ANTI-INFLAMMATORY INFLUENCE OF ADRENERGIC AGONISTS & ANTAGONISTS & THEIR INTERACTION WITH ASPIRIN IN WISTAR RATS

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Summary

The vascular changes in inflammation consist of an initial brief vasoconstriction followed by vasodilation, increased capillary permeability leading to leucocytic infiltration in the inflamed tissue. Hence vasodilators & vasoconstrictors are likely to influence the process of inflammation. Controversial reports with respect to anti-inflammatory activity of sympathomimetics & sympatholytics prompted the present study. Phenyephrine, terbutaline, prazosin, propranolol & atenolol were evaluated for their action on the acute & subacute models of inflammation & also their interaction with aspirin at subtherapeutic doses in Wistar rats. All the above mentioned drugs except atenolol showed anti-inflammatory effect in both the models of inflammation at therapeutic doses whereas only phenylephrine, prazosin & terbutaline showed the anti-inflammatory effect in combination with aspirin at subtherapeutic doses.

Key words: Phenylephrine, Terbutaline, Prazosin, Propranolol, Atenolol, Wound Healing

Introduction

The process of inflammation, a common clinical entity consists of two phases viz a vascular phase & cellular phase. The vascular events consist of an initial brief vasoconstriction followed by vasodilation & increased capillary permeability leading to leucocytic infiltration into the inflamed tissue. These events of changes are due to changes in the vascular caliber, permeability & blood flow[1]. Therefore vasoactive substances both vasodilators & vasoconstrictors are likely to influence the process of inflammation.

Both sympathomimetics & sympatholytics are well known to exert their pharmacological actions on the vascular smooth muscles leading to vasoconstriction & vasodilation.

Catecholamines like adrenaline, noradrenaline & isoprenaline potentiate thermic edema in the rat paw by reducing blood flow to the paw[2]. Another study reported that adrenaline, noradrenaline by virtue of its vasoconstrictor effect & isoprenaline by reducing vascular permeability have been reported to have anti-inflammatory action[3] . Alpha receptor blockers like phenoxybenzamine exert anti-inflammatory effect[4] whereas dihydroergotamine has been reported to have no anti-inflammatory property[5]. Beta-2 agonists like salmetrol is a potent inhibitor of inflammatory mediator release[6] while salbutamol inhibits early phase of carrageenan inflammation in marine model of plerisy[7].

Paradoxically beta blockers like propranolol is known to potentiate the anti-inflammatory activity of adrenaline in carrageenan induced rat paw edema[8]. Interestingly a clinical study with propranolol also showed anti-inflammatory activity in catheter induced urethral inflammation[9]. Some studies also indicate that propranolol potentiates inflammation by inhibiting the uptake of histamine & serotonin[10]

It is very difficult to conceive that both sympathomimetics & sympatholytics produce similar effects. The present study was thus planned to reinvestigate the effect of alpha-1 agonist phenylephrine & antagonist prazosin; beta-2 agonist terbutaline & antagonists atenolol & propranolol on acute & subacute inflammation in albino rats. Also in the present study the above mentioned agonists & antagonists have been explored for their possible interaction with aspirin, a commonly used non steroidal anti-inflammatory drug (NSAID).

Materials & Methods.

Animals & drug treatment

Healthy male Wistar rats of either sex weighing 150±20g were housed individually & acclimatized to the laboratory for a week under 12:12 light dark cycle. The animals were fed on standard pellet diet (Amrut brand) & water ad lib, where as they were starved overnight the day prior to experimentation. The study was approved by the institutional animal ethics committee constituted as per CPCSEA guidelines. For anti–inflammatory activity two models of inflammation viz carrageenan induced paw edema(acute inflammation) & foreign body (grass pith & cotton pellet) induced granuloma formation (sub-acute inflammation) were employed .

Wound Models: Acute inflammation was produced by injecting 0.05ml of 1 % carrageenan in one of the hind paw as described by Winter et al[11]. Modified standard technique of D'Arcy[12] as described in earlier literature was employed to induce subacute inflammation by implanting two cotton pellets (10 mg each) & two sterile grass piths (25X3mm) subcutaneously in axillae/groin randomly under light ether anesthesia with strict aseptic precautions .

Considering the maximum human therapeutic equivalent dose, rat doses for each was calculated using Paget & Barnes[13] conversion table.

In acute inflammation studies, aspirin was administered in the dose of 200mg/kg as therapeutic dose & 54mg/kg as subtherapeutic dose in the form of suspension in 2% gum acacia, single dose. Phenylephrine 0.72mg/kg, terbutaline 0.9mg/kg, prazosin 0.9mg/kg, propranolol hydrochloride 9mg/kg & atenolol 1mg/kg was administered as therapeutic doses & phenylephrine 0.35mg/kg, terbutaline 0.45mg/kg, prazosin 0.11mg/kg, propranolol 4.5mg/kg & atenolol 1mg/kg were administered as subtherapeutic doses in combination with aspirin as a single dose.
All the drugs were dissolved or suspended in water for oral administration in the volume of 5mg/kg body weight. Phenylephrine, terbutaline, prazosin & propranolol were given 1 hour, while atenolol 2 hr prior & aspirin 30 mins prior to carrageenan injection in each group (n=6 animals)
In subacute studies all the treatments were repeated every 24hrs for 10 days.

Carrageenan injected paw volume was measured at 0 (immediately after injection), 1, 3, & 5 hours after injection in all groups with the help of a plethysmometer (mercury displacement) & the actual edema volume was calculated by subtracting initial (0 hr) reading from subsequent corresponding readings. The percentage inhibition of paw edema was calculated using the formula

Percentage inhibition = 100(1- Vt/Vc)
Vc- edema volume in control while Vt – edema volume in treated groups.

The foreign body implanted animals were sacrificed on day 11 by over dose of ether anesthesia to dissect out the foreign body granulomas. Ten day old cotton pellet granulomas were dried overnight at 60^0C in an incubator to note their dry weight & the same was expressed as mg/100gm of body weight as suggested by Di Pasquale & Meli[14]. Grass pith induced granulomas were preserved in 10% formalin for their histopathological studies. The preserved granulomas were sectioned & stained with hematoxylin & eosin (H & E) for microscopic quantification of granulation tissue.

Statistical analysis

The results were analysed by ANOVA followed by Dunnet's posthoc test & $p \leq 0.05$ was considered as significant.

Results

In the acute model of inflammation, as expected aspirin showed significant ($p \leq 0.05$) anti-inflammatory activity from 1st hour onwards & similar results were seen with phenylephrine, prazosin, terbutaline, propranolol & atenolol at therapeutic doses (Table I). The above mentioned drugs also showed anti-inflammatory effect at subtherapeutic doses when administered singly. (Table II) Combination treatment with subtherapeutic anti-inflammatory doses showed significant anti-inflammatory activity, indicating mutual synergistic activity.(Table III)

In foreign body induced inflammation (subacute model) all the above mentioned drugs except atenolol showed significant ($p \leq 0.001$) anti-inflammatory activity as denoted by reduction in granuloma formation.

The present study revealed abundant granulation tissue (macroscopically) surrounding the grass pith in control animals & microscopic studies revealed reduced number of fibroblasts, decreased collagen content & fibrous tissue in all treated groups as compared to saline treated controls.(Figures I-VI)

Table I : Effect of adrenergic agonists & antagonists on acute & subacute inflammation in therapeutic doses

Group	Drug mg/kg	Paw volume (ml) Mean ± SEM				Granuloma dry weight
		0.5 hr	1 hr	3 hr	5hr	
1	Control	0.53± 00.05	1.4± 0.144	1.06± 0.28	0.46± 0.2	41.66± 2.61
2	Aspirin (200)	0.27± 0.1*	0.18± 0.1***	0.066± 0.042**	0.016± 0.016**	28.16± 1.35***
3	Phenylephrine 0.72	0.21± 0.02***	0.21± 0.02***	0.36± 0.06**	0.43± 0.061	16.16± 1.58***
4	Prazosin 0.9	0.26± 0.12*	0.31± 0.10***	0.26± 0.11*	0.41± 0.11	32.61± 1.15**
5	Terbutaline 0.9	0.3± 0.05**	0.56± 0.11***	0.6± 0.13*	0.41± 0.13	34.84± 1.08*
6	Propranolol 9	0.16± 0.1**	0.33± 0.11***	0.23± 0.1**	0.11± 0.04*	21.55± 2.46***
7	Atenolol 4.5	0.21± 0.08**	0.183± 0.05***	0.55± 0.1*	0.45± 0.12	33.5± 2.46

* $p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$

Table II: Effect of adrenergic agonists & antagonists on acute inflammation at subtherapeutic doses

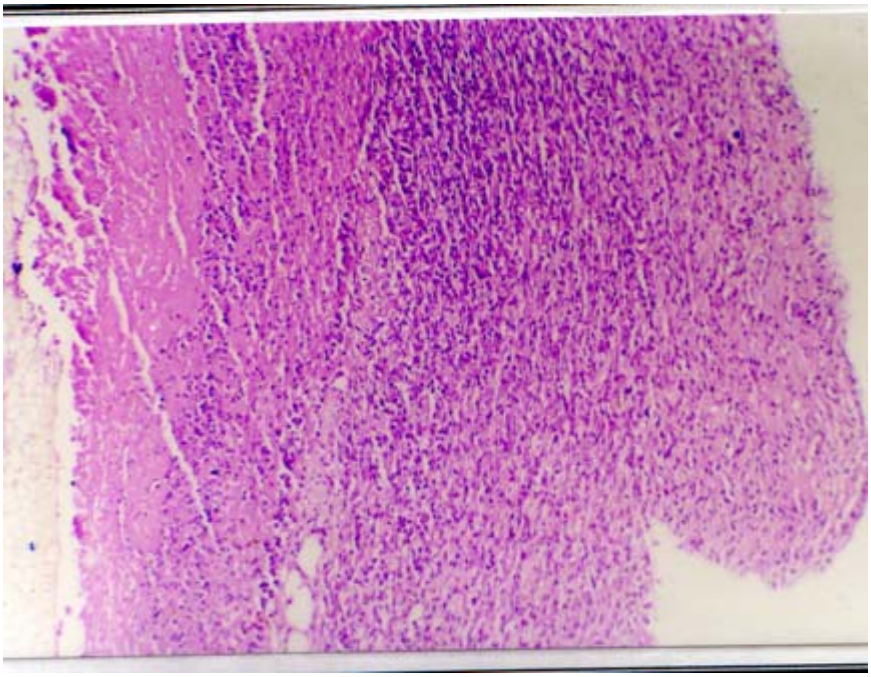
Group	Drug mg/kg	Paw volume (ml) Mean ± SEM			
		0.5hr	1hr	3 hr	5hr
1	Control	0.53± 0.05	1.4± 0.14	1.06± 0.3	0.46± 0.2
2	Phenylephrine 0.35	0.15± 0.04***	0.26± 0.04***	0.61± 0.14	0.53± 0.08
3	Prazosin 0.11	0.39± 0.03*	0.68± 0.07**	1.13± 0.15	0.81± 0.16
4	Terbutaline 0.45	0.21± 0.02***	0.31± 0.04***	0.96± 0.16	0.51± 0.16
5	Propranolol 4.5	0.18± 0.04***	0.35± 0.07***	0.55± 0.04	0.17± 0.08
6	Atenolol 1	0.50± 0.05**	0.66± 0.05***	0.85± 0.04	0.70± 0.1

$p \leq 0.05$, ** $p \leq 0.01$ & *** $p \leq 0.001$

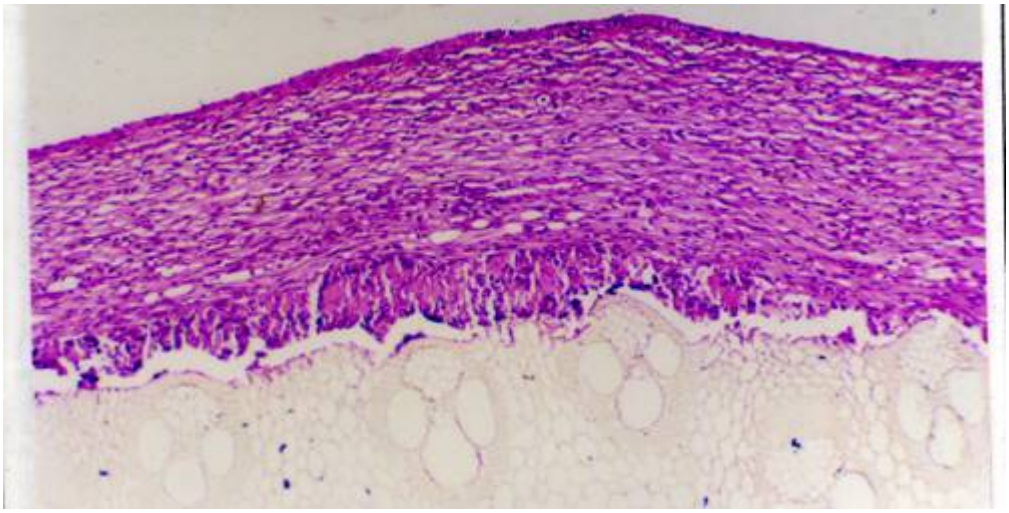
Table III: Effect of adrenergic agonists & antagonists on acute inflammation in combination with aspirin at subtherapeutic doses

Group	Drug mg/kg	Paw volume (ml) Mean ± SEM			
		0.5hr	1hr	3 hr	5hr
1	Control	0.53± 0.05	1.4± 0.14	1.06± 0.3	0.46± 0.2
2	Phenylephrine 0.35+ Aspirin54	0.15± 0.06***	0.31± 0.10***	0.33± 0.1*	0.26± 0.1
3	Prazosin 0.11+Aspirin 54	0.13± 0.05***	0.25± 0.07***	0.38± 0.1*	0.36± 0.1
4	Terbutaline 0.45+ Aspirin54	0.01± 0.02***	0.13± 0.04***	0.31± 0.06*	0.18± 0.05
5	Propranolol 4.5+ Aspirin 54	0.12± 0.04***	0.15± 0.03***	0.5± 0.11	0.12± 0.05
6	Atenolol 1+ Aspirin 54	0.02± 0.02***	0.07± 0.05***	0.68± 0.15	0.61± 0.13

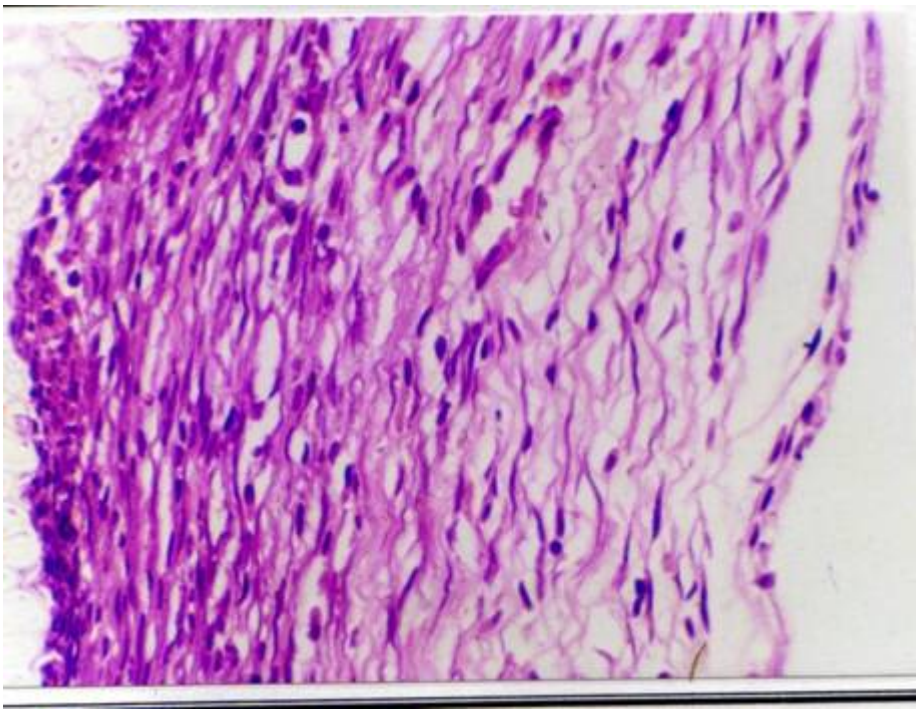
$p \leq 0.05$, $^{**}p \leq 0.01$ & $^{***}p \leq 0.001$



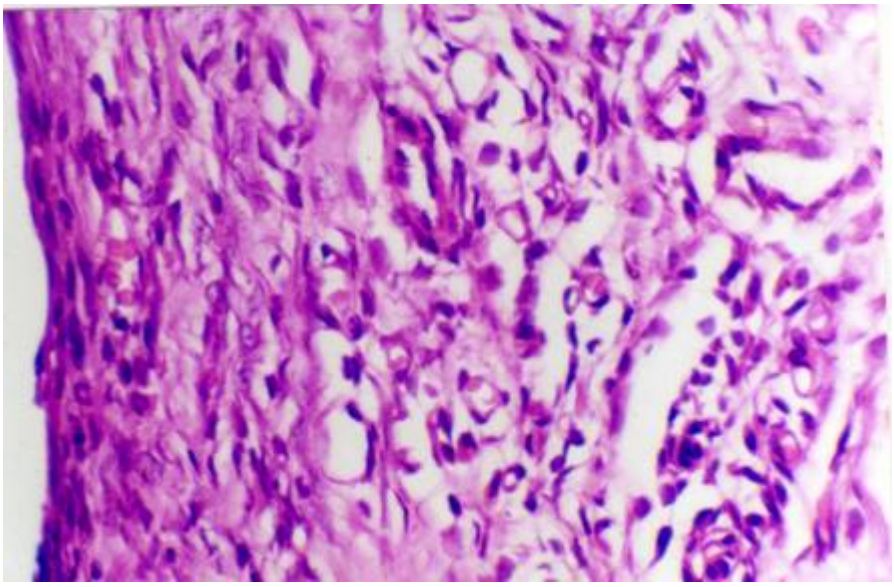
(I) Control: Abundant granulation tissue & collagen content



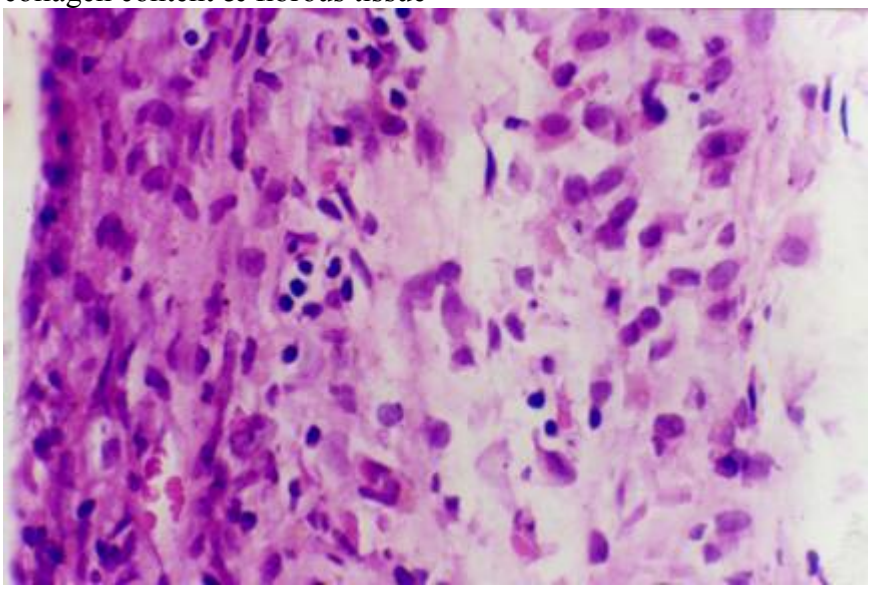
(II) Aspirin: reduced number of fibroblasts, decreased collagen content & fibrous tissue



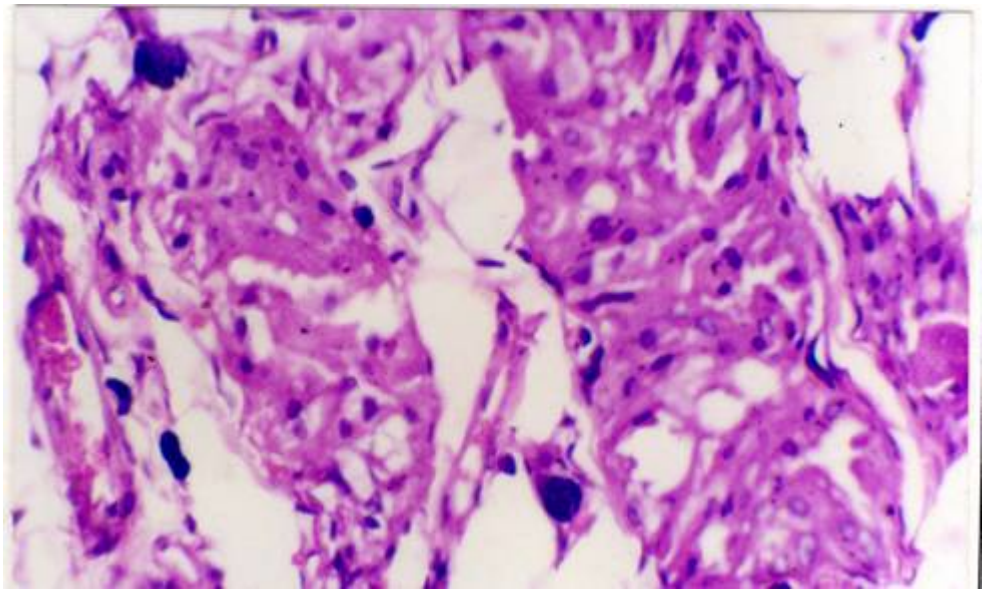
(III) Phenylephrine: reduced number of fibroblasts, decreased collagen content & fibrous tissue



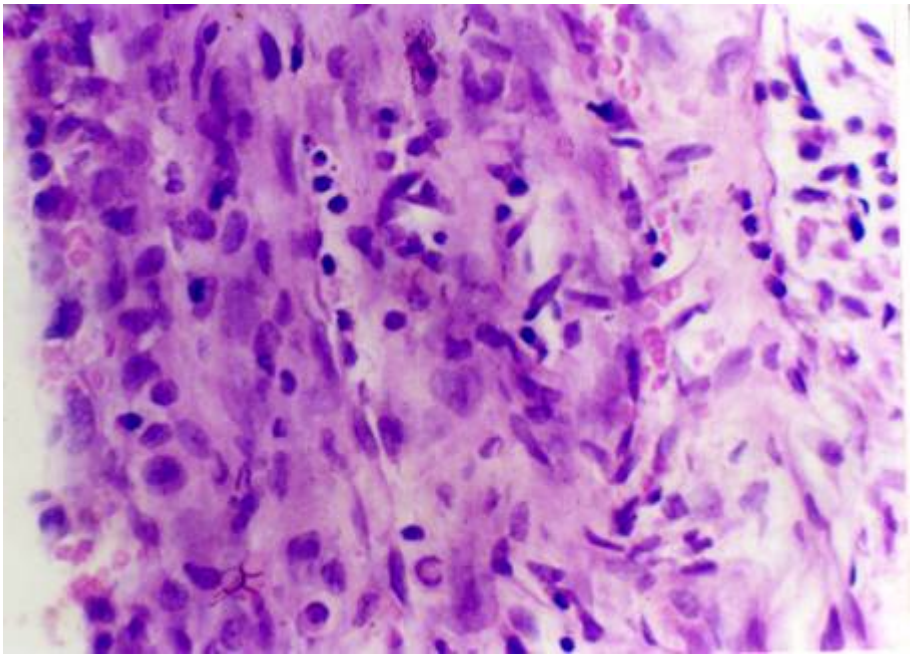
(IV) Terbutaline: Reduced number of fibroblasts, decreased collagen content & fibrous tissue



(V) Prazosin: reduced number of fibroblasts, decreased collagen content & fibrous tissue



(VI) Propranolol: reduced number of fibroblasts, decreased collagen content & fibrous tissu



(VII) Atenolol: No significant reduction in number of fibroblasts, collagen content & fibrous tissue

Discussion

Inflammation, a common clinical entity is said to be a complex protective reaction in the vascularised connective tissue due to a variety of exogenous & endogenous stimuli causing cell injury & is characterized by the reation of the blood vessels, leading to accumulation of fluids & leucocytes in the extracellular tissue[15]. .The results of the present study indicate that phenylephrine in therapeutic equivalent doses shows significant anti-inflammatory activity in the acute model of inflammation which is in agreement with an earlier report[16] wherein the drug was administered intracerebroventricularly, but contrary to a report where it is known to potentiate thermic edema[2].
It also showed anti-inflammatory action in the foreign body granuloma model & when combined with aspirin in subtherapeutic doses which have not been documented in literature. Prazosin also showed significant anti-inflammatory activity in both the models of inflammation which is agreement with the reports with phenoxybenzamine which is also an alpha-1 blocker[4].
Failure of dihydroergotamine to have a similar action can be explained on the basis of non selective alpha blocking & direct vasoconstrictor activity[5].

Terbutaline also showed anti-inflammatory action in both the models used & also potentiated the action of aspirin which corroborated with the earlier reports with other beta-2 agonists[6,7]. Both propranolol & atenolol showed significant anti-inflammatory activity in the acute model of inflammation. Literature survey reported only propranolol to possess anti-inflammatory activity[8,9] not atenolol. Subacute studies showed only propranolol to have anti-inflammatory activity whereas both propranolol & atenolol potentiated the action of aspirin in subtherapeutic doses.

From the findings of the present study it can be concluded that adrenergic mechanisms are involved in anti-inflammatory effects of the above drugs which is contrary to the earlier reports[17].

Vasoconstrictive action of alpha-1 agonist & reduced vascular permeability due to direct effect on the vascular endothelium by beta agonist are the proposed anti-inflammatory mechanisms. Another mechanism which can be hypothesized is that it is mainly the beta receptor activation by endogenous catecholamines which contributes for anti-inflammatory activity of alpha blockers & beta agonists by reducing the vascular permeability & subsequent events of inflammation. Anti-inflammatory activity of phenylephrine could be attributed to peripheral alpha adrenoceptor stimulation leading to vasoconstriction.

Anti-inflammatory activity of propranolol has been ascribed not to its beta blocking activity but due to other actions like anti-prostaglandin[18] & activation of adrenal pituitary system[19]. This is further supported by the present findings where only propranolol & not atenolol significantly inhibited chronic inflammation. It is enigmatic that atenolol failed to suppress subacute inflammation contrary to acute inflammation. Atenolol by blocking beta-1 receptors in the kidney can be expected to decrease angiotensin-II formation resulting in vasodilation. Vasodilators like isoprenaline have been shown to suppress carrageenan induced inflammation[3] by decreasing vascular permeability. The extent to which renin is inhibited by atenolol for its observed anti-inflammatory effect is difficult to speculate.

Conclusion

Adrenergics agonists & antagonists are widely used in clinical practice & hence the findings of the present study especially may have immense clinical relevance especially due to their anti-inflammatory action in subtherapeutic doses with aspirin. Though further clinical studies are required to extrapolate the above findings to the human population.

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