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Enhanced Absorption of Phenobarbital from Suppositories containing Phenobarbital-β-Cyclodextrin Inclusion Complex[1]

Reiko Iwaoku,*,[a] Kazuhiko Arimori,[a] Masahiro Nakano,[a] and Kaneto Uekama[b]

Department of Pharmacy, Kumamoto University Hospital,[a] 1-1-1 Honjo, Kumamoto 860 Japan and Faculty of Pharmaceutical Sciences, Kumamoto University,[b] 5-1 Oe-honmachi, Kumamoto 862, Japan

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Phenobarbital was dissolved faster from a β-cyclodextrin complex than from phenobarbital powder, and it was released faster from suppositories containing its β-cyclodextrin complex than from those containing phenobarbital powder. After rectal administration of the suppository containing the β-cyclodextrin complex to rabbits, the blood concentration of the drug was higher than that following administration of the phenobarbital suppository. Since β-cyclodextrin tended to retard absorption of the drug in solution from the rectum, enhanced absorption of the drug from the complex was attributed to fast release from the suppository due to rapid dissolution.

Keywords——phenobarbital; β-cyclodextrin inclusion complex; Witepsol S55; macrogol; suppository; dissolution rate; release rate; blood level; rabbit; rectal absorption

Introduction

Phenobarbital and phenytoin have been used for the prevention of convulsion. The use of phenobarbital and diazepam for the prevention or management of febrile convulsion in infants has been proposed.[2] To prevent further convulsion after a febrile convulsion, rapid absorption of the drug is required. Since in some infants the drug cannot be administered orally, either injection or rectal administration has to be considered.

For the rapid absorption of phenobarbital from the rectum, the use of phenobarbital sodium has been suggested.[3] The use of an inclusion complex for the enhancement of drug solubility has been reported,[4] and the use of inclusion complex for the enhancement of absorption after oral administration has been examined.[5] In the present report, we describe enhanced absorption of phenobarbital following rectal administration of the drug in the form of a β-cyclodextrin complex.

Experimental

Materials——Phenobarbital (pharmacopoeial grade) with an original average particle size of 30 μm, (which was reduced to 5 μm by trituration prior to dispersion into a suppository base), β-cyclodextrin, Witepsol S55 (Vosco S55) and macrogol 1000 and 4000 were purchased from Maruishi Seiyaku, Nihon Shokuhinkako, Maruishi Seiyaku, and Wako Junyaku, respectively. β-Cyclodextrin was recrystallized from water. Other chemicals were of reagent grade.

A phenobarbital–β-cyclodextrin complex was prepared according to the following procedure. Phenobarbital and β-cyclodextrin in a molar ratio of 1:1 were dissolved in hot water. The solution was then filtered and left to crystallize. The stoichiometry of the complex was estimated to be 1:1 based on the phenobarbital content in the complex. The average particle size of the complex was 17 μm.

Preparation of Suppositories——The macrogol suppository base was a mixture of macrogol 1000 and 4000 in a weight ratio of 3:1. The drug or the complex was suspended in the suppository bases after the bases had been melted. The molten mass was poured into a suppository mold (Erweka). In each batch, sufficient drug was suspended to give 50 mg of the drug in each suppository.

Measurements of Physical Characteristics of the Suppository——a) Drug Content: Phenobarbital in a suppository was dissolved in 0.01 N NaOH or saline, then a 1 ml aliquot was diluted with 2 ml of ethanol and 7 ml of 0.05 M borax solution. The absorbance at 241 nm was measured to calculate the drug content.

b) Hardness: After storage of the suppositories in a refrigerator overnight, both ends of each suppository were cut off to obtain a 1 cm portion of the central part of the suppository. This was stored in the central part of a refrigerator for 30 min, then laid on an Erweka tablet tester. Hardness was measured by applying the force perpendicular to the side surface of the suppository.

c) Liquefaction Time: A suppository, pointed end down, was put into a tube containing 12 ml of water, which was kept at 37°C by means of a thermostated water bath, and the time at which the suppository completely liquefied was measured as determined by the time when a rod which was initially placed on top of the suppository touched the bottom of the tube when the suppository had melted.

Dissolution Rate Measurement——Powder containing 50 mg of the drug was put into 300 ml of saline in a flask kept at 37°C by means of a thermostated water bath. The suspension was stirred by a magnetic bar at a rate of 100 rpm. One ml of sample solution was pipeted through a cotton plug and its absorbance at 241 nm was measured after dilution with ethanol and 0.05 M borax solution.

Release Rate Measurement——An apparatus for the measurement of drug release from suppositories, model TMS-103, Toyama Sangyo Co., was employed according to the procedures reported by Muranishi *et al.*[6] Saline was employed as a release medium since the release rate of phenobarbital was not very dependent on the pH of the medium as long as it was smaller than the pK_a value (7.3) of the drug. A Millipore filter, SSWP 04700, was employed. The rotation rate of the steel rod was 25 rpm. At predetermined intervals one ml of sample solution was pipeted through a cotton plug and its absorbance at 241 nm was measured following dilution with ethanol and 0.05 M borax solution.

***In Vivo* Absorption Experiment**——Male albino rabbits weighing 2.7—3.1 kg were fasted for 24 h prior to drug administration. The animal was secured in a supine position, and a test suppository was inserted into the rectum, then the anus was closed with a clip to prevent leakage. At predetermined intervals, a 0.5 ml blood sample was withdrawn from the ear vein. An interval of more than ten days was allowed prior to the next experiment.

Assay of phenobarbital in blood was performed according to the gas chromatographic procedure.[7] A gas chromatograph (Hitachi 063) equipped with a flame ionization detector and a glass column packed with 3% OV-17 on Chromosorb WAW 80—100 mesh was used.

***In Situ* Recirculation Experiment**——Male albino rabbits weighing 2.0—2.75 kg were employed following a 24 h fast. Under urethan (1.2 g/kg) anesthesia, the proximal part of the rectum, 15 cm from the anus, was excised. After a glass cannula had been inserted into the anus, the rectum was washed with saline, and another glass cannula was inserted into the other end of the rectum. A 120 ml aliquot of test solution containing 300 μg/ml of phenobarbital and 0—6 mM β-cyclodextrin was circulated at a rate of 20 ml/min by means of a glass pump (GM-24, Tokyo Rikakikai). Samples (0.5 ml each) were pipeted out at predetermined intervals, and the absorbance at 241 nm was measured after dilution with ethanol and 0.05 M borax solution. Apparent absorption rate constants were calculated with allowance for the volume of circulating solution.[8]

Membrane Permeation——Permeation of phenobarbital through a cellophane membrane (type 24/32, Visking Co.) in the presence and absence of β-cyclodextrin was examined by using a permeation cell.[9] Forty ml of phenobarbital solution (1.29 mM) in the presence or absence of 1.29 mM β-cyclodextrin was put in a donor compartment while the same volume of isotonic phosphate buffer was put in a receptor compartment. The permeation cell was agitated horizontally in the thermostated water bath maintained at 37°C. At predetermined intervals, a sample was pipeted from the receptor solution and the concentration of phenobarbital that had permeated into the receptor solution was measured spectrophotometrically.

Results and Discussion

Physical Characteristics of Suppositories

Weight variation among suppositories, drug content and its variance, hardness as measured by a tablet hardness tester, and liquefaction time are shown in Table I. Because of the dissolution of the macrogol base in water, the liquefaction time of macrogol suppositories could not be determined.

Table I. Physical Characteristics of Suppositories (Mean ± S.D. ($n=5$))

Suppositories	Weight of suppository (g)	Drug content (mg)	Hardness (kg/cm²)	Liquefaction time (min)
Phenobarbital in Witepsol S55	1.1358±0.0202	49.4±0.6	4.56±0.32	7.5±0.3
Complex in Witepsol S55	1.2120±0.0243	50.9±0.5	5.07±0.35	6.4±0.1
Phenobarbital in macrogol base	1.4056±0.0359	53.5±1.4	3.68±0.25	
Complex in macrogol base	1.4563±0.0508	53.1±1.3	6.72±0.34	

Dissolution Profiles

Dissolution patterns of phenobarbital from the complex and from phenobarbital powder are shown in Fig. 1. Dissolution from the complex was complete in 10 min while 75% of the drug was dissolved from the phenobarbital powder in 20 min.

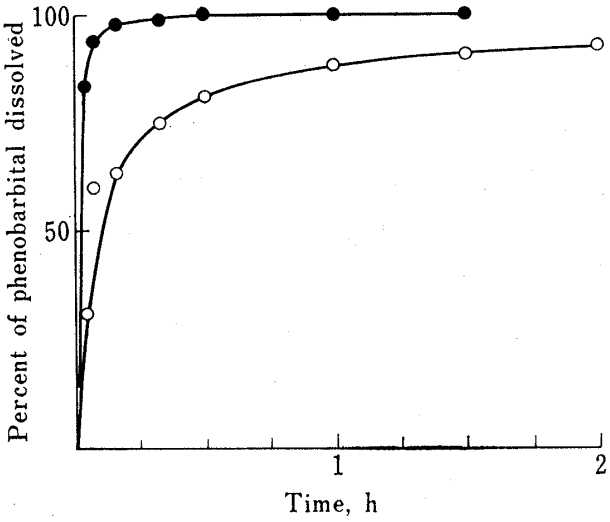

Fig. 1. Dissolution Patterns of Phenobarbital from Phenobarbital–β-Cyclodextrin Complex (●) and Phenobarbital (○) Powder in Saline at 37°C

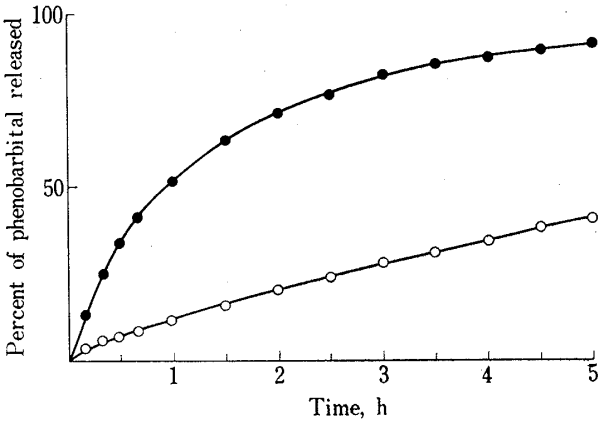

Fig. 2. Release Patterns of Phenobarbital from Phenobarbital–β-Cyclodextrin Complex (●) and Phenobarbital (○) Suppositories of Witepsol S55 in Saline at 37°C ($n=5$)

Release Profiles

Release patterns of phenobarbital from Witepsol S55 suppositories containing the complex or phenobarbital powder in saline are shown in Fig. 2. Twenty-five percent of the drug was released from the suppositories containing the complex in 20 min, while the same amount of the drug was released from the phenobarbital suppositories in 2.5 h. Release patterns of the drug in phosphate buffer, pH 7.4, were very similar to those in saline and therefore they are not shown here.

Release patterns of the drug from the macrogol suppositories are shown in Fig. 3. Release rates of the drug from suppositories containing phenobarbital powder were much greater than those from the corresponding Witepsol suppositories, while those from suppositories containing the complex were only somewhat greater than those from the corresponding Witepsol suppositories.

In order to examine the role of β-cyclodextrin in enhancing drug release from suppositories, the effect of a carbohydrate-type additive on the release characteristics of the drug from the suppositories was examined. Namely, suppositories were prepared from a physical mixture of phenobarbital and lactose in a weight ratio of 1:4.9 using Witepsol S55 as a base. Release patterns of the drug from those suppositories (not shown here) were essentially the same as those from suppositories containing only phenobarbital. Thus, enhanced release of the drug from suppositories containing the complex may be attributed to enhanced dissolution of the drug from the complex rather than to a nonspecific effect due to the presence of bulk-increasing agents.

Rectal Absorption

Blood levels of phenobarbital following rectal administration of Witepsol S55 suppositories to rabbits are shown in Fig. 4. Blood levels of the drug following administration of suppositories containing the complex were much higher during the initial 3 h period than those

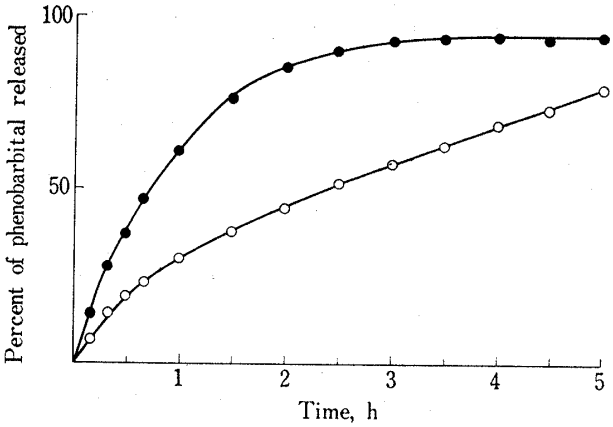

Fig. 3. Release Patterns of Phenobarbital from Phenobarbital–β-Cyclodextrin Complex (●) and Phenobarbital (○) Suppositories of Macrogol in Saline at 37°C ($n=5$)

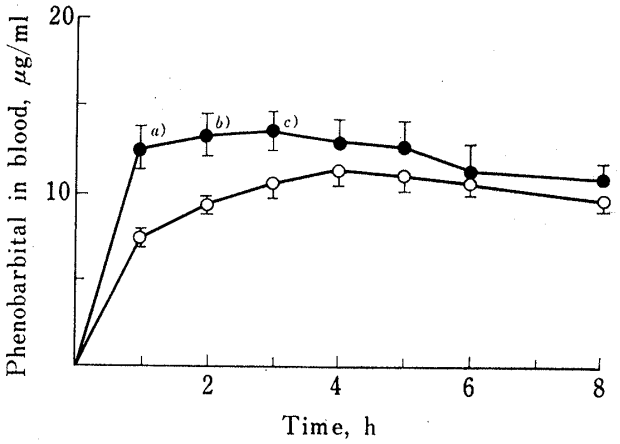

Fig. 4. Blood Levels of Phenobarbital following Rectal Administration of Phenobarbital–β-Cyclodextrin Complex (●) and Phenobarbital (○) Suppositories of Witepsol S55 in Rabbits

Mean ± standard error of 5 experiments.
Significantly different at p levels of *a*) 0.002, *b*) 0.05 and *c*) 0.01.

following administration of phenobarbital suppositories. The results may be attributed to faster release of the drug in the rectum from the suppositories containing the complex.

Blood levels of phenobarbital following rectal administration of macrogol suppositories to rabbits are shown in Fig. 5. The difference in blood levels produced by the two suppositories was small. The small difference may be rationalized in terms of a smaller difference in release rates between the two macrogol suppositories (Fig. 3) as compared with the two Witepsol S55 suppositories (Fig. 2). The results of a comparison of blood levels following rectal administration of β-cyclodextrin complex suppository and sodium phenobarbital suppository will be published elsewhere.

Effect of β-Cyclodextrin on Permeation

The reason for the smaller difference in absorption rate than that expected from the difference in release rates was examined. Absorption profiles of phenobarbital from solution

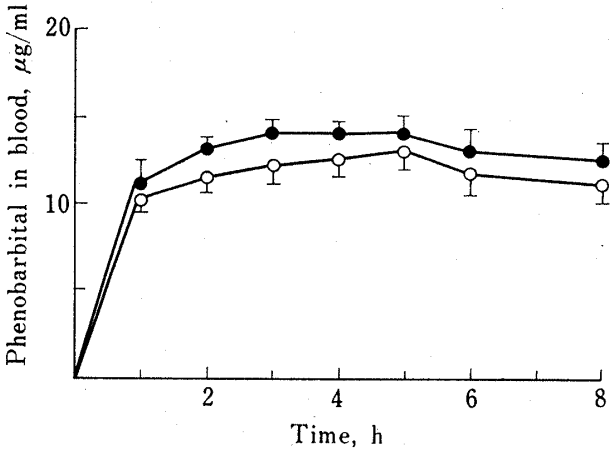

Fig. 5. Blood Levels of Phenobarbital following Rectal Administration of Phenobarbital–β-Cyclodextrin Complex (●) and Phenobarbital (○) in Macrogol Suppositories in Rabbits

Mean ± standard error of 5 experiments.

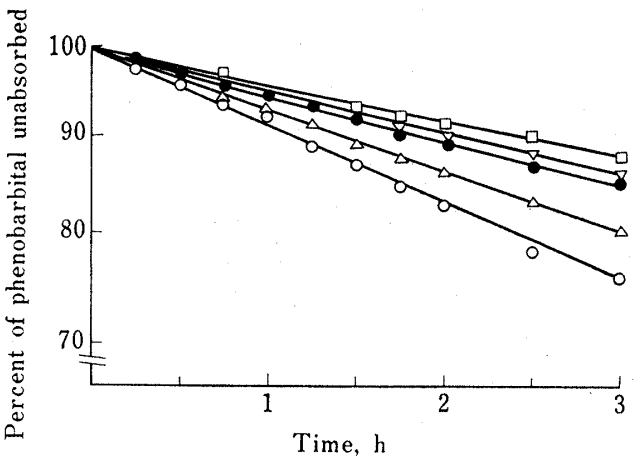

Fig. 6. Absorption Patterns of Phenobarbital in the Presence of 6 mM (□), 3 mM (▽), 1.29 mM (●), 0.5 mM (△) and 0.0 mM (○) of β-Cyclodextrin *in Situ* in Rabbits ($n=1$—3)

in the presence of various concentrations of β-cyclodextrin are shown in Fig. 6. The rate of absorption decreased with increasing concentration of β-cyclodextrin. Thus, the phenobarbital–β-cyclodextrin complex is expected to be absorbed much more slowly than the uncomplexed form of the drug.

The dependency of apparent absorption rate constants on β-cyclodextrin concentration is shown in Fig. 7. In the presence of 6 mM β-cyclodextrin, the apparent absorption rate constant was reduced to 41% of the rate constant observed in its absence.

Retarded permeation of phenobarbital through a cellophane membrane in the presence of β-cyclodextrin (Fig. 8) provided additional evidence of poor permeability of the complex, although the mechanism of permeation through a cellophane membrane is by dialysis through pores.

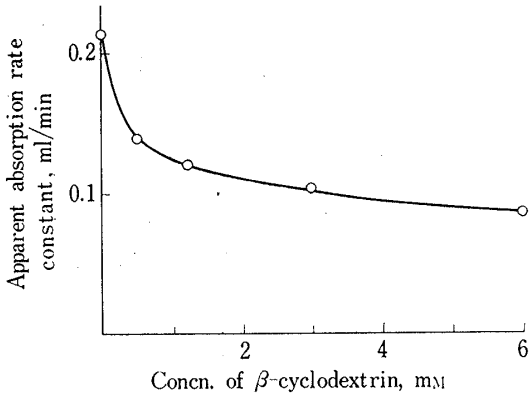

Fig. 7. Relationship between Apparent Absorption Rate Constant of Phenobarbital from the Rectum *in Situ* in Rabbits and Concentration of β-Cyclodextrin

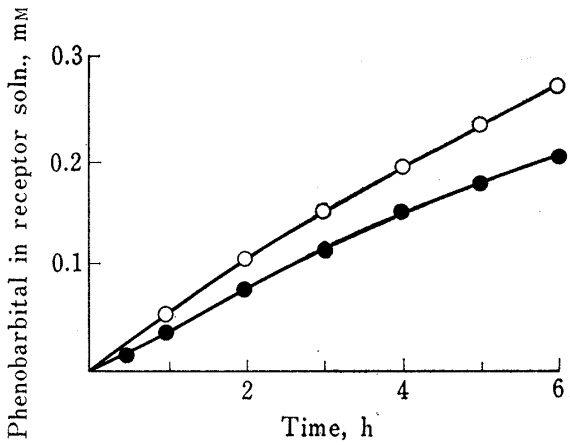

Fig. 8. Permeation Patterns of Phenobarbital in the Presence (●) and Absence (○) of 1.29 mM β-Cyclodextrin ($n=3$)

Conclusion

Although β-cyclodextrin in solution retards the absorption of phenobarbital due to poor permeability of the complex, a greatly enhanced release rate of the drug from suppositories containing the complex more than cancels out the negative effect and produces a net increase in absorption rate. Other means to increase the release rate of phenobarbital from suppositories are being examined.

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References and Notes

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