EFFECT OF β-CYCLODEXTRIN ON DISPOSITION OF HEXOBARBITAL AND PHENOBARBITAL IN MICE

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ABSTRACT. The effect of β-cyclodextrin (β-CD) on the disposition of hexobarbital (HBA) and phenobarbital (PhBA) after intravenous administration ,with the simultaneous administration with β-CD, was investigated in mice.

In the case of HBA, the whole blood concentration was slightly heightened, the brain and liver concentrations were significantly lowered, and the kidney concentration was significantly heightened. Moreover, β-CD also influenced the disposition and formation of 3'-hydroxy- and 3'-keto-HBA. On the other hand, in the case of PhBA, the whole blood concentration was slightly lowered, the time at maximum brain concentration (t-max) was prolonged about 30 to 60 min, the kidney concentration was significantly heightened at the initial stage, while the liver concentration did not show a clear difference by the simultaneous administration with β-CD. These results suggest that the disposition of drugs might be modified by the use of CD.

1. INTRODUCTION

In a previous paper (1), it was reported that the barbituric acid derivatives (BAD)-induced sleeping time, that is, the time until the recovery from the loss of righting reflex, was shortened by the simultaneous administration with CDs.

Ohata reported that the simultaneous intramuscular administration of aminopyrine and barbital resulted in a high plasma level by the formation of a molecular compound compared with single administration of the components (2,3). Further, Pitha et al. reported that the toxic effect increased and set in rapidly without symptoms of hypervitaminosis A when dimethyl- β-CD was administered simultaneously with retinoic acid, and the survival rate was improved when dimethyl-β-CD was administered alone after the hypervitaminosis A had been established (4). It has also been reported that CDs have such advantages as removal of local irritation induced by intramuscular injection (5), protection of a drug-induced haemolysis (6), and so on.

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However, the influence of CD on the potency and pharmacokinetics of intravenously administered drugs has not been known so much in detail.

From the view point mentioned above, the present study was attempted to investigate the effect of CD on disposition of BAD in mice after intravenous administration, and also to obtain fundamental informations for application of CD to injections as additives. Hexobarbital and phenobarbital were chosen as a high and low lipid soluble BAD, respectively.

2. EXPERIMENTAL

2.1. Materials

β-Cyclodextrin (β-CD), generously supplied by Nihon Shokuhin Kako Co., Ltd., was used after recrystallization from water. Hexobarbital (HBA) was purchased from Tokyo Kasei Industrial Co., Ltd. Phenobarbital (PhBA) and PhBA sodium were generously supplied by Fujinaga Pharmaceutical Co., Ltd. The other materials used were of reagent grade.

2.2. Preparation of Injections

HBA and PhBA-Na were dissolved in pH 12 disodium hydrogenphosphate-NaOH buffer solution and in water, respectively. The drug concentration in injections was 215.3 μmol/10 ml. The stability constants of drug-β-CD systems, and pH and osmotic pressure values of injections are listed in Table I.

Table I.

Drugs	Additives	A/D [a]	K(M^{-1}) [b]	pH	OP [c] (mOsm/kg)
HBA	None	0/1	-	11.4	179
	β-CD	1/1	451	11.4	186
PhBA-Na	None	0/1	-	9.8	46
	β-CD	1/1	95	10.1	49

a) Molar ratio of Additive/Drug
b) Stability constant of drug-CD system in pH 7.4 phosphate buffer solution at 37°, estimated by solubility method.
c) Osmotic pressure determined by Fiske OM ® osmometer.

2.3. Disposition Study of BAD in Mice

Male ddY mice, weighing 24-28 and 20-26 g were used for HBA and PhBA, respectively. They were housed in aluminum cages on soft wood bedding at a constant temperature under about 12 h dark-light cycle. They had free access to standard granulated food and tap water. Aqueous solutions of HBA and PhBA sodium in a dose of 215.3 μmol/kg in the absence and in the presence of equimolar β-CD were administered intravenously

to mice. The mice were killed by cervical dislocation at appropriate time intervals after the administration. The truncus blood was collected into the heparinized test tubes, and the brain, liver and kidneys were rapidly excised, rinsed in cooled saline, dried on filter papers and stored at -10°.

2.4. Determination of Barbituric Acid Derivatives
The concentration in whole blood and tissues of HBA and PhBA was determined by high-performance liquid chromatography (HPLC) according to the method reported by Bocker (7) with a slight modification. The analysis was carried out using a Shimadzu LC-3A solvent delivery system equipped with absorbance detector SPD-1 and a Chromatopac C-R1B.

3. RESULTS AND DISCUSSION

3.1. Elimination from the Whole Blood

The time-concentration curves of HBA and PhBA in whole blood of male ddY mice after intravenous administration of 215.3 μmol/kg of corresponding BAD with or without β-CD are shown in Figure 1.

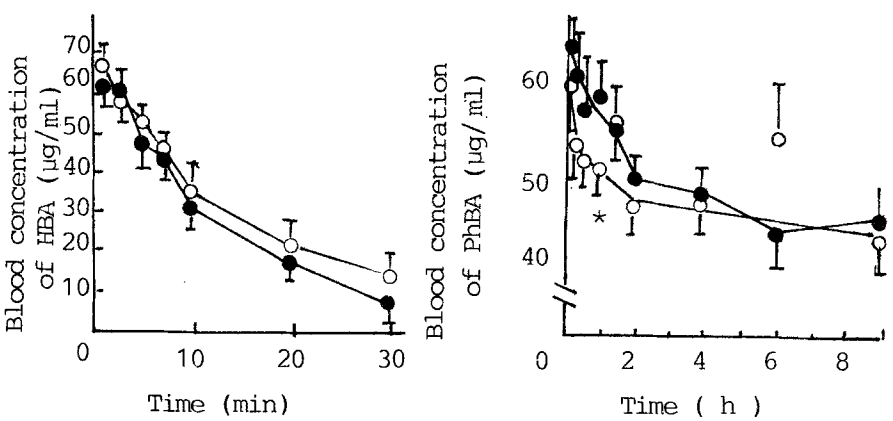

Figure 1. Time-Concentration Curves of HBA and PhBA in Whole Blood of Male ddY Mice after Intravenous Administration of 215.3 μmol/kg of Corresponding BAD with (○) or without (●) β-CD

Each point represents the mean ± S.E. of 4-7 determinations. Significantly different from the group without β-CD (*; $P<0.05$, by t-test)

The whole blood concentration of HBA slightly increased, while that of PhBA decreased by the simultaneous administration of β-CD. β-CD increases the whole blood concentration of BAD by suppressing the ditribution to liver and brain, and decreases the whole blood concentration of BAD by promoting the delivery to kidneys as will be shown

below. The material balances of HBA and PhBA in blood were not strongly affected by the simultaneous administration with β-CD.

3.2. Distribution to Brain

To obtain more detailed information concerning the disposition of BAD in the presence of β-CD, the time courses in concentration on target organ (brain), metabolic organ (liver) and excretive organ (kidneys) were determined in mice. Figure 2 shows the effect of β-CD on the time courses of brain concentration of HBA and PhBA.

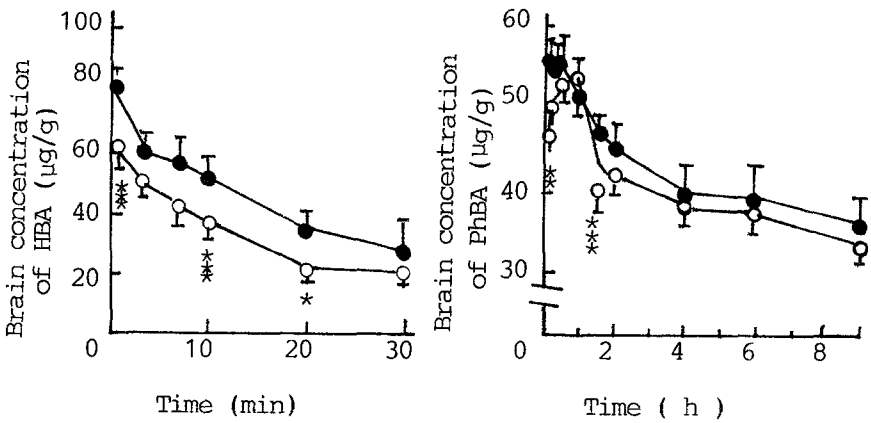

Figure 2. Time-Concentration Curves of HBA and PhBA in Brain of Male ddY Mice after Intravenous Administration of 215.3 μmol/kg of Corresponding BAD with (○) or without (●) β-CD

Each points represents the mean ± S.E. of 4-7 determinations. Significantly different from the group without β-CD (*; $p<0.05$; ** ; $p<0.02$; ***; $p<0.01$, by t-test)

The brain concentration of HBA was lowered by the simultaneous administration with β-CD, and the significant difference from the group without β-CD was recognized at 1, 10 and 20 min after administration at 1, 1 and 5 % level, respectively. On the other hand, the brain concentration of PhBA was significantly lowered at 10 and 90 min after administration at 2 and 1 % level, respectively. The distribution rate of HBA was more rapid than that of PhBA. The time at maximum concentration (t-max) in the brain of HBA was within 1 min, and the alternation by β-CD was not observed. The t-max of PhBA in the brain was prolonged from about 30 to 60 min by β-CD.

It was reported that the BAD-induced sleeping time and the sleeping lag were shortened and prolonged by the simultaneous administration of CDs (1). The shortening in sleeping time and the prolongation in sleeping lag by the simultaneous administration of CDs could be explained by the lowering in blood BAD concentration and the delay in t-max, respective-

-ly. Both the undissociated and dissociated BAD exist under the pH condition in blood. The distribution of undissociated and dissociated BAD to brain were very easy and hard, respectively (8). It is known that the undissociated BAD forms more stable complex with CD than the dissociated BAD. Although the apparent stability constants of the BAD/ β-CD inclusion complex in pH 7.4 aqueous buffer solution are small, it is considered that a part of undissociated BAD also forms an inclusion complex with CD in vivo. It was reported that the binding effect of chlorpromazine to human erythrocytes membrane in vitro decreased with an increase of concentration of β-CD (9). It is expected that β-CD decrese the affinity of drug to various biomembrane.

The lowering of brain concentration of HBA might be caused by the decrease of the free type of undissociated BAD, and by the increase of its CD complex having a low affinity to BBB and biomembrane. The prolongation of t-max in brain of PhBA by β-CD is probably due to the delay of release of PhBA from inclusion complex.

3.3 Distribution to Liver

Figure 3 shows the effect of β-CD on the time courses of liver concentration of HBA and PhBA.

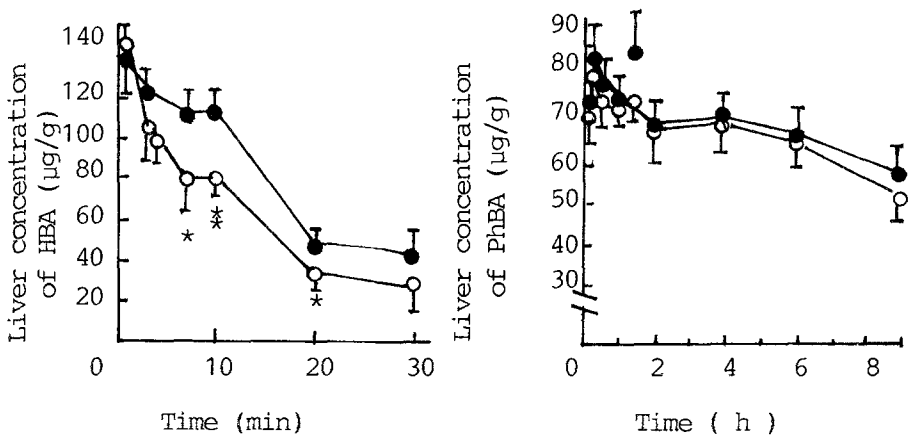

Figure 3. Time-Concentration Curves of HBA and PhBA in Liver of Male ddY Mice after Intravenous Administration of 215.3 μmol/kg of Corresponding BAD with (○) or without (●) β-CD

Each point represents the mean ± S.E. of 4-7 determinations. Significantly different from the group without β-CD (*; $p<0.05$, **; $p<0.02$, by t-test).

The liver concentration of HBA was lowered by the simultaneous administration of β-CD, and this was similar in tendency to that in brain. The significant differences from the cases without β-CD was recognized at 7, 10 and 29 min after administration at 5, 2 and 5 %, respectively.

On the other hand, the liver concentration of PhBA was not altered significantly.

3.4. Distribution to Kidneys

Figure 4 shows the effect of β-CD on the time courses of kidney concentrations of HBA and PhBA

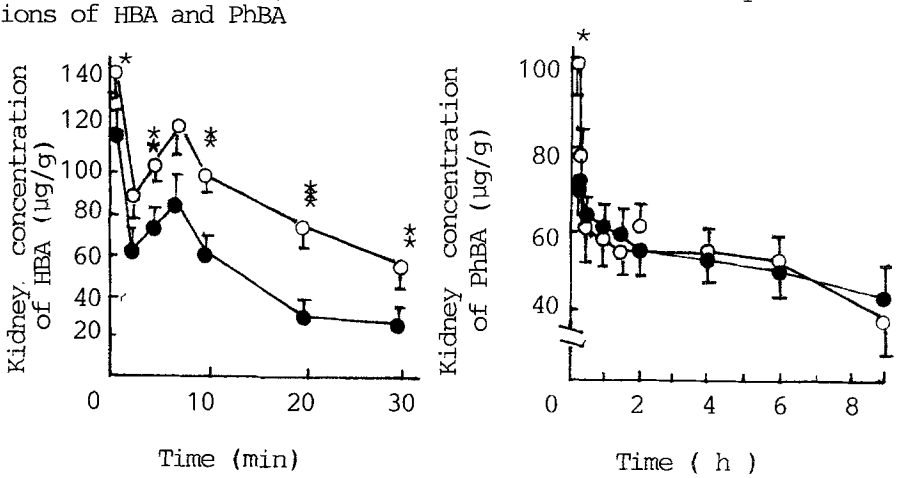

Figure 4. Time-Concentration Curves of HBA and PhBA in Kidneys of Male ddY Mice after Intravenous Administration of 215.3 μmol/kg of Corresponding BAD with (○) or without (●) β-CD

Each point represents the mean ± S.E. of 4-7 determinations. Significantly different from the group without β-CD (*; $p<0.05$, **; $p<0.01$, ***; $p<0.001$, by t-test)

The HBA concentration in kidneys was markedly heightened by β-CD, and was different from that in brain and liver. The significant difference from the group without β-CD was recognized at 1, 5, 10, 20 and 30 min after administration at 5, 1, 1, 0.1 and 1 % level, respectively. The PhBA concentration in kidneys was significantly heightened at the initial stage. The pore size of glomeruli membrane is larger than that of BBB and capillary vessel membrane (CVM). The compounds that the molecular weight is more than 1000, can little permeate through BBB and CVM, while can permeate through glomeruli membrane (10). It is considered that the complex of BAD with CD (M.W. >1000)can also permeate through glomeruli membrane, however, the permeation rate of the complex owing to high molecular weight, is low compared to that of BAD alone. It is considered that the enhanced HBA concentration in kidneys occurred by the accumulation of HBA/β-CD inclusion complex to nephron. The concentration ratios of brain, liver and kidneys to the whole blood were abbreviated as Br/B, L/B and K/B, respectively, and the simultaneous administration with β-CD decreased Br/B and L/B of HBA, and markedly increased K/B of HBA, as shown in Figure 5.

With regarded to the distribution of the drugs to kidneys in a high concentration, the following factors also should be taken into consideration for an explanation of the reason, in addition to the complex formation between BAD and β-CD. One may be a promoting effect on the extraction of drug from tissues to distribute to excretive tissues. CDs were also recomended as additives to a washing solution used for peritoneal dialysis after BAD poisoning(11). Moreover, Pitha et al. reported that the injectional administration of dimethyl β-CD to mice in hypervitaminosis A resulted in an increase of the survival rate and eventually recovered from the hypervitaminosis A symptoms(4). Additionally, they suggested that CDs extracts a drug from tissues where the drug is at a high concentration, distributing it to excretive tissues. In this case, the apparent stability constant of the complex of vitamin A with dimethyl β-CD is not so large, as is approximately equal to that of the complex of HBA with β-CD in the present study. Another factor may be concerned with some modifying effect of β-CD on the function of biomembrane, resulting in a change in the permeation. As a dose of β-CD was somewhat high, β-CD administered might modify the function of the biomembrane and tissues, consquently the disposition of BAD might be altered.

Considering the results in existing reports on the toxicity of CDs in intravenous administration(12-14), this may be considered to be different from the toxicity of β-CD. We also confirmed that the male ddY mice survived for more than 10 days after single intravenous administration of 215.3 μmol/kg BAD with 0.244 g/kg β-CD. Regarding the change of the function of biomembrane in kidneys, Serfozo et al. reported that CDs were continuously reabsorved from the glomerular filtrate and accumulate in the epithelial cells of the proximal tubule (15), when BAD might t end to stay by the complex formation with β-CD interfering with the distribution of BAD to the tissues. A functional change of biomembrane may be caused also by a removal of components from the surface of the biomembrane by β-CD. However, the direct effects of the functional change in the biomembrane on the permeation of BAD is not known clearly.

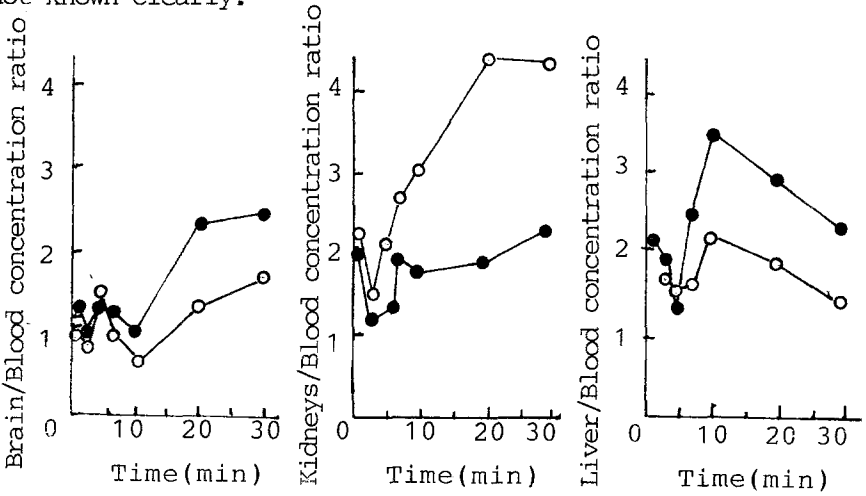

Figure 5. Brain/Blood, Kidneys/Blood, and Liver/Blood Concentration Ratios of HBA after Intravenous Administration of 215.3 μmol/kg of HBA with (○) or without (●) β-CD in Male ddY Mice

3.5 Formation of Metabolite

Table II shows the effect of additive on peak area ratios of HBA and its metabolites to PhBA as an internal standard at 10 min after administration in pregnant mice. The change of peak area ratio in whole blood and kidneys suggests that the formation rate or distribution of metabolites of HBA was modified by the simultaneous administration with β-CD.

Table II. Effect of Additives on Peak Area Ratios of HBA and Its Metabolite to PhBA as an Internal Standard

Additives	Peak area ratio[a]		
	3-hydroxy HBA / PhBA	3-keto HBA / PhBA	HBA / PhBA
(Blood)			
None	0.232	0.364	0.708
β-CD	0.177	0.380	0.794
DM-β-CD[b]	0.172	0.172	0.855
(Kidneys)			
None	0.074	0.256	1.579
β-CD	0.099	0.344	2.645
DM-β-CD	0.191	0.505	3.491

a) Each value represents the mean of 3-4 determinations.
b) Heptakis 2,6-di-O-methyl-β-CD.

The present results suggest that β-CD could modify a disposition of drug and might useful as carrier for the delivery to kidneys of such drugs containing anti-tumour agent.

4. ACKNOWLEDGEMENT

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