Effect of Chlorpromazine on the Pharmacokinetics and Pharmacodynamics of Pentobarbital in Rats

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The effects of chlorpromazine (4 mg/kg i.v.) on the disposition and duration of loss of the righting reflex (LRR, sleeping time) produced by intravenous pentobarbital (5 to 50 mg/kg) were studied in rats. The plasma concentration time profile following i.v. administration of pentobarbital alone was reasonably well described by a three compartment open model with Michaelis-Menten type elimination kinetics. The brain to plasma concentration ratio of pentobarbital was 1.5 and was almost constant during the experiment. Co-administration of chlorpromazine significantly reduced the systemic clearance of pentobarbital. Since pentobarbital is eliminated from the body mainly by hepatic metabolism, reduction of systemic clearance reflects the reduction of hepatic metabolism of pentobarbital. The hepatic intrinsic clearance of pentobarbital was decreased from 0.438 to 0.331 1/h by chlorpromazine co-administration. Hepatic blood flow was also decreased significantly, whereas the plasma protein binding and the distribution to the red blood cell were not appreciably altered. The profile of duration of LRR versus the logarithm of the dose of pentobarbital was linear over a 20 to 70 mg/kg dose range irrespective of chlorpromazine co-administration. The awakening plasma and brain concentrations of pentobarbital without chlorpromazine were estimated as 12.4 μg/ml and 17.8 μg/g, respectively. The sleeping time versus the logarithm of pentobarbital dose under chlorpromazine co-administration was shifted to the left and the slope of the linear portion was also decreased. There was no single value of awakening plasma or brain concentration. Plasma concentration at the end of the action decreased with decreasing dose. These facts indicated that the sensitivity of the central nervous system to pentobarbital might be increased by chlorpromazine. In conclusion, chlorpromazine inhibited the hepatic metabolism of pentobarbital, resulting in significant increases in plasma and brain concentrations. However, this pharmacokinetic change could not fully explain the pharmacodynamic alternation.

Keywords — pentobarbital; chlorpromazine; pharmacokinetics; pharmacodynamics; loss of righting reflex; sleeping time; hepatic intrinsic clearance; hepatic blood flow

Introduction

Pentobarbital (PB), which is known as one of the short acting barbiturates, is widely used as a sedative, hypnotic and analgesic and exerts its activity by depressing the central nervous system (CNS), especially the cerebral cortex region. The basic distribution, metabolism and pharmacokinetics of barbiturates, including PB, have been extensively presented in the literature. In recent years it has become generally recognized that the sleeping time induced by barbiturates is closely related to brain, cerebrospinal fluid (CSF) or plasma concentrations. Chlorpromazine (CPZ), which is also known as one of the phenothiazine derivatives of neuroleptics, has a wide range of activity arising from its depressant action on the autonomic nerve center of the hypothalamus and is often used concomitantly with barbiturates in the fields of anesthesiology and psychiatry.

It has been reported that certain phenothiazines including CPZ can markedly prolong the sleeping time induced by barbiturates. Although much effort has been directed to the investigation of pharmacodynamic and pharmacokinetic interaction between barbiturates and phenothiazines, almost all of these reports failed to determine the precise mechanisms responsible for the altered pharmacologic response i.e. pharmacokinetic, pharmacodynamic or both. The purpose of this investigation was to clarify

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the mechanism of the effect of CPZ on the pharmacokinetics of PB and to analyze quantitatively the effect of CPZ on the relationship between drug concentration and sleeping time induced by PB in rats.

**Materials and Methods**

**Chemicals** — PB (Tokyo Kasei Co., Ltd., Tokyo, Japan) and CPZ (Nakarai Chemical Co., Kyoto, Japan) were purchased commercially and used without further purification. These drugs were dissolved in isotonic sodium chloride solution (JP XI, Otsuka Pharmaceuticals, Tokyo, Japan) and were administered intravenously. All other chemicals were of reagent grade and were obtained commercially (Nakarai Chemicals Co., Kyoto, Japan).

**Animal Experiments** — Seven week-old male Wistar rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used. The rats were allowed free access to water and laboratory rat chow (CE-10, Nippon Clea Co., Tokyo, Japan) and were housed in a room with a 12 h light–12 h dark cycle for at least 2 weeks before the day of the experiment. Two days before the experiment, rats were cannulated in the right jugular vein for blood sampling and drug administration under light ether anesthesia. The catheter (Silastic Medical Grade Tube, Dow Corning Corp., Midland, MI and Intramedic PE-50, Clay Adams, Parsippany, NJ) was exteriorized at the back of the neck, filled with heparinized saline and plugged with a stainless-steel pin. After 19 h fasting, the rats were placed in individual metabolic cages (Type: TP-85M, Toyo Riko Co., Tokyo, Japan) and PB, CPZ, and/or PB+CPZ were administered intravenously. The blood samples were collected just prior to the drug administration (blank) and at 1, 2, 3, 5, 15, 30, 60, 120, 180 and 240 min after the dosing. The plasma sample was obtained immediately after collection by centrifuging at 10000 rpm for 2 min. The spontaneous urine excreted within 24 h after the dosing was also collected as the urine sample. The plasma and urine samples were stored at —20 °C until analysis. In a different experiment, at 1, 2, 3, 5, 15, 30, 60, 120, 180 and 240 min after the drug administration, the animals were sacrificed by decapitation and their whole brains were quickly excised, rinsed with cold physiological saline and blotted. The brain samples were also stored at —20 °C until analysis.

**Assay Methods for PB** — The plasma, brain and urine concentrations of PB were determined by the method of Martin and Driscoll with a minor modification as follows: 100 μl of plasma or urine were placed in a test tube and diluted with 200 μl of distilled water containing barbital as the internal standard. Adding 0.5 g of NaH₂PO₄, the mixture was extracted with 7 ml of ether by mechanical shaking for 15 min. After centrifugation (3000 rpm for 10 min, at 15 °C), a 4 ml sample of the ether was transferred to another test tube and evaporated to dryness at 50 °C. The residue was dissolved in 10% water methanol solution which was saturated previously with potassium carbonate. After adding 100 μl of dimethylsulfoxide, the mixture was refluxed at 60 °C for 4 min. The methanol was removed by evaporation (80 °C), the remaining material re-extracted into 100 μl of heptane and 2 μl were injected into a gas chromatograph. The brain sample was homogenized with a 3-fold weight of physiological saline containing an internal standard in a glass Potter-Elvehjem tissue grinder. The homogenate was acidified by adding 400 μl of 1 N HCl and was extracted into 10 ml of ether by mechanical shaking for 30 min. A 7 ml sample of ether extract was transferred into a test tube containing 4 ml of 1 N NaOH and shaken for 3 min. After centrifugation, a 3 ml sample of NaOH layer was transferred into another test tube containing 0.8 ml of 6 N HCl and the mixture was extracted with 5 ml of ether. A 4 ml sample of the ether extract was assayed as described above. A shimadzu Model GC-7AP gas chromatograph equipped with a nitrogen detector (FTD-7, Shimadzu Ltd., Kyoto, Japan) was used. The column was a 2 m by 3 mm (i.d.) glass tube containing a packing of 1.5% silicone SE-30 on chromosorb W (AW-DMCS), 80–100 mesh (Wako Pure Chemical Ind., Ltd., Osaka, Japan). Chromatographic conditions were as follows: column temperature 170 °C; injection port temperature 250 °C; carrier gas (nitrogen) flow rate 40
ml/min; hydrogen flow rate 10 ml/min; air flow rate 250 ml/min.

**Analytical Determination of 3'-Hydroxy-pentobarbital (3'-HP)** — Plasma 3'-HP level was determined by high performance liquid chromatography (HPLC) according to the method of Cary and Pape.6) A Shimadzu model LC-6A liquid chromatograph equipped with a variable wavelength ultraviolet (UV) detector (Shimadzu Ltd., Kyoto, Japan) was used. HPLC was performed on a column (150 x 3.5 mm i.d.) packed with Licrosorb RP-18 (particle size: 5 μ, E. Merk, Darmstadt, West Germany) with tetrahydrofuran: H₂O (5:95) as the mobile phase at a flow rate of 1 ml/min and the column temperature of 50°C. 3'-HP was detected at 215 nm against a peak area of hydroxymethyl tolbutamide, as the internal standard, which was synthesized by the method of Shibasaki et al.7)

**Determination of Plasma Protein Binding of PB** — (1) Equilibrium Dialysis: Pooled plasma from 3 to 4 rats was used to determine the plasma unbound fraction (fₚ) of PB by equilibrium dialysis using a semi-micro Plexiglas cell (surface area: 706.5 mm²) and a semi-permeable cellulose membrane (36/32, Union Carbide Corp., Chicago, Ill). One ml of plasma and 1 ml of PB buffer solution (pH 7.4 isotonic phosphate buffer) were placed in appropriate side of the cell, and the dialysis was carried out at 37°C for 8 h with gentle mechanical agitation (50 strokes/min). PB concentration of the plasma side (Cₚ) as well as the buffer side (Cₚᵣ) was determined and the fₚ value was calculated (fₚ = Cₚᵣ/Cₚ).

(2) Ultrafiltration: The in vivo plasma protein binding of PB was also determined with the Amicon Centriflo System (CF25, Amicon Corp., Danvers, Mass). After administration of PB to a rat, a 2.5 ml blood sample was withdrawn and the plasma separated by centrifugation. Before ultrafiltration, the total plasma concentration of PB and the plasma protein concentration were determined. The ultrafiltration was carried out at 3°C, 1000 x g for 15 min, under a 5% CO₂ in 95% O₂ atmosphere, and the plasma free concentration (Cₚᵣ) was determined. Plasma protein concentration was determined by the Biuret’s method (A/G B-test Wako, Wako Pure Chemical Ind., Co., Osaka, Japan).

**Determination of Red Blood Cell-Plasma Distribution Coefficient (D)** — After administration of PB to a rat, the blood concentration (Cₜₕ), plasma concentration (Cₚ) and the hematocrit value (Hct) were measured. The free fraction of PB in the blood (fₚ) was calculated by following equation.

\[ fₚ = \frac{fₚ Cₚ}{Cₜₕ} \]  

The red blood cell (RBC)-to-plasma distribution coefficient was calculated by the following equations:

\[ D = \frac{Cₜₕ}{Cₚ fₚ} \]  

\[ D = \frac{l}{fₚ} \left( \frac{Cₜₕ}{Cₚ Hct} - \frac{1}{Hct} + 1 \right) \]

where Cₜₕ is the concentration of PB in RBC and Cₚ fₚ is the free concentration of PB in plasma.

**Measurement of Hepatic Blood Flow** — The hepatic blood flow in rats was determined by the sulfobromophthalein (BSP) clearance method of Denis et al. 9) Briefly, under light ether anesthesia, the rat was cannulated in the right jugular vein (PE-50 and Silastic Medical Grade Tube), in the hepatic vein (PE-10) and in the left carotid artery (PE-50). The cannulas were exteriorized to the back of the neck and all the incisions were sutured by a surgical thread. After recovery from the anesthesia (at least 2 h after the surgery) BSP was administered by an i.v. bolus injection (7.5 mg/kg) followed by a constant infusion (1.5 mg/min/kg). After reaching the steady-state level of BSP, PB, CPZ or physiological saline (control) was injected intravenously. The arterial blood samples as well as the hepatic venous blood samples were withdrawn at 30, 60, 120, 180 and 240 min after the injection. Plasma BSP concentrations were determined spectrophotometrically as follows: One hundred μl of plasma were placed in a test tube, 50 μl of 3.5 N NaOH and 1.5 ml of 1.5 M phosphate buffer (pH 6.7)
were added and the optical density (580 nm) was recorded using an UV-visible recording spectrophotometer (UV-240, Shimadzu, Kyoto, Japan). The hepatic blood flow (HBF) was calculated by the plasma concentration of BSP in the arterial blood ($C_a$), the plasma concentration of BSP in the hepatic venous blood ($C_h$), the hematocrit value of the arterial blood (Hct) and by the BSP infusion rate ($Q$), using the following equations:

$$\text{HBF} = \frac{\text{Cl}}{E} (1 - \text{Hct})$$ \hspace{1cm} (4)

$$\text{Cl} = \frac{Q}{C_a}$$ \hspace{1cm} (5)

$$E = \frac{(C_a - C_h)}{C_a}$$ \hspace{1cm} (6)

where Cl is the hepatic clearance and $E$ is the hepatic extraction ratio of BSP.

**Determination of Sleeping Time** — After i.v. administration of PB, CPZ or both, the rats were placed on their backs and closely observed until they spontaneously righted themselves, the righting reflex (LRR). This was taken as sleeping time. All of the experiments were started at 11 a.m. to minimize the diurnal variation\(^{10}\) and the ambient temperature was maintained at 23 °C.

**Statistics** — The difference between the various experimental conditions were compared using the paired Student’s t-test. The 0.05 level of probability was used as the level of significance.

**Computer Programs for the Estimation of Pharmacokinetic Parameters** — The concentration-time or amount-time data were analyzed by a non-linear regression program based on the algorithm of Gauss–Newton and Berman\(^{11}\) using a PDP11/34 mini-computer (Digital Equipment Corp., Mass.). The inverse value of each data was used as the weighting value of the least squares method. Convergence was assumed complete when the iteration for the relative change in the sum of weighted squares was less than $10^{-6}$.\(^{12}\)

**Results and Discussion**

(1) Disposition of PB

The time courses of plasma and brain concentration of PB after i.v. administration (5, 20, 50 mg/kg) are shown in Fig. 1, as the semilogarithmic plots. The plasma concentrations of PB showed a multi-exponential decline for each dose, however, the slope of the terminal phase of 50 mg/kg dose was significantly smaller than that of the lower dosage. The brain concentration was eliminated in parallel to the corresponding plasma concentration. The brain to the plasma ratio was 1.5 and was almost constant, except during the first few minutes. Statistical moment analysis\(^{13}\) was applied to the plasma concentrations of PB at each dose and the systemic clearance (Cl) and the distribution volume at steady-state ($V_{dss}$) were calculated according to the following equations.

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} = \frac{\int_0^\infty C_p \, dt}{\int_0^\infty C_p \, dt}$$ \hspace{1cm} (7)

$$\text{Cl} = \frac{X_0}{\text{AUC}}$$ \hspace{1cm} (8)

$$V_{dss} = \text{Cl} \times \text{MRT} = \frac{X_0 \times \text{AUMC}}{(\text{AUC})^2}$$ \hspace{1cm} (9)

Where $C_p$ is the plasma concentration of PB, MRT is the mean residence time, AUC is the area under the plasma concentration–time curve, AUMC is the area under the first moment curve and $X_0$ is the dose of PB. The results are listed in Table I. Although the values of $V_{dss}$ were similar for among the doses used, the systemic clearance showed a significant dose dependency. This fact indicated that there was a non-linear kinetics in the elimination phase but not in the distribution phase of PB. Accordingly, the plasma concentrations and the brain concentrations for all doses were fitted simultaneously to a three compartment model with a Michaelis–Menten elimination kinetics as shown in Fig. 2. The brain concentration of PB was calculated according to the conventional blood flow rate limited model.\(^{14}\)
Fig. 1. Plasma and Brain Concentrations of PB after Intravenous Administration of PB and CPZ.
Each value of the data is shown as the mean ± S.E.M. The lines in the figure represent nonlinear least-squares fit of data to a three compartment open model with Michaelis-Menten elimination kinetics shown in Eqs. 10-13 in the text.

- ■ - PB 50 mg/kg (n = 3); - △ - PB 20 mg/kg (n = 5); - ● - PB 5 mg/kg (n = 5); - △ - PB 20 mg/kg + CPZ 4 mg/kg (n = 5); - ○ - PB 5 mg/kg + CPZ 4 mg/kg (n = 5).

<table>
<thead>
<tr>
<th>Dose of PB (mg/kg)</th>
<th>5</th>
<th>20</th>
<th>50</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of CPZ (mg/kg)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>$V_{d0}$ (1/100 g b.w.)</td>
<td>0.154±0.027 (5)</td>
<td>0.120±0.015 (5)</td>
<td>0.134±0.017 (3)</td>
<td>0.120±0.009 (5)</td>
</tr>
<tr>
<td>Cl (l/h/100 g b.w.)</td>
<td>0.126±0.023 (5)</td>
<td>0.081±0.012 (5)</td>
<td>0.045±0.016 (3)</td>
<td>0.058±0.014 (5)</td>
</tr>
<tr>
<td>$f_P^{b)}$</td>
<td>0.483±0.031 (12)</td>
<td>0.433±0.027 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$f_B^{b)}$</td>
<td>0.440±0.013 (12)</td>
<td>0.396±0.004 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct $^{b)}$</td>
<td>0.400±0.014 (9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBF $^{c)}$ (l/h/100 g b.w.)</td>
<td>0.390±0.047</td>
<td>0.407±0.020</td>
<td>0.442±0.035</td>
<td>0.354±0.050</td>
</tr>
<tr>
<td>Cl$_{HI}^{d)}$ (l/h/100 g b.w.)</td>
<td>0.404</td>
<td>0.223</td>
<td>0.110</td>
<td>0.143</td>
</tr>
</tbody>
</table>

$a)$ Each value of the data is shown as the mean ± S.E.M. and the number of animals used is indicated in parentheses.

$b)$ All data without CPZ coadministration were averaged and shown in the table.

c) The HBF values obtained before and after the drug administration in each experiment were averaged and listed in the table. In a separate experiment, the control value of HBF (without any medication) was obtained and was 0.369 ± 0.024 l/h/100 g b.w.

d) Since the hepatic intrinsic clearance (Cl$_{HI}$) was calculated by Eq. 14 in the text, no S.E.M. was indicated.
Chlorpromazine Pentobarbital Interaction

Fig. 2. Diagram of a Pharmacokinetic Model of PB Disposition in Rats

The plasma concentration of PB is described by a three compartment open model with non-linear elimination kinetics and the brain concentration of PB is expressed as the perfusion limited model.

\[
\frac{dX_1}{dt} = \left( - (k_{12} + k_{13} + \frac{V_{\text{max}}}{K_m + X_1}) + X_1 \right) + k_{21} X_2 + k_{31} X_3 \quad (10)
\]

\[
\frac{dX_2}{dt} = k_{12} X_1 - k_{21} X_2 \quad (11)
\]

\[
\frac{dX_3}{dt} = k_{13} X_1 - k_{31} X_3 \quad (12)
\]

\[
\frac{dX_4}{dt} = Q_{\text{BR}} \left( \frac{X_1}{V_p} - \frac{X_4}{V_{\text{BR}} K_p} \right) \quad (13)
\]

Where \(X_1, X_2, X_3\) and \(X_4\) are the amount of PB in the plasma compartment, in the shallow compartment, in the deep compartment and in the brain compartment, respectively. \(k_{12}, k_{21}, k_{13}\) and \(k_{31}\) are the first order rate constants and \(V_{\text{max}}\) and \(K_m\) are the maximum rate and the constant for the Michaelis-Menten kinetics. \(V_p\) is the volume of plasma and \(K_p\) is the distribution ratio of brain to plasma. For calculation, the plasma flow rate \(Q_{\text{BR}}\) and the plasma volume \(V_{\text{BR}}\) of the rat brain were taken from the literature \(^1\text{d}, 15\) and the brain weight of the rats was the average value of experimental data. The differential equations shown in Eqs. 10 to 13 were solved numerically by the Runge-Kutta-Gill procedure. The solid lines in Fig. 1 are the fitted values. The estimated parameters are shown in Table II. Although the calculated values for 5 mg/kg were slightly smaller than observed data, other results indicated that the three compartment model with a Michaelis-Menten elimination kinetics adequately described the disposition of PB in rats. Garrett et al.\(^1\text{d}\) and Reilly et al.\(^1\text{c}\) reported that the plasma concentrations of metabolized barbiturates such as amobarbital or pentobarbital, after i.v. administration (5–40 mg/kg) in dogs, were described reasonably well.

| Table II. Values of Pharmacokinetic Parameters of Pentobarbital in Rats Obtained from Computer Fitting of Plasma and Brain Concentration Data after Intravenous Administration of PB and CPZ |
|-------------------------------------------------|-----------------|-----------------|
| \(k_{12}\) (h\(^{-1}\))                       | 223 ± 13        | 223             |
| \(k_{21}\) (h\(^{-1}\))                       | 13.5 ± 1.4      | 13.5            |
| \(k_{13}\) (h\(^{-1}\))                       | 20.6 ± 6.9      | 20.6            |
| \(k_{31}\) (h\(^{-1}\))                       | 1.37 ± 0.47     | 1.37            |
| \(V_{\text{max}}\) (mg/h)                      | 2.19 ± 0.41     | 1.75 ± 0.36     |
| \(K_m\) (mg)                                    | 0.0593 ± 0.0199 | 0.0785 ± 0.0308 |
| \(Q_{\text{BR}}\) (l/h)                        | 0.0246          | 0.0246          |
| \(V_p\) (l)                                     | 0.0111          | 0.0111          |
| \(K_p\)                                         | 1.38 ± 0.07     | 1.38            |
| \(V_{\text{BR}}\) (kg)                          | 0.00174         | 0.00174         |

\(^a\) These parameters were estimated from PB 5, 20 and 50 mg/kg i.v. data. \(^b\) Only the parameter values of \(V_{\text{max}}\) and \(K_m\) were estimated by model fitting and other parameters were set to the control values. \(^c\) These parameter values were taken from the literatures.
by a kinetic model involving saturable elimination kinetics. The present results indicated internal consistency with these reports. High lipid solubility and high concentration of the unionized form of PB (pKₐ = 8.11) in plasma reflect the high brain concentration of PB. The Kₚ value shown in Table II was the computer estimated value. If the terminal phase of plasma concentration of PB was linear, the Kₚ value would be calculated directly by using the apparent brain to plasma ratio, by the slope of the terminal phase of plasma concentration (λ₂), by Qₜ and by Vₚ, according to the method of Chen et al.¹⁴c)

(2) Elimination Kinetics of PB

The urinary excretion of unchanged PB 24 h after administration (20 or 50 mg/kg i.v.) was less than 1% of dose, as shown in Table III. This result indicated that the contribution of urinary excretion to the total clearance of PB was extremely small. It has been generally agreed that PB is metabolized only by the liver and that the main metabolic pathway is the microsomal ω-1 oxidation resulting in 3'-HP as the main metabolite in mammals.¹⁷) Since hepatic metabolism was the major route of elimination of PB from plasma,¹³ the time course of plasma 3'-HP levels after intravenous administration of PB was determined. As shown in Fig. 3, the time required for the peak plasma concentration of

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**Table III. Urinary Excretion of PB after Intravenous Administration of PB and CPZ a)**

<table>
<thead>
<tr>
<th></th>
<th>PB 20 mg/kg</th>
<th>PB 50 mg/kg</th>
<th>PB 20 mg/kg</th>
<th>CPZ 4 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary excretion ratio (% of dose)</td>
<td>0.646 ± 0.063</td>
<td>0.767 ± 0.035</td>
<td>1.18 ± 0.38</td>
<td></td>
</tr>
</tbody>
</table>

*a) Each value of the data is shown as the mean ± S.E.M. (n = 3).*

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Fig. 3. Plasma Concentration of 3'-HP Following Intravenous Administration of PB and CPZ.

Each value of the data is shown as the mean ± S.E.M. (n = 4). Since only a small amount of authentic 3'-HP was available, the time course of 3'-HP is shown as peak area ratio to the internal standard rather than as 3'-HP concentration. ■ PB 50 mg/kg; ● PB 20 mg/kg; ○ PB 20 mg/kg + CPZ 4 mg/kg.

Fig. 4. Plasma Free Fraction of PB after Intravenous Administration of PB with or without CPZ.

Plasma free fraction of PB was almost constant in the concentration range of 0.1 to 100 µg/ml. Coadministration of CPZ (open symbols) did not alter the plasma protein binding of PB.
3'-HP was about 30 min for 20 mg/kg dose and 60 min for 50 mg/kg dose. The slope of the terminal phase for the higher dose was significantly smaller than that for the lower dose. This fact suggested that hepatic metabolism has a nonlinear characteristic which may play a major role in the nonlinearity of systemic clearance.

The hepatic metabolic clearance (Cl_H) is described by the following Eq. 14

\[
\text{Cl} \equiv \text{Cl}_H = \frac{f_B \text{Cl}_{H1}}{\text{HBF} + f_B \text{Cl}_{H1}}
\]  \hspace{1cm} (14)

where Cl_H1 is the intrinsic hepatic clearance of PB. This equation indicates that the hepatic clearance was influenced by the plasma protein binding, the distribution to RBC, the hepatic blood flow and the metabolic activity of the liver. Figure 4 shows the profile of the free concentration versus the corresponding total concentration of PB in plasma obtained by equilibrium dialysis and the ultrafiltration. There was no significant change in the protein binding of PB in the concentration range of 0.1 to 77 \(\mu\)g/ml. The mean value of the plasma free fraction (and standard deviation) was 0.447 (0.026, \(n = 21\)) by equilibrium dialysis and 0.483 (0.031, \(n = 12\)) by ultrafiltration. Figure 5 shows the distribution coefficient, \(D\), for the ratio of PB concentration in the RBC to the plasma free concentration of PB, versus plasma total concentration. The \(D\) values did not vary significantly with plasma total concentrations and gave a mean value of 5.71 with a standard deviation of 0.70. The HBF before (at time 0) and after PB administration (5, 20, 50 mg/kg i.v.) is shown in Fig. 6. There was no appreciable change in HBF. These results indicated that the dose dependent systemic clearance of PB was due to the capacity limited hepatic metabolism of PB. Using the average values of HBF, the intrinsic hepatic clearances (Cl_H1) for the three doses were calculated and are listed in Table I.

![Fig. 5. Effect of CPZ on the Distribution Coefficient of PB into RBC](image)

The distribution coefficient of PB (\(D\) value) was calculated by Eq. 3 described in the text after intravenous administration of PB without (solid circles) or with (open circles) CPZ.

![Fig. 6. Effect of PB or CPZ Administration on the Hepatic Blood Flow](image)

Each value of the data is shown as the mean ± S.E.M. a) Significantly different (\(p < 0.05\)) from the pre-medication value. Upper figure: PB 5 mg/kg (\(n = 3\)). Middle figure: PB 20 mg/kg (\(n = 3\), solid symbols), PB 20 mg and CPZ 4 mg/kg (\(n = 3\), open symbols). Lower figure: PB 50 mg/kg (\(n = 3\)).
(3) The Relationship between Sleeping Time and Plasma or Brain Concentration

The relationship between the sleeping time and the log dose of PB is illustrated in Fig. 7. After i.v. administration of PB, the rats lost their righting reflex immediately and recovered in a dose dependent manner. Theoretically, the sleeping time induced by PB is associated with a minimum concentration of the drug necessary to elicit the loss of the righting reflex in the active site, namely in the brain. Since onset of the action of PB is instantaneous, the time necessary for the PB concentration to decline to the minimum effective concentration is considered to be the duration of response. There are many instances where the sleeping time induced by barbiturates was correlated with their concentrations in the brain or CSF rather than in the plasma. However, in the case of PB, the brain-to-plasma concentration ratio is constant except during the first few minutes of disequilibrium state. This fact indicated that the plasma concentration as well as the brain concentration at the end of the pharmacologic effect (i.e. recovery of righting reflex) can be used as the awakening concentration of PB. The awakening concentration in the plasma and brain was calculated from the sleeping time data shown in Fig. 7 and from the calculated values of the brain and plasma concentrations shown in Fig. 1. The average value for the awakening plasma concentration was 12.5 ± 2.9 μg/ml and the awakening brain concentration was 17.8 ± 3.6 μg/g. The solid line in Fig. 7 is the theoretical sleeping time versus the logarithm of the dose curve, using this awakening plasma concentration. The sleeping time of PB in rats was reasonably related to the time course of plasma concentration using a single minimum effective concentration. The value for the awakening brain concentration obtained in this study was similar to that in the report of Jori et al.

(4) Effect of CPZ on the Disposition of PB

The plasma and brain concentrations of PB after intravenous coadministration of CPZ (4 mg/kg) are shown in Fig. 1 as open symbols. Plasma concentration of PB declined multieexponentially in the same manner as the case for PB control (without CPZ, shown in solid symbols in Fig. 1), however, the slope of the terminal phase (λ2) decreased significantly. The brain-to-plasma concentration ratio was constant (about 1.5). The plasma and brain concentrations were also analyzed by the hybrid model described in Fig. 2. In calculation, all the parameter values except K m and V max were set to the control values. The results are also shown in Fig. 1, as the dotted lines, and the parameter values for V max and K m are listed in Table II. The K m value was increased by CPZ coadministration, whereas V max was decreased. In the present study, the values of Q BR and K p after CPZ administration were assumed to be identical with the respective control values. As mentioned above, K p value is influenced by V BR, Q BR, λ2 and the apparent brain to plasma ratio (K papp). It was evident that the apparent value of V BR K papp λ2/Q BR was much smaller than unity and that the K papp value of PB was not influenced by CPZ. Consequently, the effect of CPZ on K p value was considered to be negligible in this study. Since there has been no report with respect to the effect of CPZ on the brain blood flow, we regarded Q BR as a constant in the calculation.
However, our preliminary experiment suggests that CPZ itself slightly reduces the brain blood flow in the rat. Details of the result will be discussed in a subsequent paper.

(5) Effect of CPZ on the Elimination Kinetics of PB

The amount of urinary excretion of PB at 24 h after CPZ and PB coadministration (CPZ: 4 mg/kg, PB: 20 mg/kg) is listed in Table III. The amount of PB excreted in urine was increased significantly by CPZ coadministration. Since less than 2% of dose was excreted in urine, the renal clearance of PB did not contribute to the systemic clearance, irrespective of CPZ coadministration. The time course of 3'-HP concentration after intravenous CPZ (4 mg/kg) and PB (20 mg/kg) is shown in Fig. 3 as open symbols. The time required for the peak concentration was delayed considerably and the terminal slope was also decreased by CPZ coadministration. As shown in Fig. 4 and Fig. 5, neither the plasma protein binding of PB nor the RBC-to-plasma distribution coefficient of PB was affected by CPZ coadministration. The plasma total protein concentration was not influenced by CPZ (data were not shown), while, hepatic blood flow was markedly decreased by CPZ administration (Fig. 6). The hepatic blood flow at 2 h after CPZ (4 mg/kg) and PB (20 mg/kg) administration was reduced by half compared to that in PB control. This fact suggested that the reduction of hepatic blood flow is one of the reasons for the decrease of systemic clearance of PB when CPZ was coadministered. The intrinsic hepatic clearance under CPZ coadministration was calculated and is also listed in Table I. The decrease of hepatic intrinsic clearance suggested that the hepatic metabolism is inhibited by CPZ.

Recently, we reported that CPZ exerts its hypothermic activity mainly by increasing the tail blood flow to increase the conductive heat loss in the body of rats. Inversely, it is reasonable that the blood flow of the liver, the heat producing organ, might decrease by CPZ administration. The details of this mechanism are unknown and further investigation is required.

Recent clinical and human investigations indicate that phenothiazines and other neuroleptics inhibit the elimination of a wide variety of drugs. The present result is consistent with these reports. CPZ is known to be extensively metabolised in the liver and excreted in the urine in the forms of various metabolites. The metabolic pathways of CPZ include hydroxylation and conjugation with glucuronic acid, oxidation of sulfur atom, dealkylation and N-oxidation. Since the main metabolic pathway of PB is oxidation, CPZ may inhibit the metabolism of PB by competing for the hepatic cytochrome P-450 system.

(6) Effect of CPZ on the Sleeping Time Induced by PB

The sleeping time versus the logarithm of PB with CPZ (4 mg/kg) coadministration is shown in Fig. 7 as open symbols. The dose–response curve of PB under CPZ coadministration was shifted to the left and the slope of the curve was also decreased as compared with that of the PB control. The broken line in Fig. 7 is the theoretical dose response curve under CPZ coadministration, assuming that the awakening plasma concentration was 12.5 μg/ml. The predicted sleeping time of PB under CPZ coadministration was shorter than experimental results. This fact indicated that the prolongation of the sleeping time by CPZ coadministration could not be explained only by the change of the plasma or brain concentration of PB itself. As shown schematically in Fig. 8A, the end of sleeping is assumed to occur when the plasma concentration of PB is less than a particular concentration, namely the awakening concentration. In the case of CPZ coadministration, the drug concentration in plasma at the end of action was not constant but decreased with the decreasing dose of PB, as shown in Fig. 8B. This fact suggested that the effect of CPZ on the sleeping time is greater in the lower dose than in the higher dose of PB. The phenomenon observed may be explained as an increased sensitivity of the central nervous system to PB.

CPZ has been reported to block pentobarbital metabolism either in vitro or in vivo, when given shortly before the barbiturates. Jori et al. reported that the low dose (2.5 mg/kg i.p.) of CPZ caused both an increase in sleeping time and in PB brain levels and that the increase in levels alone might explain the sleeping time
Fig. 8. The Relationship between Plasma Concentration of PB and the Recovery of Righting Reflex (A) Calculated values for plasma concentration of PB without CPZ. (B) Calculated values for plasma concentration of PB with CPZ (4 mg/kg i.v.). The numerical numbers in the figure are doses of PB (mg/kg) and solid lines are the theoretical values of plasma concentration of PB. The arrows depicted in the figure are the average values of the sleeping time. The awakening plasma concentrations were calculated from the sleeping time data and the theoretical plasma concentration data. The awakening plasma concentrations of each dose of PB without CPZ were almost identical and gave the average concentration of 12.5 ± 2.9 μg/ml (shown in the dotted line). In the case of CPZ coadministration, the awakening concentration did not show a constant value.

potentiation in rats. They also reported that the higher dose (4 mg/kg i.p.) of CPZ caused a prolonged sleeping time of PB without any change in the brain level. In the present study, coadministration of CPZ caused both an increase in sleeping time and in the brain level of PB, even in the 4 mg/kg i.v. dose of CPZ. This result is inconsistent with the report of Jori et al. They determined PB brain level only once at 90 min after CPZ coadministration and could not detect any differences.

In the present investigation, the sleeping time was determined by LRR. Normal physiological sleep is composed of 2 alternative phases: orthodox or slow wave sleep and paradoxical or rapid eye movement (REM) sleep. Most hypnotics, including pentobarbital, reduce the duration and intensity of REM sleep, whereas neuroleptics, such as CPZ, may inhibit or enhance REM sleep, depending on dosage. Although LRR is frequently used as a pharmacodynamic index of the hypnotic or general anesthetic drugs on rodents, LRR may not be sensitive to detect any qualitative differences in sleep. Therefore, the sleeping time observed in this study included the depressant action of CPZ on the central nervous system although CPZ 4 mg/kg i.v. showed little or no sleeping response in rats. Further investigation might be required in this respect.
In conclusion, CPZ inhibited the hepatic metabolism of PB by reducing both hepatic blood flow and hepatic enzyme activity. The increased plasma and brain concentrations of PB by CPZ coadministration was partially responsible for the prolongation of sleeping time, however, the pharmacodynamic changes still played a major role in the PB and CPZ interaction.

References


