The Pharmacodynamic and Pharmacokinetic Interaction of Pentobarbital and Chlorpromazine in Rats

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The effect of chlorpromazine on the duration of loss of righting reflex (LRR, sleeping time) of pentobarbital and vice versa in a various dosage ranges were studied in rats. The logarithm of the dose versus sleeping time profile of pentobarbital was shifted to the left and the slope of the profile was decreased as the dose of chlorpromazine was increased. The logarithm of chlorpromazine dose versus duration of LRR during pentobarbital coadministration also showed a distinct dose-dependent profile. However, chlorpromazine itself showed ambiguous duration of LRR because the terminal point of the pharmacologic effect, i.e., the recovery of the righting reflex (RRR, the awakening time), was often difficult to determine clearly.

The isobolographic method was introduced to describe the drug interaction of pentobarbital and chlorpromazine quantitatively. Assuming that the sites of action of chlorpromazine and pentobarbital were in the brain, the brain concentrations of pentobarbital at RRR were plotted against the brain concentration of chlorpromazine at RRR. The plots showed a hyperbola-like curve, indicating that there was a supra-additive interaction. In order to clarify the relationship between brain concentrations of the two drugs at RRR, a theoretical consideration was made under the following assumptions: (1) chlorpromazine and pentobarbital have a common central depressant effect, (2) the concentration-effect relationship is described by Hill’s equation and (3) the mode of interaction of these drugs is simple additive. The results indicated that the isobolographic plot of pentobarbital and chlorpromazine was reasonably described by the theory and that chlorpromazine enhanced the effect of pentobarbital at least in an additive manner.

Keywords — pentobarbital; chlorpromazine; loss of righting reflex; sleeping time; drug interaction; recovery of righting reflex; brain concentration; isobolographic method; simple additive

Introduction

It has been reported that many drugs, including certain phenothiazines, can markedly prolong the sleeping time of barbiturates in several animal species, and various explanations have been presented for these interactions. Vestal et al. suggested that most kinetic drug interactions may result from altered drug absorption, plasma protein binding, metabolism and liver blood flow. The suggestion has been made that the interaction between chlorpromazine (CPZ) and pentobarbital (PB) could be due to inhibition of the metabolism of PB. In the previous paper we reported that the hepatic metabolism of PB was significantly inhibited by CPZ coadministration; however, the prolongation of sleeping time of PB could not be explained by this pharmacokinetic alteration itself.

In order to clarify the mechanism of pharmacodynamic interaction of PB and CPZ, the influence of CPZ on the duration of loss of righting reflex (LRR) of PB and vice versa were studied in various dosage ranges in rats. The purpose of this investigation was to verify the applicability of the isobolographic method of Gessner to the interaction of PB and CPZ.

Materials and Methods

Chemicals — Pentobarbital sodium (PB, Tokyo Kasei Co., Ltd., Tokyo, Japan) and chlorpromazine hydrochloride (CPZ, Nakarai Chemical Co., Kyoto, Japan) were purchased commer-
cially and used without further purification. All other chemicals were of reagent grade and were also obtained commercially.

**Animal Experiment** — Seven-week-old male Wistar rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used. Two days before the experiment, rats were cannulated in the right jugular vein for blood sampling and drug administration, under light ether anaesthesia. After 19 h fasting, PB or CPZ or a combination of PB and CPZ was dissolved in physiological saline (JP XI, Otsuka Pharmaceutical Co., Tokyo, Japan) and was administered via the jugular vein. The total volume of administration was adjusted to 0.5 ml. The details of the animal experiment were described previously.\(^4\)

**Analytical Method of CPZ in Plasma** — Plasma CPZ level was determined by high performance liquid chromatography (HPLC) according to the method of Sato et al.\(^6\) A Shimadzu model LC-6A liquid chromatograph equipped with a variable wavelength ultraviolet detector (SPD-6A, Shimadzu Ltd., Kyoto, Japan) was used. HPLC was performed on a column (150 × 3.5 mm i.d.) packed with Lichrosorb RP-18 (particle size: 5 \(\mu\)m, E. Merk, Darmstadt, West Germany) with acetonitrile : methanol : 0.1 M acetate buffer (pH 4.2) = 820 : 80 : 100 including 0.02 M of 1-heptanesulfonate as the mobile phase at the flow rate of 1.3 ml/min. CPZ was detected at 215 nm against the peak area of trifluoperazine (internal standard).

**Determination of the Pharmacologic Effect of PB and CPZ** — The pharmacologic effect of CPZ and PB in the rat was evaluated by the duration of LRR, as described previously.\(^4\)

**Estimation of Model Parameters** — A nonlinear least squares regression program FKDM, which is based on the algorithm of Gauss–Newton and Berman et al.,\(^7\) was used on a digital computer (PDP-11/34, Digital Equipment Corp., Mass.). The weighting values and the conditions for the convergency were as described previously.\(^4\)

**Results and Discussion**

(1) Disposition of CPZ

The time courses of plasma concentration of CPZ after intravenous administration (4 mg/kg) without or with PB (20 mg/kg) are shown in

![Graph 1](image1.png)

**Fig. 1.** The Time Course of Plasma Concentration of CPZ with or without PB Administration

Data are shown as mean ± S.E. ⋄, CPZ 4 mg/kg alone; ○, CPZ 4 mg/kg + PB 20 mg/kg.
Pharmacodynamics of PB and CPZ

Fig. 2. Effect of CPZ on the Logarithm of Dose versus Duration of LRR Curve of PB

Data are shown as mean ± S.E. ●, without CPZ; □, CPZ 0.5 mg/kg i.v.; △, CPZ 2 mg/kg i.v.; ○, CPZ 4 mg/kg i.v.; ◇, CPZ 8 mg/kg i.v.

Fig. 3. Effect of PB on the Logarithm of CPZ Dose versus Duration of LRR Curve as Rats

Data are shown in mean ± S.E. ○, PB 5 mg/kg i.v.; ◇, PB 10 mg/kg i.v.; △, PB 20 mg/kg i.v.; □, PB 50 mg/kg i.v. The broken line in the figure is the calculated value without PB coadministration.

Fig. 1 as semilogarithmic plots. It is evident that plasma concentration of CPZ was not influenced by intravenous PB administration. Although the terminal phase of the plasma concentration was slightly faster than that of the serum concentration in the previous result, the disposition kinetics of CPZ were fundamentally identical. Accordingly, all of the subsequent data on serum and brain concentrations of CPZ as well as the pharmacokinetic parameters were adopted from the previous report.

(2) Effect of CPZ on the Sleeping Time of PB

The effect of i.v. administration of CPZ on the logarithm of PB dose versus sleeping time profile is shown in Fig. 2. The dose sleeping time profile of PB under CPZ coadministration was shifted to the left, and the slope of the profile was decreased as the dose of CPZ was increased.

(3) Effect of PB on the Pharmacologic Response of CPZ

The effect of i.v. administration of PB on the logarithm of the CPZ dose versus duration of LRR profile is shown in Fig. 3. The experimental data without PB are not shown here because most of the rats used in the study did not show a distinct recovery of righting reflex. Consequently, a distinct value of duration of LRR could not be determined, though CPZ itself showed relatively clear LRR just after administration in rats. In spite of the ambiguity in the duration of LRR in CPZ alone, the duration of LRR under PB coadministration showed a dose-dependent pattern. It was evident that the log-dose versus duration of LRR profile shifted to the left and the slope decreased as the dose of PB was increased.

(4) Evaluation of CPZ and PB Interaction

The PB–CPZ interaction was characterized using the isobolographic method. An isobolograph which was constructed by plotting the brain concentration of PB at awakening time (recovery of righting reflex, RRR) on the abscissa and the brain concentration of CPZ at RRR on the ordinate is shown in Fig. 4. Since the site of action of CPZ or PB is considered to be in the brain, brain concentrations of the drugs instead of doses (i.e., ED50 or LD50) were used in this study. The brain concentration of PB was calculated by the pharmacokinetic parameters in the previous report, assuming that there was no pharmacokinetic interaction between PB and CPZ. The brain concentration of CPZ was also calculated according to the report of Sato et al.
The isobolographic plots (open symbols) showed a hyperbola-like curve rather than a straight line, as if there were a supra-additive effect (as defined by Fingl et al.\textsuperscript{(9)}) between PB and CPZ.

Originally, the isobolographic method was developed to clarify the mode of interaction of two drugs by plotting the ED\textsubscript{50} (or LD\textsubscript{50}) of a drug on the abscissa and the ED\textsubscript{50} (or LD\textsubscript{50}) of another drug on the ordinate.\textsuperscript{(9)} Theoretically, the isobolograph can also be drawn using a particular pharmacologic response intensity and the drug concentration at the site of action, instead of using 50\% of the maximum effective intensity and dose (ED\textsubscript{50}). In the present study, we determined only duration of LRR but not intensity of pharmacologic response as the continuous value. Therefore, only two points, the onset of action (at the time point of LRR) and the end of action (at the time point of RRR), were available for determination of the pharmacologic response intensity. Since onset of LRR of PB was instantaneous, the exact time required to onset could not be determined. Consequently we used the end of the pharmacologic effect for the construction of the isobolograph. The plots in Fig. 4 were obtained by this method. Although the duration of LRR in CPZ alone was ambiguous, it was assumed that CPZ had a pharmacologic response in common with PB, namely the central depressant effect. If the respective central depressant effects of PB and CPZ could be expressed by a Hill’s equation, the following equations were derived:

\[
R_{PB} = \frac{V_{m1} C_{PB}^a}{1/Q_1 + C_{PB}^a} \quad (1)
\]

\[
R_{CPZ} = \frac{V_{m2} C_{CPZ}^b}{1/Q_2 + C_{CPZ}^b} \quad (2)
\]

where \(R_{PB}\) and \(R_{CPZ}\) are central depressant effects of PB and CPZ, respectively. \(V_{m1}\) and \(V_{m2}\) are the maximum responses, and \(Q_1, Q_2, a\) and \(b\) are the Hill’s constants. \(C_{PB}\) and \(C_{CPZ}\) are brain concentrations of PB and CPZ, respectively. If the total central depressant effect \((R)\) was expressed as a simple addition of respective pharmacologic activity, \(R\) was explained by Eq. 3:

\[
R = \frac{V_{m1} C_{PB}^a}{1/Q_1 + C_{PB}^a} + \frac{V_{m2} C_{CPZ}^b}{1/Q_2 + C_{CPZ}^b} \quad (3)
\]

If the awakening took place at a central response \(R_c\), \(C_{PB}\) and \(C_{CPZ}\) at awakening have to be sufficiently small compared to the corresponding values of \(1/Q\). Then, Eq. 3 reduces to Eq. 4:

\[
R_c = V_{m1} Q_1 C_{PB}^a + V_{m2} Q_2 C_{CPZ}^b \quad (4)
\]

Dividing both sides of Eq. 4 by \(R_c\) and simplifying the results gives

\[
A C_{PB}^a + B C_{CPZ}^b = 1 \quad (5)
\]

where

\[
A = \frac{V_{m1} Q_1}{R_c} \quad \text{and} \quad B = \frac{V_{m2} Q_2}{R_c} \quad (6)
\]
TABLE I. Computer Estimated Parameters of Pharmacodynamic Interaction between PB and CPZ

<table>
<thead>
<tr>
<th></th>
<th>Without PK interaction</th>
<th>With PK interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>0.2222</td>
<td>0.1711</td>
</tr>
<tr>
<td>$a^{a)}$</td>
<td>0.4836</td>
<td>0.3913</td>
</tr>
<tr>
<td>$B$</td>
<td>0.3710</td>
<td>0.3710</td>
</tr>
<tr>
<td>$b^{a)}$</td>
<td>0.4646</td>
<td>0.4646</td>
</tr>
<tr>
<td>$C_{PB0}$ ($\mu g/g$)</td>
<td>22.43</td>
<td>91.10</td>
</tr>
<tr>
<td>$C_{CP20}$ ($\mu g/g$)</td>
<td>8.451</td>
<td>8.451</td>
</tr>
</tbody>
</table>

$a^{a)} = \frac{-\ln A}{\ln (C_{PB0})}, \quad b = \frac{-\ln B}{\ln (C_{CP20})}$

If the effect of PB during CPZ coadministration (or the effect of CPZ during PB coadministration) can be described by simple addition of the respective pharmacologic effects, the isobolographic plots of PB and CPZ will lie on a single line as expressed by Eqs. 5 and 6. It is notable here however that the isobologram of a simple additive interaction is not always a straight line but shows a concave or convex curve depending on the values of $a$ and $b$. Moreover, it may also be noted that the isobolographic plots of experimental data fall significantly below the line; this is evidence of supra-additivity (as defined by Fingl et al. $^{8)}$), provided that $A$, $B$, $a$ and $b$ were adequately obtained. Where the plots rise markedly above the line, the interaction suggests infra-additivity.

The isobolographic plots of PB and CPZ interaction were fitted to Eqs. 5 and 6 using a least squares method, and the parameters $A$, $B$, $a$, and $b$ were obtained. The calculated values shown as the solid line in Fig. 4 describe the observed data pretty well. This result indicated that the pharmacodynamic interaction of PB and CPZ could be expressed by the simple additive effect if there was no pharmacokinetic interaction between the two drugs. The estimated parameters were listed in Table I. The intercept to the ordinate represents the awakening brain concentration of CPZ without PB coadministration ($C_{CP20}$), while the intercept to the abscissa represents the awakening brain concentration of PB without CPZ coadministration ($C_{PB0}$). The brain concentrations of PB at awakening time without CPZ was estimated to be 22.43 $\mu g/g$. This value was almost identical with the experimental data reported previously (the actual value for PB was 17.78 $\pm$ 3.55 $\mu g/g$). The brain CPZ concentration at awakening time without PB was estimated to be 4.45 $\mu g/g$.

We reported in the previous paper $^{4)}$ that the hepatic clearance of PB was significantly decreased by CPZ coadministration. The solid symbols in Fig. 4 are the isobologram obtained under CPZ 4 mg/kg coadministration using actual pharmacokinetic parameters. All of the plots rose above the regression line and thus the effect of PB during 4 mg/kg CPZ coadministration was smaller than expected on the basis of a simple additivity of the central depressant effect of the two agents.

Since we did not measure the $R$ value of Eq. 3 in the present study, the parameter values obtained here were comparative values rather than absolute values. Consequently, the mode of interaction evaluated here was comparative. The broken line in Fig. 4 is a regression line using Eqs. 5 and 6 and using the experimental data shown in solid symbols. The estimated parameters are also listed in Table I. The parameter values also indicated that the effect of PB under CPZ 4 mg/kg coadministration was apparently diminished compared with the expected value. For instance, the $C_{PB0}$ value involving pharmacokinetic (PK) interaction with CPZ was about 4 times greater than that without PK interaction. This indicates that the rats awoke at about 4 times higher brain concentration of PB than would be expected from simple additive interaction alone.

The broken line in Fig. 3 shows the values calculated using the estimated awakening brain concentration of PB without CPZ.
concentration and using the time course of CPZ brain concentrations in the dose range of 4 to 20 mg/kg i.v. As mentioned previously, we could not obtain the distinct value of duration of LRR for CPZ alone because of ambiguous recovery of the righting reflex. It is well known clinically that the sedative effect of CPZ differs from that of barbiturates in that the phenothiazine causes little ataxia and incoordination and the patient may be easily aroused. This fact indicates that the pharmacologic effect of CPZ may be easily disturbed by factors such as noise, light, temperature and so on, and this may be one of the causes of difficulty in determining the effect of CPZ on the loss of righting reflex. Although the dose response curve of CPZ shown in Fig. 3 was obtained under several assumptions, this dose response profile may describe the net effect of CPZ.

Previously we reported that the PB concentration at RRR under CPZ coadministration did not show a single value, but showed variable values. This phenomenon is now easily explained by the fact that the prolongation of sleeping time was the result of an additive effect of CPZ on the sleeping time (duration of LRR) of PB. The isobolographic method could be described by the summation of Hill’s equations representing the concentration–effect relationship of PB and CPZ. If we could obtain the precise values of the pharmacodynamic parameters in Eqs. 1 and 2, PB–CPZ interaction might be more distinctly characterized. Further investigation may be required in this respect.

In conclusion, the isobolographic method presented in this paper was useful for quantitative understanding of drug–drug interaction, especially when pharmacokinetic and pharmacologic interaction may occur simultaneously.

References


