Enhancement of Bioavailability of Cinnarizine from Its β-Cyclodextrin Complex on Oral Administration with DL-Phenylalanine as a Competing Agent

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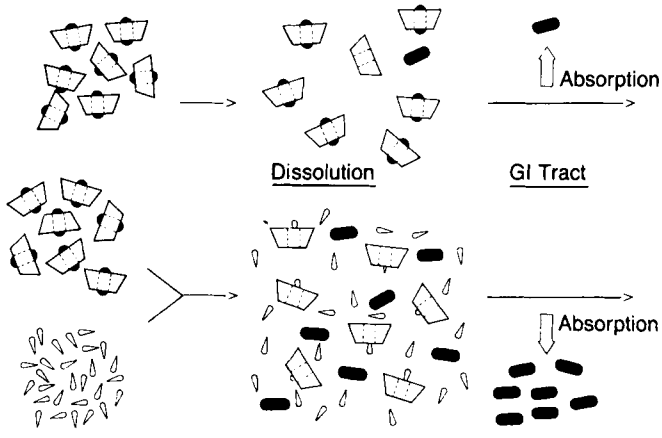
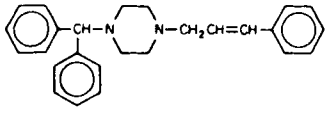
Abstract □ The present investigation is concerned with an improvement of the bioavailability of cinnarizine by administering its β-cyclodextrin complex together with another compound which competes with the β-cyclodextrin molecule in complex formation in aqueous solution (competing agent). The bioavailability of cinnarizine on oral administration of the cinnarizine–β-cyclodextrin inclusion complex was enhanced by the simultaneous administration of DL-phenylalanine as a competing agent, e.g., the AUC was 1.9 and 2.7 times as large as those of the cinnarizine–β-cyclodextrin complex alone and cinnarizine alone, respectively. The enhancement of AUC and C_{max} completely depended on the dose of DL-phenylalanine. It was found from these results that DL-phenylalanine acted as a competing agent in the GI tract and the minimum effective dose required of DL-phenylalanine might be 1 g for 50 mg of cinnarizine in the cinnarizine–β-cyclodextrin complex. Evaluating the competing effect of DL-phenylalanine in vitro using an absorption simulator, it was found that the decreased penetration rate of cinnarizine through the artificial lipid barrier with addition of β-cyclodextrin was restored with the addition of DL-phenylalanine.



Scheme I—The process of drug absorption from cyclodextrin complexes and the role of a competing agent. Key: ( ) cyclodextrin; ( ) drug–cyclodextrin complex; ( ) drug; ( ) competing agent; ( ) competing agent–cyclodextrin complex.

It was reported briefly in a previous communication[1] that the bioavailability on oral administration of cinnarizine [1-(diphenylmethyl)-4-(3-phenyl-2-propenyl)piperazine (1)] was enhanced when its inclusion complex with β-cyclodextrin (2) was administered together with another compound, which competes with 2 in complex formation in aqueous solution (competing agent). The present study attempts to investigate this finding in more detail.



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An inclusion-complex formation of a drug with cyclodextrin is known to bring about an enhancement of the solubility,[2,3] dissolution rate,[4,5] and bioavailability[6–9] of the drug. The enhancement of bioavailability is generally attributed to the increase in solubility and dissolution rate of the drug due to the cyclodextrin-complex formation. However, the drug-absorption rate itself may generally be decreased by complex formation, because only free drug can be absorbed.

Therefore, if the stability constant is large, the cyclodextrin complex formation is not as effective an enhancer of the bioavailability. In this connection, there may be a possibility of an enhancement of bioavailability of such a drug in an inclusion complex by combined administration with a competing agent. Scheme I outlines the process of drug absorption from the cyclodextrin complex and the role of a competing agent. In the case of the complex alone, as in the upper process in the scheme, the concentration of free drug which is available for absorption may be low. When the complex and the competing agent are administered together, as in the lower process in the scheme, the concentration of free drug may be increased by the action of the competing agent after both components dissolve.

In the series of studies of the inclusion complexes of 1 with 2,[10–12] it was found that the solubility of 1 was enhanced by the inclusion-complex formation with 2, while the bioavailability of 1 was not enhanced.[11] This result was considered to be due to the large stability constant of the 1–2 complex.[12] Therefore, the present study was performed in an attempt to enhance the bioavailability of 1 in the 2 complex using (DL)-phenylalanine (3) as a competing agent. DL-phenylalanine (3) was chosen because it has been reported that L-phenylalanine forms an inclusion complex with 2 with a stability constant of 1×10^3 M^{-1}.[13] Moreover, DL-phenylalanine (3) seemed to be a pharmaceutically acceptable excipient. The absorption study was performed with beagle dogs. In addition to the absorption study, the competing effect of 3 in vivo was evaluated using an absorption simulator. The penetration rate of 1 through the artificial lipid barrier was determined in the presence of 2 and both 2 and 3.

Experimental Section

Materials—Cinnarizine (1) and β-cyclodextrin (2) were obtained from Eisai Co., Ltd., and Nippon Shokuhin Kako Co., Ltd., respectively. All other chemicals and solvents used were of analytical reagent grade. De-ionized water was used in all experiments. The

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samples of the 1–2 complex, with a molar ratio of 1:2, was prepared by a previously described method.[10]

Absorption Study—*Experiment 1*—The results of the first experiment confirmed the enhancement of bioavailability of 1 on the administration of the 1–2 complex with 3. Two tablets of 1 or the 1–2 complex, containing 25 mg/tablet (6.78×10^{-5} mol) of 1, were administered alone or together with 2 g (1.21×10^{-2} mol) of 3 in four gelatin capsules with 30 mL of water to three male beagle dogs which had been fasted for 24 h. The dose administered was the maximum experimentally possible one, as a minimum effective amount of the competing agent was not known. The beagle dogs fasted for 4 h following the administration of drug. At a given time, a 2.5-mL blood sample was taken and the plasma concentration of 1 was determined following the method described in a previous paper.[1]

Experiment 2—The second experiment was a study of the effect of the dose of 3 on the bioavailability of 1 following the administration of the 1–2 complex with 3. The formulas of the preparations administered are shown in Table I. Crystalline cellulose (Avicel PH102, Asahikasei, Tokyo), a typical inert excipient for powder preparations, was added to adjust the weight of the sample, because the total weight of the dosage form may influence the secretion of stomach acid which affects the oral bioavailability of 1.[11] The components of each formula were mixed in a mortar and packed into five gelatin capsules. Five different preparations, *a–e*, were administered to five male beagle dogs which had been fasted for 18 h. This experiment was carried out using a crossover method. The administration, sampling, and determination of 1 in plasma were carried out as for experiment 1.

In Vitro Study in an Absorption Simulator—The penetration rate of 1 through an artificial lipid membrane was determined employing a Sartorius absorption simulator. The membrane filter (Sartorius; diameter: 9 cm), which contained the lipid-barrier substance M1, was used as the artificial lipid membrane. The membrane filter increased in weight by 100 ± 5% following treatment with M1. A type A diffusion chamber, with an effective area of 40 cm^2, was used. Test solution (100 mL, pH 1.2) and 100 mL of the Japanese Pharmacopoeia X[14] (JP X) first fluid (pH 1.2) were put in phase I and phase II, respectively. The penetration rate of 1 was determined for five different test solutions which were prepared by dissolving the following components in JP X first fluid (final pH, 1.2): (1) 2 mg/mL (5.4 mM) solution of 1: (2) 2 mg/mL solution of 1 containing 108.0 mM 3; (3) 2 mg/mL solution of 1 containing 10.8 mM 2; (4) 2 mg/mL solution of 1 containing 10.8 mM 2 and 54.0 mM 3; (5) 2 mg/mL solution of 1 containing 10.8 mM 2 and 108.0 mM 3. A 1.5-mL aliquot of the sample solution was taken from phase II at appropriate intervals and the same amount of the first fluid was added. The concentration of 1 was determined by the UV-absorption method at 254 nm using a Hitachi UV-200 spectrophotometer.

Results and Discussion

Competing Agent—Figure 1 shows the mean plasma levels of 1 after oral administration of 1 and the 1–2 complex with or without 3 to dogs. As shown in Table II, when 1 was administered with 3 there was no clear difference in plasma level and bioavailability parameters compared with the administration of 1 alone. This result indicates that 3 does not act as an absorption promoter of 1. On the other hand, the administration of the 1–2 complex with 3 brought about a clear increase in the plasma level and the AUC; the AUC was 1.9 and 2.7 times as large with 3 as for the 1–2 complex alone and 1 alone, respectively. Here, for the administration of the 1–2 complex with 3, the time of the peak blood concentration was somewhat later than in the administration of the 1–2 complex alone. The reason for this result is not clear at present, but it might be that different methods of administration were used, i.e., the 1–2 complex was administered as tablets and 3 was administered as capsules (two tablets were used to administer the 1–2 complex alone; two tablets and five capsules were used to administer the 1–2 complex with 3). These results indicate that 3 acts as a competing agent for 1 in the 1–2 complex and promotes a higher bioavailability of 1.

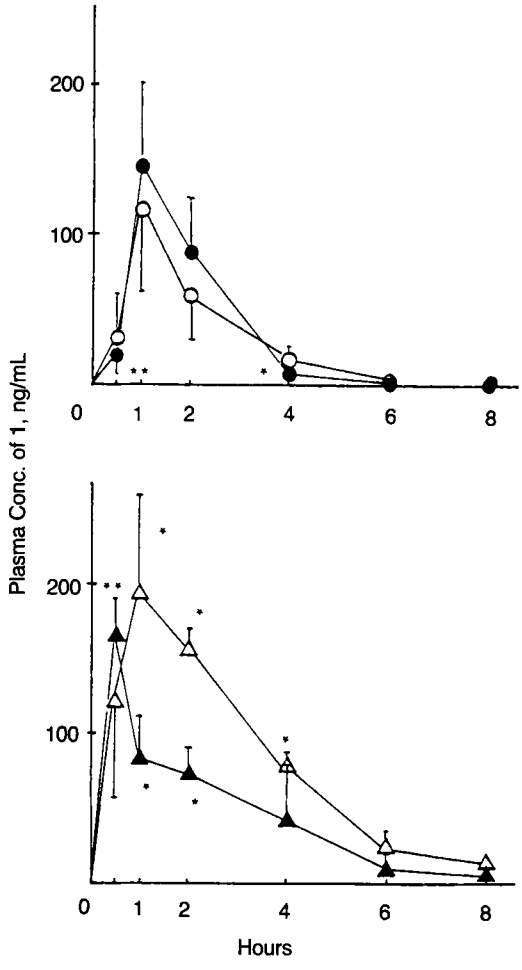

Figure 1—*Plasma concentration of cinnarizine after oral administration to dogs of cinnarizine (50 mg) and cinnarizine–β-cyclodextrin complex (equivalent to 50 mg of cinnarizine) with or without 2 g of* DL*-phenylalanine. Each point represents the mean ± SEM of three dogs. Key:* (●) *cinnarizine alone;* (○) *cinnarizine with* DL*-phenlalanine;* (▲) *β-cyclodextrin complex alone;* (△) *β-cyclodextrin complex with* DL*-phenylalanine.* (**) $p < 0.01$; (*) $p < 0.05$.

Table I—Ingredients in the Preparations for Oral Administration to Dogs[a]

	Formulations, mg[a]				
	a	*b*	*c*	*d*	*e*
1	50	—	—	—	—
1–2 Complex	—	465	465	465	465
3	—	—	500	1000	2000
Crystalline cellulose	2450	2035	1535	1035	35

[a] 2500 mg per formulation.

Table II—Bioavailability Parameters for Oral Administration of Cinnarizine (1) or Its β-Cyclodextrin Complex (2) with or without DL**-Phenylalanine (3)[a]**

Administration Sample	AUC_{0-8h}, ng · h/mL	C_{max}, ng/mL	t_{max}, h
1[b]	267.2 ± 102.9[c]	145.3 ± 55.3	1.0
1 + 3	224.1 ± 106.7	123.9 ± 70.7	1.7 ± 0.3
1–2 Complex	374.2 ± 97.2[c]	166.9 ± 22.4	0.5
1–2 Complex + 3	709.3 ± 57.4[c]	270.8 ± 41.1	0.8 ± 0.2

[a] Each value represents the mean ± SEM of three dogs. [b] Data from ref. 11. [c] $p < 0.05$.

Effect of Dose—Figure 2 and Table III show the results of the absorption study of formulas *a–e*. There is no difference in the plasma levels and the bioavailability parameters between the results of formulations *a* and *b*, which is in agreement with previous results.[11] It is clearly indicated in Fig. 2 that the plasma levels of **1** increase with an increase in the dose of **3**. The values of C_{max} in formulations *c*, *d*, and *e* were 1.5, 1.8, and 2.4 times as large as that of formulation *b*, and 2.0, 2.5, and 3.2 times as large as that of formulation *a*. The values of AUC of formulations *c*, *d*, and *e* were 1.6, 1.8, and 2.0 times as large as that of formulation *b*, and 2.1, 2.3, and 2.6 times as large as that of formulation *a*. On the other hand, t_{max} was not changed by the simultaneous administration of **3**. Considering that crystalline cellulose has been known to have no influence on the oral bioavailability of drugs in powdered dosage forms, the enhancement of the bioavailability thereby achieved may depend on the addition of **3**.

The results of the absorption study of formulations *a–e* indicate that the increase in C_{max} and AUC depend completely on the dose of **3** and, accordingly, that **3** acts as a competing agent in the GI tract. In this experimental system, the minimum effective dose of **3** seemed to be 1 g for 50 mg of **1** in the **1–2** complex.

Figure 3 shows the effect of the dose of **3** on C_{max} and AUC in formulations *b–e* on the basis of the data in Table III. Apparently, there is a linear relationship between C_{max} and the dose of **3** ($r = 0.992$); the AUC increased hyperbolically with an increase in the dose of **3**. It is difficult to explain this difference in profile between C_{max} and AUC; this result indicates that the increase in C_{max} and AUC may be related to the concentration of **1** available for absorption.

Absorption Simulator—Figure 4 shows the penetration of **1** through the artificial lipid barrier. The apparent penetration rate constant k was calculated from the slope of the linear part of each plot in Fig. 4 by:[15]

$$k = \frac{AV}{CF}\ (\text{cm} \cdot \text{min}^{-1}) \qquad (1)$$

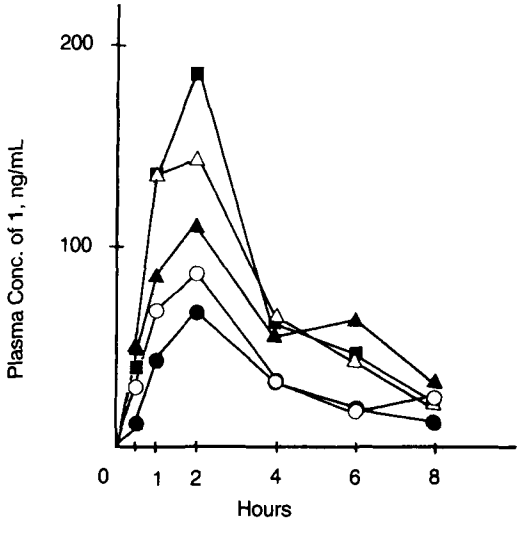

Figure 2—*Time courses of plasma cinnarizine concentrations in dogs following oral administration of formulations a–e.* Key: (●) *formulation a;* (○) *b;* (▲) *c;* (△) *d;* (■) *e. (see Experimental Section for formulations). Each point represents the mean of the determinations from five dogs.*

Table III—Bioavailability Parameters for Oral Administration of Formulations *a–e*[a]

Formulation	AUC_{0-8h}, ng · h/mL	C_{max}, ng/mL	t_{max}, h
a	251.0 ± 78.9	70.4 ± 18.0	1.7 ± 0.3
b	329.9 ± 52.1	96.0 ± 6.0	1.9 ± 0.6
c	520.0 ± 175.2	140.5 ± 21.7[c]	2.4 ± 0.9
d	578.8 ± 78.4[c,d]	175.8 ± 20.1[d]	2.4 ± 0.9
e	645.6 ± 127.0[c]	228.7 ± 26.4[d,e]	1.8 ± 0.2

[a] Each value represents the mean ± SEM of five dogs. [b] $p < 0.01$ (compared with formulation a). [c] $p < 0.05$ (compared with formulation a). [d] $p < 0.05$ (compared with formulation b). [e] $p < 0.05$ (compared with formulation c).

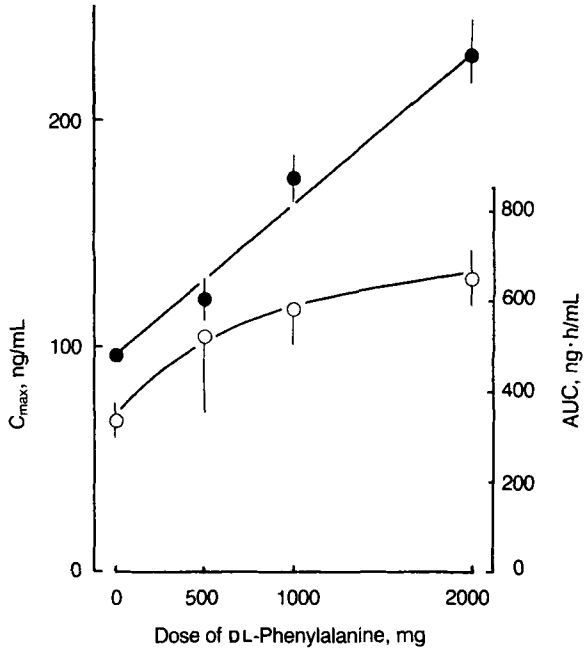

Figure 3—*Effect of the dose of DL-phenylalanine on C_{max} (●) and AUC (○) on oral administration of cinnarizine–β-cyclodextrin complex. Each point represents the mean ± SEM of the determinations for five dogs.*

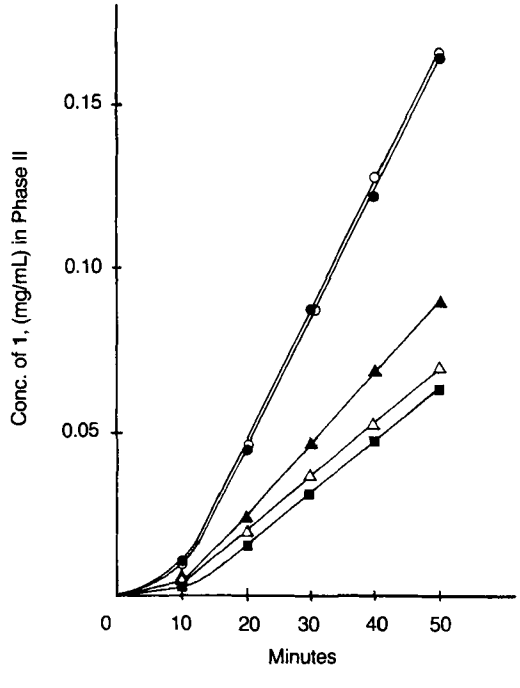

Figure 4—*Concentration of cinnarizine in phase II in an absorption simulator. Key: (●) test solution (1); (○) (2); (■) (3); (△) (4); (▲) (5).*

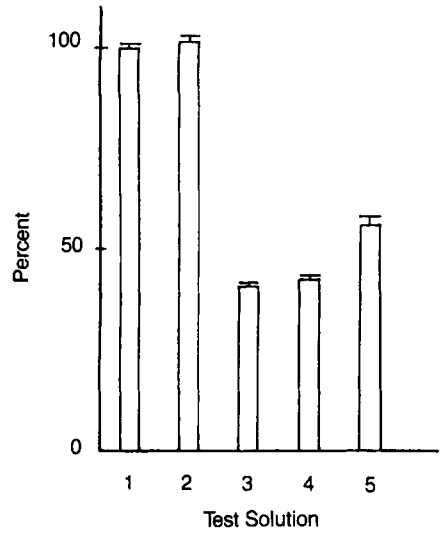

Figure 5—*Effect of β-cyclodextrin and DL-phenylalanine on the apparent penetration rate constant of cinnarizine. The y-axis shows the percent of the rate constant compared with cinnarizine alone. Each bar represents the mean ± SEM of three determinations.*

where A is the slope, C is the initial concentration of phase I, V is the volume of phase II, and F is the effective area of the barrier. Figure 5 shows the apparent penetration rate constant as a percent of the apparent penetration rate constant of 1 alone. The penetration rate of 1 decreased with addition of 2. This is considered due to the decrease in the concentration of free drug by the inclusion-complex formation, because only free drug can pass through the membrane. When 3 was added to the experimental system, the value of the penetration rate constant was restored. This was especially true in test solution (5), which contained 108.0 mM 3, i.e., 20 times the molar ratio of 1. The reason that test solution (4) did not restore the value of the rate constant might be that the value of the stability constant of the 1–2 complex was large[12] compared with the 3–2 complex. From this result, it was considered possible that the concentration of free 1 increased with the addition of 3, which acted as a competing agent in the solution; this is in agreement with the result of the in vivo absorption study. On the other hand, the result of using test solution (2) indicated that 3 did not affect the penetration rate of 1 directly. A precise discussion of the association constants between 1, 2, and 3, under the conditions in the present experiments using an absorption simulator, would be necessary to explain the mechanism of the competing action on the penetration rate although there is an experimental difficulty in determining such association constants (stability constants). It has been suggested that this experimental system affords a useful method for an in vitro evaluation of competing agents.

As described above, enhancement of the bioavailability of 1 on oral administration of the 1–2 complex was achieved by the simultaneous administration of 3 as a competing agent. In order to enhance the bioavailability of a drug on oral administration by complexation with 2, the enhancement of the dissolution rate of the drug should be first taken into consideration. The present method, which is concerned with an increase in the concentration of free drug from a complex in the GI tract by simultaneous administration of a competing agent, may be a useful means of enhancing the bioavailability of drugs. Such use of a competing agent can apply not only to an oral administration of the 1–2 complex but also to other cyclodextrin complexes of drugs, and can be used as an advanced methodology to study the controlled release of drugs.

References and Notes

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