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Enhancement of the Hypnotic Potency of Barbiturates by Inclusion Complexation with β-Cyclodextrin[1]

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The 50% effective doses of five barbiturate–β-cyclodextrin complexes on oral administration to mice were compared with those of the corresponding barbiturates. In all cases tested, the complex gave a smaller ED_{50} than the intact drug. ED_{50} of phenobarbital, which forms the most stable complex and consequently shows the greatest enhancement in solubility, was reduced most markedly by complexation.

With the exception of barbital, sleeping lag (the time from oral administration to loss of righting reflex) on administration of the complex to mice was shorter than that on giving an equimolar amount of the intact drug ($p<0.001$), and sleeping time (the time from loss to recovery of righting reflex) was significantly increased by inclusion complexation with β-cyclodextrin.

Keywords——barbiturates; β-cyclodextrin (cycloheptaamylose); barbiturate–β-cyclodextrin complexes; ED_{50}; sleeping lag; sleeping time; oral administration to mice

Barbiturates (BA) form stable inclusion complexes with β-cyclodextrin (β-CyD) in aqueous solution,[3] and the 1:1 complexes can each be isolated as a pure microcrystalline powder.[3c] It was shown in a previous paper[4] that β-CyD is useful for increasing the solubility of BA, that it hardly interferes with the permeation of BA through everted rat small intestine, and that the improved solubility of BA due to inclusion complexation results in an enhancement of the bioavailability of BA in rabbits.

Following these findings, the present study was carried out to compare the hypnotic potencies of BA–β-CyD complexes with those of BA on oral administration to mice.

Experimental

Materials—— Amobarbital (Amo, mp 157.5—158.3°), allobarbital (Allo, mp 173.2—174.1°), barbital (Bar, mp 191.0—191.9°), and phenobarbital (Pheno, mp 176.3—176.8°), of Japanese Pharmacopoeia standard, were purchased and purified by recrystallization. Pentobarbital (Pento, mp 132.0—133.5°) was prepared from sodium pentobarbiturate injection solution (Somnopentyl, Pitman-Moore, Inc.) in the usual manner[4] and purified by recrystallization. β-CyD was used after recrystallization from water, $[\alpha]_D^{18}$ +165.5°.

Preparation of Complexes[3c]——Equimolar amounts of BA and β-CyD were dissolved in a minimum volume of hot water and left at room temperature. The complex precipitated as a microcrystalline powder, which was filtered off, washed with a small amount of water, and then dried under a vacuum at 100° for 24 hours. The composition of the dried substance was confirmed by spectrophotometry and gravimetry as described previously. All the barbiturates tested were included in a 1:1 molar ratio.

1) This paper forms Part VI of "Studies on Inclusion Compounds." This work was presented at the 28th Annual Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Nishinomiya, October 1978, Abstracts of Papers, p. 89.
2) Location: *4-16 Edagawa, Nishinomiya 663, Japan.*
3) *a*) K. Koizumi and K. Fujimura, *Yakugaku Zasshi*, **92**, 32 (1972); *b*) A.L. Thakker, P.B. Kuehn, J.H. Perrin, and W.L. Wilham, *J. Pharm. Sci.*, **61**, 1841 (1972); *c*) K. Koizumi, K. Mitsui, and K. Higuchi, *Yakugaku Zasshi*, **94**, 1515 (1974); *d*) M. Otagiri, T. Miyaji, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.*, **24**, 1146 (1976); *e*) T. Miyaji, K. Kurono, K. Uekama, and K. Ikeda, *ibid.*, **24**, 1155 (1976).
4) K. Koizumi and Y. Kidera, *Yakugaku Zasshi*, **97**, 705 (1977).

Animals——Male ICR mice, 3 weeks old, were purchased and housed in temperature-regulated quarters (22—24°) on an artificial day-night cycle of 12 hours (light on from 19: 00 to 7: 00[5]) for a minimum of 10 days before use. The average weight of mice used in all experiments was 22±2 g.

Administration of Drugs——The mice were given water but no food for 24 hours prior to administration of the drugs. The drugs (100—200 mesh powder) suspended in 1% CMC–Na solution were orally administered through a stomach sonde.

Estimation of ED_{50}——The 50% effective dose (ED_{50}) was estimated according to the probit method[6] using fifty mice per an experiment, and the 95% confidence limits were calculated using Fieller's equation.[7]

Measurement of Sleeping Time——The animals were divided into groups of seven mice each. One group of mice was given BA orally and another group of mice received an equimolar amount of the corresponding BA–β-CyD complex. The sleeping lags (the times from oral administration to loss of righting reflex) of both groups and their sleeping times (the times from loss to recovery of righting reflex) were compared. The doses administered were confirmed to exhibit sufficient hypnotic activity in preliminary experiments using two or three mice each: 1.2 times ED_{50} of intact BA in the case of long-acting types, Pheno and Bar, 1.4—1.6 times in the case of intermediate-acting types, Amo and Allo, and 2.5 times in the case of the short-acting type, Pento. Results are shown as the mean values of seven mice±standard error and statistical significances were determined by Student's *t*-test.[8]

Determination of Dissolution Rate and Solubility——Dissolution rates were determined by the dispersed amount method[9]: 30 ml of 0.1 N HCl prewarmed at 37° was added quickly to a flask, containing an excess (about twice the saturated concentration) of the sample as a 100—200 mesh powder. The dissolution medium was maintained at 37° and stirred vigorously. At appropriate intervals, the solution was sampled with a 0.5 ml pipette fitted with a cotton filter. Each sample was suitably diluted with distilled water, and analyzed spectrophotometrically at the ultraviolet (UV) absorption maximum of each BA in 0.4 N NaOH. The concentration at the saturation point was regarded as the solubility in 0.1 N HCl at 37°.

Estimation of the Stability Constants of BA–β-CyD Complexes——Stability constants of the complexes were estimated by the solubility method.[10]

Result and Discussion

50% Effective Dose

In all cases tested, a BA–β-CyD complex gave a smaller ED_{50} than the corresponding BA (Table I). The ratio of ED_{50} of BA–β-CyD to ED_{50} of BA was the smallest in the case of Pheno, which showed the most marked solubility improvement on inclusion complexation.

Table I. ED_{50} Values (μmol/10 g) of BA–β-CyD and BA on Oral Administration to Mice

Drug	ED_{50}	Ratios[b]
Pheno–β-CyD	2.91 (2.75—3.03) [a]	0.78
Pheno	3.72 (3.27—3.85)	
Pento–β-CyD	0.91 (0.85—0.96)	0.85
Pento	1.07 (0.99—1.19)	
Amo–β-CyD	3.00 (2.68—3.18)	0.87
Amo	3.44 (3.33—3.62)	
Allo–β-CyD	1.61 (1.03—1.70)	0.92
Allo	1.76 (1.70—1.82)	
Bar–β-CyD	8.02 (7.79—8.39)	0.92
Bar	8.73 (8.50—8.81)	

a) Values in brackets are 95% confidence limits.
b) ED_{50} of BA-β-CyD/ED_{50} of BA.

5) In order to avoid natural sleep, a reversed day-night cycle was used.
6) A. Sakuma, "Bioassay–Design and Analysis," University of Tokyo Press, Tokyo, 1964, p. 255.
7) A. Sakuma, "Bioassay–Design and Analysis," University of Tokyo Press, Tokyo, 1964, p. 259.
8) A. Sakuma, "Bioassay–Design and Analysis," University of Tokyo Press, Tokyo, 1964, p. 19.
9) H. Nogami, T. Nagai, and T. Yotsuyanagi, *Chem. Pharm. Bull.*, **17**, 499 (1969).
10) K. Koizumi, J. Tatsumi, M. Ohae, H. Kumagai, and T. Hayata, *Yakugaku Zasshi*, **89**, 1594 (1969); Y. Hamada, H. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **23**, 1205 (1975).

Table II. Solubilities and Dissolution Rates in 0.1 N HCl, and Stability Constants of BA–β-CyD at 37°

Drug	S (M×10²)	$T_{1/2}$ (min)	K (M^{-1})
Pheno–β-CyD	7.0	0.2	787
Pheno	0.8	1.7	
Pento–β-CyD	3.8	0.2	619
Pento	0.7	2.3	
Amo–β-CyD	1.4	0.2	562
Amo	0.4	2.2	
Allo–β-CyD	1.9	0.2	98
Allo	1.2	1.2	
Bar–β-CyD	9.8	0.1	62
Bar	5.3	0.7	

S: Solubility (saturated concentration).
$T_{1/2}$: Time required for the concentration to reach half the saturated concentration.
K: Stability constant of BA-β-CyD, estimated by the solubility method.

Table III. Effect of Inclusion Complexation with β-Cyclodextrin on Barbiturate-Induced Sleeping Time in Mice

Drug	Dose (μmol/10 g)	Sleeping lag* (min)	Sleeping time* (min)
Pheno–β-CyD	4.5	27.0±3.6[a]	241.0±29.8[b]
Pheno		75.7±7.0	122.9±20.4
Pento–β-CyD	2.7	5.0±0.5[a]	63.9± 9.8[b]
Pento		14.3±1.0	18.0± 5.9
Amo–β-CyD	4.9	6.7±1.3[a]	86.3± 6.4[b]
Amo		20.1±2.4	49.7± 3.3
Allo–β-CyD	2.9	12.6±1.0[a]	136.1±11.8[c]
Allo		23.3±1.9	93.6±10.8
Bar–β-CyD	10.9	31.6±2.8	128.4±16.6
Bar		31.3±1.1	122.4±13.8

* Sleeping lag: time from oral administration to loss of righting reflex.
Sleeping time: time from loss to recovery of righting reflex.
a, *b*, *c*): significantly different from that of intact BA by the two-tailed Student's *t*-test. *a*) $p<0.001$, *b*) $p<0.01$, *c*) $p<0.05$.

In the case of Allo or Bar, where the solubility was not much improved by complexation, the ratio was closest to 1 (Table II). It appears that the more stable the complex formed, the greater the reduction in ED_{50}.

BA-Induced Sleeping Time

To evaluate the effects of inclusion complexation on BA-induced sleep, sleeping lag and sleeping time were measured. As shown in Table III, the sleeping lags in all cases except Bar were shortened by complexation. There was a significant difference between each inclusion complex and the corresponding intact drug as regards sleeping lag at the 0.1% level. Although a decrease in the membrane permeability of the complex due to the poor lipophilicity of the β-CyD molecule cannot be excluded, Sjögren *et al.*[11] reported that the rate-limiting step in absorption was usually not passage through the gastrointestinal mucosa, but the dissolution and dispersal of the drug in the gastrointestinal contents. The complexes dissolve in 0.1 N HCl much faster than the intact drugs do, and moreover the solubilities

11) J. Sjögren, L. Sölvell, and I. Karlson, *Acta Med. Scand.*, **178**, 553 (1965).

of the complexes are greater than those of the intact drugs (Table II). Therefore it appears that the absorption rate of BA in the stomach was accelerated by complexation. This view is supported by the finding[4] that the blood level of BA rises faster on oral administration of BA–β-CyD complex than on that of the intact BA in rabbits. Since Bar has a sufficiently high solubility and dissolves rapidly even in the intact form, no appreciable difference between the complex and the intact drug was observed.

With the exception of Bar, complexation significantly increased the sleeping time. Pheno-induced sleeping time was markedly prolonged by complexation. In contrast, in the case of Bar, a long-acting hypnotic of the same type as Pheno, complexation had little effect on the sleeping time. These results might be explained by assuming that this difference arises from the very different stabilities of their complexes; that is to say, the stability constant of Pheno–β-CyD complex is more than 10 times that of Bar–β-CyD complex. A similar tendency was observed with Amo and Allo. Perrin *et al.*[12] reported that the complexation of BA by β-CyD can be regarded as in competition with the drug-albumin interaction. It is suspected that complexation increases the amount of BA reaching the brain and tends to maintain the brain level of BA for a longer time.

Although detailed studies of the absorption mechanism, distribution, and excretion of BA–β-CyD complexes in mice are required, it is clear that the hypnotic potency of BA is enhanced by inclusion complexation with β-CyD, so the use of BA-β-CyD rather than increasing the dose of BA should result in faster appearing and more prolonged effective hypnotic action in therapeutic use.

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12) J.H. Perrin, F.P. Field, D.A. Hansen, R.A. Mufson, and G. Torosian, *Res. Commun. Chem. Pathol. Pharmacol.*, **19**, 373 (1978).