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Enhancement of the Bioavailability of Cinnarizine from Its β-Cyclodextrin Complex on Oral Administration with L-Isoleucine as a Competing Agent[1)]

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This investigation was aimed at the improvement of the bioavailability of cinnarizine (CN) by administering its β-cyclodextrin (β-CD) complex together with a competing agent. The ability of L-leucine (L-Leu) and L-isoleucine (L-Ileu) to act as competing agents was evaluated by determining the penetration rate of CN, employing a Sartorius absorption simulator. L-Ileu showed a stronger competing action than L-Leu. The bioavailability of CN, upon oral administration of the CN–β-CD inclusion complex, was enhanced by the simultaneous administration of L-Ileu as a competing agent; C_{max} was 1.9 and 2.7 times those of CN–β-CD complex alone and CN alone, respectively. L-Leu showed no clear effect on the bioavailability. The *in vivo* competing effects of L-Leu and L-Ileu appeared to be in agreement with the *in vitro* evaluations.

Keywords——cinnarizine; β-cyclodextrin; inclusion complex; L-leucine; L-isoleucine; plasma level; competing agent; oral administration; beagle dog; bioavailability

Inclusion complex formation of a drug with cyclodextrin (CD) is known to bring about an enhancement of the drug's bioavailability.[2–5)] However, the bioavailability of cinnarizine (CN) after oral administration was not enhanced by inclusion complex formation with β-cyclodextrin (β-CD).[6)] This was considered to be due to the large stability constant of CN–β-CD complex. Therefore, we tried to enhance the bioavailability of CN from the complex by the simultaneous administration of a competing agent. An improvement of the bioavailability was found upon the simultaneous administration of DL-phenylalanine as a competing agent.[7)] In addition, measurement of the penetration rate of CN under various conditions, employing a Sartorius absorption simulator, was found to be a useful method for *in vitro* evaluation of a competing agent.[8)]

In this study, the ability of L-leucine (L-Leu) and L-isoleucine (L-Ileu) to act as competing agents was examined by employing a Sartorius absorption simulator. The *in vitro* data were compared with the *in vivo* data obtained from an absorption study in beagle dogs.

Experimental

Materials——CN and β-CD were obtained from Eisai Co., Ltd., and Nippon Shokuhin Kako Co., Ltd., respectively. All other chemicals and solvents used were of analytical reagent grade. Deionized water was used in all experiments. A sample of CN–β-CD complex of molar ratio 1 : 2 was prepared by the method described in a previous paper.[9)]

***In Vitro* Study Employing a Sartorius Absorption Simulator**——The penetration rate of CN through an artificial lipid membrane was determined by employing a Sartorius absorption simulator. A Sartorius membrane filter (ϕ =

TABLE I. Formulae of Preparations I—VIII for Oral Administration

	Preparation							
	I[a]	II[a]	III	IV	V	VI	VII	VIII
CN	50	—	—	—	—	—	—	50
CN–β-CD complex	—	465	465	465	465	465	465	—
L-Leu	—	—	1000	2000	—	—	—	—
L-Ileu	—	—	—	—	500	1000	2000	2000
Crystalline cellulose	2450	2035	1035	35	1535	1035	35	450
Total (mg)	2500							

a) Data from the previous paper.[8]

9 cm) containing a lipid barrier substance M1 was used as the artificial lipid membrane. The percent increase in weight of the membrane filter, after treatment with the lipid barrier substance M1, was 100±5%. A Type A diffusion chamber with an effective area of 40 cm^2 was used, and 100 ml of test solution (pH 1.2) and 100 ml of the Japanese Pharmacopoeia X (JP X) 1st fluid (pH 1.2) were used in phase I and phase II, respectively. The CN penetration rate was determined for the following 4 different test solutions, which were prepared by dissolving the components in the JP X 1st fluid, pH 1.2: 1) 2 mg/ml (5.4 mM) solution of CN; 2) 2 mg/ml solution of CN containing 10.8 mM β-CD; 3) 2 mg/ml solution of CN containing 10.8 mM β-CD and 108.0 mM L-Leu; 4) 2 mg/ml solution of CN containing 10.8 mM β-CD and 108.0 mM L-Ileu. Aliquots of 1.5 ml of the sample solution were taken from phase II at appropriate intervals and the same amount of the 1st fluid was added at the same time. The concentration of CN was determined by the ultraviolet (UV) method at 254 nm using a Hitachi UV-200 spectrophotometer.

Absorption Study——The formulae of the preparations administered are shown in Table I. The components of each formula were mixed in a mortar and packed into 5 gelatin capsules. Eight different preparations, I—VIII, were administered to 9 male beagle dogs, which had been fasted for 18 h. The administration, sampling and determination of CN in plasma were conducted according to the methods described in a previous paper.[6]

Results and Discussion

In Vitro Evaluation of L-Leu and L-Ileu as Competing Agents

Figure 1 shows how CN penetrates through the artificial lipid barrier. The apparent penetration rate constant, k, was calculated, as described in a previous paper,[8] from the slope of the linear part of each plot in Fig. 1.

Figure 2 shows the apparent penetration rate constant as a percentage, on the assumption that the apparent penetration rate constant of CN alone is 100%. The penetration rate of CN decreased with the addition of β-CD. The reason for this was explained in a previous paper.[8] When L-Leu or L-Ileu was added to the experimental system, the penetration rate constant was restored. This phenomenon depends on the increase of free CN in the solution, because only the "free" CN molecule can pass through the membrane. L-Leu and L-Ileu are completely ionized under these experimental conditions, and β-CD can not pass through the membrane. Therefore, these results suggest that L-Leu and L-Ileu act as competing agents. The data in Fig. 2 indicate that the action of L-Ileu was stronger than that of L-Leu, so L-Ileu is expected to be a more effective competing agent.

Effects of L-Leu and L-Ileu on the Bioavailability of CN

Tables II and III show the plasma concentrations of CN and the bioavailability parameters after oral administration of preparations I—VII, respectively. The plasma levels of CN in the cases of preparations III and IV were increased compared with those of preparations I and II. However, no significant difference was found by the t-test. The area under the blood concentration curve (AUC) and C_{max} of preparations III and IV were also increased, but the difference was not significant. These results indicate that L-Leu acts as a

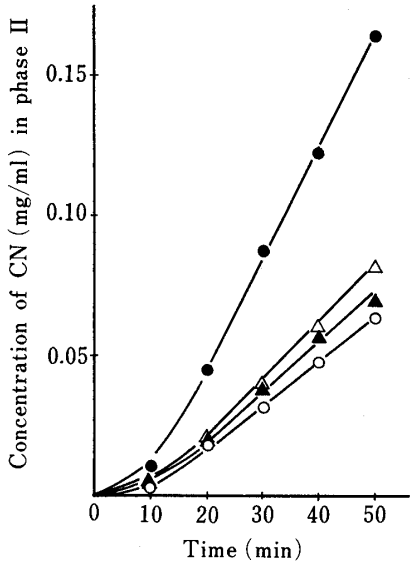

Fig. 1. Concentration of CN in Phase II in a Sartorius Absorption Simulator

●, test solution 1[a]; ○, test solution 2[a]; ▲, test solution 3; △, test solution 4. *a*) Data from the previous paper.[8]

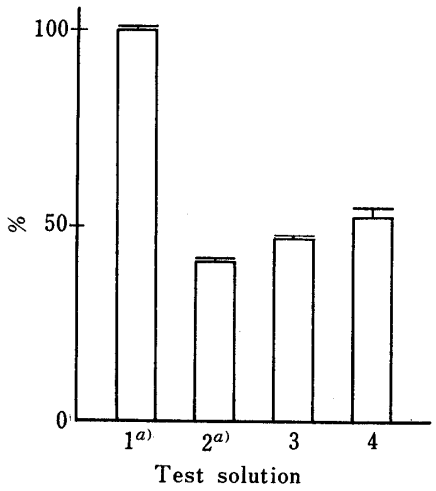

Fig. 2. Effects of L-Leu and L-Ileu on the Apparent Penetration Rate Constant of CN

The Y axis shows the rate constant as a percentage of that obtained with CN alone. Each bar represent the mean ± S.E. of three determinations. *a*) Data from the previous paper.[8]

TABLE II. Plasma Concentration of CN after Oral Administration of Preparations I—VIII

Preparation	Time (h)					
	0.5	1	2	4	6	8
I[a]	11.9± 7.8[b]	43.8±17.0[b,c]	68.1±19.9	30.3±11.2	18.1± 8.2	13.3± 7.1
II[a]	30.7±17.7	68.3± 6.7[b,c]	87.7± 5.8[c]	36.4±18.3	16.0± 8.1	26.9±11.2
III	25.1±12.7	142.4±54.4	85.4±17.8	33.1±14.4	3.6± 1.9	5.7± 2.6
IV	1.6± 1.6	78.4±47.7	147.3±70.8	53.3±12.3	6.7± 6.7	0
V	78.2± 4.0[b]	118.4±21.6[c]	63.4± 8.4	6.7± 3.6	10.1±10.1	1.7± 1.7
VI	68.8±65.2	98.3±12.8	141.2±19.5[c]	36.6±14.2	13.1± 8.8	2.8± 2.0
VII	58.8±40.2	187.0±24.2[b]	113.2±22.9	34.1± 8.6	31.1±10.8	42.0±35.5
VIII	12.1± 9.7	83.8±66.0	60.0±37.9	8.7± 5.9	2.0± 2.0	0

a) Data from the previous paper.[8] *b*) $p<0.01$, *c*) $p<0.05$ (compared with preparations I or II). Each value represents the mean ± S.E. of 3—5 dogs.

competing agent, but it is very weak.

The plasma levels in the cases of preparations V and VII were significantly increased compared with those in the cases of preparations I and II, and the plasma level with preparation VI was significantly increased compared with that in the case of preparation II. The *AUC* values of preparations VI and VII were increased, but the difference was not statistically significant. The values of C_{max} for preparations VI and VII were increased significantly, and were 2 and 2.7 times that of preparation I and 1.5 and 1.9 times that of preparation II, respectively. No regular variation of T_{max} in preparations II, V, VI and VII was observed. This result indicates that L-Ileu does not affect T_{max} and is in agreement with the result for DL-phenylalanine.[8] L-Ileu had no effect as an absorption promoter on CN, judging from the result with preparation VIII. These results indicate that the L-Ileu acts as a competing agent in the gastro-intestinal tract.

Figure 3 shows the effect of the L-Ileu dose on C_{max} and *AUC* with preparations II, V, VI

TABLE III. Bioavailability Parameters for the Oral Administration of Preparations I—VIII

Preparation	AUC_{0-8h} (ng·h/ml)	C_{max} (ng/ml)	T_{max} (h)
I[a]	251.0± 78.9	70.4±18.0[b,c]	1.7±0.3
II[a]	329.9± 52.1	96.0± 6.0[b,c]	1.9±0.6
III	326.1± 59.1	148.3±51.0	1.5±0.3
IV	400.5±183.0	176.2±57.8	2
V	258.4± 37.7	118.4±21.6	1
VI	422.2± 75.4	141.2±19.5[c]	2
VII	530.8± 74.1	187.0±24.2[b]	1
VIII	180.2±120.9	88.0±64.6	1.5±0.4

Each value represents the mean±S.E. of 3—5 dogs. *a*) Data from the previous paper.[8] *b*) $p<0.01$, *c*) $p<0.05$ (compared with preparations I or II).

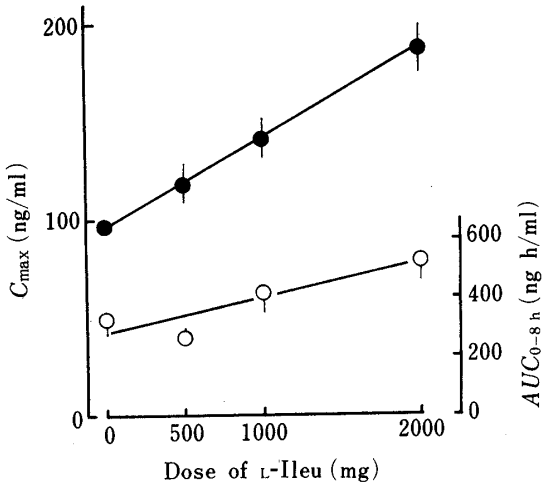

Fig. 3. Effect of the L-Ileu Dose on C_{max} and AUC_{0-8h} after Oral Administration of CN–β-CD Complex

Each point represents the mean±S.E. of the determinations for 3 or 5 dogs.
●, C_{max}; ○, AUC_{0-8h}.

and VII on the basis of the data in Table III. There is a linear relationship between C_{max} and the L-Ileu dose ($r=0.992$). However, a clear linear relationship between AUC and the L-Ileu doses was not observed ($r=0.875$). It seems difficult to explain this difference in profile between C_{max} and AUC; however, it is possible that an increase in the dose affects C_{max} more directly than AUC, because the latter is a more complicated parameter than the former.

As described above, an enhancement of CN bioavailability, after oral administration of CN–β-CD complex, was achieved by the simultaneous administration of L-Ileu. However, the simultaneous administration of L-Leu did not cause a clear enhancement of CN bioavailability. These *in vivo* results are consistent with the conclusions based on the *in vitro* data. This indicates that the determination of the penetration rate of a drug by employing a Sartorius absorption simulator is a useful method for the evaluation of competing agents. The utilization of a competing agent may be applicable not only to the oral administration of CN–β-CD complex but also to CD complexes of other drugs as an advanced method to control the drug release. From this point of view, the finding of new competing agents other than DL-phenylalanine is very important, because a suitable competing agent should be chosen from among various competing agents by taking into consideration the degree of competition and the release rate of the drug from the complex.

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References and Notes

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2) N. Nambu, M. Shinoda, Y. Takahashi, H. Ueda and T. Nagai, *Chem. Pharm. Bull.*, **26**, 2952 (1978).
3) M. Tsuruoka, T. Hashimoto, H. Seo, S. Ichimasa, O. Ueno, T. Fujinaga, M. Otagiri and K. Uekama, *Yakugaku Zasshi*, **101**, 360 (1981).
4) K. Uekama, T. Fujinaga, M. Otagiri, H. Seo and M. Tsuruoka, *J. Pharmacobio-Dyn.*, **4**, 726 (1981).
5) K. Uekama, N. Matsuo, F. Hirayama, H. Ichibagase, K. Arimori, K. Tsubaki and K. Satake, *Yakugaku Zasshi*, **100**, 903 (1980).
6) T. Tokumura, Y. Tsushima, K. Tatsuishi, M. Kayano, Y. Machida and T. Nagai, *Chem. Pharm. Bull.*, **33**, 2962 (1985).
7) T. Tokumura, Y. Tsushima, M. Kayano, Y. Machida and T. Nagai, *J. Pharm. Sci.*, **74**, 496 (1985).
8) T. Tokumura, M. Nanba, Y. Tsushima, K. Tatsuishi, M. Kayano, Y. Machida and T. Nagai, *J. Pharm. Sci.*, in press.
9) T. Tokumura, Y. Tsushima, K. Tatsuishi, M. Kayano, Y. Machida and T. Nagai, *Yakuzaigaku*, **45**, 1 (1985).