Research Paper

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The Study of Relative Potency of First Generation and Second Generation Antihistamines on Contraction of Atropinised Isolated Guinea Pig Ileum

Medical Science

KEYWORDS : Histamine, Pheniramine maleate, Cetirizine, Guinea pig ileum, Potency, DRC, sub maximal dose, IC50, PSS

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ABSTRACT

Objectives: 1.To study the effect of Histamine (standard drug) & test drugs i.e. 1st generation antihistamine (Pheniramine) 2nd generation antihistamine (cetirizine) in graded doses on isolated guinea pig ileum.

2. Comparison of the potency of both 1st & 2nd generation antihistamines.

Materials & methods:

Drugs: Histamine, Pheniraminemaleate & Cetirizine

Tyrode solution, Kymograph , Dales organ bath, Frontal writing lever.

Four ileal tissues from each of 3 guinea pigs were used for the study. Out of 12 ileum preparations, 6 were used for Pheniramine maleate &6 for Cetirizine. After taking DRC with histamine till saturation, followed by DRC with anti histamine by adding antihistamine to the inner organ bath in incremental doses along with sub maximal dose of Histamine. Then IC50 values were calculated for 12tissues by graphical method and mean values were compared.

Statistical analysis: was done using student's unpaired t-test for comparing IC50 values of Pheniramine maleate & Cetirizine.

Introduction

Histamine an autacoid, is present in mast cells and non mast cells. It is released whenever antigen antibody reaction occurs and is responsible for skin allergy, nasal allergy, respiratory allergy including bronchial asthma[1].So H_1 antihistamines(1st & 2nd gen.) are used in above conditions. The first generation drugs have more CNS depressant and ant cholinergic side effects which is lacking in second generation drugs [3]. Pheniramine maleate is 1st generation antihistamine and Cetirizine is 2nd generation antihistamine. The present study is undertaken to evaluate the potency of first generation and second generation antihistamines in relation with that of in vivo effect.

Materials& methods[2,3,4]:

3 Guinea pigs (4 tissues from each animal) were used as experimental animal as these are highly sensitive to histamine [3]. Dales organ bath [5]. Tyrode solution, Frontal writing lever, Kymographs were used.

Overnight fast guinea pig was sacrificed by cervical dislocation, abdomen was opened by medial incision then terminal part of ileum was isolated. It was kept in tyrode solution for acclimatization for 5min after removing fecal matter by gentle wash &then cut into 5-6 cm segments.12 ileum preparations were used (6 for Pheniramine & 6for Cetirizine).Then one end of the segment was tied securely to the tissue holder and was transferred to the organ bath [6] containing Tyrode [6] solution at 37^{0}C in which aeration was allowed. The contraction of the preparation was recorded on a smoked drum by a frontal writing lever [2], using same magnification by adding the drug. DRC with Histamine was traced for 12 total tissues with incremental doses. After attaining saturation, the dose less than maximal dose was taken as sub maximal dose & DRC was again repeated after pretreatment of 10min with antihistamines (6 with Pheniramine & 6 with Cetirizine). These tracings were fixed by dipping in a solution of resin (colophony) in methylated spirit [10]. Two concentrations were selected (one inhibiting 70%& other inhibiting 30% 0f sub max. concentration response) in order to calculate IC50 values. The load required is 0.5 gm, magnification [2] is 5-7 throughout the experiment. Dose cycle : total 3min (baseline 15sec, contact period 30 sec)[6].

Observations & calculation:

TABLE No. 1 DRC of Histamine on isolated guinea pig ileum & inhibitory effect of Pheniramine maleate on Histamine induced contractions in isolated guinea pig ileum against sub.max dose of Histamine(40 µg). (Tissue-1)

Si.no	Dose of Histamine (µg)	Height of contraction (mm)
1	1	16
2	2	17
3	4	19
4	8	21
5	10	23
6	20	25
7	40(sub max. dose)	27
8	80	29
9	160	25

TABLE.NO.2 Inhibitory effect of Pheniramine maleate & Cetirizine on Histamine induced contractions in isolated guinea pig ileum against sub maximal dose of Histamine(40 µg n=11, 80ug n=1).

Si. no.	tissue1		tissue2		tisse3		tissue4		tissue5		tissue6	
1	DC	DR	DC	DR	DC	DR	DC	DR	DC	DR	DC	DR
2	H 40ug	27	40ug	24	80ug	26	40ug	17	40ug	15	40ug	20
3	PM 0.04ug	17	0.04ug	15	0.04ug	16	0.04ug	11	0.04ug	10	0.04ug	14

4	PM 0.08 ug	9	0.08ug	8	0.08ug	11	0.08ug	6	0.08ug	5	0.08ug	8
Si. no.	tissue7		tissue8		tissue9		tissue10		tissue11		tissue12	
1	DC	DR	DC	DR	DC	DR	DC	DR	DC	DR	DC	DR
2	H 40ug	40	40ug	30	40ug	36	40ug	30	40ug	24	40ug	14
3	C 0.1ug	28	0.1ug	28	0.2ug	23	0.1ug	25	0.1ug	19	0.1ug	9
4	C 0.4ug	16	0.4ug	16	0.4ug	9	0.6ug	13	0.3ug	11	0.4ug	6

C: Cetirizine concentration/ 20ml of inner organ bath Tyrode solution.
DC: drug concentration (ug), DR: drug response (mm)
H: Histamine, PM: Pheniramine Maleate concentration /20ml of inner organ bath Tyrode solution.

IC50 calculation: IC_{50} values for each tissue response was calculated graphically, log dose of test drug(Pheniramine maleate / Cetirizine) concentration were potted on X-axis and height of contraction(mm) of Histamine were plotted on Y-axis and a line was drawn by joining test drug concentration with relative contractions. The point where half the height of sub maximal dose of Histamine intersects the plotted line that point is extended towards X-axis concentration. The log value of this concentration was considered as IC_{50}. (Fig.no.1& 2).

TABLE No.3 IC50 (µg /20ml of Tyrode) of Pheniramine maleate (Tissue 1-6) 40 µg n=5 or 80 µg n=1. & Cetirizine (Tissue 7-12) 40 ug n=6 against sub max. dose of Histamine

Tissue	IC 50 values ug/20ml Tyrode	Tissue	IC 50 values ug/20ml tyrode
tissue-1	0.055	tissue-7	0.25
tissue-2	0.055	tissue-8	0.25
tissue-3	0.06	tissue-9	0.3
tissue-4	0.071	tissue-10	0.27
tissue-5	0.074	tissue-11	0.28
tissue-6	0.052	tissue-12	0.25
MEAN	0.06116	MEAN	0.2666
STANDARD DEVIATION	0.009196	STANDARD DEVIATION	0.0206559
STANDARD ERROR	0.003754	STANDARD ERROR	0.0084327

t-value= 17.46 , p value < 0.001* * significant

Statistical analysis: Statistical analysis was done by student's t-test (unpaired) for comparison of mean IC50 value of Pheniramine maleate & Cetirizine.

$$t = \frac{\text{Mean IC50 of Cetirizine} - \text{Mean IC50 of Pheniramine}}{\sqrt{\text{S.E of Cetirizine}^2 + \text{S.E of Pheniramine maleate}^2}}$$

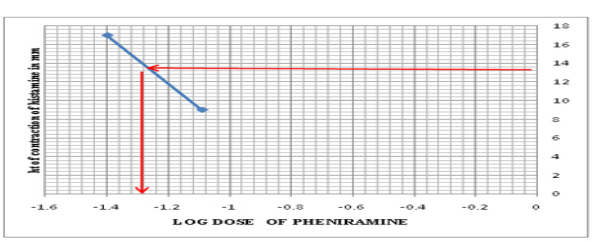
df=6+6-2= 10, the calculated t -value 17.46 lies beyond the recorded value 4.587 at probability level 0.001 which indicates that the difference in the mean IC50 values of the 2 groups are highly significant.

RESULTS: Histamine in the graded concentrations of 1µg, 2µg, 40µg, 8µg, 10 µg, 20µg, 40µg, 80µg,160µg per 20 ml organ bath containing Tyrode solution produced concentration dependent increase in the contraction of guinea pig ileum(table. no.1).

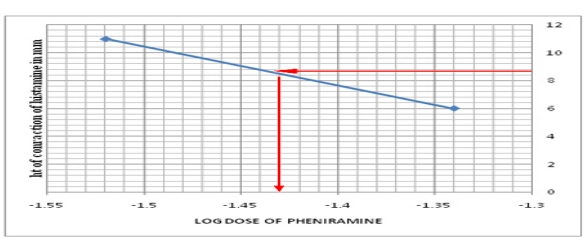
Pretreatment with Pheniramine in the concentrations of 0.03, 0.04, 0.06, 0.08 µg per 20ml reduced the histamine induced (40ug or 80ug-submaximal concentration) contraction of guinea pig ileum(table no.2).

Pretreatment with Cetirizine in the concentrations of 0.1, 0.3, 0.4, 0.6 µg per 20ml tyrode reduced the histamine induced contraction (40ug-submaximal dose)(table no.2).

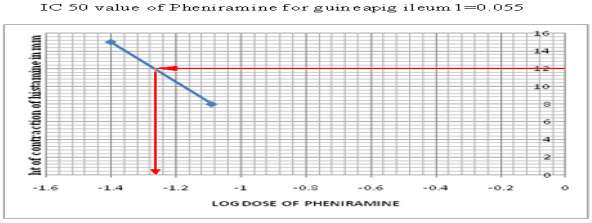
Fig.no1 IC50 CALCULATION BY GRAPHICAL METHOD-PHENIRAMINEMALEATE



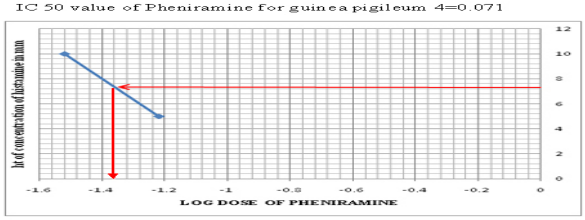
IC 50 value of Pheniramine for guinea pig ileum 1=0.055



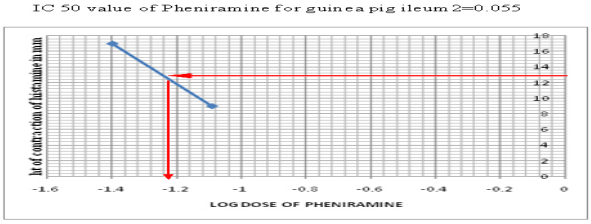
IC 50 value of Pheniramine for guinea pig ileum 4=0.071



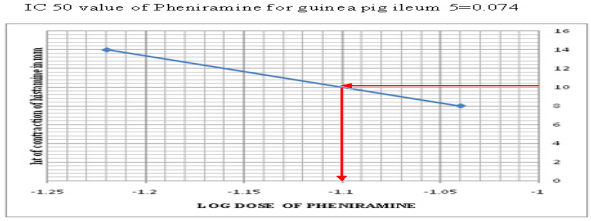
IC 50 value of Pheniramine for guinea pig ileum 2=0.055



IC 50 value of Pheniramine for guinea pig ileum 5=0.074



IC 50 value of Pheniramine for guinea pig ileum 3=0.060



IC 50 value of Pheniramine for guinea pig ileum 6=0.052

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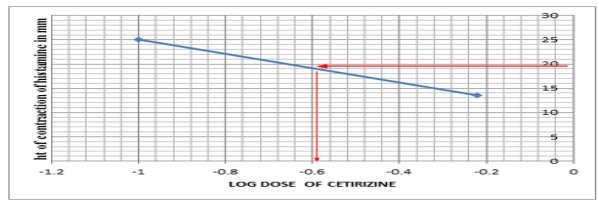
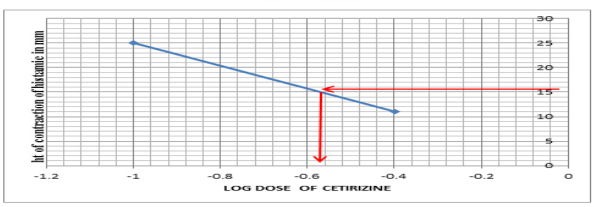
Fig.no.2 IC 50 CALCULATION BY GRAPHICAL METHOD FOR CETIRIZINE



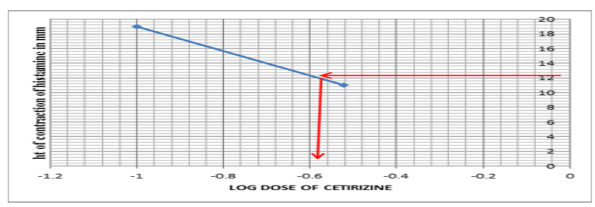
IC 50 value of cetirizine for guinea pig ileum1=0.025



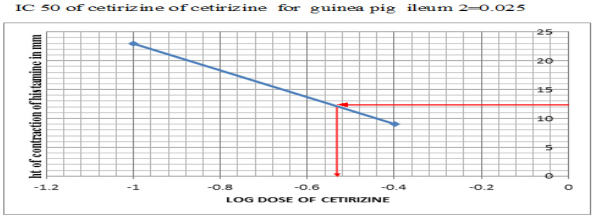
IC 50 of cetirizine of cetirizine for guinea pig ileum 4=0.027



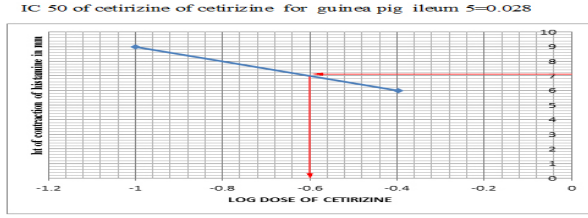
IC 50 of cetirizine of cetirizine for guinea pig ileum 2=0.025



IC 50 of cetirizine of cetirizine for guinea pig ileum 5=0.028



IC 50 of cetirizine of cetirizine for guinea pig ileum 3=0.03



IC 50 of cetirizine of cetirizine for guinea pig ileum 6=0.025

The IC50 value of Pheniramine & Cetirizine against histamine induced contraction of guinea pig ileum in the 6 samples was recorded(table no.3).The mean± SE IC 50 value of Pheniramine against the histamine contraction in guinea pig ileum was 0.061 ±0.003 µg /20ml where as for Cetirizine it was 0.266±0.008 µg /20ml (table no.3). The mean IC 50 value of Cetirizine is significantly more in comparison to mean IC50 value of Pheniramine..

DISCUSSION: The first generation H1 receptor blockers produce adverse effects like sedation and anti cholinergic effects [1,5] (dry mouth, constipation, tachycardia etc). The second generation H_1 blockers are devoid of sedation [1]. The effective therapeutic doses of H_1 anti histamincs vary among the first generation H_1 blockers (2mg to 50mg/ day) and hence classified as less potent and more potent. The effective therapeutic doses of 2nd generation H_1 anti histaminics like cetirizine, loratidine, mezolastine, ebastine, levo cetirizine is same in quantity i.e. 10mg daily [7].

In vivo models suggest that cetirizine is more potent than Pheniramine maleate as so it is used in low doses whereas my study results proved to be cetirizine is less potent. Further studies in other in vitro animal models and in vivo models are required to conform the results of my present study.

CONCLUSION:

The MEAN ± SE IC 50 value of Cetirizine against histamine induced contractions in guinea pig ileum is found to be significantly more ($p< 0.001$) than that of Pheniramine suggesting that Cetirizine is less potent than the Pheniramine as antihistaminic in the in vitro model i.e. isolated guine apig ileum.

REFERENCE

1.GOODMAN & GILMAN'S, Joel G.hardman, lee E.limbird. "The pharmacological basis of therapeutics" 10th edition (2001)McGraw- Hill –medical publishing division Newyork. | | 2. Ghosh M N. Fundamentals of Experimental Pharmacology. Scientific book agency, Calcutta. 3ed 106-133. | | 3. Murali krishna R .Vademeum of experimental pharmacology. ESPE Media & printing.visakhapatnam. Feb 1999. P: 21. | | 4. Perry.W L M. Pharmacological experiments on isolated preparations. 2nd ed. p.106-133. | 5. Sharma H L, Sharma K K. Principles of Pharmacology. 2nd ed Hyderabad:Paras medical publisher;2011. p:200-13. | 6. Kulkarni S K. Hand book of experimental pharmacology.3rd ed. Vallabh prakashan; 1999 p.3-11. | | 7. Tripathi K D. Essentials of pharmacology.6th ed. New Delhi. Jaypee brothers medical publishers(p) ltd 2006 ;p 153-4. | 8. CarlLudwig.Kymograph.(online). (7screens).Available from; URL:http://en.wikipedia.org/wiki/kymograph. |