kg and 0.9 mg/kg.
- 3) **Propranolol Hydrochloride**: (Sigma laboratories, USA) dissolved in water was administered in doses of 0.35 mg/kg and 0.72 mg/kg.
- 4) **Atenolol**: (Wockhardt India Ltd.) was used in the dose of 1mg/kg and 4.5mg/kg
- 5) **Carrageenan** (Sigma St. Louis, USA) was used in the dose of 0.1 ml at 1-% suspension in normal saline to induce rat paw edema.

In interaction studies, aspirin, terbutaline. propranolol and atenolol were used in their sub effective doses as given in **Table 1**.

All the procedures were performed in accordance with the guidelines issued by the Institutional Animal Ethical Committee.

### Statistical analysis

Data were expressed as mean  $\pm$ SEM and were analysed by ANOVA followed by student's 't' test. P value  $\leq$  to 0.05 was considered significant.

## RESULTS

### Acute Studies

In the present study, aspirin (200mg/kg) indicating significant ( $P < 0.05-0.001$ ) inhibition of edema as compared to that of the control group up to 5<sup>th</sup> h. Similarly terbutaline in the dose of (0.9 mg/kg) showed significant ( $P < 0.05 - 0.001$ ) inhibition of paw edema (0.5<sup>th</sup> h, 1<sup>st</sup> h and 3<sup>rd</sup> h). Propranolol in the dose of (9mg/kg) produced significant ( $P < 0.05 - 0.001$ ) inhibition of inflammation (0.5<sup>th</sup> h, 1<sup>st</sup> h, 3<sup>rd</sup> and 5h respectively). Atenolol in the dose of 4.5mg/kg produced significant ( $P < 0.05 - 0.001$ ) inhibition of inflammation (0.5<sup>th</sup> h, 1<sup>st</sup> h, 3<sup>rd</sup> and 5h respectively).

To study possible interaction between aspirin and adrenergic agonist and antagonists series of experiment carried out to determine sub anti-inflammatory dose of all above mentioned drugs.

**Table 1: Drug treatment Schedule for anti-inflammatory studies (Acute and subacute)**

Sr. No.	Treatment	Dose (mg/kg)
1*	Control (Saline)	--
2*	Aspirin	200
3*	Terbutaline	0.9
4*	Propranolol	9
5*	Atenolol	4.5
6	Terbutaline+Aspirin	0.45+54
7	Propranolol+Aspirin	4.5+54
8	Atenolol+Aspirin	1+54

1. All the mentioned drugs were given one hour while aspirin 30 min. prior to carrageenan injection.
2. \*Similar groups were included for sub-acute studies and drugs were given once daily for 10 days  
All drugs were administered orally.

**Table 2: Effect of  $\beta$  - adrenergic agonist and antagonist on carrageenan edema**

Group	Drug (mg/kg)	Paw Volume in ml Mean $\pm$ SEM			
		0.5 h	1 h	3 h	5 h
1	Control	0.53 $\pm$ 0.05	1.4 $\pm$ 0.14	1.06 $\pm$ 0.28	0.46 $\pm$ 0.2
2	Aspirin (200)	0.27* $\pm$ 0.01	0.18 *** $\pm$ 0.1	0.066** $\pm$ 0.04	0.016** $\pm$ 0.016
3	Terbutaline (0.9)	0.21*** $\pm$ 0.02	0.31*** $\pm$ 0.03	0.36** $\pm$ 0.06	0.43 $\pm$ 0.061
4	Propranolol (9)	0.26* $\pm$ 0.12	0.31*** $\pm$ 0.10	0.26* $\pm$ 0.11	0.41 $\pm$ 0.11
5	Atenolol(4.5)	0.15*** $\pm$ 0.06	0.18*** $\pm$ 0.07	0.33** $\pm$ 0.10	0.26 $\pm$ 0.1
6	Terbutaline(0.450 + Aspirin (54)	0.01*** $\pm$ 0.02	0.13*** $\pm$ 0.04	0.31* $\pm$ 0.06	0.18 $\pm$ 0.05
7	Propranolol(4.5) + Aspirin (54)	0.12*** $\pm$ 0.04	0.15*** $\pm$ 0.03	0.5 $\pm$ 0.11	0.12 $\pm$ 0.05
8	Atenolol(1) + Aspirin (54)	0.02*** $\pm$ 0.02	0.07*** $\pm$ 0.05	0.68 $\pm$ 0.15	0.61 $\pm$ 0.13

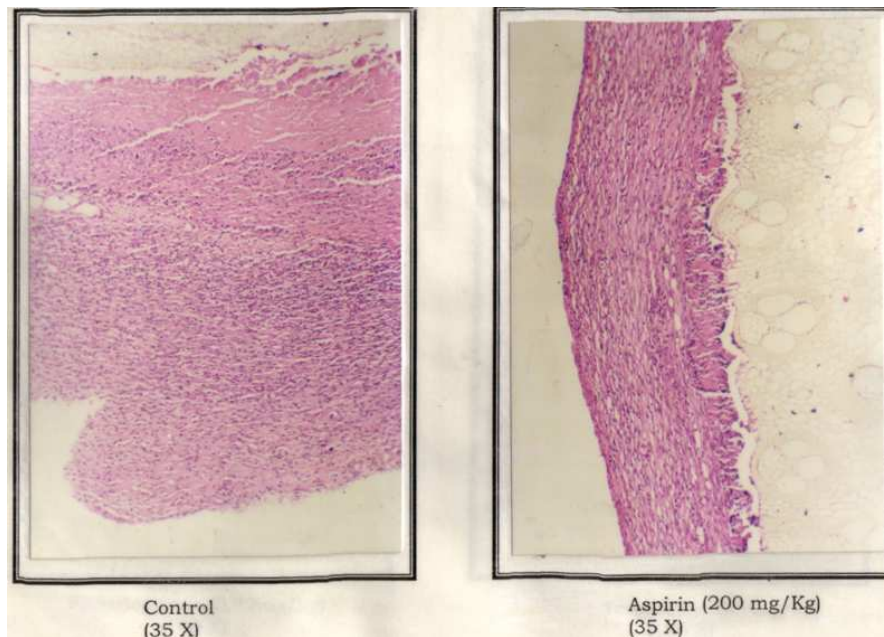
#### ANOVA

$F_{5, 30} = 8.71 (0.5hr), 15.26 (1hr) \text{ and } 5.92 (3hr), 4.37 (3hr) \text{ and } 2.88(5h) > 2.37 = 0.05$   
\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (Student 't' test). N = 6 Animals in each group.

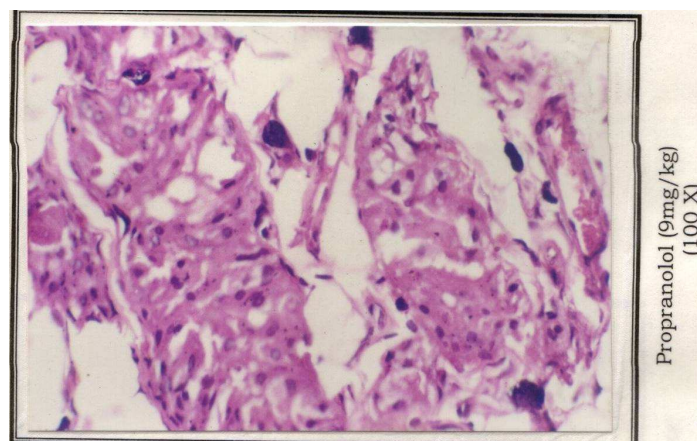
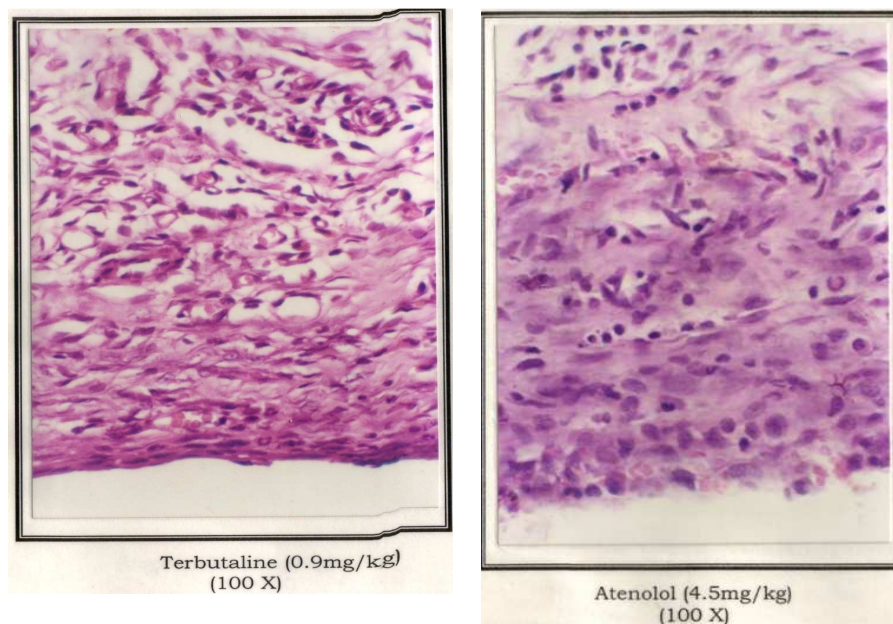
**Table 3: Effect of  $\beta$  adrenergic agonist and antagonist on foreign body induced granuloma**

Group	Dose (mg/kg)	Granuloma dry wt. (mg % body wt)	't' value	'P' value
1	Control	41.66 $\pm$ 2.61	--	--
2	Aspirin (200)	28.16*** $\pm$ 1.35	4.59	< 0.001
3	Terbutaline (0.9)	34.84* $\pm$ 1.08	2.41	< 0.05
4	Propranolol (9)	21.55*** $\pm$ 2.46	5.60	< 0.001
5	Atenolol(4.5)	33.50 $\pm$ 2.46	1.79	N.S

Values are mean  $\pm$  SEM. All the drugs were administered p.o. once daily for 10 days. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  as compared to control. ANOVA  $F_{6, 35} = 14.62 > 3.36 = 0.01$ . N = 6 Animals in each group



Microphotographs of granulation tissues stained with H and E



Microphotographs of granulation tissues stained with H and E



Sub anti-inflammatory dose of aspirin (54mg/kg) when given with sub-effective (low dose) dose of terbutaline (0.45 mg/kg) kg produced significant ( $P < 0.05$  -0.001) inhibition of inflammation (0.5<sup>th</sup> h, 1<sup>st</sup> h, 3<sup>rd</sup> hand respectively). However propranolol and atenolol in the dose of (4.5 mg/kg) and (1mg/kg) respectively failed to potentiate anti-inflammatory activity of aspirin (up to 0.5h and 1h). These results clearly indicate that terbutaline potentiated anti-inflammatory activity of aspirin while beta blockers used in the present study failed to do so when co-administered with aspirin. All these results are given in **Table 2**.

#### **Sub acute studies**

Mean granuloma dry weights in aspirin, terbutaline, propranolol and atenolol groups were found to be lower than the control group, indicating significant ( $P < 0.001$ ) anti-inflammatory property of aspirin, terbutaline, propranolol and atenolol. These observations were further confirmed by histopathological studies of haematoxyline and eosin stained granulation tissue sections in the various treated groups. There was abundant granulation tissue (Macroscopically) surrounding the grass pith in control animal and microscopic studies revealed reduced number of fibroblasts, decreased collagen contents and fibrous tissue in all treated groups as compared to saline treated control.

### **DISCUSSION**

As expected, aspirin showed significant anti-inflammatory activity in both the models of inflammation i.e. with carrageenan induced, paw edema and foreign body induced granuloma. Significant anti-inflammatory activity of terbutaline in the present study, also corroborated with earlier report about anti-inflammatory activity of salmeterol and salbutamol like agonist [7].

Terbutaline also potentiated anti-inflammatory activity of aspirin in acute model of inflammation and no such interaction studies are reported in literature. Both the beta blockers showed significant anti-inflammatory activity in both the models of inflammation. Though there are no reports regarding anti-inflammatory activity of atenolol, as observed in the present study propranolol has been reported to possess anti-inflammatory activity in earlier experimental and clinical studies [9, 10].

Both propranolol and atenolol significantly potentiated the anti-inflammatory activity of aspirin for about one hour in acute inflammation and such interaction studies between beta blockers and aspirin appears to be scanty. In subacute inflammation propranolol but not atenolol showed significant anti-inflammatory activity in present study. Literature survey indicates paucity of such study. Based on earlier reports and present finding, it reveals that reduced vascular permeability due to direct effect on vascular endothelium has been attributed as mechanism of action for beta agonist.

Anti-inflammatory activity of propranolol has been ascribed not to its beta blocking activity but due to its other activities like antiprostaglandin and activation of adrenal pituitary system [15, 16]. This is supported by the present findings that only propranolol but not atenolol inhibited significantly chronic inflammation. It is enigmating that atenolol which failed to suppress sub-acute inflammation significantly inhibited acute inflammation.

Atenolol by blocking beta-1 receptors in the kidney can be expected to decrease the rennin release leading to decrease angiotensin-2 formation resulting into vasodilatation. Vasodilator like isoprenaline have been shown to suppress carrageenan induced inflammation by decreasing vascular permeability.

### **CONCLUSION**

The findings of the present studies with regard to anti-inflammatory activity of adrenergic agonist and antagonist indicate need for further studies to ascertain their mechanism of action.

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