J. Pharm. Pharmacol. 1981, 33: 707–710
Received March 17, 1981

0022–3573/81/110707–04 $02.50/0

Effects of cyclodextrins on chlorpromazine-induced haemolysis and central nervous system responses

K. UEKAMA,* T. IRIE, M. SUNADA, M. OTAGIRI, K. IWASAKI, Y. OKANO, T. MIYATA, AND Y. KASÉ

Faculty of Pharmaceutical Sciences, Kumamoto University, 5–1, Oe-honmachi, Kumamoto 862, Japan

Cyclodextrins (CyDs) protected the human erythrocytes from haemolysis induced with chlorpromazine (CPZ) in isotonic solution, depending upon the magnitude of the stability constant of CPZ-CyD complexes (β-> γ-> α-CyD). From the observations of CPZ uptake into erythrocytes and changes in surface activity of CPZ, the protective effects of CyDs in vitro appeared to be due to the decrease in effective haemolytic concentration of CPZ through inclusion complex formation rather than the direct interaction of CyDs with the erythrocyte membrane. The effect of β-CyD on some central nervous system (c.n.s.) actions of CPZ in rats was also investigated to see if there were any advantages in the use of β-CyD complexes given by injection. The results suggest that β-CyD does not alter the time-course or magnitude of the effects of CPZ on the c.n.s.

Drug-induced haemolysis may limit clinical trial of compounds having excellent pharmacological activity, yet the protection of human erythrocytes against drug-induced haemolysis has received little attention (Ogiso et al 1978). α-, β-, and γ-Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of six, seven, and eight glucose units, produced by the enzymatic partial hydrolysis of starch. One of their characteristics is the formation of inclusion complexes with various drug molecules in the solid phase and in solution, in which the drug molecules are included in the relatively hydrophobic cavity of CyDs (Saenger 1980). Although CyD complexes are relatively stable in the solid phase, they dissociate in aqueous solution to release the drug molecules to an extent depending upon the magnitude of the stability constant of the complex (Uekama et al 1979). Thus, CyDs have been successfully applied pharmaceutically, to improve the physical and chemical properties of the drug molecules through inclusion complex formation (Uekama 1979; Saenger 1980). Preliminary studies have indicated that β-CyD significantly protects human erythrocytes from haemolysis and shape changes induced by amphiphilic drugs including chlorpromazine (CPZ) in isotonic solution (Uekama et al 1981). The present paper deals with the effects of CyDs on CPZ-induced haemolysis in vitro and discusses the protective mechanism of CyDs on the basis of inclusion complexation. The effect of β-CyD on some central nervous system (c.n.s.) actions of CPZ was also examined to see if the CyD complex offered any advantages when given by injection.

* Correspondence.

MATERIALS AND METHODS

Materials

Chlorpromazine HCl, a gift from Yoshitomi Co. Ltd, was used without further purification. α- and β-CyDs were purchased from Nakarai Chemicals, Ltd and Nippon Shokuhin Kakō Ltd, and recrystallized from water. Pure γ-CyD was a gift from Zeria Pharm. Co. Ltd. All other materials and solvents were analytical reagent grade. Deionized double-distilled water was used throughout.

CPZ-induced haemolysis

Human blood was collected from healthy donors, using sodium citrate (0·47%) as an anticoagulant. Erythrocytes were separated by centrifugation at 1000 *g* for 10 min and washed 3 times with 10 mM isotonic phosphate buffer (pH 7·4) and then resuspended in the buffer solution to give an haematocrit value of 10%. To 4 ml of the buffer solution containing CPZ at various concentrations, 0·4 ml of the erythrocyte suspension was added. The mixture was incubated for 5 min at 37 °C and then centrifuged at 250 *g* for 3 min. The percent haemolysis was expressed as the ratio of the absorbance at 543 nm of haemoglobin in the supernatant to the absorbance after the complete haemolysis of erythrocytes in water.

Uptake of CPZ into erythrocytes

To 4 ml of the buffer solution containing CPZ at various concentrations, 0·4 ml of the erythrocyte suspension was added. The mixture was incubated for 5 min at 37 °C and centrifuged at 250 *g* for 3 min. The concentration of CPZ in the supernatant was

analysed spectrophotometrically at 255 nm. The control was 0·4 ml of the buffer solution. The uptake of CPZ into erythrocytes was calculated from the difference in the absorbance of the control from that of supernatant solution, and expressed as mol ml^{-1} of erythrocytes. The correction was made for the absorption change of CPZ upon addition of CyDs.

Surface tension measurements

Surface activities of varying aqueous concentrations of CPZ ($1·0 \times 10^{-5}$ to $8·0 \times 10^{-4}$ M) in the absence and presence of CyDs ($1·0 \times 10^{-3}$ M) were determined at 25 °C. A duNouy surface tensiometer with an accuracy of 0·5 mN m^{-1} was used for the estimation of the surface tension. The compounds to be examined were diluted with 10 mM isotonic phosphate buffer (pH 7·4) to a volume of 10 ml. At least three measurements were made of each concentration of the samples.

In vivo activities

Male Wistar rats (200 ± 5 g, 7 week-old) were maintained in a light-dark cycled room for 12 h with constant temperature and humidity. They were divided in 3 groups of 6 rats. Group 1 and group 2 were given i.m. (M. biceps femolis), CPZ (10 mg kg^{-1}) with or without β-CyD (32 mg kg^{-1}) at 9.00 a.m. and time-course of the action on psychomotor activities such as locomotor activity, grooming and rearing (upright standing on the hind legs) and cataleptogenic action were studied. Group 3, the control group, was administered 0·2 ml of 0·9% NaCl (saline) and similarly examined. Locomotor activity was recorded for 10 min at appropriate times after administration of the samples using an Animex activity meter (A. B. Farad, Type S) and simultaneously counts for grooming and rearing were made by two unbiased observers. The degree of catalepsy was also examined by the two observers according to the scoring method of Wirth et al (1958).

RESULTS AND DISCUSSION

Effects of CyDs on CPZ-induced haemolysis

Fig. 1 shows the haemolytic effects of CPZ on human erythrocytes in the absence and presence of three CyDs in isotonic solution. Haemolysis was evident at $3·0 \times 10^{-4}$ M CPZ and was complete above $7·0 \times 10^{-4}$ M. The haemolytic activity of CPZ was significantly decreased by addition of β- and γ-CyD, but no appreciable effect was observed with α-CyD. This dependency on molecular size implies complementarity between host and guest molecules and

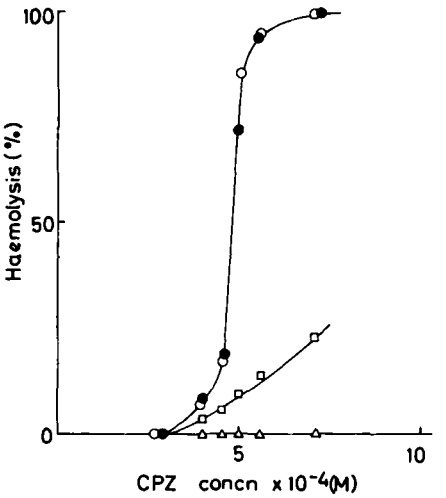

FIG. 1. Haemolytic effect of CPZ on human erythrocytes in the absence and presence of CyDs in 10 mM isotonic phosphate buffer (pH 7·4) at 37 °C. All the points are the average of three determinations. Concentrations of CyDs were $1·0 \times 10^{-3}$ M. ○ CPZ alone. ● CPZ + α-CyD. △ CPZ + β-CyD. □ CPZ + γ-CyD.

suggests that to prevent the haemolysis the CPZ molecule is preferably included in the β-CyD cavity. Fig. 2 shows the effects of CyD concentrations on the haemolysis induced by $7·0 \times 10^{-4}$ M CPZ. It suggests that the β-CyD is the most effective in protecting against haemolysis. Since the haemolysis was reduced as a function of CyD concentration, attention was directed toward the stability constant, K_c of inclusion complex. Taking into account the free and complexed species in the CPZ-CyD systems, the free CPZ concentration, $(CPZ)_f$ was calculated from equation (2), using previously reported K_c values (Uekama et al 1978: 200 M^{-1} for α-CyD complex, 12000 M^{-1} for β-CyD complex, 1000 M for γ-CyD complex), where (complex) and $(CyD)_f$ are concentrations of complex

$$CPZ + CyD \overset{K_c}{\rightleftharpoons} complex \qquad (1)$$

$$K_c = \frac{(complex)}{(CPZ)_f(CyD)_f} = \frac{(complex)}{[(CPZ)_t - (complex)][(CyD)_t - (complex)]} \qquad (2)$$

and free CyD, and $(CPZ)_t$ and $(CyD)_t$ are total concentrations of CPZ and CyD, respectively. It is evident from Table 1 that the extent of haemolysis is well correlated with $(CPZ)_f$, indicating that the complexed CPZ may be essentially inactive and thus

Table 1. Relationship between the extent of haemolysis and free CPZ concentration in CPZ-CyD systems. Initial concentration of CPZ $7{\cdot}0 \times 10^{-4}$ M.

Initial concn. of CyD $\times 10^{-4}$ (M)	α-CyD system		β-CyD system		γ-CyD system	
	Haemolysis (%)	$(CPZ)_f$ $\times 10^{-4}$ (M)	Haemolysis (%)	$(CPZ)_f$ $\times 10^{-4}$ (M)	Haemolysis (%)	$(CPZ)_f$ $\times 10^{-4}$ (M) †
0·0	100	7·0	100	7·0	100	7·0
2·0	100	6·8	92	5·3	100	6·2
4·0	100	6·5	22	3·7	97	5·6
6·0	100	6·3	0	2·5	81	5·0
8·0	100	6·1	0	1·7	60	4·5
10·0	100	5·9	0	1·2	23	4·1
12·0	100	5·8	0	0·9	15	3·7

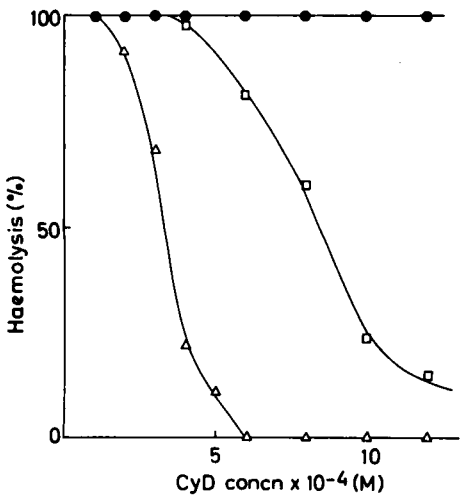

FIG. 2. Effects of CyD concentrations on the haemolysis induced with CPZ at $7{\cdot}0 \times 10^{-4}$ M in 10 mM isotonic phosphate buffer (pH 7·4) at 37 °C. All the points are the average of three determinations. ● CPZ + α-CyD. △ CPZ + β-CyD. □ CPZ + γ-CyD.

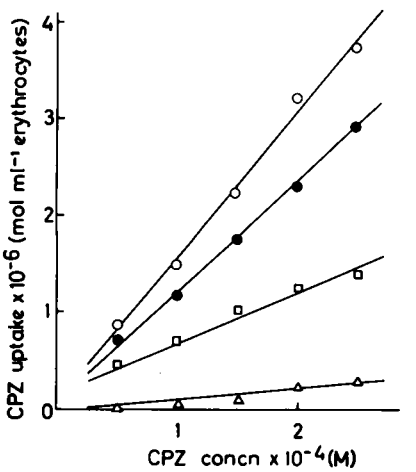

FIG. 3. Effects of CyDs on the uptake of CPZ into erythrocytes in 10 mM isotonic phosphate buffer (pH 7·4) at 37 °C. All the points are the average of three determinations. ○ CPZ alone. ● CPZ + αCyD. △ CPZ + β-CyD. □ CPZ + γ-CyD.

unable to induce the haemolysis. Complete inhibition was achieved by β-CyD at concentrations above $6{\cdot}0 \times 10^{-4}$ M, where $(CPZ)_f$ values were smaller than the effective haemolytic concentration of CPZ ($3{\cdot}0 \times 10^{-4}$ M).

To gain insight into the protective mechanism of CyD, some factors responsible for CPZ-induced haemolysis (Ahtee 1966) were investigated. Fig. 3 shows the effects of CyDs on the uptake of CPZ into erythrocytes. The amounts of CPZ uptake were found to decrease on addition of CyDs in the order of β-> γ-> α-CyD. This may be due to the reduction of hydrophobicity and membrane permeability of the CPZ molecule by a sheath of hydrophilic CyD. Fig. 4 shows the effects of CyDs on the surface tension of CPZ in isotonic phosphate buffer. The surface activity of CPZ was reduced by CyDs in the same order β-> γ-> α CyD, as that in CPZ-induced haemolysis. The addition of CyDs effected no noticeable changes in osmotic pressure and viscosity of the CPZ solution under the experimental conditions used. Moreover, CyDs showed a little protective or accelerative effect against the osmotic haemolysis. These results clearly indicate that the protective effect of CyD was probably due to the decrease in effective haemolytic concentration of CPZ through inclusion complex formation rather than the direct interaction of CyD with the erythrocyte membrane.

Effect of β-CyD on some central nervous system actions of CPZ

The therapeutic effects of phenothiazines, including CPZ, are known to relate to the extent of haemolysis as well as the interactive abilities of the drugs at the erythrocyte membrane (Seeman 1972). Since β-CyD was found to significantly protect the human erythrocytes from haemolysis induced by CPZ, its effect on the pharmacological activity of CPZ in vivo was examined. Locomotor activity of the rats in the

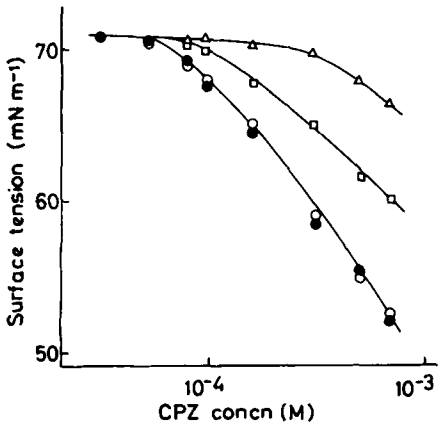

FIG. 4. Effects of CyDs on the surface activity of CPZ in 10 mM isotonic phosphate buffer (pH 7·4) at 25 °C. Concentrations of CyDs were $1·0 \times 10^{-3}$ M. ○ CPZ alone. ● CPZ + α-CyD. △ CPZ + β-CyD. □ CPZ + γ-CyD.

control group given saline decreased and fluctuated when measured at different times of the day. Locomotor activities in the groups administered CPZ or its complex were almost completely suppressed for 8–11 h; the suppression began 15 min after their administration. As with locomotor activity, grooming and rearing markedly decreased 15 min after administration of CPZ or its complex. The behaviours were completely suppressed for at least 8 h and returned 11·13 h after the drug had been given. Neither qualitative nor quantitative difference was observed between drug and its complex in their psychosedative actions but there was a slight difference in the time course of their cataleptogenic actions. Pronounced catalepsy (score 3) appeared in all the animals by within 30 min of administration of CPZ, while with the complex, the same degree of catalepsy was reached by 1 h. The cataleptogenic actions of both drug and complex diminished and disappeared 11 h and 13 h after administration, respectively.

The results suggest that β-CyD does not alter the time course or magnitude of the studied effects of CPZ on the c.n.s. This might be explained by the dissociation equilibrium of the complex. After the injection is completed the complexed CPZ may be quickly dissociated by the circulating blood. If this were so, no appreciable difference in the pharmacological activities of CPZ and its β-CyD complex would be observed.

These limited data suggest that β-CyD may have a use in alleviating injury to erythrocyte membranes at the injection site.

REFERENCES

Ahtee, L. (1966) Ann. Med. Exp. Fenn. 44: 453–457

Ogiso, T., Masuda, H., Oue, S. (1978) Biochem. Pharmacol. 27: 1263–1268

Saenger, W. (1980) Angew. Chem. Int. Ed. Eng. 19: 344–362

Seeman, P. (1972) Pharmacol. Rev. 24: 583–655

Uekama, K., Irie, T., Hirayama, F. (1978) Chem. Lett.: 1109–1112

Uekama, K. (1979) Jpn. J. Antibiot. 32: S-103-111

Uekama, K., Hirayama, F., Esaki, K., Inoue, M. (1979) Chem. Pharm. Bull. 27: 76–79

Uekama, K., Irie, T., Sunada, M., Otagiri, M., Tsubaki, K. (1981) J. Pharm. Dyn. 4: 142–144

Wirth, W., Gossawald, R., Horlein, U., Risse, Kl.-H. Kreiskott, H. (1958) Arch. Int. Pharmacodyn. 115: 1–31