ALLEVIATION OF THE CHLORPROMAZINE-INDUCED MUSCULAR TISSUE DAMAGE BY β-CYCLODEXTRIN COMPLEXATION

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ABSTRACT. From the gross and histological examinations, β-cyclodextrin (β-CyD) was found to reduce the muscular tissue damage produced by chlorpromazine hydrochloride (CPZ) on *M. vastus lateralis* in rabbits. The protective effect of β-CyD may be attributable to the decrease in affinity of CPZ to the tissue membrane through inclusion complexation. There was no appreciable difference in the pharmacokinetic and pharmacodynamic behaviors between CPZ and its β-CyD complex in rabbits. These results suggest that β-CyD is useful to reduce the local tissue toxicity of CPZ without altering the pharmacological efficacy.

1. INTRODUCTION

In clinical practice, chlorpromazine hydrochloride (CPZ), which is widely used in the treatment of psychiatric disorders, has been known to cause frequently pain response or topical tissue damage when given by intramuscular injection (1,2). However, few attempts to reduce these irritancies have been made from the pharmaceutical and toxicological points of view. Recently, we reported that cyclodextrins (CyDs) protect the human erythrocytes from the hemolysis induced by phenothiazine neuroleptics including CPZ *in vitro* (3,4), suggesting that the CyD complexation is potentially useful in alleviating the membrane damage by these drugs *in vivo*. In these continuing investigations, we report here the effect of β-CyD on the local tissue toxicity of CPZ following the intramuscular injection to rabbits by means of gross and histological examinations. Moreover, the effect of β-CyD on the pharmacological efficacy of CPZ was examined to see if the CyD complex offered any advantages when given by injection. Although γ-CyD may be generally acceptable as an injecting agent on account of its highly aqueous solubility and safety (5,6), β-CyD was adequately chosen here because it elicits the largest protective effect to human erythrocytes and the greatest stability constant of inclusion complex with CPZ among the CyD homologs (3).

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2. MATERIALS AND METHODS

2.1. Materials

CPZ, a gift from Yoshitomi Pharmaceutical Industries Ltd. (Fukuoka, Japan), was used without further purification. β-CyD was purchased from Nihon Shokuhin Kako Ltd. (Tokyo, Japan), and recrystallized from water. All other materials and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout the study.

2.2. Apparatus

The osmotic pressure and surface tension of sample solutions were measured with a Kyoto Daiichi Kagaku OM-6010 Osmotic Pressure Auto & Stat (Kyoto, Japan) and a Shimadzu Du Noüy Surface & Interfacial Tensionmeter (Kyoto, Japan), respectively. The selected ion monitoring quantification of CPZ was performed on a JEOL JMS Modified D-100 equipped with JMA-2000S mass data analysis system (Tokyo, Japan). The rectal temperature in rabbits was recorded with a Shibaura Electronics MGSIII-269 Thermometer (Tokyo, Japan).

2.3. Intramuscular Irritation Studies

The irritation studies were carried out according to the method of Shintani et al. (7). The concentration of CPZ (7 μmol) was chosen here by considering the dose intended for clinical use. The complex solution was prepared by dissolving 7 μmol CPZ with 14 μmol β-CyD in 1 ml normal saline, in which the most portion of CPZ (more than 98 %) exists as a complexed form, estimated from the stability constant reported previously (3). Male albino rabbits weighing 2.5 to 3.0 kg were used throughout the study. The test solutions were injected into the one side of *M. vastus lateralis* in rabbits and the other side of the muscle served as control. The animals were sacrificed at 2, 5, and 10 days after the injection. The dissected muscles were cut longitudinally and the lesions were then observed grossly. Some of the muscles served as sample for histological examinations in which these were selected from the results of gross findings. These muscles were fixed in 10 % neutral formalin and the muscle blocks were embedded in paraffin and then the sections were stained with hematoxylin and eosin.

2.4. Pharmacokinetic Studies

The experimental conditions and the injection procedure were essentially the same as those in the irritation studies. Blood samples were collected from the ear vein at appropriate time intervals following treatment and centrifuged to obtain the serum for analysis. The extraction procedure of CPZ in the serum was performed according to the modified method of Mckay et al. (8). The quantification of CPZ was carried out on a gas chromatograph-mass spectrometer-computer system. The instrument was used in the selected ion monitoring mode.

Separations were made on a 100 x 2 mm (ID) glass column packed with 3 % OV-17 on 80-100 mesh Chromosorb W HP (Tokyo, Japan). The fragment ion used for the quantification was m/z 318 for CPZ. Full details of the analysis will be reported elsewhere.

3. RESULTS AND DISCUSSION

3.1. Effect of β-CyD on the CPZ-induced Local Tissue Toxicity

Table I shows some physicochemical properties of the test solutions injected intramuscularly to rabbits. There are no significant differences in the pH and osmotic pressure of the solutions, and

TABLE I. Some physicochemical properties of test solutions[a] used in the intramuscular irritation studies

System	pH	Osmotic pressure (mOsm)	Surface tension (mN/m)
Control	5.9	286	70.9
β-CyD	5.6	297	71.6
CPZ	5.3	292	48.2
CPZ + β-CyD	5.4	303	68.7

a) CPZ: 7 μmol, β-CyD: 14 μmol.

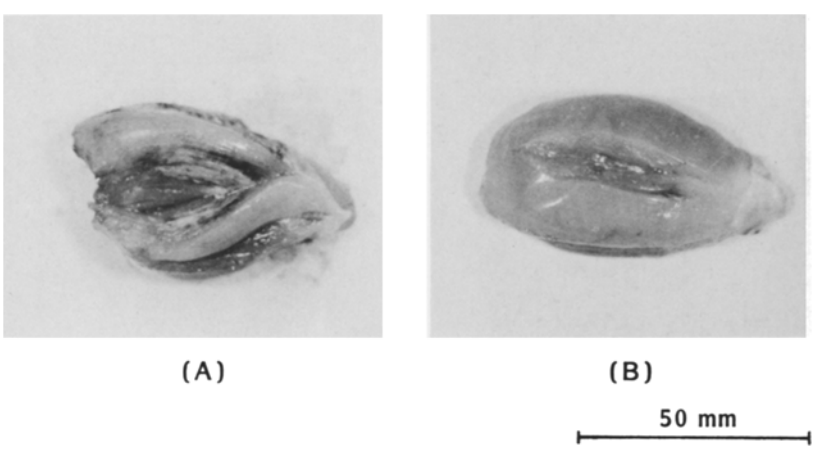

Figure 1. Macrographs of *M. vastus lateralis* at 2 days after the injection of CPZ to rabbits in the absence and presence of β-CyD
(A): CPZ (7 μmol); (B): CPZ (7 μmol) + β-CyD (14 μmol).

TABLE II. Evaluation scores of the intramuscular irritation produced by CPZ (7 μmol) in the absence and presence of β-CyD (14 μmol)

System	Average irritation score[a)]		
	2 days	5 days	10 days
Control	0	0	0
β-CyD	0.3	0	0
CPZ	3.0	3.5	2.8
CPZ + β-CyD	0.5	0	0

a) Mean obtained from 4 rabbits.

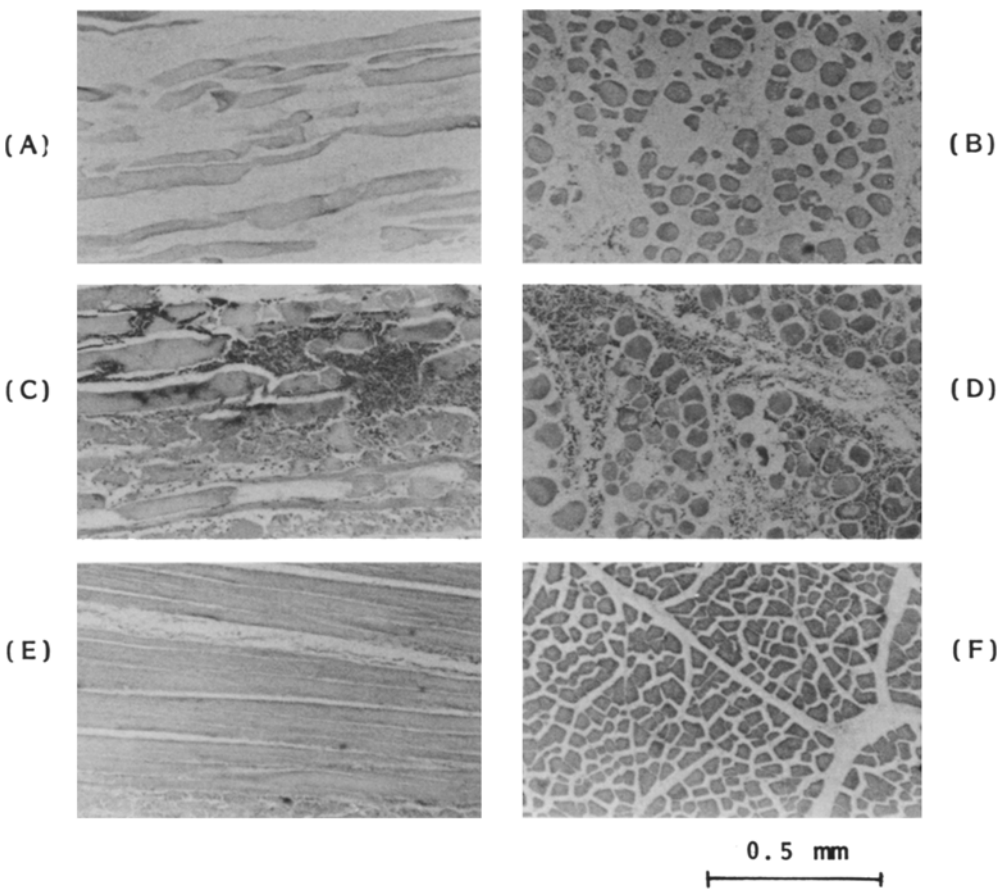

Figure 2. Micrographs of the longitudinal (A,C, and E) and cross (B,D, and F) sections of *M. vastus lateralis* at 2 days after the injection of CPZ to rabbits in the absence and presence of β-CyD
(A,B,C, and D): CPZ (7 μmol); (E and F): CPZ (7 μmol) + β-CyD (14 μmol).

these values are in the safety range on the topical irritation (7). In contrast, the intense surface activity observed for CPZ may be one of the factors responsible for local tissue toxicity (9,10). Thus, it is expected that the reduced surface activity of the β-CyD complex may provide alleviation of the local tissue damage produced by CPZ. Figure 1 shows the typical macrographs of *M. vastus lateralis* at 2 days after the injection of 7 μmol CPZ in the absence and presence of 14 μmol β-CyD. The injection of CPZ caused the distinct and extensive discoloration of the muscle along with the surrounding hemorrhage area (Fig. 1-A). These discolored loci became partly brownish at 5 days, and the recovery of the damaged muscle was scarcely observed even at 10 days after the injection. In contrast, the injection of β-CyD complex showed a slight lesion along with the needle tract at 2 days after the injection (Fig. 1-B). The remarkable irritating reactions were no longer observed at 5 days and 10 days after the injection of the complex. Under these experimental conditions, both normal saline and β-CyD solution caused no noticeable irritating reactions on gross findings. Then, these gross findings were evaluated according to the scoring method of Shintani et al.(7), and the results are summarized in Table II.

Figure 2 shows the typical micrographs corresponding to the lesions observed macroscopically. In the longitudinal section of the central portion of the lesions produced by CPZ (Fig. 2-A), the muscle fibers lost their cross striation and the collapsed sarcoplasms indicated severe degeneration or necrosis. Segmentation of the muscle fibers and stromal edema were also observable. In the cross section (Fig. 2-B), the muscle fibers lost their polygonal appearance, and vacuolation of the sarcoplasms and atrophic sarcoplasms detached from their sacrolemmas were observed. The inflammatory cells such as polymorphs and phagocytes infiltered into the stroma in the surrounding portion of the lesions, as shown in Fig. 2-C and 2-D. With the elapse of time, the damaged fibers were clearly distinguishable from the intact fibers, and the connective tissues increased slightly in the interstitium of the muscle bundles. However, the histological findings at 5 days and 10 days after the injection of CPZ were almost the same as those at 2 days. In the case of β-CyD complex, although the separation of muscle bundles and small foci of myocyte destruction were observed, the muscle fibers had almost normal structure even in the central portion of the lesions at 2 days after the injection (Fig.2-E and 2-F). In addition, the irritating reactions produced by the complex were hardly detectable at 5 days and 10 days.

It is well known that the extent of topical tissue damage produced by a series of surface active compounds are in parallel with their *in vitro* hemolytic activities (9,10). Thus, it is reasonable to assume that the muscular tissue toxicity of amphiphatic CPZ occurs mainly through the membrane disruption of the muscular tissues. Recently, we reported that β-CyD significantly protected the human erythrocytes against the membrane disruption elicited by the penetration of CPZ molecules into the cytoplasmic leaflet of the bilayer (4). Therefore, the reduction in the muscular tissue toxicity of CPZ by β-CyD may be explained by decrease in the affinity of CPZ to the tissue

membrane. The detailed studies including the dose-response relationship are now in progress.

3.2. Effect of β-CyD on the Pharmacological Efficacy of CPZ

Since the pharmacological efficacy of phenothiazine neuroleptics are known to be related to the membrane perturbating ability of these drugs (11), there is an apprehension that β-CyD may alter the pharmacokinetic and pharmacodynamic behaviors of CPZ, which is concomitant with the reduction in the local irritancy of CPZ. Figure 3 shows the mean serum levels of CPZ following the intramuscular injection of CPZ or its

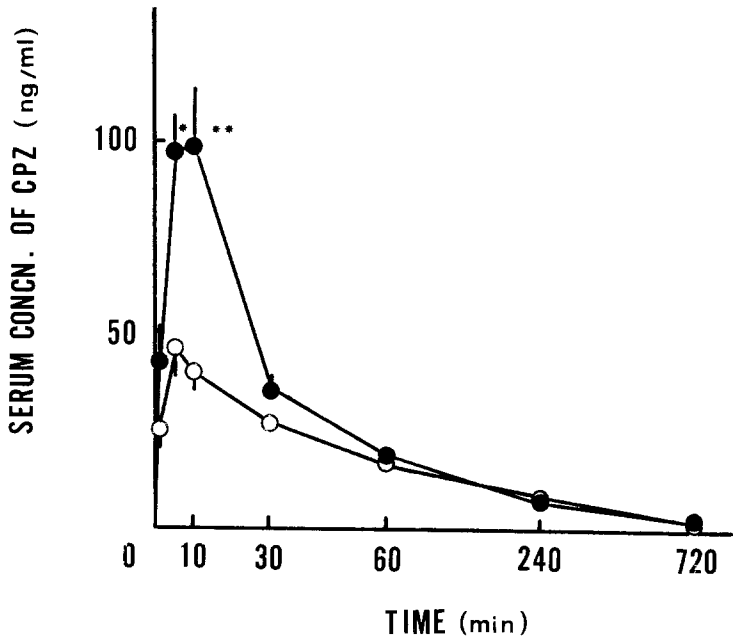

Figure 3. Mean serum levels of CPZ following the intramuscular injection of CPZ (7 μmol) to rabbits in the absence (○) and presence (●) of β-CyD (14 μmol)
Each point represents the mean ± S.E. of 5 rabbits.
*) $p < 0.01$ in (●) versus (○), **) $p < 0.05$ in (●) versus (○).

TABLE III. Bioavailability parameters of CPZ following the intramuscular injection in the absence and presence of β-CyD

System	AUC (h·ng/ml)	MRT (h)
CPZ	114 ± 13	2.8 ± 0.14
CPZ + β-CyD	127 ± 10	2.4 ± 0.15

Each point represents the mean ± S.E. of 5 rabbits.

β-CyD complex (equivalent to 7 mol CPZ) to 5 rabbits. The area under serum level-time curve (AUC) and the mean residence time of CPZ in the body (MRT) were calculated from the serum levels of CPZ up to 12 hours post administration and listed in Table III. The two statistical moments (AUC and MRT) can be representive of the extent and rate of bioavailability, respectively.(12) Although an initial increase in serum levels of CPZ was observed for β-CyD complex, both AUC and MRT of the complex were almost the same as those of CPZ alone. It is well known that both physicochemical and physiological factors affect the rate of drug absorption from the site of an intramuscular injection (13). In the case of CPZ, the strong binding to the muscular tissue (14) and the poor solubility at physiological pH may be possible determinants of the absorption rate. On the other hand, one potential determinant of β-CyD complex may be the diffusion rate through the capillary membrane. When these factors compensated each other, no appreciable difference in the serum levels between CPZ and the complex would be observed, as shown in Table III.

Furthermore, the effect of β-CyD on the CPZ-induced rectal temperature depression in rabbits was preliminarily investigated. The rabbits were placed into a constant temperature room at 25°C, and the rectal temperature was recorded up to 8 hours post administration. The temperature depression in the groups administered CPZ or its β-CyD complex (equivalent to 7 μmol CPZ) was observed immediately after the injection. The maximum response for CPZ or the complex was observed at about 2 hours after the injection, at which the extent of temperature depression for the complex was slightly larger than that for CPZ alone. However, no appreaciable difference in their hypothermic responses was observed between CPZ and complex. In addition, β-CyD did not alter the time course or the magnitude of the effects of CPZ on the central nervous system following the intramuscular (*M. biceps femoralis*) injection to rats (3).

The above results can be explained on the basis of the dissociation equilibrium of the complex in the body. When the complex is injected into the muscle, CPZ may exist predominantly as the complexed form at the injection site, owing to the large stability constant (3). This may consequently provide the reduction in the muscular tissue toxicity of CPZ. On the other hand, when the complex passes over into the systemic circulation, the equilibrium may be shifted to dissociation, because of the dilution and interference caused by biological components. In such a situation, no appreciable difference in the pharmacological efficacy between CPZ and the complex will be observed.

The present data apparently indicate that β-CyD is great utility in reducing the local tissue toxicity of CPZ without altering its pharmacological efficacy.

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