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RESEARCH PAPER

Terfenadine–β-Cyclodextrin Inclusion Complex with Antihistaminic Activity Enhancement

Han-Gon Choi,[1,2] Beom-Jin Lee,[3] Jeong-Hee Han,[1] Mi-Kyung Lee,[1] Kyung-Mi Park,[1] Chul Soon Yong,[2] Jong-Dal Rhee,[2] Yang-Bae Kim,[1] and Chong-Kook Kim[1,*]

[1]*College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Ku, Seoul 151-742, South Korea*
[2]*College of Pharmacy, Yeungnam University, 214-1, Dae-Dong, Gyongsan 712-749, South Korea*
[3]*College of Pharmacy, Kangwon National University, Chuncheon 200-701, South Korea*

ABSTRACT

Terfenadine, an antihistaminic drug, has relatively low bioavailability after oral administration due to its limited solubility in water. To enhance the antihistaminic activity of terfenadine, the terfenadine–β-cyclodextrin (1 : 2) inclusion complex was prepared by the neutralization method. The solubility and dissolution of the inclusion complex were carried out, and its antihistaminic activity was then evaluated and compared with terfenadine powder by the passive subcutaneous anaphylaxis method in rats. The formation constant of the inclusion complex was higher at lower pH, while its formation ratio was 1 : 2 irrespective of pH. For terfenadine, it improved the solubility 200 times and the dissolution rate 5 times. It gave a low histamine level at 30 min, followed by a sustained low level until 60 min, while terfenadine powder gave a low histamine level at 60 min, suggesting that it had faster and more effective antihistaminic activity than terfenadine powder in rats due to fast dissolution and absorption of terfenadine.

*Corresponding author. Fax: 82-2-873-7482; E-mail: ckkim@plaza.snu.ac.kr

It is concluded that this inclusion complex enhanced the antihistaminic activity of terfenadine following the enhanced solubility and dissolution of terfenadine.
Key Words: *Antihistaminic activity; Inclusion; Solubility; Terfenadine*

INTRODUCTION

Terfenadine [1-(4-*tert*-butylphenyl)-4-[4-(α-hydroxybenzhydryl)piperidino]butan-1-ol], an antihistaminic drug, is widely used to treat allergic rhinitis and urticaria. However, the bioavailability of terfenadine is relatively low after oral administration due to its limited solubility in water (1,2). It has been attempted to improve the solubility of terfenadine by microballoon for oral controlled release (1), gentisate hydrotropes (3), and solid dispersion with sodium carboxymethylcellulose (CMC) (4).

Cyclodextrin complexation has been extensively applied to enhance the solubility, dissolution rate, and bioavailability of slightly water soluble drugs (5–9). The cyclodextrin complexes with slightly water soluble drugs have been prepared using coprecipitation, kneading, neutralization, spray-drying, and freeze-drying methods (5–9). Among the methods, the neutralization method has been extensively applied to prepare the inclusion complex with a drug since it has many advantages—no necessity for organic solvent, good yield in a short operating period, and suitability for extension to manufacturing scale (8,10).

Thus, in this study, to enhance the antihistaminic activity of terfenadine, the terfenadine–β-cyclodextrin (1 : 2) inclusion complex was prepared by the neutralization method after the investigation of its formation properties. The solubility and dissolution of the inclusion complex were carried out, and its antihistaminic activity was then evaluated by the passive subcutaneous anaphylaxis method in rats compared with terfenadine powder.

EXPERIMENTAL

Materials

The terfenadine used was USP grade. Evans blue, histamine, and β-cyclodextrin were purchased from Aldrich Chemical Company (Milwaukee, WI). The semipermeable membrane tube (Spectra membrane tubing No. 1) was purchased from Spectrum Medical Industries, Incorporated (Los Angeles, CA). All other chemicals were reagent grade and were used without further purification.

Preparation and Physicochemical Properties of Inclusion Complex

Formation Properties

The formation properties of terfenadine–β-cyclodextrin inclusion complex were measured using the dialysis method (11). In brief, 5 ml of pH 1.2–6.8 were added in one part of the dialysis cells, while 5 ml of pH 1.2–6.8 media, in which β-cyclodextrin (1 mg/ml) and terfenadine (0.025–0.5 mg/ml) were dissolved or dispersed, were added in the other part of the dialysis cells. Thereafter, the dialysis cells were shaken at 20°C for 7 days. Samples were taken in the former part of dialysis cell, filtered, and analyzed by an ultraviolet/visible (UV/Vis) spectrophotometer (Philips, model PU8730) at 258 nm.

Preparation

Terfenadine–β-cyclodextrin (1 : 2) inclusion complex was prepared by the neutralization method (8,10). In brief, 5 g of terfenadine and 30 g of β-cyclodextrin (1 : 2 molar ratio) were dissolved in 500 ml of 0.1 N acetic acid. With continuous agitation, the pH of the resulting solution was adjusted to 11.0 by the slow addition of 0.1 N sodium hydroxide and then to 7.0 by the slow addition of 0.1 N hydrochloric acid. It was filtered and dried in vacuo at room temperature overnight, leading to terfenadine–β-cyclodextrin (1 : 2) inclusion complex. The formation of inclusion complex was conformed by differential scanning calorimetry (DSC) (Netzsch, model 200).

Solubility

Excess of terfenadine powder (0.5 g) and 3.5 g of inclusion complex (equivalent to 0.5 g terfenadine) were added to 10 ml of pH 1.2–6.8. They were shaken at 20°C for 7 days, filtered, and analyzed by UV/Vis spectrophotometer at 258 nm (11).

Dissolution

Terfenadine powder (0.27 g) and inclusion complex (1.89 g) (equivalent to 0.27 g terfenadine) were placed in a dissolution tester (DST-600, Fine Chemical, Korea). The dissolution test was performed at 36.5°C using the paddle method at 50 rpm with 500 ml of pH 1.2 as a dissolution medium. At 5-min intervals, 5 ml of the medium were sampled, filtered, and analyzed by UV/Vis spectrophotometer at 258 nm (12).

Antihistaminic Activity of Inclusion Complex

The antihistaminic activity was evaluated by the passive subcutaneous anaphylaxis method (14,15). In brief, the rats were fasted for 18 h prior to the experiments, but were allowed free access to water. The 48 male Sprague-Dawley rats, weighing 250 ± 20 g, were divided into three groups: those orally administered 1 ml/kg CMC (0.5%) as a control, those given terfenadine powder/CMC (1%/0.5%) (equivalent to 10 mg terfenadine/kg), and those given inclusion complex/CMC (7%/0.5%) (equivalent to 10 mg terfenadine/kg). After predetermined time intervals, 0.05 ml of histamine-saline solution (160 mg/ml) was subcutaneously administered, and instantly 1 ml of Evans blue–saline solution (2.5 mg/ml) was intravenously administered. After 30 min, each rat was killed in an ether-saturated chamber, and the skin was removed. The blue spot area beneath the histamine-administered skin was measured. The statistical significance of the difference between the blue spot areas on animals administered terfenadine and inclusion complex was determined by the Student t test.

RESULTS AND DISCUSSION

We investigated the formation properties of terfenadine –β-cyclodextrin inclusion complex using the following Scatchard plot (11):

$$\frac{1}{r} = \frac{1}{[vK]} \times \frac{1}{[D]} + \frac{1}{v} \quad (1)$$

where r is moles of terfenadine bound per mole of β-cyclodextrin, $[D]$ is the concentration of free terfenadine, v is the number of formation sites per mole of β-cyclodextrin, and K is a formation constant. From the plot of $1/r$ versus $1/[D]$, v and K were calculated. Fig. 1 shows that the inclusion complex gave a v value of 0.47 and a K value of $9.0 \times 10^3\,M^{-1}$ in pH 1.2. Table 1 shows that the inclusion complex gave v values of nearly 0.5, irrespective of pH, suggesting that it means the formation ratio of 1 : 2 (terfenadine–β-cyclodextrin). The K values indicate that the inclusion complex was formed more at a lower pH (Table 1). Such a larger formation constant of inclusion complex at a lower pH may be due to the higher solubility of terfenadine at a lower pH (1,12,13).

The terfenadine–β-cyclodextrin (1 : 2) inclusion complex was prepared easily by the neutraliza-

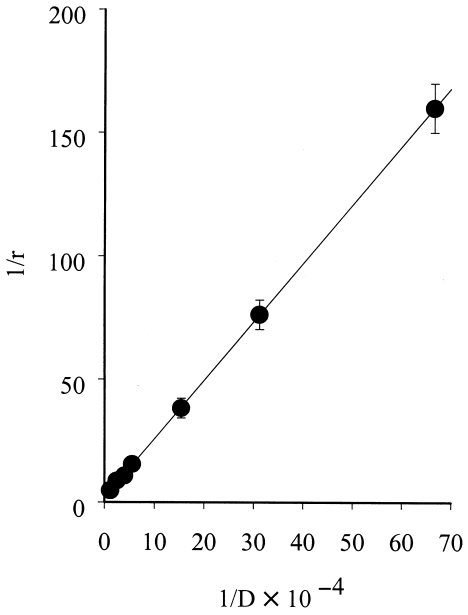

Figure 1. Scatchard plot for terfenadine-β-cyclodextrin complex at pH 1.2. Each value represents the mean ± SD ($n = 5$). $Y = 2.13 + 2.38X$ ($r = 0.988$).

Table 1

Formation Properties of Inclusion Complex

pH	Number of Formation Sites v	Formation Constant K (M^{-1})	Correlation Coefficient R
1.2	0.47	9.0×10^3	0.988
2.5	0.49	8.9×10^2	0.982
4.0	0.47	7.9×10^2	0.985
5.0	0.51	6.6×10^2	0.991
5.5	0.46	6.3×10^2	0.981
6.8	0.45	5.3	0.983

tion method using 5 g of terfenadine and 30 g of β-cyclodextrin (1 : 2 molar ratio). The DSC curve shows that the sharp peak at around 150°C, which was observed for terfenadine and the physical mixture (1 : 2), disappeared in the inclusion complex (1 : 2). Furthermore, the wide peak at 60°C, which was observed for β-cyclodextrin and the physical mixture (1 : 2), shifted to 50°C in the inclusion complex (Fig. 2). Our results suggest that the complete inclusion complex without free terfenadine should be formed (9,10).

Fig. 3 shows that the solubility of terfenadine in the inclusion complex increased with decreasing pH. This inclusion complex gave 200 times the solubility of terfenadine, irrespective of pH (at pH 1.2 it was 195.2 vs. 0.896×10^{-2} mg/ml, respectively; at pH 6.8, it was 20.3 vs. 0.094×10^{-2} mg/ml, respectively). The solubility of terfenadine in inclusion complex according to pH had a profile similar to the formation constant of inclusion complex according to pH (Table 1 and Fig. 3), indirectly proving that the improved solubility of terfenadine was due to formation of an inclusion complex with β-cyclodextrin. The dissolved amount of terfenadine from drug powder and inclusion complex was about 20% and 100% within 10 min, respectively, and there was no further significant change in dissolution rates until 30 min. Our results suggest that the inclusion complex gave five times the increased dissolution rate of terfenadine. Such an enhanced dissolution of terfenadine from inclusion complex may be by the contribution of the increased solubility of terfenadine in inclusion complex (9,10).

As described by the passive subcutaneous anaphylaxis method (14,15), the antihistaminic activity of a drug was evaluated by measuring the blue spot area beneath the histamine-administered skin on treated and control animals. The smaller blue spot area beneath the histamine-administered skin, the more effective the antihistaminic activity since the skin sites with anaphylactic reaction provoked by the histamine appear blue. However, in this study, to understand the comparative antihistaminic activity, instead of the blue spot area, we described the histamine level using the following equation.

$$\text{Histamine level (\%)} = Dt/D \times 100 \quad (2)$$

where Dt and D are the blue spot area beneath the histamine-administered skin on animals administered inclusion complex (or terfenadine powder) and control, respectively. As the histamine level decreases,

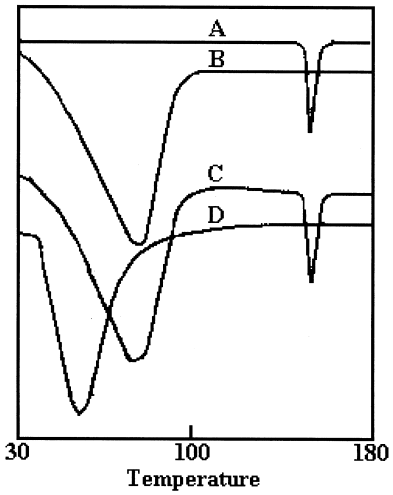

Figure 2. DSC curves: (A) terfenadine; (B) β-cyclodextrin; (C) physical mixture (1 : 2); (D) inclusion complex (1 : 2).

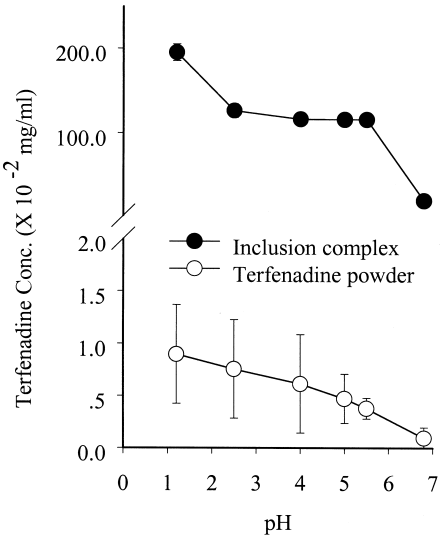

Figure 3. Solubility of terfenadine in inclusion complex and terfenadine powder at pH 1.2–6.8. Each value represents the mean ± SD ($n = 5$).

the antihistaminic activity is more effective. The histamine level of 100% means no antihistaminic activity. As shown in Fig. 4, the inclusion complex gave a histamine level of nearly 100% at 15 min and a low histamine level (81%) at 30 min, followed by a sustained low histamine level (76%–85%) until 60 min, while terfenadine powder gave a histamine level of nearly 100% until 45 min and a low histamine

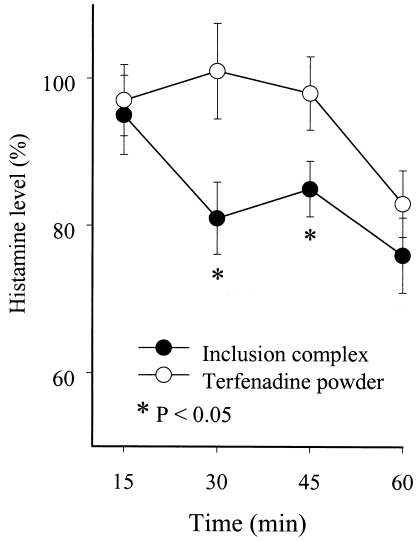

Figure 4. Comparative antihistaminic activity of inclusion complex and terfenadine powder. Each value represents the mean ± SD ($n = 4$).

level (83%) at 60 min, indicating that the inclusion complex (30 min) had faster antihistamine activity than terfenadine powder (60 min). In addition, the inclusion complex gave a lower histamine level than terfenadine powder at 30–45 min ($P < .05$). At 60 min, the inclusion complex also gave a lower histamine level than terfenadine powder, although there was no significant difference between their histamine levels ($P > .05$), indicating that the inclusion complex had a more effective antihistamine activity than terfenadine powder. Such a fast and effective antihistaminic activity of inclusion complex may be due to fast dissolution and absorption of terfenadine from inclusion complex.

Taken together, it is concluded that terfenadine–β-cyclodextrin (1 : 2) inclusion complex prepared by the neutralization method enhanced the antihistaminic activity of terfenadine following the enhanced solubility and dissolution of terfenadine.

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