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Research Article

Interaction of Ketotifen Fumarate with Anhydrous Theophylline in Simulated Gastric and Intestinal Media and Effect on Protein Binding

Mohammed Aktar Sayeed[1], Razibul Habib[2*], Mominur Rahman[1], Hasan Al Banna[1] and Sohel Rana[3]

[1]Department of Pharmacy, International Islamic University, Chittagong, [2]Department of Pharmacy, East West University, [3]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh

Abstract

Purpose: *The purpose of the present study was to investigate interaction between ketotifen fumarate and anhydrous theophylline in aqueous media of various pH.*
Methods: *By using Job's continuous-variation analysis and Ardon's spectrophotomeric methods, the values of stability constants of theophylline with ketotifen were determined at a fixed temperature (37 ^{0}C) at each of the medium pH. In vitro study of protein (bovine albumin, fraction v) binding was carried out by equilibrium dialysis method at pH 7.4 to ascertain the influence of ketotifen on the protein binding of theophylline.*
Results: *Stability constant, ranging between 5.07 and 6.35, were derived from Ardon's plot, indicating that complexes formed, as a result of interaction between the drugs, were comparatively stable. However, following theophylline interaction with ketotifen, stability constant was < 1 at gastric pH (0.4 and 2.0) and 4.12 at intestinal pH. (6.0)The highest degree of protein binding by ketotifen was 98 % and the lowest 90 %. For theophylline, the highest and lowest degrees of protein binding were 90 and 85 %, respectively.*
Conclusion: *Concurrent administration of ketotifen and theophylline would result in the formation of a stable complex and this is likely to reduce the therapeutic activities of both drugs. With regard to protein binding, the concentration of theophylline increased with decrease in ketotifen concentration.*

Keywords*: Stability constant, Job's method, Ardon's method, Ketotifen fumarate, Complex formation, Protein binding, Theophylline*

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**Corresponding author: Email: mrhjewel@gmail.com*

INTRODUCTION

Ketotifen is a benzocycloheptathiophene derivative that has been shown to possess anti-histaminic and anti-anaphylactic properties [1]. It has been demonstrated that it can block *in vitro* release of mediators from rat peritoneal mast cells [1]. The drug inhibits 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 an established place in the treatment of asthma [5,6]. Ketotifen, which is useful in the treatment of hay fever and asthma, have been found to inhibit anaphylactic histamine release from animal tissues .

Theophylline, also known as dimethylxanthine, is a xanthine with bronchodilator properties and is used in the treatment of asthma and chronic obstruvtive pulmonary disease (COPD). Moreover, theophylline has been shown to have some anti-inflammatory activities, inhibiting the activity of CD4 lymphocytes *in-vitro* and mediator release from mast cells [7]. It also inhibits bronchoconstriction produced by exercise and challenge testing, and has also been shown to have beneficial effects on the contraction of the diaphragm, an effect which may be particularly useful in patients with COPD [8,9].

Drug-drug interaction occurs when one therapeutic agent either alters the concentration (pharmacokinetic interactions) or the biological effect (pharmacodynamic interactions) of another agent [10] .The clinical significance of a specific drug-drug interaction depends on the degree of accumulation of the substrate and the therapeutic window of the substrate [11]. The combination of theophylline and ketotifen is often used for respiratory tract infection and some suggest the combination is effective [12] though others suggest the combination may be embryotoxic, with growth retardation, morphological abnormalities, etc [13]. The present study was designed to evaluate the interaction between ketotifen fumarate and anhydrous theophylline, as well as the safety of the combination therapy and their protein binding activity.

EXPERIMENTAL

Drugs and chemicals

Ketotifen fumarate (potency, 95%) and anhydrous theophylline (potency, 91%) were obtained from Square Pharmaceuticals Ltd, Dhaka, Bangladesh, as gifts, and used without further purification. Bovine serum albumin (fraction V) and semipermeable membrane (Medicell, England) were purchased from BDH (England). 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 and other reagents used were all of reagent grade.

Equipment

For the tests, we used UV-Visible spectrometer (model no. UV-1600, Shimadzu, Japan), pH meter (Mettler Toledo, Switzerland), analytical balance (model AL 204-S/01, Mettler Toledo, Switzerland), and a thermostatted water bath (Shimadzu, Japan). A Dunbuff metabolic shakimg incubator (Nickel, Electrical Company, England) was used to shake the plasma/drug mixtures to attain equilibrium.

Preparation of standard solutions

Stock solutions of ketotifen fumarate ($1X10^{-3}$ M) and anhydrous theophylline ($1X10^{-3}$ M) prepared by dissolving them in distilled water. These stock solutions were diluted to desired strengths ($1X10^{-5}$M) by buffer solutions to obtain the working standard solutions.

Absorption spectrum analysis

The absorption characteristics of ketotifen fumarate and theophylline, separately, and their 1:1, 1:2 and 2:1 mixtures in HCl/NaCl buffer solutions (pH 0.4 and 2.0) and phosphate buffer (pH 6.0), respectively, were obtained and compared with those of each of the interacting species [14,15]. The concentrations of the sample were kept at very dilute levels in each case and the measurements made using UV-VIS spectrophotometer with a constant temperature cell compartment and automatic recording unit. The stock solutions of the samples were diluted to appropriate levels with the respective buffers (1×10^{-5}M) at the desired pH and the spectra recorded between 400 - 190 nm. The spectra were compared with those of the pure samples in each case.

Job's spectrophotometric method

Based on Job's method [16], a series of solutions were prepared in which the analytical concentration of one reactant (usually the cation) was held constant while that of the other was varied. Absorbance of series of mixtures of ketotifen fumarate and theophylline in varying molar ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) were measured, keeping the total mole constant. The absorbance of each of the mixtures was subtracted from sum of the values for the free drugs. The absorbance difference (D) was then plotted against the mole fraction of the drug in the mixtures. If the two straight lines of different slopes that intersect at a mole ratio that corresponds to the combined ratio in the complex are obtained.

Ardon's spectrophotometric method [17]

In this method [18], the concentration of anhydrous theophylline was fixed (2×10^{-4}M) while ketotifen concentration was varied. The absorbance of the free drug solutions and that of the mixtures were measured spectrophotometrically at 300 nm at different pH. Based on Ardon's equation (Eq 1), the $1/(D\text{-}C_{\varepsilon A})$ was plotted against 1/drug, and the values of stability constant were calculated from the intercept/slope of the straight lines obtained.

$$(D\text{-}\epsilon_A C)^{-1} = \{KC\ (\epsilon_{com} - \epsilon_A)[B]\}^{-1} + \{C\ (\epsilon_{com} - \epsilon_A)\}^{-1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

where D = absorbance of the mixture; B = molar concentration of the ketotifen fumarate; C = molar concentration of the anhydrous theophylline; ϵ_{com} = molar extinction co-efficient of the complex; and ϵ_A = molar extinction coefficient of the ketotifen fumarate

Equilibrium dialysis method

The semi-permeable membrane (Medicell, England) was activated by digesting with 1M $NaHCO_3$ at 70^0C for 4 h, washed with de-ionised water and immersed in 0.067M phosphate buffer at pH 7.4. At first the membrane was cut into small pieces, 4 cm in length, and taken in a 500 ml beaker containing de-ionized water maintained at 65 - 70 ^{0}C. The activated membrane and drug solution (ranging from 1×10^{-5}M, to 7×10^{-5}M). The mixtures were in a ratio of 1:1 in 60 ml of phosphate buffer and then shaken gently for 6 h in a metabolic shaking incubator at 37 ^{0}C. Following completion of dialysis, the absorbance of the medium was measured at 300 nm and the concentration of bound and unbound drugs were calculated.

Protein binding (F) was computed from Eq 2 [18]

$$F = \{[B]-[A]\ /[B]\} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (2)$$

where, [A] is the molar concentration of the free drug in the buffer compartment [B] is the molar concentration of drug in the plasma compartment

Statistical analysis

The results are expressed as mean ± standard error of mean (SEM) values. Differences between the means of experimental data were analyzed by unpaired t-test. A probability value less than 0.05 ($p < 0.05$) was taken as significant.

RESULTS

Each of the drugs studied showed absorption in the UV-VIS region. The molecular species of ketotifen fumarate and theophylline, when mixed together, showed some changes in their absorption characteristics including shift in absorption maxima. The curves obtained by Job's method showed breaks at different molar concentrations for the drugs. The curve for pH 0.4 medium was exhibited downward movement to at pH 2 and 6 (see Figure 1).

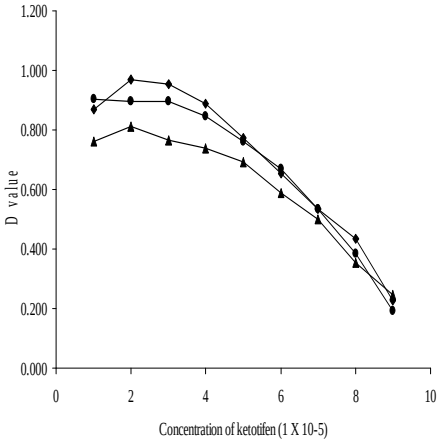

Figure 1: Absorbance difference (D value) at pH 6.0 (■), pH 2.0 (●) and pH 0.4 (▲)

Continuous variation plot gives information on the relative affinities of the complexes and it also depends on the intrinsic spectral characteristics of each complex [19].

Ardon's plots is used to evaluate the stability constants. When values of $1/(D\text{-}C_{\varepsilon A})$ were plotted against 1/Drug (see Figure 2), straight lines were obtained which followed Ardon's model.

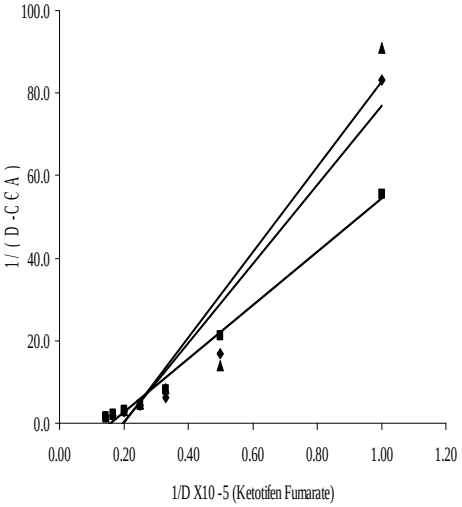

Figure 2: $1/(D\text{-}C_{\varepsilon A})$ values at pH 6.0(▲), pH 0.4 (●) and pH 2.0 (■)

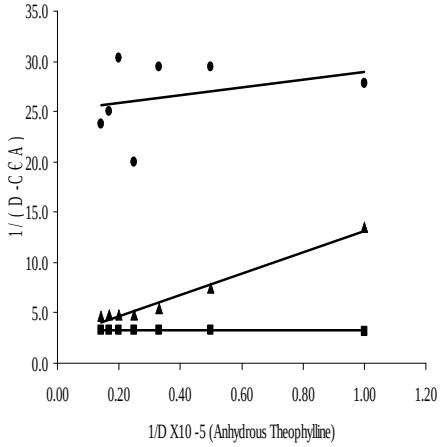

Figure 3: $1/(D\text{-}C_{\varepsilon A})$ values at pH 0.4 (●), pH 6.0 (▲) and pH 2.0 (■).

The values of the stability constants were 5.12, 6.35 and 5.07 at pH 0.4, 2.0 and 6.0, respectively, when ketotifen interacted with theophylline, .

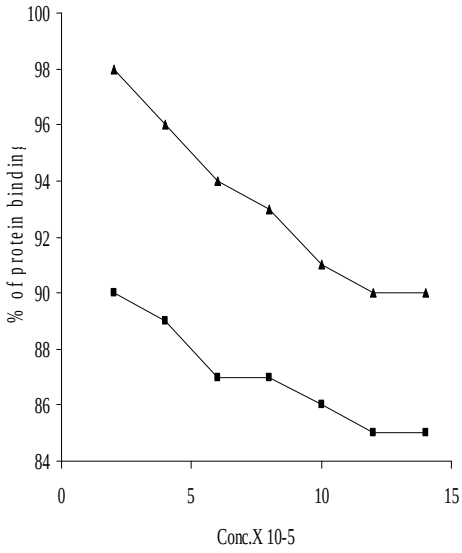

Figure 4: Protein binding (%) of ketotifen (▲) and ketotifen-theophylline mixture (■**)**

Figure 4 illustrates the protein binding characteristics of ketotifen fumarate, and that of theophylline/ketotifen mixture at pH 7.4 and 37 ^{0}C. The highest degree of protein binding of ketotifen with bovine serum albumin was 98 % and the lowest 90 %. In the presence of theophylline, the highest and lowest values were 89 and 83 %, respectively.

DISCUSSION

Initial evidence for complexation of ketotifen fumarate with theophylline came from differences between the spectra of the drugs and those of their mixtures in buffer solutions. Each compound has its unique molecular structure or electronic configuration which is responsible for absorption of light. The spectra of ketotifen fumarate at different pH showed a sharp absorption maximum at 300 nm but when theophylline was mixed with it in 2:1 ratio at pH 0.4, the intensity of ketotifen peak changed remarkably as absorption decreased but the absorption of the compound did not shift at pH 0.4. At pH 6, the intensity of the peak of ketotifen was altered as absorption increased.

Very low stability constant values mean that the complex formed due to the interaction of the drugs readily dissociates, yielding essentially the drugs in ionic form, ranging from pH as low as stomach acid (pH 0.4 to 3) to as high as physiologic pH 6.0 (pH of main extracellular body fluids such as serum and lymph). The values of the stability constant, which varied between 5.07 and 6.35, indicate not only that complexation occured between ketotifen and theophylline but also that the interaction was pronounced. It can be assumed that these two drugs should not be co-administered.

In applying Ardon,s method, theophylline was taken as the parent drug, and in its interaction with ketotifen, lower stability constant values were found, indicating readily solubility of both drugs and minimum drug-drug interaction. The degree of protein binding of ketotifen decreased with increase in the concentration of theophylline attaining a steady (plateau) state when the free drug concentration was around 5 x 10^{-5}M. On the other hand, the theophylline curve also declined significantly (p = 0.01) due to protein binding of ketotifen fumerate. .

CONCLUSION

Interaction of ketotifen with theophylline 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. Furthermore, ketotifen fumarate and theophylline lowered protein binding of theophylline, could increase the volume of distribution of theophylline. Therefore, caution should be exercised during the administration of both drugs, pending *in vivo* experiments to determine the implication of our findings.

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