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Effect of β-Cyclodextrin on Sleeping Time Induced by Barbituric Acid Derivatives in Mice[1)]

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An investigation was carried out on the effect of β-cyclodextrin (β-CD), which is an excellent complexing agent, on the hypnotic potency of four kinds of barbituric acid derivatives (BAD) after intravenous and intraperitoneal administration in mice. Among BAD, hexobarbital (HBA), pentobarbital (PBA), phenobarbital (PhBA) and thiopental (TPA) were chosen. To evaluate the hypnotic potency, the sleeping lag (the time until the onset of the righting reflex from administration) and the sleeping time (the time until recovery from the loss of righting reflex) were determined.

In the cases of intravenous administration at the dose of 215.3 μmol/kg weight, the sleeping times were significantly shortened, but the sleeping lags were not affected by the simultaneous administration of β-CD equivalent (in molar amount) to BAD. However, the brain concentration of BAD at the time of awakening was little affected by the simultaneous administration of β-CD. The solubility of HBA in pH 7.4 phosphate buffer solution with rat serum increased sigmoidally with increasing amount of β-CD.

The present results suggest that the shortening in sleeping time may be due to a decrease in the distribution of BAD to the brain, at least partly as a result of complex formation of BAD with β-CD, resulting in a shortening of the BAD-induced sleeping time.

Keywords——β-cyclodextrin inclusion complex; hexobarbital; pentobarbital; phenobarbital; thiopental sleeping lag; sleeping time; brain distribution

Barbituric acid derivatives (BAD) have been widely used clinically as analgesics. It has been reported that the pharmacological activity and pharmacokinetics of BAD were affected by biological[2,3)] and physical[4,5)] interactions with various kinds of drugs, including BAD.[6-8)] Koizumi *et al.* reported that the sleeping time[9)] and tissues concentrations[10)] after oral administration of BAD–cyclodextrin (CD) solid inclusion complex were longer and higher, respectively, than those after BAD alone. β-CD itself is poorly absorbed from the gastrointestinal tract, being degraded to glucose by the intestinal flora.[11)] Accordingly, it is expected that the pharmacological activity and pharmacokinetics after intravenous administration of inclusion complex will differ from those after oral administration. Arimori *et al.* reported that the plasma concentration–time curve of thiopental (TPA) in rabbits was little affected by simultaneous intravenous administration of γ-CD.[12)] However, relatively little is known about the effect of β-CD inclusion complexation on pharmacological activity and pharmacokinetics after intravenous administration of drugs in general.

The present study was designed to investigate the effect of β-CD on hypnotic potency after intravenous administration of BAD to mice.

Experimental

Materials——β-CD, generously supplied by Nihon Shokuhin Kako Co., Ltd., was used after recrystallization from water. Hexobarbital (HBA) and pentobarbital (PBA) sodium were purchased from Tokyo Kasei Industrial Co., Ltd. TPA sodium for injection (marketed as "Ravonal") was purchased from Tanabe Seiyaku Co., Ltd. TPA and

PBA were isolated from the corresponding sodium salts according to the method in JP X and recrystallized from aqueous ethanol. Phenobarbital (PhBA) was generously supplied by Fujinaga Pharmaceutical Co., Ltd. Rat serum was purchased from Charles River Co., Ltd. The other materials used were of reagent grade.

Preparation of Drug Solutions——Each drug was dissolved in pH 12, 1/10 N phosphate buffer solution with or without β-CD in a molar equivalent amount. The drug solution was used within 1 h after preparation. HBA, PBA, PhBA and TPA were administered into the tail vein at doses of 50.0, 47.9, 49.1 and 51.3 mg/kg weight (215.3 μmol/kg weight), respectively. The formation of inclusion complexes of BAD with CD at physiological pH was confirmed by the solubility method and the proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy method.[13]

Measurement of Sleeping Time——Male ddY mice weighing 20—26 g were used throughout the study. They were housed in alminium cages on wood chip bedding at a constant temperature and humidity under a dark–light cycle of about 12 h for several days. They had free access to standard granulated food and tap water. The animals were randomly divided into groups of a given number of mice. Aqueous solutions of BAD with or without β-CD were administered intravenously and intraperitoneally to the mice at the dose of 215.3 μmol/kg weight of BAD. The sleeping lag (the time unitl the onset of loss of righting reflex after drug administration) and the sleeping time (the time unitl recovery from the loss of righting reflex) were measurd under the standard laboratory conditions.

Determination of Whole Blood and Brain Concentration of BAD——Male ddY mice weighing 20—26 g were killed by cervical dislocation at the time of awakening after the intravenous administration of 215.3 μmol/kg weight of BAD with or without β-CD.

The concentrations of BAD in whole blood and brain were determined by high performance liquid chromatography (HPLC) after an extraction procedure as shown in Chart 1. The analysis was carried out using a Waters M-45 solvent delivery system equipped with a Waters 440 type absorbance detector. A reverse phase column (25 cm × 4.6 mm i.d.), Zorvax ODS (Du Pont), was used for analysis. A mixture of 0.01 M potassium dihydrogen phosphate and methanol was used as the mobile phase.

whole blood (0.2 ml) with water (1 ml), and homogenate of brain (0.2—0.3 g wet weight) with 1.15% KCl (3 ml)
- (1) add 2 ml of 1 M acetate buffer solution (pH 5.0), 2 g of NaCl, and 25 ml of *n*-heptane containing 1.5% isoamyl alcohol
- (2) add 1 ml of *n*-heptane containing HBA (for TPA and PBA) or PhBA (for HBA) as an internal standard
- (3) agitate for 20 min
- (4) centrifuge for 10 min at 3000 rpm

organic layer
- (5) add 4 ml of 0.4 M phosphate buffer (pH 11)
- (6) agitate for 10 min
- (7) centrifuge for 5 min at 3000 rpm

aqueous layer
- (8) add 2 ml of 1 N HCl
- (9) add 6 ml of ethyl ether
- (10) aditate for 10 min
- (11) centrifuge for 5 min at 3000 rpm
- (12) repeat (9), (10) and (11)

organic layer
- (13) evaporate to dryness under a gentle stream of N_2 gas
- (14) dissolve in eluent

10 μl portion for HPLC

Chart 1. Extraction Procedure for Determination of Hexobarbital (HBA), Pentobarbital (PBA) and Thiopental (TPA) in Whole Blood and Brain

Solubility of Hexobarbital——An excess amount of hexobarbital and various amounts of β-CD were added to 5 ml of pH 7.4 phosphate buffer solution with or without 20 v/v% rat serum and shaken in an incubator for 1 d at 22 ± 0.5 °C. The mixture was centrifuged for 15 min at 6000 rpm (22 ± 1 °C). Then 2 ml of 1 M acetate buffer solution (pH 5.0), 2 g of NaCl, and 25 ml of *n*-heptane containing 1.5% isoamyl alcohol were added to a 50 ml centrifuge tube containing 200 μl of the supernatant. The mixture was agitated for 20 min and centrifuged for 10 min at 3000 rpm. Then, 20 ml of the organic layer was placed in a 50 ml centrifuge tube with a Pasteur pipette and 4 ml of 0.4 M phosphate buffer (pH 11) was added. The mixture was agitated for 10 min and centrifuged for 5 min at 3000 rpm. The

concentration of hexobarbital in the aqueous layer was determined spectrophotometrically at the maximum wavelength (244 nm).

Results and Discussion

Sleeping Time Induced by BAD in the Presence of β-CD

To assess the effect of β-CD on the distribution of BAD to the brain, we evaluated the change in the BAD-induced sleeping time in mice. The sleeping times induced by three short-acting BADs (HBA, TPA and PBA) were found to be significantly shortened by the concurrent intravenous administration of β-CD, as shown in Table I. On the other hand, the sleeping lags of HBA, TPA and PBA were within 5, 10 and 60 s, respectively, and were little affected by the simultaneous administration of β-CD. Shortening of the sleeping time induced by BAD may be caused by a decrease of the sensitivity of the brain to BAD, a decrease of the distribution of BAD to brain, and so on. Thus, the BAD concentrations in whole blood and brain at the time of awakening from sleep were examined. The importance of the BAD concentration in the brain in determining the pharmacological activity has been reported by Joli *et al.*[14] At the time of awakening, the brain concentration of BAD in mice concurrently treated with β-CD was approximately equal to that in mice given the drug alone, whereas the whole blood concentration of BAD was increased by the simultaneous administration of β-CD, as shown in Table II. The constant concentration of brain BAD at the time of awakening from sleep indicate a constant sensitivity of the brain to BAD.[14] These results suggest that β-

Table I. Effect of β-CD on Sleeping Lags and Sleeping Times after Intravenous Administration of HBA, TPA, PBA and PhBA to Mice

Drug (215.3 μmol/kg)	Sleeping lag (s)		Sleeping time[a] (min)	
	Without β-CD	With β-CD (215.3 μmol/kg)	Without β-CD	With β-CD[b] (215.3 μmol/kg)
HBA	<5	<5	13.88±1.43	2.60±0.33[c]
TPA	<10	<10	24.67±2.16	14.97±1.08[c]
PBA	<60	<60	44.05±4.40	26.00±3.70[d]
PhBA	—	—	0.00±0.00	0.00±0.00

a) Each value represents the mean±S.E. of more than 7 determinations. *b*) Significant difference from the group without β-CD. *c*) $p<0.001$. *d*) $p<0.01$.

Table II. Effect of β-CD on BAD Concentration in Whole Blood and Brain at the Time of Awakening

Drug (215.3 μmol/kg)	Additive (215.3 μmol/kg)	Time until awakening after BAD administration[a] (min)	BAD concentration[a]	
			Whole blood (μg/ml)	Brain (μg/g)
HBA	None	12.52±2.60 (6)	33.8±1.7 (6)	39.7±0.6 (6)
	β-CD	3.99±0.59 (5)[b]	52.3±2.4 (5)[b]	40.6±0.8 (5)
TPA	None	21.75±2.14 (6)	38.5±2.4 (5)	32.6±1.5 (6)
	β-CD	11.80±1.15 (6)[b]	52.8±2.3 (5)[b]	34.7±1.3 (6)
PBA	None	43.22±2.17 (6)	29.9±1.4 (6)	29.5±1.0 (6)
	β-CD	26.30±2.23 (6)[b]	33.2±3.6 (6)	28.5±2.2 (4)

a) Each value represents the mean±S.E. with the number of determinations in parentheses. *b*) Significant difference from the group without β-CD. $p<0.01$.

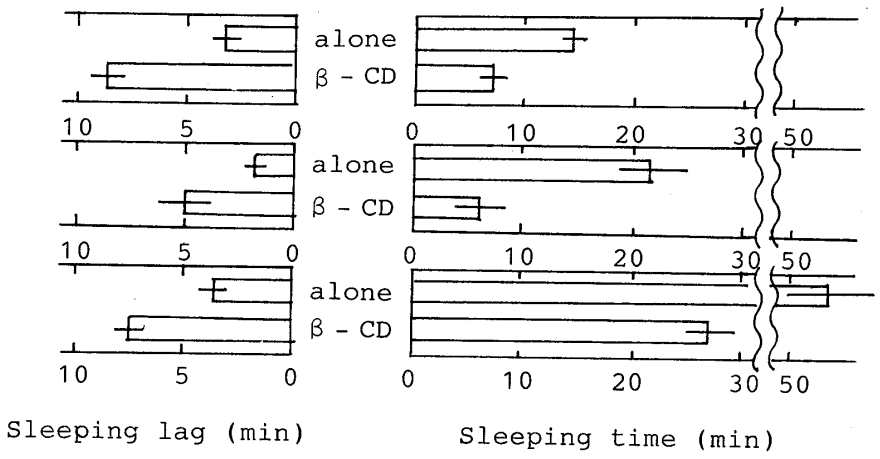

Fig. 1. Effect of β-CD on Sleeping Lag (Left) and Sleeping Time (Right) Induced by HBA (Top), TPA (Middle) and PBA (Bottom) in Mice

BAD (215.3 μmol/kg) with or without β-CD (215.3 μmol/kg) was intraperitoneally administered to mice. Each bar represents the mean ± S.E. of more than 5 determinations.

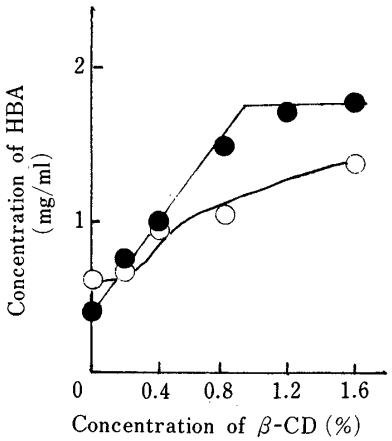

Fig. 2. Phase Solubility Diagrams of HBA–β-CD Systems in pH 7.4 Phosphate Buffer Solution with (○) or without (●) 20 v/v% Rat Serum at 22 °C

CD may not affect the sensitivity of the brain to BAD, but may not affect the distribution of BAD to the brain.

It was reported that the permeation of acetohexamide[15] and BAD[13] through cellulose membrane was decreased in the presence of β-CD. Figure 1 shows the effect of β-CD on the sleeping lags and sleeping times in mice after intraperitoneal administration of HBA, TPA or PBA at the dose of 215.3 μmol/kg weight. The sleeping lags of BAD were significantly prolonged and the sleeping times induced by BAD were significantly shortened in the presence of β-CD. Thus, the hypnotic potency after intraperitoneal administration was also found to be lowered by β-CD. Since similar effects of β-CD on the intestinal absorption of drugs in cases where a dissolution process was not involved have been reported,[15,16] this suggests that the permeation of BAD through biomembranes might be suppressed by β-CD.

It has been reported that β-CD forms stable inclusion complexes with BAD.[6-8,13] The lowering of the permeation of BAD through various membranes by β-CD may thus arise mainly from the inclusion complex formation. Both CD and its inclusion complex with BAD, having molecular weights of more than 1000, can hardly permeate through the blood brain barrier (BBB).[17] Therefore, the distribution of BAD across the BBB might be suppressed by inclusion complex formation. However, the inclusion complex formation of BAD with β-CD in blood is considered to be competitively inhibited by various biocomponents.

Figure 2 shows the phase solubility diagram of the HBA–β-CD system in pH 7.4

phosphate buffer solution with or without 20 v/v% rat serum at 22 °C. The solubility of HBA in buffer solution with rat serum increased sigmoidally with increasing concentration of β-CD, but was low compared to that in buffer solution without rat serum. This suggests that, although the apparent stability constants of BAD–β-CD inclusion complex are not very large, β-CD might form an inclusion complex with BAD in the blood after intravenous administration of the test solutions.

With regard to the shortening of BAD-induced sleeping time, other factors should also be taken into consideration, in addition to complex formation between BAD and β-CD. One such factor may be a promoting effect on the extraction of the drug from tissues for distribution to excretive tissues.[18] Another factor may be some modifying effect of β-CD on the function of the BBB, resulting in a change in the drug distribution. As the dose of β-CD was rather high, β-CD administered might modify the function of the BBB and tissues, and consequently the disposition of BAD might be altered; however, there is no evidence for this at present.

In view of the existing data on the toxicity of CDs given by intravenous administration,[19,20] this may be considered to be mild. We confirmed that male ddY mice survived for more than 10 d after single intravenous administration of 215.3 μmol/kg BAD with 0.244 g/kg β-CD.

The modifying effect on the disposition of BAD should also be taken into consideration.[21] Overall, the present results suggest that the shortening in sleeping time may be brought about by a decrease in the distribution of BAD to the brain. One factor causing the decrease in distribution to the brain may be complex formation of BAD with β-CD.

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