International Journal of Pharmaceutics, 16 (1983) 327–338
Elsevier

Improvement of dissolution and absorption characteristics of benzodiazepines by cyclodextrin complexation

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(Received January 4th, 1983)
(Accepted March 30th, 1983)

Summary

Inclusion complexes of 13 benzodiazepines with 3 cyclodextrins (α-, β-, γ-CyDs) in aqueous solution and in the solid phase were studied by solubility methods, spectroscopy (UV, CD, IR), thermal analysis, and X-ray diffractometry, and their modes of interaction were assessed. The importance of the hydrophobicity of the guest molecule and the spatial relationship between host and guest molecules were clearly reflected in the magnitude of the stability constant of the inclusion complexes. The solid complexes of some benzodiazepines with γ-CyD were obtained in a variety of molar ratios, and dissolution and membrane permeation behaviors were examined. The rates of dissolution and permeation of benzodiazepines through a cellophane membrane were significantly increased by γ-CyD complexation. As an example, the rapid dissolving form of diazepam–γ-CyD complex was found to significantly increase the serum levels of drug after oral administration to rabbits.

Introduction

Benzodiazepines are widely used as anticonvulsants, sedatives and hypnotics in psychotherapy (Bellantuono et al., 1980; Lader, 1980). Many biopharmaceutical studies on benzodiazepines showed that a rapid plasma appearance of benzodiazepines is therapeutically essential, particularly in the treatment of acute convulsive attacks. In this respect, a fast-dissolving form of benzodiazepines with high aqueous solubility is desirable for rapid absorption in oral benzodiazepine therapy. Cyclodextrins (CyDs) have been extensively employed to increase the solubility, dissolution rate, and absorption characteristics of poorly-soluble drugs (Saenger, 1980;

Uekama, 1981; Szejtli, 1982). The present study deals with inclusion complexations of 13 benzodiazepines with 3 CyDs (α-, β-, γ-CyDs) in anticipation of improving solubility and dissolution behavior of the benzodiazepines. In addition, the soluble complex of diazepam with γ-CyD was prepared and its absorption behavior was compared with the drug itself after oral administration to rabbits.

Materials and Methods

Materials

Benzodiazepines (see Table 1) were kindly donated by Sumitomo Chemicals (Osaka, Japan). CyDs were purchased from Nippon Shokuhin Kako (Tokyo, Japan) and recrystallized twice from water. All other materials and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout the study.

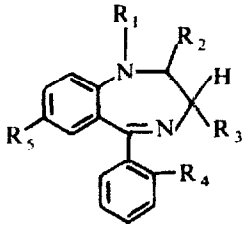
Apparatus

The ultraviolet (UV) and circular dichroism (CD) spectra were taken by a Hitachi 556S double-beam spectrophotometer (Tokyo, Japan) and a Jasco J-40S recording

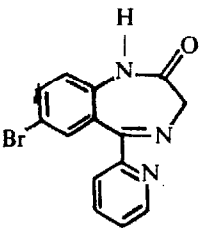
TABLE 1

BENZODIAZEPINES USED IN THIS STUDY

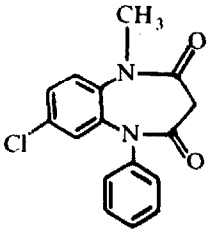
Compound	Substituent				
	R_1	R_2	R_3	R_4	R_5
(1) Diazepam	CH_3	=O	H	H	Cl
(2) Medazepam	CH_3		H	H	Cl
(3) Fludiazepam	CH_3	=O	H	F	Cl
(4) Nitrazepam	H	=O	H	H	NO_2
(5) Nimetazepam	CH_3	=O	H	H	NO_2
(6) Flunitrazepam	CH_3	=O	H	F	NO_2
(7) Clonazepam	H	=O	H	Cl	NO_2
(8) Flurazepam	$CH_2CH_2N(C_2H_5)_2$	=O	H	F	Cl
(9) Lorazepam	H	=O	OH	Cl	Cl
(10) Oxazepam	H	=O	OH	H	Cl



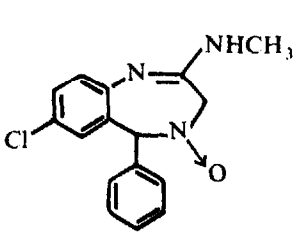
(1)–(10)



(11) Bromazepam



(12) Clobazam



(13) Chlordiazepoxide

spectropolarimeter (Tokyo, Japan), respectively, at 25 ± 0.5°C. The CD spectra were expressed as a molar ellipticity, $[\theta]$, (deg · cm^2 · dmol^{-1}). The infrared (IR) spectra were measured as a KBr disc, using a Jasco DS-701G double-beam spectrophotometer (Tokyo, Japan). The powder X-ray diffraction patterns were taken by a Rigaku Denki Geiger Flex-2012 diffractometer (Tokyo, Japan). The differential thermal analysis (DTA) was carried out using a scanning rate of 10°C/min on a Shimadzu DT-20B thermal analyzer (Kyoto, Japan). The high-performance liquid chromatography (HPLC) was performed on a Hitachi 635A chromatograph (Tokyo, Japan).

Solubility studies

Solubility measurements were carried out according to the methods of Higuchi and Connors (1965). Excess amounts of benzodiazepines were added to aqueous solutions containing various concentrations of CyDs and shaken at 25 ± 0.5°C. After equilibrium was attained (approximately 7 days), an aliquot was centrifuged and pipetted through a cotton filter. A portion of the sample was adequately diluted with water and analyzed spectrophotometrically. The 1 : 1 stability constant (K) was calculated from the initial straight line portion of phase-solubility diagrams according to the method of Higuchi and Connors (1965).

Measurements of partition coefficients

Partition coefficients between water and *n*-octanol were determined in the following manner. A 25 ml aliquot of water containing benzodiazepine was added to 2 ml of *n*-octanol, and the mixture was agitated vigorously for 2 h at 25 ± 1°C. The initial concentrations of the benzodiazepines in water were $1-2 \times 10^{-4}$ M. After equilibration the aqueous phases were centrifuged for 10 min to obtain clear solutions and then assayed spectrophotometrically. The partition coefficient is defined as the ratio of the concentration in organic phase to that in the aqueous solution.

Preparation of solid complexes

The solid complexes were prepared by mixing appropriate amounts of the desired CyD and the benzodiazepines in water. Amounts were calculated from the descending curvature of the phase solubility diagram (see Fig. 1). For example, 210 mg of diazepam and 7.8 g of γ-CyD were added to 30 ml of water, sealed in a flask, and the mixture was stirred at 25°C for 7 days. The complex, which precipitated as a microcrystalline powder, was filtered and dried under vacuum at room temperature for 48 h. This powder corresponded to a 2 : 3 diazepam–γ-CyD complex which had a molecular weight of 4461 ± 5%.

Dissolution and membrane permeation studies

Dissolution rates of benzodiazepines and their γ-CyD complexes were measured by the method of Nogami et al. (1969). For example, the sample powder (100 mesh) of diazepam (54 mg) or its equivalent amount of the γ-CyD complex was put into 25 ml of water in a dissolution cell which was kept at 25°C, and the dissolution medium was stirred at 91 rpm. At appropriate intervals, 0.5 ml samples were removed from the flask, diluted with water and assayed spectrophotometrically. A correction was

applied for cumulative dilution caused by replacement of sample by equal volumes of the original medium.

Permeation behavior of benzodiazepines through a cellophane membrane (type 36/32, Visking) was examined using a permeation cell apparatus described previously (Uekama et al., 1980). The sample powder (100 mesh) of benzodiazepines (216 mg) or its equivalent amount of the γ-CyD complex was put into 100 ml of water in a donor cell compartment while the same volume of water was placed in an acceptor compartment. The solutions in the permeation cell were stirred by a magnetic bar at 241 rpm at 25°C. At appropriate intervals, a sample was pipetted from the receptor solution and the concentration of benzodiazepines which had permeated from donor cell was measured spectrophotometrically. A correction was again applied for the cumulative dilution.

In vivo absorption studies

Five male albino rabbits, weighing 2.5–3.0 kg, were employed in the absorption studies. Intervals of at least 2 weeks were taken in a cross-over manner to minimize the residual or cumulative effect of the preceding dose. The stomach-emptying rate of the rabbits was controlled prior to drug administration according to the method of Maeda et al. (1979). A test powder (10 mg/kg of body weight as diazepam, 100 mesh) was administered orally along with 80 ml of water by means of stomach catheter. A 2 ml blood sample was taken from the ear vein at the predetermined time and centrifuged to obtain 0.5 ml of serum for analysis. Diazepam in serum was determined by the HPLC method suggested by Kabra et al. (1978). The chromatograph was operated at flow rate of 2 ml/min, and the eluent was monitored spectrophotometrically at 240 nm. The separation utilized a Partisil-10 ODS column

Fig. 1.

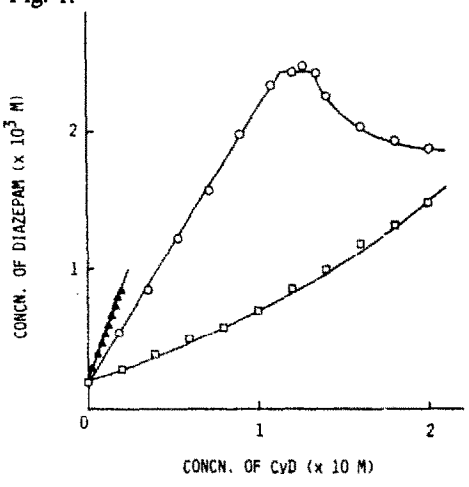


Fig. 2.

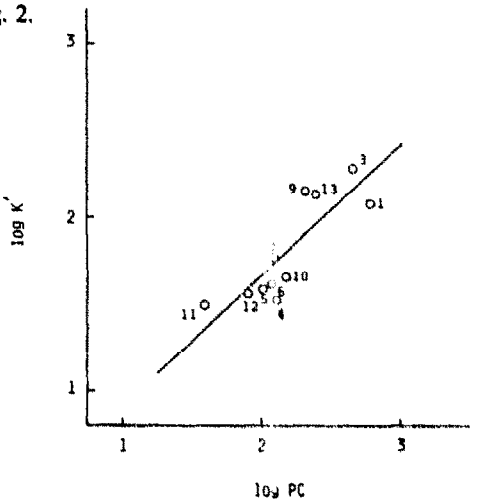


Fig. 1. Phase solubility diagrams of diazepam–CyD system in water at 25°C. □, α-CyD; ▲, β-CyD; ○, γ-CyD.

Fig. 2. Relationship between logarithm of stability constants of benzodiazepine–γ-CyD complexes and partition coefficients of benzodiazepines. Numbers refer to the benzodiazepines listed in Table 1.

(10 μm particle size, 4 mm × 25 cm; Whatman, U.S.A.), with an acetonitrile–0.01 M sodium acetate buffer (33 : 67 v/v) as mobile phase. Components were quantitated by measuring peak heights and comparing the height with that of known amounts of the internal standard, prazepam.

Results and Discussion

Inclusion complexation in aqueous solution and in solid state

Fig. 1 shows examples of the phase solubility diagrams obtained for diazepam with 3 CyDs. The differences in the solubility curves are substantiated and obvious. The solubility of diazepam increased with increasing concentrations of α- and β-CyDs, showing A_p- and A_L-type phase-solubility diagrams (Higuchi and Connors, 1965), respectively. On the other hand, the γ-CyD system showed a typical B_S-type solubility curve (Higuchi and Connors, 1965) with precipitation of microcrystalline diazepam–γ-CyD complex (2 : 3) occurring at high γ-CyD concentrations. Calculation of the stoichiometry of the complex based on the data in the plateau region in Fig. 1 was in good agreement with that obtained by isolation and analysis of the crystalline complex. Solid complexes of some benzodiazepines with γ-CyD were also obtained in the molar ratio of 1 : 2 or 2 : 3 (guest : host) as listed in Table 2. In sharp contrast, the α- and β-CyD systems did not yield any solid complexes. The 1 : 1 stability constant (K′), a tentative measure of inclusion complexation, was estimated on the basis of the assumption that 1 : 1 complex is initially formed. In the case of

TABLE 2
APPARENT 1 : 1 STABILITY CONSTANTS, K′, (M^{-1}), STOICHIOMETRIES[a], AND TYPES OF PHASE SOLUBILITY DIAGRAMS FOR BENZODIAZEPINE–CyD SYSTEMS AT 25°C

Compound	α-CyD system		β-CyD system		γ-CyD system		
	K′	Type[b]	K′	Type[b]	K′	Molar ratio[c]	Type[b]
(1) Diazepam	24(2)[d]	A_p	220	A_L	120	2 : 3	B_S
(2) Medazepam	46(2)[d]	A_P	260	A_L	160	2 : 3	B_S
(3) Fludiazepam	27	A_L	220	A_L	190	2 : 3	B_S
(4) Nitrazepam	26	A_L	96	A_L	33	–	A_L
(5) Nimetazepam	24	A_L	55	A_L	38	–	A_L
(6) Flunitrazepam	21	A_L	77	A_L	40(2)[d]	–	A_P
(7) Clonazepam	22	A_L	80	A_L	58	–	A_L
(8) Flurazepam	10	A_L	120	A_L	130	1 : 2	B_S
(9) Lorazepam	27	A_L	320	A_L	140	1 : 2	B_S
(10) Oxazepam	44	A_L	170	A_L	45	–	A_L
(11) Bromazepam	60	A_L	55	A_L	31	–	A_L
(12) Clobazam	10	A_L	49	A_L	36	1 : 2	B_S
(13) Chlordiazepoxide	44	A_L	23(450)[d]	A_P	140	–	A_L

[a] For the complexes precipitated at high CyD concentrations in B_S-type phase-solubility diagrams.
[b] The type of phase-solubility diagrams was defined according to Higuchi and Connors (1965).
[c] Benzodiazepine: CyD.
[d] The values in parentheses represent the 1 = 2 stability constant.

TABLE 3
UV AND CD SPECTRAL DATA [a] OF VARIOUS BENZODIAZEPINE–CyD SYSTEMS

Compound	Without CyDs [b]		With CyDs											
			α-CyD system				β-CyD system				γ-CyD system			
	UV		UV		CD		UV		CD		UV		CD	
	λ_{max} (nm)	ϵ ($\times 10^{-4}$)	λ_{max} (nm)	ϵ ($\times 10^{-4}$)	λ_{max} (nm)	$[\theta]$ ($\times 10^{-4}$)	λ_{max} (nm)	ϵ ($\times 10^{-4}$)	λ_{max} (nm)	$[\theta]$ ($\times 10^{-4}$)	λ_{max} (nm)	ϵ ($\times 10^{-4}$)	λ_{max} (nm)	$[\theta]$ ($\times 10^{-4}$)
Diazepam	229	3.62	229	3.67	253	−0.31	228	3.53	230	1.67	229	3.48	227	1.21
	310	0.26	310	0.27	310	0.07	310	0.25	258	1.49	310	0.26	258	0.82
									310	−0.23			310	−0.34
Medazepam	231	2.10	230	1.95	260	−0.23	226	1.80	223	2.00	227	1.87	244	−0.93
									270	1.06			270	1.93
Fludiazepam	231	3.30	231	3.01	260	−0.26	229	2.51	228	1.39	230	2.84	259	1.14
	311	0.43	312	0.40					258	0.89	311	0.40	310	−0.15
									318	−0.17				
Nitrazepam	258	1.70	258	1.63			256	1.63	250	−0.91	259	1.61		
	307	1.04	307	1.01			308	0.99	292	0.83	307	1.00		
Nimetazepam	260	1.63	260	1.59	290	−0.09	260	1.56	250	−0.16	260	1.58	250	−0.05
	310	0.97	310	0.95			310	0.94	290	0.30	310	0.95	340	0.09
									330	0.21				

Flunitrazepam	253	1.58	253	1.53	310	−0.07	253	1.49			253	1.51	243	−0.08
	309	0.97	309	0.96			309	0.94			309	0.94	340	0.14
Clonazepam	308	1.05	308	1.05			308	1.02	246	0.17	308	1.04	280	−0.11
									288	−0.10				
Flurazepam	229	3.43	229	3.16			228	2.96	230	0.81	230	3.09		
	307	0.22	307	0.18					315	−0.11	307	0.17		
Lorazepam	231	3.61	231	3.38			231	3.05	290	0.17	231	3.31	280	0.12
	315	0.36	315	0.35			313	0.33	310	−0.05	315	0.32		
Oxazepam	230	3.74	230	3.37			229	3.04	226	4.02	230	3.32		
	313	0.26	313	0.23					256	2.53	313	0.21		
									314	−0.62				
Bromazepam	235	3.62	234	3.41	251	−0.46	232	3.28	250	0.13	234	3.41	260	0.08
	317	0.22	317	0.20	320	0.06	317	0.18	320	−0.04	317	0.20		
Clobazam	229	4.40	229	3.99			229	3.68	238	0.56	229	3.86	230	0.30
													260	−0.51
Chlordiazep-oxide	243	3.66	244	3.67			246	3.34	248	2.55	245	3.57	249	0.76
	259	3.64	260	3.66			262	3.33	313	−0.27	260	3.56		

[a] Concentrations of benzodiazepines and CyDs were 2.7×10^{-5} M and 5.0×10^{-3} M, respectively, in 0.1 M phosphate buffer (pH 7.0) at 25°C, measured with duplicated runs.

[b] Since the benzodiazepines are optically inactive, no CD data are possible.

Fig. 3.

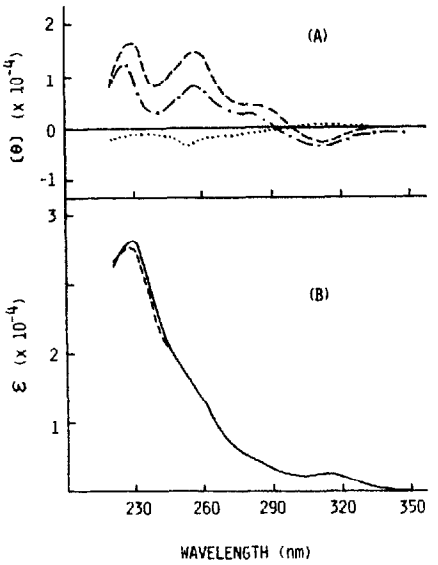


Fig. 4.

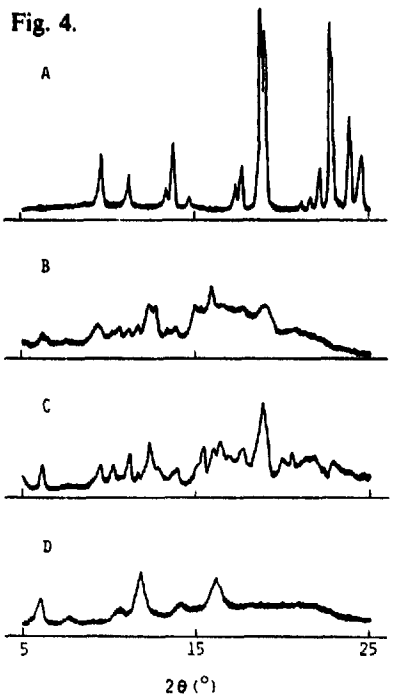


Fig. 3. Circular dichroism spectra (A) of diazepam complexes with CyDs, and UV absorption spectra (B) of diazepam in the absence and presence of CyD in 0.1 M phosphate buffer (pH 7.0) at 25°C. $[CyDs]_0 = 5 \times 10^{-3}$ M, $[Diazepam]_0 = 2.73 \times 10^{-5}$ M. ———, without CyD; ·····, with α-CyD; - - - - - - - -, with β-CyD; –·–·–·–·–, with γ-CyD. The UV absorption spectra of diazepam in the presence of α- and γ-CyDs were not shown here because no appreciable spectral changes were observed for either systems.

Fig. 4. Powder X-ray diffraction patterns of diazepam–γ-CyD system. A, diazepam; B, γ-CyD; C, physical mixture of diazepam and γ-CyD; D, complex of diazepam with γ-CyD.

A_P-type phase-solubility diagram, 1 : 1 and 1 : 2 stability constants were calculated according to the method of Higuchi and Kristiansen (1970). The results of the solubility studies are listed in Table 2. In many cases, the magnitude of K values increased in the order of β-> γ-> α-CyD, suggesting that in aqueous media the cavity size of β-CyD most favorably accommodates the benzodiazepine molecules. As shown in Fig. 2, a good correlation was found between stability constant of the complex and partition coefficient of drug molecule, indicating that the more hydrophobic guest molecule exhibited the stronger binding to CyDs. These findings imply that the hydrophobic nature of the guest molecule and steric factors between the host and guest molecules were responsible for these interactions.

Inclusion complexation in aqueous solution was further examined by both UV and CD spectroscopy. The effects of CyDs on the UV and CD spectra of benzodiazepines are summarized in Table 3. Since the 3 CyDs themselves show no CD band at longer wavelength than 220 nm under these experimental conditions, the observed optical activities can be attributed to the induced Cotton effects of benzodiazepines by the binding to CyDs. Fig. 3 shows typical examples of the CD curves of the

Fig. 5.

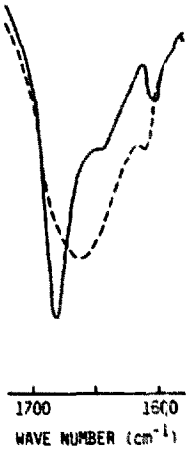


Fig. 6.

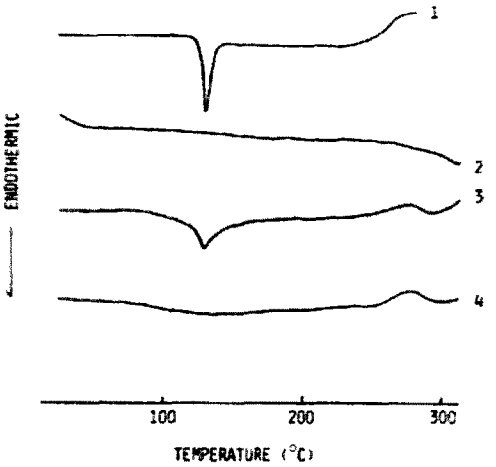


Fig. 5. IR spectra of diazepam–γ-CyD system, measured by KBr disk method. ———, physical mixture of diazepam and γ-CyD; - - - - - - -, complex of diazepam with γ-CyD.

Fig. 6. DTA thermograms of diazepam–γ-CyD system. 1, diazepam; 2, γ-CyD; 3, physical mixture of diazepam and γ-CyD; 4, complex of diazepam with γ-Cyd.

diazepam–CyD systems and the changes in UV spectra of diazepam in the presence and absence of CyD. The interaction of diazepam with the β- or γ-CyDs altered the CD spectra of the CyDs as evidenced by the appearance of two positive and one negative peak at 230, 258 and 130 nm, respectively. In contrast, the α-CyD system showed very weak negative and positive peaks at 253 and 310 nm, respectively, suggesting that the inclusion mode of the α-CyD complex might be somewhat

Fig. 7.

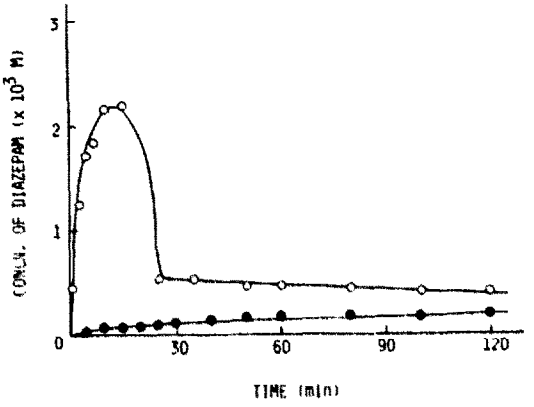


Fig. 8.

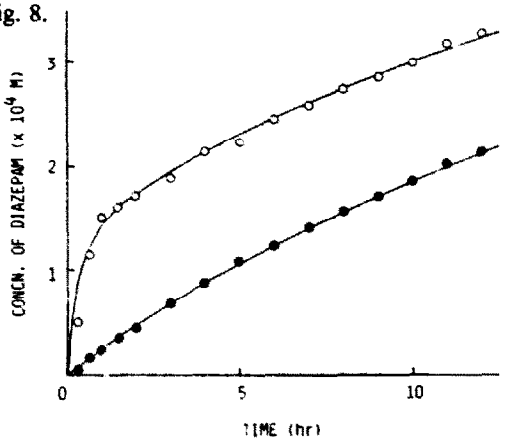


Fig. 7. Dissolution profiles of diazepam and its γ-CyD complex in water at 25°C, measured by dispersed amount method. ●, diazepam; ○, γ-CyD complex.

Fig. 8. Permeation profiles of diazepam and its γ-CyD complex through a cellophane membrane in water at 25°C. ●, diazepam; ○, γ-CyD complex.

different from those of β- and γ-CyD complexes. An intense UV maximum peak of diazepam at 229 nm was shifted to shorter wavelength with decrease in molar absorptivity by the addition of β-CyD, while α- and γ-CyDs did not have any effect on the UV spectra of diazepam. Since the magnitude of these spectral changes was well correlated with that of K values, the diazepam molecule may be included in the cavity of CyDs.

The solid complexes of some benzodiazepines with γ-CyD were isolated on the basis of the B_s-type solubility diagrams, and their interactions were examined by X-ray diffractometry, IR spectroscopy and DTA measurement in comparison with the corresponding physical mixtures in the same molar ratio. Fig. 4 shows the powder X-ray diffraction pattern of the diazepam–γ-CyD complex and that of physical mixture. The diffraction pattern of physical mixture was simply the superposition of each component, while that of γ-CyD complex was apparently different from each constituent and constitutes a new solid phase. The γ-CyD complex gave a somewhat diffuse diffraction pattern, indicating that it is much less crystalline than the physical mixture. Fig. 5 shows the IR spectra of diazepam–γ-CyD systems in the carbonyl-stretching region. In the case of the γ-CyD complex, the 1685 cm^{-1} band shifted to 1665 cm^{-1}, suggesting the formation of the intermolecular hydrogen bonding between diazepam and γ-CyD (Camerman et al., 1972). Fig. 6 shows the DTA thermograms of diazepam–γ-CyD systems. The physical mixture showed an endothermic peak around 130°C and the broad exothermic and endothermic peaks around 290°C corresponding to the melting temperature of diazepam and to the decomposition temperature of γ-CyD, respectively. In the case of the γ-CyD complex, the peak due to the melting of diazepam disappeared completely. Similar results for powder X-ray diffraction patterns, IR spectra and DTA thermograms were observed for other benzodiazepine–γ-CyD solid complexes. These data clearly indicate that the benzodiazepine–γ-CyD complexes exist in the solid state.

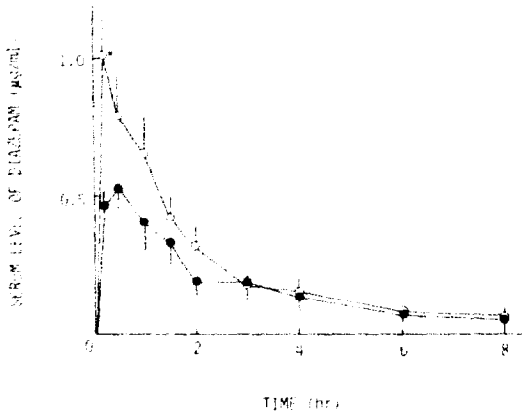


Fig. 6. Serum levels of diazepam following the oral administration of diazepam and its γ-CyD complex (equivalent to 10 mg/kg diazepam) to rabbits. ●, diazepam; ○, γ-CyD complex. Values represent the mean ± S.E. of 5 rabbits. $^*P < 0.02$ in ○ versus ●.

Dissolution and permeation behaviors of the complexes

Fig. 7 shows the dissolution profiles of diazepam from γ-CyD complex and diazepam powders in water at 25°C. It is evident that the γ-CyD complex dissolved much rapidly than diazepam. Rapid dissolution of other benzodiazepines from their γ-CyD complexes were also observed. The enhanced dissolution rate may be due to the increase in solubility and the decrease in crystallinity of the drug by the inclusion complexation as expected from Fig. 1 and Fig. 4, respectively. It is interesting to note that the diazepam concentration following the dissolution from the γ-CyD complex reached supersaturation and then decreased. This anomalous behavior may be explained on the basis of the dissociation equilibrium of the complex in the dissolution medium. That is, the γ-CyD complex having a small stability constant (120 M^{-1}) dissociates extensively upon dissolution, and this results in the precipitation of the free drug during the dissolution process.

Fig. 8 shows the permeation profiles of diazepam through a cellophane membrane, following the dissolution from diazepam or its γ-CyD complex powder in a donor cell. Unfortunately, a reliable value for the permeation rate of γ-CyD was not obtainable because no sensitive assay is currently available for such a low γ-CyD concentration. As shown in Fig. 8, the faster dissolution rate of the γ-CyD complex resulted in an increase in the net amount of diazepam permeating into the acceptor cell. However, the increase in permeation rate of the complex was rather small compared with that expected from the dissolution profiles. This may be due to the poorer permeability of the bulky complex (relative to diazepam) since the permeation mechanism is mainly pore size controlled (Iwaoku et al., 1982). In the case of the γ-CyD complex, however, the rapid dissolution more than cancels out the negative effect due to the poor permeability and produces a net increase in a drug permeation.

It was also found that no appreciable effect of γ-CyD on the ring-opening and ring-closure reactions of diazepam (Nakano et al., 1979) was observed in the acidic medium (pH 1.3) at 37°C. Thus, the remarkable increase in dissolution rate together with the enhanced membrane permeation suggested that the complexed form of diazepam might improve oral bioavailability.

Bioavailability of diazepam–γ-CyD complex

Fig. 9 shows the mean serum levels of diazepam following the oral administration of diazepam or its γ-CyD complex to 5 rabbits. When the equivalent dose of diazepam (10 mg/kg) was administered to rabbits, the serum levels of drug during an initial 30 min period were much higher when the drug was administered as the complex rather than the drug alone. In the case of diazepam, the maximum serum levels (C_{max}) of 0.59 ± 0.08 μg/ml was observed at 30 ± 8 min. On the other hand, the γ-CyD complex resulted in the rapid appearance of diazepam in the serum, showing the C_{max} of 1.05 ± 0.16 μg/ml at 24 ± 9 min. The area under serum concentration–time curve (AUC) of the γ-CyD complex up to 8 h post-administration was about 1.4 times as much as that from diazepam alone.

The present approach of using a rapid dissolving form of γ-CyD complex seems to be promising for improving the oral bioavailability of diazepam. Furthermore, the

2 : 3 complexation of diazepam with γ-CyD results in 7.8-fold increase in molecular weight of the drug, which should facilitate the pharmaceutical preparation of the tablets, particularly from the viewpoint of content uniformity.

Acknowledgements

The authors are grateful to Professor M. Nakano and Mr. N. Inotsume, Kumamoto University Hospital, for their helpful discussions throughout this work.

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