Pharmacokinetic Analysis of Chlorpromazine in Rat Serum, Cerebrospinal Fluid and Striatum

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The time courses of the total concentration of chlorpromazine (CPZ) and its S-oxide (CPZSO) in serum after i.v. administration of CPZ are described by a two compartment model and a simple metabolism model, respectively. The time course of the free CPZ concentration in serum is predicted by the correlation between the ratio of CPZSO/CPZ and the free fraction of CPZ in serum which was established in the previous study. The time course of CPZ concentration in cerebrospinal fluid (CSF) is described by a basic physiological model in which the CSF compartment is connected with the serum compartment (free drug) by the apparent diffusion clearance. Equilibrium dialysis of the striatum homogenate was carried out to clarify the CPZ disposition in the striatum. Since the binding curves of CPZ in the striatum on the Langmuir plot and the Scatchard plot were a sigmoidal and an upward curve, the time course of the drug's concentration was analyzed on a simple kinetic model designed in conformity with a blood-flow limited model. The time course was described by a simple kinetic model for up to 8 h after CPZ administration. These pharmacokinetic models for CSF and the striatum will be used to analyze the relationship between the pharmacokinetics and pharmacodynamics of CPZ.

Key words chlorpromazine; chlorpromazine metabolite; cerebrospinal fluid; striatum; pharmacokinetics

Many investigators have studied the relationship between the pharmacokinetics of chlorpromazine (CPZ) and clinical responses.¹⁾ Wode-Helgodt *et al.* studied the correlation of CPZ concentrations in cerebrospinal fluid (CSF) with the clinical response, and indicated there is a good correlation between them.²⁾ The antipsychotic effect of CPZ is believed to be closely related to the dopamine receptor blocking activity.³⁾ However, comprehensive pharmacokinetic studies of CPZ in CSF and the striatum have not been made. A previous report showed that the free fraction of CPZ in serum after CPZ i.v. administration increased with time, and that the free fraction in serum was significantly correlated with the ratio of total concentration of chlorpromazine S-oxide (CPZSO) and CPZ in serum in *in vitro* and *in vivo* studies.⁴⁾ The purpose of this investigation was to describe the pharmacokinetics of the total and free CPZ concentrations in serum using the correlation between the ratio of CPZSO/CPZ and the free fraction of CPZ in serum, and to quantitatively describe the pharmacokinetics of CPZ concentrations in CSF and the striatum using a basic physiological model and a simple kinetic model.

MATERIALS AND METHODS

Chemicals Chlorpromazine hydrochloride (CPZ, JP grade, Nakarai Chemical Co., Kyoto, Japan) was purchased commercially and CPZ metabolite standards were the kind gift of Dr. Manian at the National Institute of Mental Health (Washington, DC, U.S.A.); these metabolites were used without further purification. All other chemicals were of reagent grade and were obtained commercially (Wako Pure Chemical Industries, Osaka, Japan).

Animal Experiment on CPZ The rats used in this study were the same as those used in the previous study.⁴⁾ To characterize the disposition of CPZ and CPZ metabolites in serum, CSF and striatum, 4 mg/kg of CPZ (as free base) was injected rapidly into the right jugular vein. At 2, 5,

10, 15, 30, 45, 60, 120, 240, 360, 480, 600 and 720 min after CPZ injection, the rats were lightly anesthetized with ether and samples of CSF, blood and striatum were obtained. CSF was obtained by cisternal puncture,⁵⁾ as much blood as possible was obtained from the abdominal aorta, the entire brain was quickly excised, rinsed with cold physiological saline and dissected into corpus striatum by a modified method of Glowinski and Iversen.⁶⁾ Collected blood was centrifuged (10 min, 3000 rpm) and separated serum was used for the binding experiments. The samples of serum, CSF and striatum were stored at -80°C until analysis.

Animal Experiment on CPZSO To characterize the CPZSO disposition in serum, 2 mg/kg of CPZSO was injected rapidly into the right jugular vein. Blood samples (0.2 ml) were collected at 5, 10, 15, 30, 45, 60, 90, 120 and 180 min after the dosing from the right jugular vein cannula and the blood was replaced by injection of an equal volume of citrated blood from a donor rat. The samples of serum were stored at -80°C until analysis.

Equilibrium Dialysis for the Striatum Homogenate The protein binding of CPZ in the striatum homogenate was measured by equilibrium dialysis using the same cell and membrane as in the previous study.⁴⁾ The striatum homogenate (5%) was prepared by a polytron homogenizer (PT 10-35, Kinematica, Switzerland) at 15000 rpm for 10 s in pH 7.4 isotonic phosphate buffer. Three-tenths ml of the striatum homogenate samples and 0.3 ml of CPZ buffer solution (pH 7.4 isotonic phosphate buffer) were placed on the appropriate side of the cell and the dialysis was carried out at 37°C for 4 h with gentle mechanical agitation. The cells were protected from light by wrapping them in aluminum foil to prevent breakdown of CPZ. CPZ concentrations on both the homogenate side (CHT : $\mu\text{g/ml}$) and the buffer side (CHF : $\mu\text{g/ml}$) were determined and the percentage of the striatum unbound fraction was calculated by the following equation: $FF = CHF / (CHF + CHB \times df) \times 100$, where CHB and df are the bound con-

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centration of CPZ in the striatum homogenate ($\mu\text{g/ml}$) and the dilution factor of the striatum homogenate, respectively. The total (C_T : $\mu\text{g/g}$), free (C_F : $\mu\text{g/g}$) and bound (C_B : $\mu\text{g/g}$) concentrations of CPZ in the striatum were calculated by the following equations: $C_T = CHT \times df$, $C_F = C_T \times FF/100$ and $C_B = C_T - C_F$, respectively.

Assay Methods for CPZ and CPZ Metabolites in CSF and Striatum CPZ and CPZ metabolite concentrations in CSF and the striatum were determined by a modification of the method used in the previous study.⁴⁾ The sample of CSF and the dialysis homogenate buffer sample were collected in a glass tube containing internal standard and the weight of samples was measured in the tube. Extraction of these samples was performed by the procedure used previously.⁴⁾ Extraction of the striatum sample was done by a modification of the solid phase extraction method of Smith *et al.*⁷⁾ To the glass tube containing internal standard, 0.1 ml of the striatum homogenate sample, 0.9 ml of water and 0.05 ml of phosphoric acid (3 M) were added and mixed well. An extraction column (Analytichem International, Harbor City, CA, U.S.A.) was prepared by sequential washing with 1 ml of 0.2 M phosphoric acid, 0.25% sodium carbonate, methanol, and then water. The prepared striatum homogenate sample was pipetted into a prepared extraction column and centrifuged for 30 s at 3000 rpm. The column was then washed with 1 ml water, centrifuged briefly, washed with 0.5 ml methanol, and centrifuged again. The column was further washed with 1 ml sodium carbonate (0.25%) and centrifuged for 30 s at 3000 rpm. The analytes were eluted with two 0.2 ml aliquots of methanol, centrifuged after each addition and collected in a glass tube. To the glass tube containing the eluted sample, 0.6 ml of water and 0.2 ml of saturated sodium carbonate were added and mixed well. The mixture was extracted using procedure reported previously.⁴⁾

Pharmacokinetic Analysis A simple kinetic model was constructed to describe the time courses of the serum concentrations (total and free drug) of CPZ and CPZSO and the CSF concentration of CPZ (Fig. 1). Since a metabolism model from CPZ to CPZSO was necessary to predict the free concentration of CPZ in serum after i.v. administration of CPZ, the time courses of CPZ and CPZSO concentrations in serum after i.v. administration of these drugs were measured and these data were fitted to a two compartment model as follows:

$$\frac{dX_1}{dt} = k_{21}X_2 - (k_{12} + k_{10})X_1 \quad (1)$$

$$\frac{dX_2}{dt} = k_{12}X_1 - k_{21}X_2 \quad (2)$$

$$C_1 = X_1/V_d \quad (3)$$

where X_1 and X_2 are the amount of drug in the serum compartment and the peripheral compartment ($\mu\text{g/kg}$); k_{12} , k_{21} and k_{10} are the intercompartmental transfer rate constants (h^{-1}); C_1 is the drug concentration in the serum compartment ($\mu\text{g/l}$); and V_d is the apparent volume distribution of the drug in the serum compartment (l/kg). The computer program FKDM⁸⁾ was used for the nonlinear least squares analysis using a digital computer (PC-9821 Ae, NEC Corp., Tokyo, Japan) and the parameters

Fig. 1. Diagrammatic Representation of Pharmacokinetic Model for Metabolism, Protein Binding and Distribution of CPZ in Rat

of k_{12} , k_{21} , k_{10} and V_d for CPZ and CPZSO were estimated by this program.

Assuming that the metabolism from CPZ to CPZSO in serum after i.v. administration of CPZ can be described by linear metabolism kinetics, a simple metabolism model from CPZ to CPZSO in serum was constructed with the following equations:

$$\frac{dX_{1cp}}{dt} = k_{21cp}X_{2cp} - (k_{12cp} + k_{ecp} + k_{mcp})X_{1cp} \quad (4)$$

$$\frac{dX_{2cp}}{dt} = k_{12cp}X_{1cp} - k_{21cp}X_{2cp} \quad (5)$$

$$C_{1cp} = X_{1cp}/V_{dcp} \quad (6)$$

$$\frac{dX_{1so}}{dt} = k_{mcp}X_{1cp} + k_{21so}X_{2so} - (k_{12so} + k_{10so})X_{1so} \quad (7)$$

$$\frac{dX_{2so}}{dt} = k_{12so}X_{1so} - k_{21so}X_{2so} \quad (8)$$

$$C_{1so} = X_{1so}/V_{dso} \quad (9)$$

where X_{1cp} , X_{2cp} , X_{1so} and X_{2so} are the amount of CPZ and CPZSO in the serum compartment and the peripheral compartment ($\mu\text{g/kg}$), respectively; k_{12cp} , k_{12so} , k_{21cp} , k_{21so} , k_{ecp} and k_{10so} are the intercompartmental transfer rate constants (h^{-1}); k_{mcp} is the metabolism rate constant from CPZ to CPZSO (h^{-1}); C_{1cp} and C_{1so} are the CPZ and CPZSO concentrations in the serum compartment ($\mu\text{g/l}$), respectively; and V_{dcp} and V_{dso} are the apparent volume distribution of the CPZ and CPZSO in the serum compartment (l/kg), respectively. The values of the parameters of k_{12cp} , k_{21cp} , k_{10cp} , V_{dcp} and V_{dso} were set as those obtained by Eqs. 1, 2 and 3. The values of the parameters of k_{mcp} , k_{12so} , k_{21so} , and k_{10so} were estimated by the nonlinear least squares regression program, FKDM, using the initial values of parameters obtained by Eqs. 1, 2 and 3, and the value of the parameter of k_{ecp} was obtained by the following equation: $k_{ecp} = k_{10cp} - k_{mcp}$.

The correlation between the ratio of CPZSO/CPZ and the free fraction of CPZ in serum which was established in the previous report⁴⁾ was used to predict the values of the free concentrations of CPZ in serum after i.v. ad-

ministration of CPZ. These procedures are expressed as the following equations:

$$R_{s/c} = C_{1so}/C_{1cp} \quad (10)$$

$$FF_{cp} = SR_{s/c} + I \quad (11)$$

$$fC_{1cp} = FF_{cp} C_{1cp} \quad (12)$$

where $R_{s/c}$ is the ratio of CPZSO/CPZ in serum; FF_{cp} is the free fraction of CPZ in serum; S and I are the slope and the intercept of the relationship between the ratio of CPZSO/CPZ and the free fraction of CPZ; and fC_{1cp} is the free concentration of CPZ in serum. The values of the parameters of S and I were set at the values obtained in the previous study.⁴⁾

The time course of CPZ concentration in CSF was analyzed on a basic physiological model in which the CSF compartment is connected with the serum compartment (free drug) by the apparent diffusion clearance. The time course of the CPZ concentrations in CSF could be expressed as follows:

$$V_{CSF} \frac{dC_{CSF}}{dt} = CL_{in} fC_{1cp} - CL_{out} C_{CSF} \quad (13)$$

where V_{CSF} is the volume of the CSF compartment; C_{CSF} is the CPZ concentration in CSF ($\mu\text{g/l}$); CL_{in} is the apparent diffusion clearance from serum to CSF including the bulk flow rate of CSF (Q_{CSF}) and the diffusion clearance of drug between serum and CSF (PA); and CL_{out} is the apparent diffusion clearance from CSF to serum including the bulk flow rate of CSF (Q_{CSF}), the diffusion clearance of drug between serum and CSF (PA) and the efflux clearance from CSF to serum (CL_{eff}).⁹⁾ The parameters of CL_{in} and CL_{out} were estimated as the k_{in} and k_{out} which are the apparent diffusion constants from serum to CSF and from CSF to serum ($k_{in} = CL_{in}/V_{CSF}$, $k_{out} = CL_{out}/V_{CSF}$; h^{-1}) using the nonlinear least squares regression program, FKDM.

sion program, FKDM.

The time course of CPZ concentration in the striatum was analyzed on a simple kinetic model designed in conformity with the blood-flow limited model in accordance with the previous report.¹⁰⁾ The changes in the concentration of CPZ in serum and the striatum were expressed as follows:

$$C_{SR} = A \exp(-\alpha t) + B \exp(-\beta t) + C \exp(-\gamma t) \quad (14)$$

$$V_{BR} \frac{dC_{BR}}{dt} = Q_{BR}(C_{SR} - C_{BR}/K_p) \quad (15)$$

where C_{SR} is the serum concentration of CPZ ($\mu\text{g/l}$) at time t ; A , B , C , α , β and γ are constants ($\mu\text{g/l}$ and h^{-1}); V_{BR} is the brain weight (g); and C_{BR} represents the concentrations of CPZ in the striatum ($\mu\text{g/l}$). Q_{BR} is the serum flow rate to the brain (l/h), K_p is a partition coefficient relating the drug concentration in the striatum to the drug concentration in the serum at equilibrium. The value of the parameter of the initial CPZ concentration in serum (C_0) was fixed according to the CPZ dose (4 mg/kg) and the physiological value of the serum compartment (V_{SR} : $V_{SR} = 44 \times (\text{body weight})^{0.99}$)¹¹⁾: $C_0 = \text{dose}/V_{SR}$. The parameters of V_{BR} and Q_{BR} were also set at the literature values,¹²⁾ and the value of the serum flow rate constant to the brain (Q_{BR} : $Q_{BR} = Q_{BR}/V_{BR}$, h^{-1}) was used in this analysis. The values of the parameters of A , B , C ($C_0 = A + B + C$), α , β , γ and K_p were estimated by the nonlinear least squares regression program, FKDM.

RESULTS AND DISCUSSION

Disposition of CPZ and CPZSO in Serum CPZ is rapidly metabolized in mammals, and there are a large number of CPZ metabolites after the drug's administration.¹³⁾ It is well known that S-oxidation is a major route

Fig. 2. Time Courses of CPZ and CPZSO Concentrations in Serum after Intravenous Administration of CPZ and CPZSO

(a) CPZ 4 mg/kg ($n=3$); (b) CPZSO 2 mg/kg ($n=4$). The plotted points represent the observed data and the solid lines represent the calculated values using Eqs. 1, 2 and 3 in the text. Each experimental point is shown as mean $\pm$ S.D.

for CPZ in mammals. The previous study showed that the alteration of the free fraction of CPZ after i.v. administration of CPZ is closely related to the disposition of CPZSO.⁴⁾ Therefore, to estimate the parameters for the CPZSO disposition in serum, the time course of the CPZSO concentration in serum after its i.v. administration was measured. The time course of total CPZSO concentrations in serum after i.v. administration of 2 mg/kg of CPZSO in rats is shown in Fig. 2b, and Fig. 2a shows the time course of total CPZ concentration in serum after i.v. administration of 4 mg/kg of CPZ. The total concentrations of CPZ and CPZSO in serum declined biexponentially with time. The solid lines in Fig. 2 represent the calculated values obtained by the two compartment model (Eqs. 1, 2 and 3). The time courses of CPZ and CPZSO concentrations in serum after i.v. administration of those drugs were described quantitatively by this model. The pharmacokinetic parameters were computed by the nonlinear least squares method and are listed in Table 1.

Total Concentrations of CPZ and CPZSO and Free Concentrations of CPZ in Serum The time courses of total concentrations of CPZ and CPZSO and free concentrations of CPZ in serum after i.v. administration of

Table 1. Values of Pharmacokinetic Parameters of CPZ and CPZSO in Rats Obtained from Computer Fitting of Serum Concentration Data after Intravenous Administration of CPZ 4 mg/kg and CPZSO 2 mg/kg

Characteristics	CPZ	CPZSO
k_{12} (h^{-1})	5.538 ± 3.273	4.049 ± 1.640
k_{21} (h^{-1})	2.653 ± 0.928	4.464 ± 1.008
k_{10} (h^{-1})	1.374 ± 0.408	1.192 ± 0.175
V_d (l/kg)	3.274 ± 1.013	4.073 ± 0.573

The pharmacokinetic parameters of CPZ and CPZSO in serum were estimated by fitting the data to Eqs. 1, 2 and 3, using the computer program FKDM. All values are expressed as the mean $\pm$ S.D. of the estimated parameter.

4 mg/kg of CPZ in rats are shown in Fig. 3. The total CPZSO concentration in serum declined monoexponentially with the peak concentration occurring at 1 h after i.v. administration of CPZ. The time course of free CPZ concentrations in serum did not parallel that of the total CPZ concentration. The previous study showed that the relationship between the ratio of CPZSO/CPZ and the free fraction of CPZ was in good correlation in *in vivo* studies, and there was no statistically significant difference between the population regression coefficients of the *in vitro* and *in vivo* studies; the values of the slope and the intercept were almost the same as values in the *in vivo* studies when combined with CPZNO, and the influence of CPZNO on this correlation was very small.⁴⁾ In the current study, therefore, the free concentration of CPZ in serum after i.v. administration of CPZ was predicted using this correlation. The dotted lines in Fig. 3 represent the predicted values. The results indicated that the time course of CPZSO concentrations in serum after i.v. administration of CPZ can be described quantitatively by the simple metabolism model ($k_{\text{mcp}} = 0.733 \pm 0.044 \text{ h}^{-1}$); however, the predicted values of the free CPZ concentrations in serum could not be described by the observed data. The characteristic of the protein binding of CPZSO was earlier found not to be stable and the free fraction of CPZSO fluctuated from 11% to 57%.⁴⁾ Therefore, in this analysis, assuming that there is difference between the disposition of CPZSO after its i.v. administration and its disposition after i.v. administration of CPZ, the values of parameters for this disposition were reestimated by the nonlinear least squares program. The solid lines in Fig. 3 represent the predicted values obtained using Eqs. 4 to 12. The predicted values of the free CPZ concentrations in serum described the observed data reasonably well. Thus, the free CPZ concentrations in serum after i.v. administration of CPZ can be predicted using the correlation between the ratio

Fig. 3. Time Course of CPZ and CPZSO Concentrations in Serum and CSF after Intravenous Administration of CPZ

(a) Total (●) and free (○) concentration of CPZ in serum; (b) total CPZSO concentration in serum (●) and CPZ concentration in CSF (○). The plotted points represent the observed data and the solid lines represent the calculated values using from Eqs. 4 to 13 in the text. Each experimental point is shown as mean $\pm$ S.D. ($n=3$).

of CPZSO/CPZ and the free fraction of CPZ in serum. The pharmacokinetic parameters for CPZ and CPZSO are listed in Table 2.

CPZ Concentration in CSF The time course of CPZ concentration in CSF after the drug's i.v. administration is shown in Fig 3b. The CPZ metabolites were not detected

Table 2. Values of Pharmacokinetic Parameters of CPZ and CPZSO in Rats Obtained from Computer Fitting of Serum and CSF Concentration Data after Intravenous Administration of 4 mg/kg of CPZ

Characteristics	
k_{12cp} (h^{-1})	5.538 ^{a)}
k_{21cp} (h^{-1})	2.653 ^{a)}
V_{dcp} (l/kg)	3.274 ^{a)}
k_{mcp} (h^{-1})	1.221 ± 0.177 ^{b)}
k_{ecp} (h^{-1})	0.153 ^{c)}
k_{12so} (h^{-1})	2.698 ± 1.029 ^{b)}
k_{21so} (h^{-1})	0.837 ± 0.254 ^{b)}
k_{10so} (h^{-1})	2.239 ± 0.345 ^{b)}
V_{dso} (l/kg)	4.073 ^{a)}
S	6.517 ^{d)}
I	0.675 ^{d)}
k_{in} (h^{-1})	10.558 ± 2.120 ^{e)}
k_{out} (h^{-1})	12.982 ± 2.963 ^{e)}
V_{CSF} (ml)	0.15 ^{f)}
Q_{CSF} (ml/h)	0.132 ^{f)}
CL_{in} (ml/h)	1.584 ^{g)}
CL_{out} (ml/h)	1.947 ^{g)}
PA (ml/h)	1.452 ^{g)}
CL_{eff} (ml/h)	0.363 ^{g)}

a) The values of these parameters were set at the estimated values in Table 1. b) The values of these parameters were estimated by fitting the data from Eqs. 4 to 9, using the computer program FKDM. All values are expressed as the mean $\pm$ S.D. of the estimated parameter. c) The value of this parameter was calculated by the following equation: $k_{ecp} = k_{10cp} - k_{mcp}$. d) The values of these parameters were set at the estimated values in the previous report.⁴⁾ e) The values of these parameters were estimated by fitting the data to Eq. 13 using the computer program FKDM. All values are expressed as the mean $\pm$ S.D. of the estimated parameter. f) The values of these parameters were those in the literature.^{9d)} g) The values of these parameters were calculated by the following equations: $CL_{in} = k_{in} \times V_{CSF}$, $CL_{out} = k_{out} \times V_{CSF}$, $PA = CL_{in} - Q_{CSF}$, $CL_{eff} = CL_{out} - PA - Q_{CSF}$.

in CSF. The CPZ concentrations in CSF were almost the same as the free concentrations of CPZ in serum, and declined monoexponentially with the peak concentration occurring at 30 min. Several kinetic models have been used to explain the disposition of drugs in CSF.⁹⁾ The basic physiological model of CPZ for CSF was constructed in conformity with these pharmacokinetic models. The time course of CPZ concentration in CSF was analyzed on the basic physiological model in which the CSF compartment is connected with the serum compartment (free drug) by the apparent diffusion clearance, Eq. 13. The solid line in Fig. 3b represents the calculated values of the CPZ concentrations in CSF, and indicates that the time course of CPZ concentration in CSF can be described by the basic physiological model. The pharmacokinetic parameters (k_{in} and k_{out}) were computed by the nonlinear least squares method and other parameters (CL_{in} , CL_{out} , Q_{CSF} , PA and CL_{eff}) were calculated using the literature values^{9d)}; these parameters are listed in Table 2.

CPZ and CPZ Metabolites Concentrations in the Striatum The time courses of CPZ, CPZSO and desmethylchlorpromazine (nor1-CPZ) concentrations in the striatum after i.v. administration of CPZ are shown in Fig. 4. The CPZ concentration in the striatum was 20 times higher than the total concentrations of CPZ in serum. The CPZSO and nor1-CPZ concentrations in the striatum were very low compared with the CPZ concentrations in the striatum, and the chlorpromazine *N*-oxide (CPZNO) and chlorpromazine sulfone (sul-CPZ) were not detectable in the striatum. It is well known that 7-hydroxychlorpromazine (7-OH-CPZ) and nor1-CPZ have antischizophrenic activity¹⁴⁾ and dopamine receptor blocking activity.¹⁵⁾ The concentration of 7-OH-CPZ in serum after i.v. administration of CPZ was found to be very low, less than 50 ng/ml.⁴⁾ In this study, the nor1-CPZ concentrations were very low compared with the CPZ concentration in the striatum after

Fig. 4. Time Course of CPZ, CPZSO and nor1-CPZ Concentrations in Striatum after Intravenous Administration of CPZ

(a) Total CPZ concentration in striatum (●) and serum (○), (b) CPZSO (●) and nor1-CPZ (○) concentration in striatum. The plotted points represent the observed data and the solid lines represent the calculated values using Eqs. 14 and 15 in the text. Each experimental point is shown as mean $\pm$ S.D. ($n=3$).

CPZ i.v. administration. These results seem to indicate that there is little possibility of an effect by 7-OH-CPZ or nor1-CPZ on the pharmacological response after i.v. administration of CPZ in rats.

The time courses of CPZ concentrations in serum and the striatum were analyzed on a simple kinetic model designed in conformity with the blood-flow limited model,¹⁰⁾ Eqs. 14 and 15. The solid line in Fig. 4 represents the calculated values of the CPZ concentrations in serum and the striatum. The time course of the concentration in the striatum could be described by the simple kinetic model for up to 8 h after CPZ administration. The pharmacokinetic parameters (A , B , C , α , β , γ and K_p) were computed by the nonlinear least squares method and the physiological parameters (V_{SR} , V_{BR} and Q_{BR}) were fixed literature values^{11,12)}; these parameters are listed in Table 3. CPZ

Table 3. Values of Pharmacokinetic Parameters of CPZ in Rats Obtained from Computer Fitting of Serum and Striatum Concentration Data after Intravenous Administration of CPZ 4 mg/kg

Characteristics		
C_0	($\mu\text{g/ml}$)	89.71 ^{a)}
A	($\mu\text{g/ml}$)	$0.402 \pm 0.079^b)$
B	($\mu\text{g/ml}$)	$0.155 \pm 0.049^b)$
C	($\mu\text{g/ml}$)	89.15 ^{c)}
α	(h^{-1})	$1.246 \pm 0.389^b)$
β	(h^{-1})	$0.311 \pm 0.037^b)$
γ	(h^{-1})	$156.62 \pm 10.83^b)$
Q_{BR}	(h^{-1})	37.2 ^{d)}
K_p		$22.336 \pm 1.786^b)$

a) The value of this parameter was calculated by the following equation: $C_0 = \text{dose}/V_{SR}$; dose = 1038 μg (rat body weight = 259.5 ± 19.90 g ($n=39$)), $V_{SR} = 11.57$ ml ($V_{SR} = 44 \times (\text{body weight})^{0.99}$).¹¹⁾ b) The values of these parameters were estimated by fitting the data to Eqs. 14 and 15, using the computer program FKDM. All values are expressed as the mean $\pm$ S.D. of the estimated parameter. c) The value of this parameter was calculated by the following equation: $C = C_0 - A - B$. d) These parameters were calculated by the literature values: $Q_{BR} = Q_{BR}/V_{BR}$; $Q_{BR} = 64.728$ ml/h, $V_{BR} = 1.74$ ml.¹²⁾

concentrations in the striatum at 10 h and 12 h after i.v. administration of the drug, however, could not be described by the simple kinetic model. Moreover, the three compartment model which included the physiological value of the serum volume for the CPZ disposition in serum was needed to predict the time course of CPZ concentration in the striatum. To explore the possibility of another model for the prediction of the time course of CPZ concentration in the striatum, equilibrium dialysis for the striatum homogenate (5%) was carried out using the *in vivo* samples of the striatum. Figure 5 shows the time courses of total and free CPZ concentrations and the free fraction of CPZ in the striatum after i.v. administration of CPZ. The time course of free CPZ concentration in the striatum did not parallel that of the total CPZ concentration, and the free fraction of CPZ in the striatum increased with time. These results were similar to those on CPZ disposition in serum. Characteristics of the CPZ protein binding on the rat striatum were determined using the Langmuir plot and the Scatchard plot of CPZ and these results are shown in Fig. 6. The binding curves of CPZ in the striatum on the Langmuir and the Scatchard plots were a sigmoidal curve and an upward curve, respectively. The results indicated that analysis of the CPZ disposition in the striatum is very difficult using Hill's equation or the competitive inhibition equation on the multiple binding sites for a drug. Therefore, in this analysis, the time course of CPZ concentration in the striatum was analyzed on a simple kinetic model designed in conformity with the blood-flow limited model. The time course of CPZ concentration in the striatum was described by the simple kinetic model for up to 8 h after CPZ administration.

In conclusion, calculations of CPZ concentrations in serum (total and free drug), CSF and the striatum after i.v. administration of CPZ were made using the simple

Fig. 5. Time Course of Total and Free CPZ Concentration and Free Fraction of CPZ in Striatum after Intravenous Administration of CPZ

(a) Total (●) and free (○) CPZ concentration in striatum; (b) free fraction of CPZ in striatum. The plotted points represent the observed data. Each experimental point is shown as mean $\pm$ S.D. ($n=3$).

Fig. 6. Langmuir Plot and Scatchard Plot of CPZ Protein Binding on Rat Striatum *in Vivo*

(a) The Langmuir plot of CPZ; (b) the Scatchard plot of CPZ. Each experimental point is shown as mean $\pm$ S.D. ($n=3$).

kinetic model. The time course of free CPZ concentration in serum was predicted by the correlation between the ratio of CPZSO/CPZ and the free fraction of CPZ in serum. The results indicated that the correlation between these two factors is very useful for predicting the free CPZ concentration in serum after CPZ administration. The time courses of CPZ concentration in CSF and the striatum were well explained by the basic physiological models. These pharmacokinetic models for CPZ will be used to analyze the relationship between the pharmacokinetics and pharmacodynamics of CPZ.

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