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Anti-inflammatory activity of Loratidine in animal models

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Abstract:

Aim: To compare the Anti-inflammatory activity of Loratidine with Diclofenac sodium in animal models. **Material & Methods**: Xylol induced Mouse ear edema: 5 animals (3groups) received orally 4% Gum acacia, Diclofenac and Loratidine respectively, 1 hour before the application of one to two drops of xylol applied to one of the ears on both sides through a syringe. The mouse ear edema was measured at the end of 40 minutes and percentage inhibition of edema was calculated in each group. Rexin pellet granuloma method: Four rexin pellets were implanted in to dorsum skin of each rat of 3 groups (n=5), which include Control, Diclofenac and Loratidine respectively. The animals were treated with fixed doses of drugs once a day for 7 days including the day of implantation of pellets and on 8th day rexin pellets were removed after sacrificing animals. Rexin pellets were kept in incubator at 60^0 C overnight, weighed and percent inhibition of granuloma tissue was calculated. Study on mast cell count. **Result:** Loratidine has shown significant anti-inflammatory activity both in acute and chronic inflammatory animal models. **Conclusion:** Loratidine may be used as an anti-inflammatory agent for both acute and chronic inflammation.

Key words: Anti-inflammatory; Loratidine; Mast cell count; Mouse ear edema; Rexin pellet granuloma

Introduction

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants [1]. Inflammation may contribute to a variety of diseases such as Atherosclerosis, Rheumatoid arthritis; Type -2 diabetes, Alzheimer disease and Cancer [2]. Histamine is a naturally occurring amine stored within the body and capable of eliciting major changes in a variety of body functions. Histamine is stored in mast cells [3]. The concentration of histamine is particularly rich in tissues that contain large number of mast cells such as skin, mucosa of bronchial tree and intestinal mucosa. Non-mast cell sites of histamine are epidermis, gastric mucosa and neurons with in the CNS. H1 receptors are found in smooth muscles, endothelial cells, adrenal medulla, heart and CNS [4].

Histamine plays a major role in acute inflammation by causing an increase in vascular permeability and vasodilatation which allows general egress of white blood cells into the extravascular space. The potential importance of the interaction of mediators was first developed based upon the

synergism between vasodilator prostaglandins and factors which increase micro vascular permeability and subsequently extended to include non-prostaglandin vasodilators. It also causes pain, itching and act as chemotactic especially for eosinophil. The role of histamine in acute inflammation is associated with mast cell degranulation in non-rodent species including man where as its role in chronic inflammation is yet to be established [5].

Evidence suggests that the continual release of histamine from lung cells of asthma patients may contribute to lung tissue inflammation and remodeling, and may affect lymphocytes, monocytes, basophils, epithelial cells, and macrophages by modulating the release of proinflammatory and immune regulatory mediators and cytokines [6-8]. Recruitment of circulating leucocytes is enhanced by H1 receptor mediated expression of adhesion molecules (e.g.: P- selectin) on endothelial cells [9]. Free radicals play an important role in the pathogenesis of inflammation [10]. Antihistamines that show anti-inflammatory effects at therapeutic concentrations may modify the effects of inflammatory mediators *in vivo* at doses comparable to those used clinically.

Keeping in view of the above ideas, the present study has been undertaken to evaluate the anti-inflammatory activity of non-sedative H1 receptor antagonists on the acute and chronic inflammatory models in albino mice and rats respectively.

Materials and Methods

The present study was conducted in the Department of Pharmacology, Mahadevappa Rampure Medical College, Gulbarga, Karnataka, after taking permission from the Institution Ethics Committee and Animal Ethics Committee of M.R. Medical College, Gulbarga, Karnataka.

Drugs used in the study:

1. Loratidine: M/S Cadila pharmaceuticals Ltd., Ahmadabad.

2. Vehicle: Normal Saline (0.9%), local purchase.

3. Gum Acacia: 4%, local purchase.

4. Diclofenac sodium: Crude powder, Biocon Pharmaceuticals, Bangalore.

5. Xylol: Local purchase.

Study design:

Albino mice of either sex weighing 20 to 30 grams were used. Total 15 mice were selected and were divided into 3 groups of 5 in each. The mice were obtained from the Central Animal House of M.R. Medical College, Gulbarga. Before starting the study, the animals were allowed to acclimatize to the laboratory environment for 1 week and they were provided with standard diet and water ad libitum as per recommendation of (CPCSEA) "Committee for the purpose of control and supervision of experiments on animals", Government of India (Reg. No.142/99, dated 11-07-1999/CPCSEA.) for laboratory animal facilities[11,12].

1. Xylol induced Mouse ear edema [13].

Group 1: (Control): 4% Gum Acacia, 2ml/kg.

Group 2: (Standard Drug): Diclofenac (6.5mg/kg mouse) in 4% Gum acacia suspension.

Group 3: (Test Drug): Loratidine(1.3mg/kg mouse) in 4% gum acacia suspension.

All the drugs were administered orally followed by constant volume of distilled water after each administration to ensure the entry of drug. One hour after the oral administration of gum acacia and drugs in control and treated group respectively, one to two drops of xylolisapplied to one of the ears on both sides through a syringe. Animals were observed for 30 to 40 minutes for appearance of edema and disappearance of translucence in the ears. At the end of 40 minutes animals were sacrificed and both the ears of each animal were cut. Control ears and treated ears of each group were separated and weighed. Same procedure was adopted for mice of all the groups (photograph1).The difference between edema of the drug treated group and control group was calculated and expressed as percent inhibition by using the formula (Table2).

$$\text{Percent inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where,

Vc = Volume of ear edema in control animals

Vt = Volume of ear edema in drug treated animals.

The dose of the drug under study was calculated by using the dose conversion table.

Figure 1: Xylol induced Mouse ear edema

2. Rexin pellet granuloma method [15]

Discs of equal size and weight were punched out from rexin sheet. Two such discs were stitched together with their rough surface exposed outside and rexin covered surfaces facing each other. Rexin pellets were sterilized using 70% ethyl alcohol. Adult albino rats, 15 in number of either sex weighing about 150 to 200gms, were selected and divided into 3 groups of 5 animals each. The first group served as a Control and was given 4% Gum acacia orally. The remaining two groups received Diclofenac 4.5mg/kg body weight and Loratidine900µg/ kg body weight respectively in 4% Gum acacia suspension.

All the rats were anaesthetized with ether. The dorsal skin was shaved and applied alcohol to maintain aseptic condition. On either side of midline of dorsal skin, four small incisions of about 1cm length were made. A curved forceps was passed through incisions to make subcutaneous pouch around it. Similarly 4 such pouches were made and sterilized rexin pellets were implanted into each pouch (Photograph 2).

Figure 2: Implanted rexin pellets.

All the rats were treated with fixed dose of drugs (as mentioned above) once in every 24hours for seven days including the day of implantation of pellets. The animals were provided with free access to food and water. During seven days, the rats were observed for any behavioral changes. On the 8th day, rats were sacrificed with ether anesthesia. The implanted pellets along with granulation tissue were removed.

All the pellets were cleaned separately. Extraneous tissue removed and dried by incubating in hot air oven at 60ºC for 24 hrs. The pellets thus dried along with adherent granulation tissue were weighed and the weight of the granulation tissue formed was obtained by deducting the weight of rexin pellets before implantation. Then the mean weight of granulation tissue for each group was calculated. The difference in weight of granulation tissue of control and drug treated group was determined and percent inhibition was calculated by using the following formula (Table3).

$$\text{Percent inhibition} = \frac{Wc - Wt}{Wc} \times 100$$

Where,

Wc = Weight of pellets in control group.

Wt =Weight of pellets in drug treated group.

3. Study of mast cell count [16].

From the control and treated groups of rats subcutaneous areolar tissue near the implanted pellet was carefully removed and thinly spread on a clean slide avoiding over stretching. The spread was fixed for 2 minutes in absolute alcohol and stained for 1 min in 0.1% aqueous solution of toluidine blue. The microscopic study has been carried out from the subcutaneous spread collected from these animals of different groups, to find out the number of mast cells in ten high power fields at random. Mean was calculated to find out S.D, S.E and P values and results were tabulated in Table 4.

Statistical analysis:

Statistical analysis of experimental data was done by using standard deviation (S.D), standard error (S.E) and P values.

Results

The results obtained from the standard and test drugs are shown in Table 2, Photograph 1. The increase in the weight of the edematous ear taken as an index of formation of edema in the control which was compared with the edema observed in different groups by different drugs. The percent inhibition in formation of edema was calculated and the results were shown in table 2. The percent inhibition of edema at the end of 40 minutes with Diclofenac is 80.40% and with Loratidine is 57.14%.

The anti-inflammatory effect was judged by noting the percentage inhibition in granuloma formation by weighing the rexin pellets 7 days after their implantation in the subcutaneous tissue as shown in Table 3. Diclofenac sodium has shown 66.36% and Loratidine 47.92% inhibition of granuloma formation. Net granuloma formation was calculated by subtracting initial weight of rexin pellet (9.25mg) from the weights noted [17].

Table 1: Dose Conversion table [14]

Drugs	**Human 70 kg**	**Conversion factor**		**Dose for Mouse (mg)**		**Dose for Rat(mg)**	
	Dose mg/kg	Mouse	Rat	For 20gm	Per kg body wt. (1000gms)	For 200gms	Per kg body wt (1000gms)
Diclofenac	50	0.0026	0.018	0.13	6.5	0.9	4.5
Loratidine	10	0.0026	0.018	0.026	1.3	0.18	0.9

Table 2: Effect of Standard and Test drugs on Xylol induced mouse ear edema

Groups	Control Group			Standard Group			Test group		
Drugs	4% Gum Acacia			Diclofenac			Loratidine		
	Ear weight (mg)			Ear weight (mg)			Ear weight (mg)		
Mouse	Control	Xylol treated	Difference	Control	Xylol treated	Difference	Control	Xylol treated	Difference
1	37	87	50	36	46	10	30	50	20
2	32	82	50	36	45	09	26	48	22
3	34	84	50	33	43	10	31	52	21
4	35	81	46	33	42	09	30	52	22
5	38	87	49	35	45	10	28	48	20
Mean			49			09.6			21
Percent inhibition of edema	----------			80.40 %			57.14%		
Standard Deviation	1.55			0.49			0.89		
Standard Error	0.69			0.22			0.40		
*p- value	----------			<0.001			<0.001		

Table 3: Effect of Standard and Test drugs on Rexin pellet granuloma method

Group (n=5)	Control Group		Standard Group		Test Group	
Drugs	4% Gum Acacia		Diclofenac		Loratidine	
Rats (4 pellets/ rat)	Gain in weight of individual rexin pellet (mg)	Mean weight gain (mg)	Gain in weight of individual rexin pellet (mg)	Mean weight gain (mg)	Gain in weight of individual rexin pellet (mg)	Mean weight gain (mg)
1	18,16,14,17	16.25	5,7,6,4	5.5	10,9,9,7	8.75
2	20,17,16,14	16.75	5,6,5,8	6	11,10,8,7	9.00
3	20,18,15,17	17.5	6,7,4,5	5.5	8,7,9,10	8.50
4	18,15,16,17	16.50	5,8,4,3	5.0	10,9,11,8	9.50
5	20,17,16,15	17.0	6,8,5,6	6.25	9,7,7,9	8.00

Total Mean		16.80		5.65		8.75
Percentage inhibition of granulation tissue	--		66.36%		47.92%	
Standard deviation	0.43		0.44		0.50	
Standard error	0.19		0.20		0.22	
p- value	--		<0.001		<0.001	

Table 4: **Mast cell count in Control, Standard and Test group**

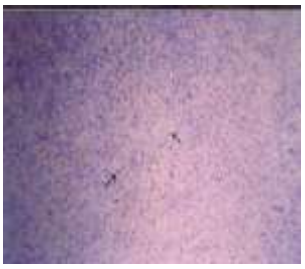
Field nos.	1	2	3	4	5	6	7	8	9	10	Mean	S.D	S.E	P value
Control	13	9	3	13	18	21	20	11	6	6	12.0	5.88	1.86	--
Diclofenac	10	10	9	6	10	15	16	6	10	7	9.9	3.21	1.02	>0.10
Loratidine	5	4	6	4	3	5	14	10	15	13	7.9	4.39	1.39	>0.10

Mast cells were counted in ten fields at random under high power and results were shown in Table 4. There was a marginal reduction in mast cells with Loratidine (Mean 7.9) as compared to Diclofenac (Mean 9.9).

Mast cells:

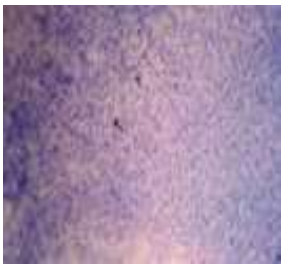
Control

Figure: 3



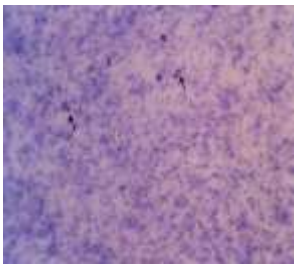
Diclofenac

Figure: 4



Loratidine

Figure: 5



Discussion

The principle indications for the use of second generation H1 receptor antagonists are in the treatment of disease with inflammatory components like allergic rhinitis, conjunctivitis, idiopathic urticarial and diseases of allergic nature like hay fever [18], Pollinosis [19], Dermographism [20] and atopic eczema[21]. These facts state that histamine plays a role as a mediator of inflammation. The inflammatory edema is mediated by histamine and 5HT during the 1st hour after which the increased vascular permeability is maintained by kinin release up to 2.5hours. Thereafter up to 6 hours the mediator appears to be prostaglandins [8], release of which is closely associated with migration of Leucocytes into the inflamed site.

The anti-inflammatory activities of second- and third-generation H_1-receptor antagonists have been evaluated in vitro. These studies have shown that many second-generation H_1-receptor antagonists (considered potentially or minimally sedating) and third-generation H_1-receptor antagonists (considered non -sedating) inhibit release or generation of multiple inflammatory mediators, including IL-4, IL-6, IL-8,IL-13,PGD_2,LTC_4,Tryptase,Histamine and the TNF-α-induced chemokine RANTES, as well as eosinophil chemo taxis and adhesion[8].All the mediators appear to be dependent upon an intact complement system for their activation and release. In-vitro effect of loratadine on human eosinophils (from allergic patients) was investigated by Eda et al [22].

Loratadine significantly inhibited Platelet Activating Factor (PAF) induced eosinophil chemo taxis at concentration that were equivalent to serum

concentration achieved after a single oral administration of 20 or 40 mg. There was no effect on PAF-induced ECP release. These findings suggest that loratadine has a direct inhibitory effect on eosinophil activation. In another study loratadine (Desloratadine) 10 µmol/l significantly inhibited expression of intercellular adhesion molecule-1 (ICAM-1) and Human Leukocyte Class II antigen (HLA-DR) in nasal epithelial cells in vitro [23]. These studies suggest that loratadine may exert an in vivo anti-inflammatory effect. Caution should be used in treating pregnant or lactating women with certain H1 antihistamines especially first generation drugs, because of their possible teratogenic effects or symptomatic effects on infants resulting from secretion of drugs into breast milk. Cetirizine and Loratidine are preferred if H1 antihistamines are required, but if they are not effective, diphenhydramine can be safely used in pregnant (but not breast feeding) women.

Xylol induced mouse ear edema is a method that detects the potency of the anti-inflammatory compounds by measuring its property of inhibiting the exudation that results due to application of an irritant on the skin. Loratidine has shown considerable anti-inflammatory activity in mouse ear edema at the end of 40 minutes and found to be maximum. It acts by inhibiting the exudation that results due to application of an irritant. In the chronic study, Rexin pellet method, formation of granuloma was minimum with Loratidine. As granuloma represents the exudative and proliferative phases of inflammation, the drug under study might be acting at these levels thereby reducing the migration of eosinophils, neutrophils and platelets [24]. In chronic model study there was a marginal reduction in mast cells count with Loratidine.

In the present study Loratidine has shown anti-inflammatory effects. If clinical anti-inflammatory effects necessitate dosages higher than those usually recommended for allergic reactions, H_1- receptor antagonists with the widest therapeutic window and the lowest potential for dose-limiting sedation and cognitive impairment may offer the greatest therapeutic potential for its clinical use [25].

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