In vitro and Invivo Drug-Drug Interaction Study between Ketotifen Fumerate and Chlorpheniramine Maleate at Gastric and Intestinal pH

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ABSTRACT

The use of two or more drugs in the same prescription is a common practice for the treatment of a patient, which may sometimes be neither safe nor effective. The main aim of the present study was to find out invitro and invivo drug- drug interaction study between ketotifen fumerate and chlorpheniramine maleate in aqueous media at various pH (1.2, 2.8 and 6.8) by using Job's continuous-variation analysis and Ardon's spectrophotomeric methods. The stability constant values of chlorpheniramine with ketotifen were determined at a fixed temperature (37 ^{0}C) at both gastric and intestinal pH. The stability constant values , ranging between 7.79 and 8.21 when ketotifen fumerate and chlorpheniramine maleate were the drug of choice, were derived from Ardon's plot as a result of interaction between the drugs, were comparatively stable. However, after interaction it was observed that, stability constant values were more than 1 at both gastric pH (1.2 and 2.8) and intestinal pH (6.8). The in vivo study indicate that the decrease in concentration of ketotifen after concurrent administration of ketotifen and chlorpheniramine was statistically insignificant.

KEY WORDS: Ketotifen fumerate, Chlorpheniramine maleate, Job's method, Ardon's method, Stability constant values.

1. INTRODUCTION

Ketotifen is a benzocycloheptathiophene derivative that has been shown to possess anti-histaminic and anti-anaphylactic properties (1). The common mechanism of ketotifen shows the release of histamine and leukotriene from basophil and lung tissue, antagonizes histamine at H_1 receptors, inhibits calcium uptake, blocks passive cutaneous anaphylactic reaction, reverses isoprenaline-induced beta-adrenoceptor tachyphylaxis, and inhibits both allergen-induced and drug-induced asthma (2) . A number of clinical trials of ketotifen have shown it to have a beneficial effect in the treatment of asthma (3-4)

equivalent to that of disodium cromoglycate, which has a common use in the treatment of asthma (5). Ketotifen has been using in the treatment of hay fever and asthma, have been found to inhibit anaphylactic histamine release from animal tissues (6). Chlorphenamine commonly marketed in the form of chlorpheniramine maleate ,Chlorphen-12 (7), is a first-generation alkylamine antihistamine used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria. In addition to being an histamine H1 receptor antagonist, chlorphenamine has been shown to work as a serotonin-norepinephrine reuptake inhibitor(7). A similar antihistamine, brompheniramine, led to the discovery of the SSRI zimelidine. Limited clinical evidence shows that it is comparable to several antidepressant medications in its ability to inhibit the reuptake of serotonin and also norepinephrine (noradrenaline).(8) However, extensive clinical trials of its psychiatric properties in humans have not been conducted. It inhibits serotonin reuptake less than norepinephrine reuptake (9). It was also noted that long term administration of Chlorphenamine reduced age-related deficits in motor function (10).The major goal of the present study was to finf out the drug-drug interactions (DDIs) as well as to determine the stability of the complexes, which could be formed after interaction between ketotifen and chlorpheniramine at various pH. The values of stability constants were determined by using Job's continuous-variation analysis and Ardon's spectrophotomeric measurement methods. However when ketotifen is interacted with chlorpheniramine, the values of stability constants were 7.79 to 8.21 at both gastric pH (1.2 and 2.8) and intestinal pH (6.8).

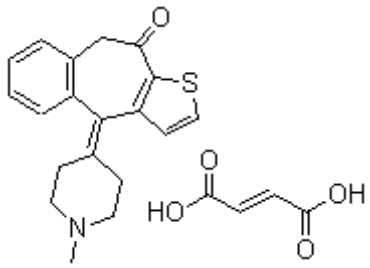

Figure 1: Chemical structure of ketotifen fumerate

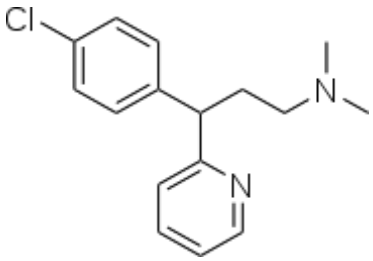

Figure 2: Chemical structure of chlorpheniramine maleate

2. MATERIALS AND METHODS:

2.1. Invitro study

2.1.1. Drugs and chemicals

Ketotifen fumerate and chlorpheniramine maleate were collected from Square Pharmaceuticals Ltd., Dhaka, Bangladesh.. Sodium dihydrogen orthophosphate and di- sodium hydrogen orthophosphate, used for the preparation of buffer solutions were purchased from Merck, Germany. Potassium chloride, sodium hydroxide, potassium hydroxide etc. were all of analytical grade.

2.1.2. Equipments

UV-Visible spectrometer (Model No. UV-1600, Shimadzu, Japan), pH meter (Mettler Toledo, Switzerland), analytical balance (Model No. AL 204-S/01, Mettler Toledo, Switzerland), and a thermostated water bath (Shimadzu, Japan) were used for the test.

2.1.3. Preparation of standard solutions

Ketotifen fumarate (1×10^{-3} M) and maleate chlorpheniramine (1×10^{-3} M) were dissolved in distilled water to prepare the stock solutions. These stock solutions were diluted to desired strengths (1×10^{-5} M) by buffer solutions to obtain the working standard solutions.

2.1.4. Absorption spectrum analysis

In observation of the spectra, the absorption characteristics of ketotifen fumerate and chlorpheniramine maleate and their 1:1, 1:2 and 2:1 mixtures in the solutions of buffers (11-12) pH 1.2, 2.8 and 6.8 were compared with those of each interacting species. The concentrations of the sample were kept at very dilute levels in each case and the measurements made using UV-VIS spectrophotometer. The spectra of the working standard solutions(1×10^{-5} M) were recorded between 400 - 190 nm. The spectra were compared with those of the pure samples in each case.

2.1.5. Job's Spectrophotometric method

According to Job's method (13) the absorbance of series of ketotifen fumerate with chlorpheniramine in different molar ratios 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 were measured by keeping the total mole constant. The observed absorbance of the mixtures at various mole fractions was subtracted from sum of the values for free drugs (ketotifen fumerate and chlorpheniramine maleate). The absorbance difference (D) was then plotted against the mole fractions of the drug in the mixtures. If the formation constant is reasonably favorable, two straight lines of different slopes that intersect at a mole ratio that corresponds to the combining ratio in the complex are obtained (13).

2.1.6. Ardon's spectrometric method:

In the Ardon's spectrophotometric method, (13) concentrations of Ketotifen fumerate was varied while keeping the concentrations of chlorpheniramine maleate fixed at 1×10^{-4} M. All the experiments were performed in buffer at pH 1.2, 2.8 and 6.8. The absorbance of solutions were measured at 300 nm using UV-VIS spectrophotometer. The Ardon's equation was used for calculation. This equation is given below-

$$\frac{1}{(D-\in_A C)} = \frac{1}{KC\,(\in_{com}-\in_A)\,[B]^n} + \frac{1}{C(\in_{com}-\in_A)}$$

Where, D = Absorbance of the mixture, B = Molar concentration of the Kitotifen fumerate. C = Molar concentration of the other drug $\in_{com}$ = Molar extinction co-efficient of the complex, $\in_A$ = Molar extinction co-efficient of the Kitotifen fumerate.

The value of n was chosen as 1, which is an essential condition for validation of the method. The value for 1 / (D - $\in_A$C) was plotted versus 1 / D to get the straight lines. The stability constant of the complex was given by the relation,

$$K = \text{intercept} / \text{slope}$$

It is to be mentioned that this method is only valid for the systems where 1:1 complexes are found (13)

2.2. In-vivo study

2.2.1. Animals

Wister rats (150-200 g) of either sex bred were used. The animals were housed under standard conditions, maintained on a 12-h light/dark cycle and had free access to food and water up to the time of experimentation. The animals were acclimatized to the laboratory environment 1h before the experiments. Experiment was conducted during the light period.

2.2.2. Preparation of drugs

Ketotifen fumerate and chlorpheniramine maleate solutions formulated in 5% Tween-80 and 0.5% carboxy methyl cellulose in Milli-Q water for in vivo study.

2.2.3. Methodology

Freshly prepared solutions of ketotifen fumerate , chlorpheniramine maleate and ketotifen fumerate + chlorpheniramine maleate were administered as single does to three groups of rats. The selection of dose levels based on efficacy dose and toxicokinetic doses. Group I received ketotifen fumerate (0.2mg/kg, p.o.), group II received chlorpheniramine maleate (1mg/kg, p.o.) and group III received ketotifen fumerate (0.2mg/kg, p.o.)+ Chlorpheniramine maleate (1mg/kg, p.o.). Serial blood samples in heparinized saline solution was collected from rats approximately 0.3 ml at each time point namely 0 min, 30 min, 60 min, 120 min and 180 min. Blood samples will be centrifuged at 6000 rpm for 6 min to obtain the plasma. Methanol was added as a protein precipitating agent to plasma samples and vortexed for 1 min and centrifuged at 1000 rpm for 5 min. The supernatant solution transferred to UV-Spectroscopy and analyzed.

3. RESULTS

3.1. Invitro study

In spectral observation analysis, each of the drugs studied showed absorption in UV-VIS region. Initial detection of complexation of ketotifen fumerate with chlorpheniramine was done from the nature of spectra of pure compounds as well as their 1:1, 1:2 and 2:1 mixtures in buffer solution of pH 1.2, 2.8 and 6.8 at a fixed concentration (1×10^{-5}) M. Continuous variation plot (Table 1 and Figure 3) gives information on the relative affinities of the complexes . The numeric values of the resulting stability constant values were between 7.79 and 8.21 when complexation occurs among the ketotifen and chlorpheniramine (Table 3).

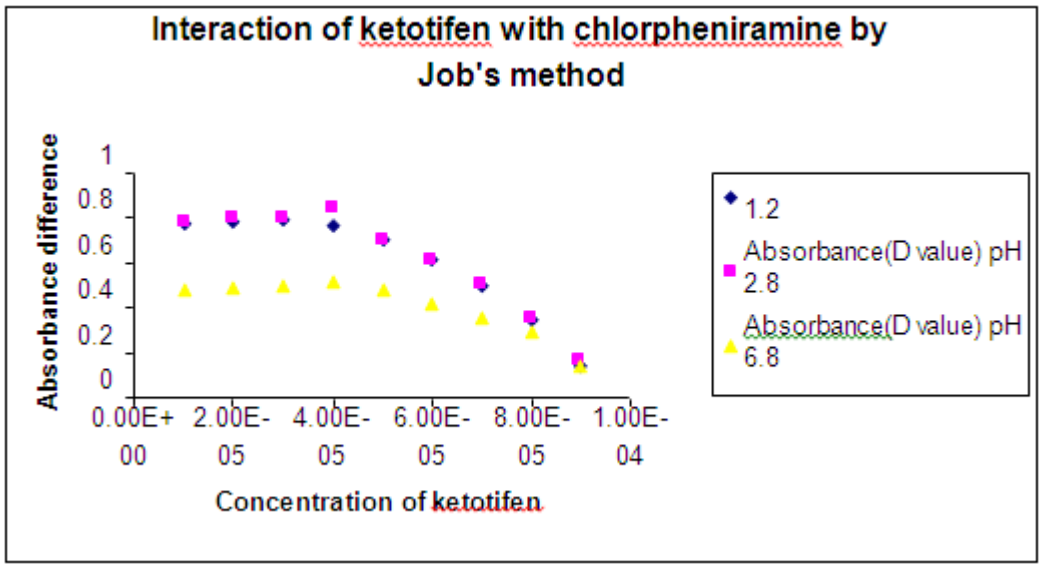

FIGURE 3 - **Job's plot for complexation of ketotifen with chlorpheniramine at 300 nm.**

TABLE 1 - Absorbance of ketotifen at different pH (using Job's method)

Conc. of ketotifen	Absorbance(D value)		
	pH 1.2	pH 2.8	pH 6.8
1.00E-05	0.774	0.785	0.478
2.00E-05	0.79	0.802	0.492
3.00E-05	0.791	0.804	0.504
4.00E-05	0.77	0.845	0.514
5.00E-05	0.704	0.701	0.483
6.00E-05	0.616	0.62	0.42
7.00E-05	0.498	0.508	0.361
8.00E-05	0.347	0.353	0.299
9.00E-05	0.143	0.172	0.14

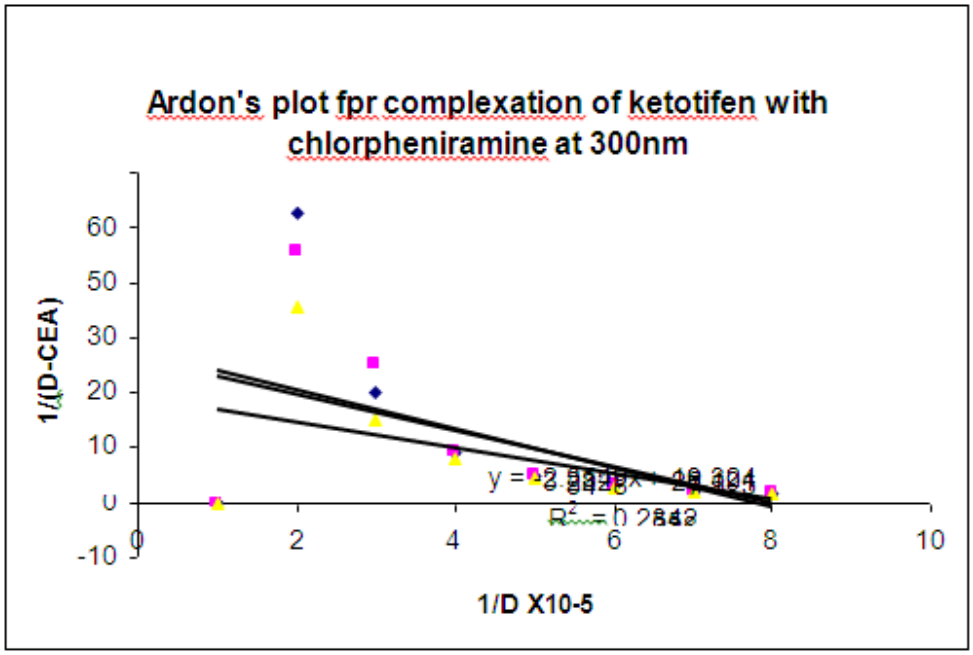

FIGURE 4 - Ardon's plot for complexation of ketotifen with chlorpheniramine at 300 nm

TABLE 2 - Absorbance of ketotifen at different pH (using Ardon's method, when conc. of chlorpheniramine is constant)

$1/D \times 10^{-5}$	$1/(D-C\epsilon_A)$		
	pH=1.2	pH= 2.8	pH= 6.8
1	52.63	45.45	35.71429
0.5	20	25	15.15152
0.33	9.259	9.25926	7.936508
0.25	4.717	4.83092	4.545455

0.2	3.012	3.11526	2.881844
0.167	2.079	2.13675	2.057613
0.143	1.524	1.55521	1.52439
0.25	4.717	4.83092	4.545455

TABLE 3 - Stability constant of ketotifen with chlorpheniramine at different pH

System	pH	Stability Constants($1X10^{-2}$)
Interaction of ketotifen with chlorpheniramine	pH=1.2	7.79
	pH=2.8	8.18
	pH= 6.8	8.21

3.2. In-vivo study

Invivo study on interaction of ketotifen fumerate with chlorpheniramine maleate was studied to find out whether the interaction occur between these two drugs or not.It was performed by using Dunnet method. The mean difference is significant at the 0.05 level except at 180 minutes when only chlorpheniramine was interacted with ketotifen at 265 nm and when 1:1 mixture of ketotifen and chlorpheniramine was interacted at 300nm and also when 1:1 mixture of chlorpheniramine and ketotifen was interacted at 265nm .

4. DISCUSSIONS

4.1. Invitro study

It is obvious that each compound has its unique molecular structure or electronic configuration which is responsible for absorption of light in the form of ultraviolet or visible form. It spectra of alone ketotifen fumerate at different pH showed a sharp absorption maximum at 300 nm. When chlorpheniramine mixed with ketotifen in 2:1 ratio the intensity of the peak of ketotifen change remarkably (absorbance decreases) i.e. absorption characteristics are altered due to interaction but the position of the compound do not shift. The Ardon's plots have been used to evaluate the stability constants and it has been observed that when values of 1 / (D - $C_{\varepsilon A}$) are plotted against 1/ Drug (Figure 4), good straight lines are obtained obeying the Ardon's equation. The values of stability constants at different pH are shown in table 3. Stability constants data showed that ketotifen-chlorpheniramine system formed relatively stronger complexes at all pH conditions..

4.2. In-vivo study
Data analysis and statistics

Data were expressed as mean ± S.E.M. Differences in mean values between experimental groups were analyzed by unpaired 't' test. A probability value less than 0.05 ($p < 0.05$) was defined to be significant. Invivo study on interaction of ketotifen fumerate with chlorpheniramine maleate by using Dunnet method. A Dunnett t-tests treat one group as a control, and compare all other groups against it.

5. CONCLUSION

Interaction of ketotifen with chlorpheniramine decreased the free drug concentration of both drugs which can result in decreased availability of the drugs at receptors. Ultimately, one or both drugs may show diminished pharmacologic activity. However, in the in vivo study, it was found that the decrease in concentration of ketotifen after co-administration of ketotifen and chlorpheniramine was statistically insignificant. We, thus, conclude that concurrent administration of ketotifen with chlorpheniramine may be safe and effective.

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