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# Evaluation of Bioavailability upon Oral Administration of Cinnarizine–β-Cyclodextrin Inclusion Complex to Beagle Dogs[1)]

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The bioavailability of cinnarizine (CN) upon oral administration of its β-cyclodextrin complex to beagle dogs was investigated in comparison with that of CN alone. The dissolution rate at pH 3.0—6.8 was increased significantly by inclusion complexation, but there was no difference in the bioavailability of CN after oral administration of β-cylodextrin complex and that of CN alone. The absorption of CN was decreased significantly when CN was administered with $NaHCO_3$. However, no decrease was observed in the case of CN–β-cylodextrin inclusion complex.

**Keywords**——cinnarizine; β-cyclodextrin; inclusion complex; dissolution rate; solubility; partition coefficient; oral bioavailability; plasma level; beagle dog

Cinnarizine (CN), an agent for increasing cerebral blood flow, is widely used orally to treat various problems in cerebral apoplexy, cerebral arteriosclerosis and post traumatic cerebral symptoms. CN is a weak base and its $pK_{a1}$ and $pK_{a2}$ were reported to be 7.47[2)] and 1.95.[3)] The solubility in water is very poor. It was already reported that the dissolution rate of CN differs among commercially available pharmaceutical preparations,[4)] and this affects the absorption of CN after oral administration.[4)]

Clinically, CN preparations are usually administered to aged persons. However, many of those patients are known to have achlorhydria or anacidity.[5)] Consequently, it is necessary to develop a preparation of cinnarizine which can dissolve well in a neutral medium. In the previous work,[6)] it was shown that the dissolution rate of CN in CN–β-cyclodextrin (β-CD) inclusion complex is 30 times or more larger than that of intact CN at pH 5.0. Thus, the present investigation was designed to investigate the bioavailability of CN after oral administration of CN–β-CD inclusion complex to beagle dogs. In order to simulate the absorption of CN when CN–β-CD complex is administered orally to patients with achlorhydria, CN–β-CD complex and $NaHCO_3$ were administered at the same time. An experiment using citric acid was also carried out, because the bioavailability of CN after oral administration were reported to be enhanced by simultaneous administration of citric acid.[7)]

The solubility, the partition coefficients and the dissolution rates of cinnarizine in buffer solutions having various pH values were determined and their importance in relation to the bioavailability of CN is discussed.

## Experimental

**Materials**——Cinnarizine (CN) and β-cyclodextrin (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.

**Solubility Studies**——One gram of CN was added to 50 ml of buffer solution in a centrifuge tube, which was then sealed and shaken at 37 °C. The buffer solutions were made up by mixing the 1st fluid and 2nd fluid of the Japanese Pharmacopoeia X (JP X), and the pH values were as follows: 1.3, 2.5, 4.0, 5.0, 5.5 and 6.8. After equilibration for 2 h, an aliquot was filtered through a Toyo No. 2 paper filter. The concentration of CN in the filtrate was determined by the ultraviolet (UV) absorption method using a Hitachi UV-101 spectrophotometer.

**Partition Coefficient**——Twenty milliliters of 2-ethylhexyl alcohol containing 10 $\mu$g/ml of CN was added to 20 ml of buffer solution at various pH values in a centrifuge tube, which was then sealed and shaken at 37 °C. The pH values of HCl–KCl buffer solutions ($\mu=0.3$) used in the experimental were as follows: 1.0, 1.5, 2.0, 2.5 and 3.0. After equilibration for 2 h, the concentration of CN in the buffer solution was determined by the UV absorption method using a Hitachi UV-101 spectrophotometer.

**Procedure for Dissolution Study**——The paddle method of JP X was used in buffer solutions of various pH values. Buffer solutions of pH 1.2, 2.0, 3.0, 4.0, 5.0, 6.0 and 6.8 were prepared by mixing the 1st fluid and 2nd fluid of JP X. A certain amount of each sample corresponding to 25 mg of CN was placed in a beaker filled with 1000 ml of medium equilibrated at 37 °C. Inclusion complex of CN with $\beta$-CD was prepared by the spray-drying method.[8] The paddle was rotated at 100 rpm, and 3 ml of the sample solution was taken through a Fine Filter F®(Iwaki Seisakusho Co., Ltd.) at appropriate intervals. The concentrations of CN were determined by the UV absorption method at 254 nm using a Hitachi UV-200 spectrophotometer.

**Absorption Study**——Six male beagle dogs were used after fasting for 24 h before drug administration. The intervals between administrations were more than one week. Two tablets of CN or CN–$\beta$-CD complex, containing 25 mg of CN in each tablet, were administered alone or together with 500 mg of $NaHCO_3$ or citric acid in gelatin capsules with 30 ml of water. At given intervals, a 2.5 ml of blood sample was taken from the cephalic vein. The blood samples were centrifuged for 10 min at 3000 rpm. The plasma layer was removed and frozen until analysis. One milliliter of plasma, 0.5 ml of 1 N HCl and 5 ml of diethyl ether were added to a glass-stoppered centrifuge tube. The tubes were shaken well and centrifuged for 10 min at 3000 rpm. After freezing of the aqueous layer, the diethyl ether layer was discarded. Five milliliters of diethyl ether was added again to the aqueous layer, and the mixture was shaken, centrifuged and frozen. The diethyl ether layer was discarded, and the aqueous layer was extracted with 5 ml of dichloromethane. The dichloromethane phase was evaporated to dryness under nitrogen on a water bath. The residue was dissolved in 100 $\mu$l of mobile phase (acetonitrile : 0.01 M $NH_4H_2PO_4=70:30$) containing 100 $\mu$g of mechlizine chloride as an internal standard, and 50 $\mu$l of the solution was injected into a Shimadzu LC-5A HPLC instrument. The chromatograph was operated at a flow rate of 2.5 ml/min and the eluate was monitored spectrofluorometrically by using a fluorescence monitor (Shimadzu RF-530). The excitation wavelength and analyzer wavelength were 260 and 315 nm, respectively. A column of Nucleosil $C_{18}$ (5 $\mu$m in 4 mm × 25 cm) was used for analysis. A standard curve was prepared by analyzing plasma samples to which CN had been added at various concentrations ranging from 25 to 400 ng/ml.

## Results and Discussion

### Solubility and Partition Coefficients of CN

In drug absorption in the gastro-intestinal tract, the first step is dissolution of the drug and the next is absorption of the drug following the pH-partition theory. Therefore, in order to consider the absorption of CN in the gastro-intestinal tract, the solubility of CN in buffer solutions and partition coefficients (parameters of the theory) were determined.

Table I shows the solubility of CN in buffer solutions of various pH values. Its solubility depended on the pH and was very low around the neutral region. Table II shows the partition

Table I. Solubility of Cinnarizine

| | mg/100 ml |
|---|---|
| pH 1.3 | 705 |
| pH 2.5 | 147 |
| pH 4.0 | 7.15 |
| pH 5.0 | 1.07 |
| pH 5.5 | 0.47 |
| pH 6.9 | 0.067 |

Table II. Partition Coefficients of Cinnarizine between 2-Ethylhexyl Alcohol and Buffer Solutions

| pH of buffer | Partition coefficient |
|---|---|
| 1.0 | 6.3 |
| 1.5 | 18.2 |
| 2.0 | 46.6 |
| 2.5 | 82.3 |
| 3.0 | 141.9 |

coefficients of CN between 2-ethylhexyl alcohol and buffer solutions of various pH values. The partition coefficients of CN were large even at such low pH values as 1.0 to 3.0. This result and the $pK_a$ value of CN suggest that ionized CN can be absorbed in the gastro-intestinal tract. From the data in Tables I and II, the general nature of absorption of CN in gastro-intestinal tract may be as follows: CN is dissolved in the normal stomach at high acidity and then absorbed rapidly in the upper part of the small intestine because of the large partition coefficient of CN. However, the acidity of the human stomach may be decreased by food, and shows inter-individual variation. Further, as described previously, many aged persons are known to show achlorhydria or anacidity.[5] From the solubility data and the problem of acidity in the stomach as mentioned above, it seems reasonable to assume that the dissolution of CN in the stomach is the rate-determining step in the absorption of CN. This assumption is supported by a report which indicated that the dissolution rate of CN preparations at pH 2.0 is correlated with the bioavailability after oral administration[9] to rabbits. If this is correct, the absorption of CN in a patient with achlorhydria might be very low due to the failure of CN to

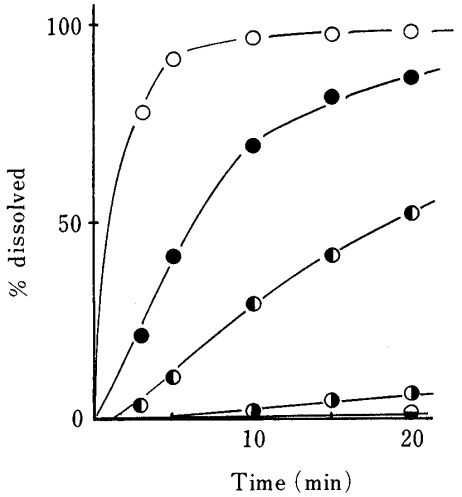


Fig. 1. Dissolution Rate Curves of CN in Buffer Solutions of Various pH Values

○, pH 1.2; ●, pH 2.0; ◐, pH 3.0; ◑, pH 4.0; ◒, pH 5.0.
Each point is the mean of three determinations.

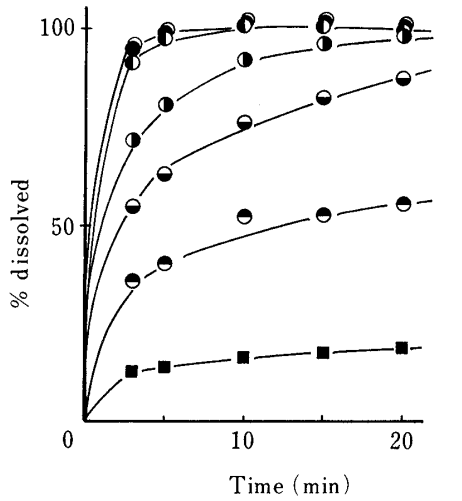


Fig. 2. Dissolution Rate Curves of CN–β-CD Inclusion Complex in Buffer Solutions of Various pH Values

○, pH 1.2; ●, pH 2.0; ◐, pH 3.0; ◑, pH 4.0; ◒, pH 5.0; ◓, pH 6.0; ■, pH 6.8.
Each point is the mean of three determinations.

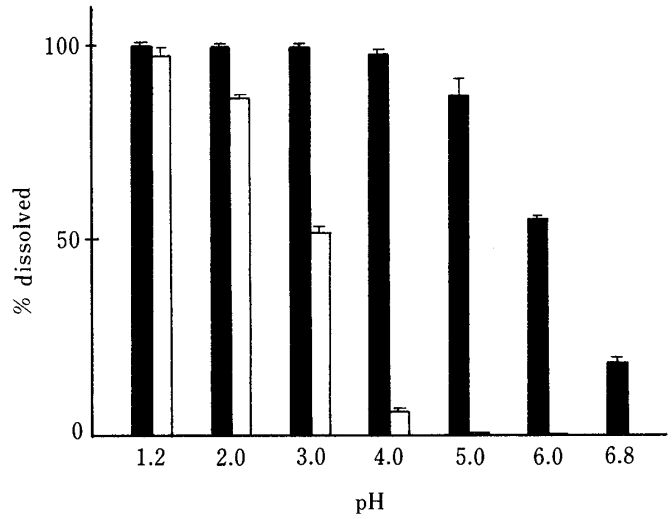


Fig. 3. Percent Dissolved of CN from Intact CN and CN–β-CD Inclusion Complex at 20 min after the Start of the Dissolution Test

□, intact CN; ■, CN–β-CD complex.
Each bar represents the mean ± S.D. of three determinations.

dissolve in the stomach.

**Dissolution Behavior of CN and CN–β-CD Inclusion Complex**

The dissolution behavior of CN and CN–β-CD complex at pH 5.0 was reported previously.[6] In this report, dissolution tests were carried out at pH 1.2—6.8 to investigate the influence of solubility on bioavailability.

Figure 1 shows the results of dissolution tests of intact CN. The dissolution of intact CN at pH 1.2 was very rapid but it decreased with increase of the pH value and at pH above 4.0, CN hardly dissolved. Figure 2 shows the dissolution behavior of CN–β-CD complex. Good dissolution was observed at pH 1.2—5.0, and CN–β-CD complex could dissolve even at pH above 5.0, where the dissolution of intact CN was not observed. Figure 3 shows the percent dissolved after 20 min in the dissolution tests of intact CN and CN–β-CD complex. The results indicate clearly that the dissolution of CN–β-CD complex is faster than that of intact CN, especially at pH 4.0—6.8. From these results, it may be assumed that sufficient dissolution of CN upon oral administration of CN–β-CD complex might occur even in achlorhydric patients.

**Bioavailability of CN–β-CD Complex**

Figure 4, based on data reported in a previous short communication,[10] shows the mean plasma levels of CN after the oral administration of CN and its β-CD complex to dogs. When

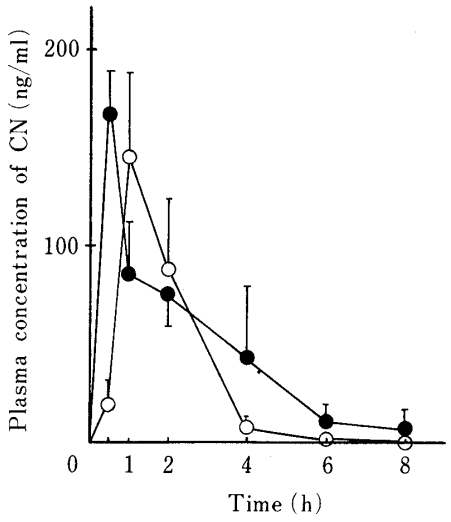


Fig. 4. Time Courses of Plasma CN Concentration in Dogs after Oral Administration of CN (50 mg) and Its β-CD Complex (Containing 50 mg CN)

○, CN; ●, CN–β-CD complex.
Each point represents the mean ± S.E. of 3 dogs.
Data from the previous paper.[10]

TABLE III. Bioavailability Parameters for Oral Administration of CN or Its β-CD Complex and Effect of the Simultaneous Administration of $NaHCO_3$ or Citric Acid on the Parameters

| Administered sample | $AUC_{0-8\,h}$ (ng h/ml) | $C_{max}$ (ng/ml) | $T_{max}$ (h) |
|---|---|---|---|
| CN[a] | 267.2 ± 102.9 | 145.3 ± 55.3 | 1.0 |
| CN–β-CD[a] | 374.2 ± 97.2[b] | 166.9 ± 22.4 | 0.5 |
| CN + $NaHCO_3$ | 23.0 ± 11.5[b,c] | 25.1 ± 15.4 | 1.25 ± 0.75 |
| CN–β-CD + $NaHCO_3$ | 390.2 ± 121.9[c] | 137.3 ± 25.3 | 0.5 |
| CH + citric acid | 293.7 ± 138.5 | 127.7 ± 40.3 | 1.67 ± 0.33 |
| CN–β-CD + citric acid | 527.2 ± 42.6 | 169.4 ± 32.2 | 2.2 ± 1.01 |

Each value represents the mean ± S.E. of 3 dogs. *a*) Data from the previous paper.[10] *b, c*) $p < 0.05$.

a dose equivalent to 50 mg of CN was administered to dogs, the CN–β-CD complex gave a maximum plasma level of 166.9 ± 22.4 ng/ml (mean ± S.E.) at 0.5 h, which is 8.6 times that of CN alone. This initial increase in drug absorption might be due to the high dissolution rate of the complex as mentioned above. However, there was no statistically significant difference in area under the blood concentration curve (*AUC*) between CN and CN–β-CD complex, as shown in Table III. This result indicates that enhancement of the dissolution rate of the complex does not affect the bioavailability of CN. This seemed very peculiar because generally the bioavailability is enhanced with increasing dissolution rate of a drug caused by complexation with CDs, *e.g.*, in the cases of phenytoin,[11] digoxin,[12] acetohexamide[13] and nonsteroidal antiinflammatory agents.[14] The absence of enhancement of bioavailability after administration of CN–β-CD complex is probably due to the large stability constant of the complex, which was estimated to be apparently $6.2 \times 10^3$ M$^{-1}$ in water at 20 °C.[6] In the case of a complex having a large stability constant, the dissolved complex cannot dissociate sufficiently, and so the concentration of free drug remains low. This may be the reason why the absorption of CN in the gastro-intestinal tract after administration of CN–β-CD complex did not exceed the absorption after administration of CN alone.

**Effects of $NaHCO_3$ and Citric Acid on the Bioavailability of CN**

Figure 5 shows the effect of $NaHCO_3$ on the absorption of intact CN and its β-CD complex. When CN was administered to dogs with $NaHCO_3$, the plasma levels of CN remained extremely low. However, in the case of CN–β-CD complex, the plasma levels of CN and other bioavailability parameters were unaffected by simultaneous administration of $NaHCO_3$ as shown in Table III. These results presumably reflect a difference of the dissolution rate of CN at around neutral pH as shown in Fig. 3, because the acid in the stomach is neutralized by $NaHCO_3$. The condition of the stomach after administration of $NaHCO_3$ can be considered to be similar to that in patients with achlorhydria. From these results, an enhancement of absorption of CN in achlorhydric patients can be expected on oral administration of CN–β-CD complex.

Figure 6 shows the results of an experiment concerning the effect of citric acid on the

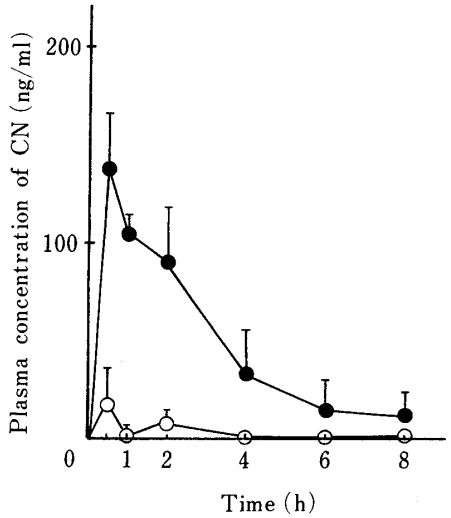


Fig. 5. Time Courses of Plasma CN Concentration in Dogs after Oral Administration of CN (50 mg) and Its β-CD Complex (Containing 50 mg CN) with $NaHCO_3$ (500 mg)

○, CN with $NaHCO_3$; ●, CN–β-CD complex with $NaHCO_3$.
Each point represents the mean ± S.E. of 3 dogs.

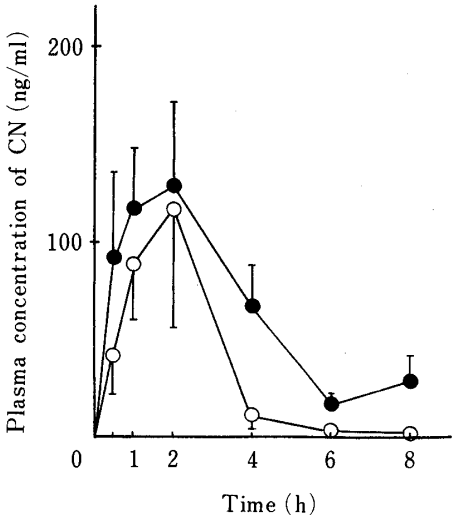


Fig. 6. Time Courses of Plasma CN Concentration in Dogs after Oral Administration of CN (50 mg) and Its β-CD Complex (Containing 50 mg CN) with Citric Acid (400 mg)

○, CN with citric acid; ●, CN–β-CD complex with citric acid.
Each point represents the mean ± S.E. of 3 dogs.

absorption of CN. It had been reported that the bioavailability of CN was enhanced by simultaneous administration of citric acid.[7] However, there was no significant difference between the administration of CN and CN–β-CD complex with citric acid in our experiment. The bioavailability parameters are shown in Table III. $AUC_{0-8\,h}$ and $C_{max}$ after oral administration of CN or CN–β-CD complex were not changed by the simultaneous administration of citric acid, as compared with those parameters of CN alone and CN–β-CD complex alone, respectively.

The reason why the simultaneous administration of citric acid did not enhance the bioavailability of CN is not clear, but may reflect the difference of dosage form or some other experimental conditions between the present study and the previous report.[7] Further experiments will be required to confirm the effect of citric acid on the bioavailability of CN, but the likelihood that CN–β-CD complex will give improved bioavailability of CN in patients with achlorhydria or anacidity has been clearly indicated by our results.

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**References and Notes**

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