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ANTI HISTAMINIC, ANTI CHOLINERGIC AND SEDATIVE ACTIVITIES OF FEXOFENADINE ANALOGUE

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ABSTRACT

The Fexofenadine analogue 4-(4-(4-(Hydroxy diphenyl methyl)-1-piperidinyl)-1-oxo butyl)-fluorobenzene (analogue I) was screened for anti-histaminic activity by guinea pig tracheal chain, anticholinergic by guinea pig tracheal chain and sedative activity. The antihistamine effect of analogue I was examined by producing cumulative log concentration response curve of histamine acid phosphate induced contraction of tracheal chains 10 minutes after exposing tissue to compound at different dose levels (20 µM, 60 µM, and 200 µM), and histamine 0.001 µM. The effect of different concentrations of Analogue I was tested on each trachea, the concentration response curve to acetylcholine was constructed by cumulative addition of acetylcholine in absence and in presence of antagonists. Analogue I was incubated with the tracheal chain preparation for 30 minutes before addition of cumulative concentration of acetylcholine. Spontaneous motor activity was performed using Actophotometer. Results of the treated groups were compared with those of control group at each time interval. SMA measurements started 30 minutes after the administration of the compound and the results were compared with those of control. analogue I exhibited significant anti-histaminic activity, anticholinergic activity and less sedative effect against various models.

KEYWORDS

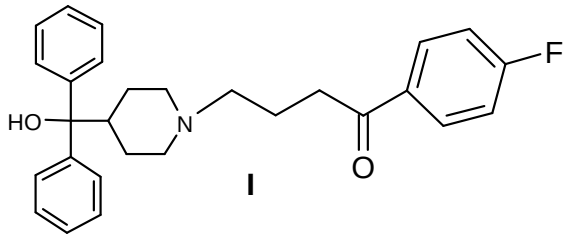
Fexofenadine analogue, Anti-histaminic, Anticholinergic and Sedative activity.

INTRODUCTION

Histamine is one of the most frequently studied local hormone and first clinical recognized as a mediator of immediate allergic response in humans. Despite the compelling evidence that suggests histamine playing an important pathological role in asthma[1]. Up to now, four of its target proteins have been cloned (H_1, H_2, H_3 and H_4 receptors) from among which H_1 and H_2 receptors are assigned to an overriding pharmaceutical importance. The H_1 receptor is mainly responsible for the inflammatory effect of histamine. Therefore, it is the target of most drugs developed against allergic rhinitis. By means of the H_2 receptors, histamine is responsible for the stomach's HCl production, which makes it possible to treat ulcer with H_2 antagonists. Several effective and selective H_2

antagonists are available, that can be used without any serious side effects[2]. The H_3 receptor can enhance some neurotransmitter's release. A few H_3 antagonists have already been developed for the treatment of neurological and cognitive disorder, but the efficiency of these compounds has been confirmed reassuringly yet[3]. The H_4 receptor was cloned just a few years ago[4], and there is not sufficient information about its physiological effects, but it seems to play a role in histaminergic receptor mediated chemotoxis[5]. Histamine is performed in cells, has a rapid onset of action and is therefore mainly responsible for the phase of allergic reactions. The first and second generation antihistamines are in use, but these agents exert non negligible adverse effects on the central nerves system such as drowsiness, new antihistamines which lack this defect therefore represent attractive target for the drug discovery in the field of antiallergic agents. Some such desired antihistamines terfenadine[6] and astemizole[7] have already been discovered, but we independently focused on the search for new antihistamines using fexofenadine as a chemical lead. Hence, there is a need to develop molecules for the treatment of allergy symptoms with fewer side effects.

Terfenadine was a selective H_1-receptor antagonist with weak anticholinergic activity, which was originally developed as a selective dopamine receptor antagonist belonging to the haloperidol and azacyclonol class. However, it led to the development of terfenadine, a peripherally acting H_1-receptor antagonist with minimal CNS depression as side effect. A structurally related product of terfenadine, ebastine has also been claimed to have less sedative effects. The novel substituted piperidine derivatives were screened for anti-histaminic activity by histamine induced isolated guinea pig ileal muscle contraction[8]. Fexofenadine analogue **I** which has been synthesized and reported for their antihistamine activity by using guinea pig ileum model.[9]



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In view of these observations and our continued interest in screening of the analogue **I** for antihistaminic, anticholinergic by Guinea pig tracheal model and sedative activity by Actophotometer.

MATERIALS AND METHODS

In-vitro inhibition of histamine induced contraction on guinea pig Trachea

Male guinea pigs (400 – 700 g) were killed by a blow on the neck and then trachea was removed. Each trachea was cut into 10 rings (each containing 2- 3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle and sutured together to form tracheal chain[10].

Tissue was suspended in 20ml organ bath containing Krebs –Hense leit solution of the following composition in mM: NaCl, $NaHCO_3$, $MgSO_4$, KH_2PO_4, KCl, $CaCl_2$ and dextrose.

The Krebs solution was maintained at 37 ºC and constantly bubbled with 95 % O_2 / 5 % CO_2. The tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for atleast one hour while it was washed with Krebs solution for every 15 minutes.

The inhibitory effect of analogue I on histamine H_1 receptors was examined by producing cumulative log concentration response curve of histamine acid phosphate induced contraction of tracheal chains 10 minutes after exposing tissue to one solution of analogue I in

20 µM, 60 µM, and 200 µM, and histamine 0.001 µM, (sigma chemical Ltd) the consecutive concentrations of histamine were added for every 2 min (range 0.1 – 1000 µM,) and the percentage of contraction due to each concentration in proportion to the maximum contraction obtained was plotted against log concentration of histamine

The concentration response curve to histamine was constructed by cumulative addition of histamine in absence and in presence of antagonists. The antagonist was added to the bath after 30min of histamine response alone, antagonist was incubated with the tracheal chain preparation for 30min before addition of cumulative concentration of histamine. Isolated tracheal muscles of guinea pig relaxant effect of this analogue I was demonstrated on isolated guinea pig tracheal chains.

In-vitro inhibition of Acetyl choline induced contraction on guinea pig trachea:

Tissue preparation: Male guinea pig 400-700G was sacrificed by a blow to the neck and the trachea were removed. Each trachea was cut in to 10 rings (each ring contained 2-3 cartilaginous rings). The rings were then cut open opposite the tracheal muscle and sutured together to form a tracheal chain (n=7)[10].

Tracheal chain tissue was then suspended in a 10 ml organ bath containing Krebs-Hense Leit solution with the following composition (mM): NaCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$, KH_2PO_4, KCl and dextrose.

The Krebs solution was maintained at 37^oC and aerated with 95% O_2 and 5% CO_2. Tissue was suspended under an isotonic force of 1g and allowed to come to equilibrium for at least 1 hour while washing with Krebs solution every 15 minutes.

The effect of different concentrations of analogue I was tested on each trachea, the concentration response curve to Ach was constructed by cumulative addition of Ach in absence and in presence of antagonists. The antagonist was added to the bath after 30 minutes of Ach response alone. Antagonist was incubated with the tracheal chain preparation for 30 minutes before addition of cumulative concentration of acetylcholine.

Spontaneous motor activity (SMA)

Spontaneous motor activity was performed using Actophotometer (Techno LE3806, India). Mice were grouped of six each and treated with saline or the analogue I (10, 30 and 100 mg/Kg i.p.) or received Mepyramine 10 mg/kg i.p. Activity was automatically recorded 30 min after treatment and at every 10 minutes. The experiments were repeated at an interval of 30 minutes, for a total of 120 minutes. Results of the treated groups were compared with those of control group at each time interval[11]. SMA measurements started 30 minutes after the administration of the compound and the results were compared with those of control.

RESULTS AND DISCUSSION

Isolated guinea pig tracheal Chain:

Shift in cumulative log concentration-response curves: Cumulative log concentration-response curves of histamine obtained in the presence of analogue I, and Mepyramine in these experimental groups showed clear rightward shift compared to histamine-response curves produced **(Figure-1).**

Anti histaminic activity in guinea pig trachea of Analogue I

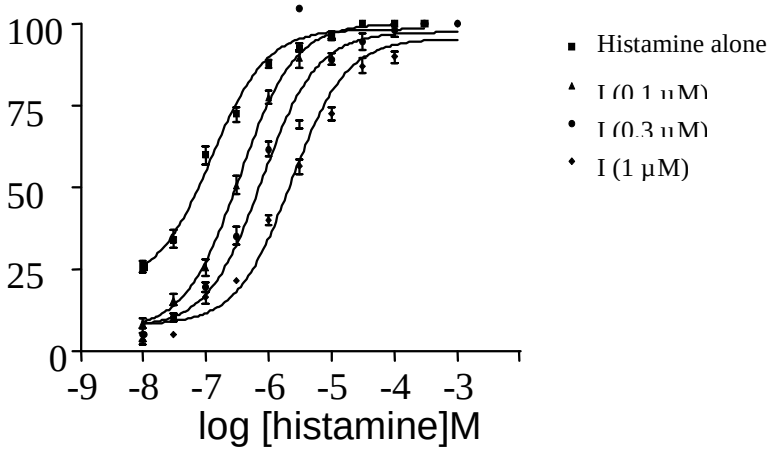

Figure-1:*Cumulative log concentration-response curves of histamine induced contraction of guinea pig tracheal chains, in the presence of compounds on incubated Preparations with three different concentrations. The shifts of histamine-response curves obtained in the presence of chlorpheniramine in all three sets of experiments were also parallel*

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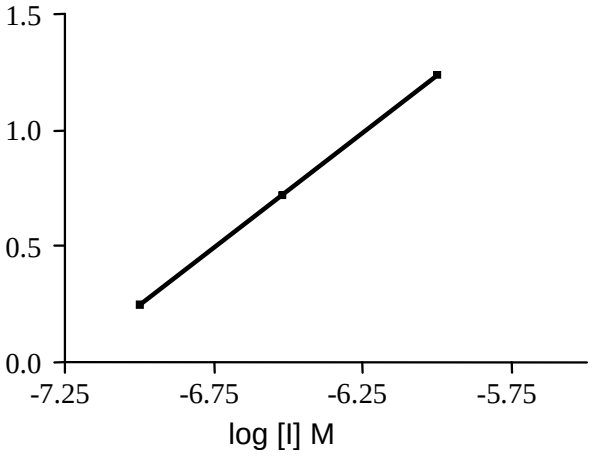


The relaxant effect of different concentration of analogue I on tracheal chains of guinea pigs might be due to several different mechanisms including stimulation of β- adrenergic receptors, inhibition of histamine H_1 receptors or an anticholinergic property. This analogue I showed the relaxant effect due to β_2 stimulatory[12, 13] and histamine H_1 receptors inhibitory[14] and anticholinergic property[15].

***Shift in cumulative concentration-response curves*:** Cumulative concentration-response curves of analogue I obtained with three different concentrations showed clear rightward shifts compared to the standard drug Atropine

Anti cholinergic activity in guinea pig trachea of Analogue I

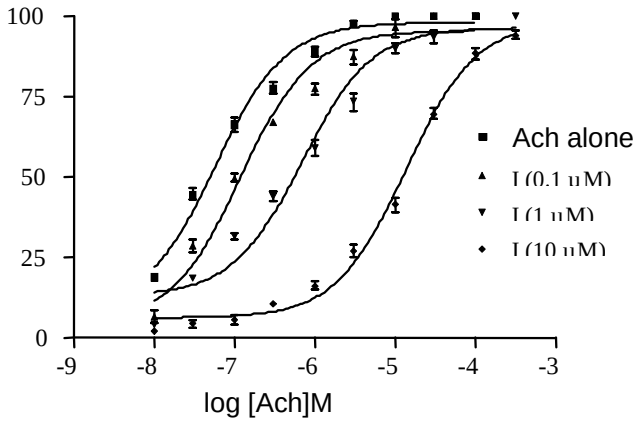
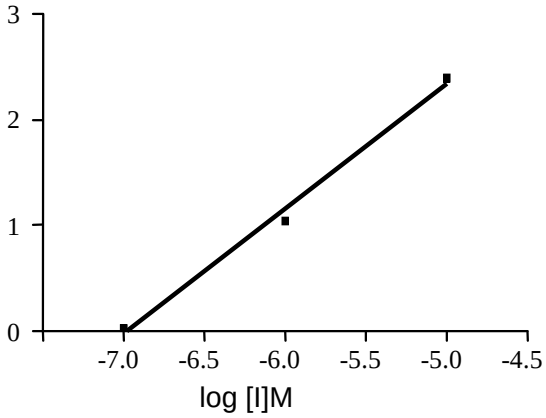


Figure-2: Cumulative log concentration-response curves of Acetyl choline induced contraction of guinea pig tracheal chains, in the presence of compounds on incubated preparations with three different concentrations. The shifts of histamine-response curves obtained in the presence of Atropine in all three sets of experiments were also parallel.



PA_2 value is 7.06 ± 0.19 (1.18 ± 0.10)

Spontaneous motor activity: analogue I produced significant decrease in the spontaneous motor activity in mice. This effect was dose dependent and the effect was observed within 30 minutes of drug administration and persisted for 120 minutes **(Table-1).** The compound was found to produced alteration in general behavior pattern, significant reduction of spontaneous motor motility. The present findings suggest that analogue I possesses CNS-depressant action. The significantly reduced spontaneous motor activity is a measure of the level of excitability of the CNS[16] and this decrease may be closely related to sedation resulting from depression of the central nervous system[17]. The analogue I possessed central nervous system depressant activity. It also showed a marked sedative effect as indicated by the reduction in motor activity. It is generally accepted that the sedative effect of drugs can be evaluated by measurement of spontaneous motor activity and pentobarbitone induced sleeping time in laboratory animal model[18]. These results corroborate those of who proposed that the enhancement of barbital hypnosis is a good index of CNS depressant activity[19].

Table-1
Sedative activity of the analogue I

analogue I	Dose in mg/kg	Activity of animal
Distilled water	---------	145.7 ± 3.42
I	3	134.2 ± 5.21
I	10	123.4 ± 4.84
I	30	108.2 ± 9.21
Mepyramine	10	104.23 ± 2.89

Results of the exploratory behavior test **(Table 1)** further support the neuro sedative activity and its possible application in anxiety condition. Further studies are planned to establish mechanism of CNS-depressant action of Compound by using various agonists and antagonists.

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