A Physiologically Based Pharmacokinetic Model for Biperiden in Animals and Its Extrapolation to Humans

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(Received May 14, 1986)

The disposition characteristics of biperiden were investigated in rats, rabbits, beagles, and humans, and a physiologically based pharmacokinetic model was established by using the hepatic intrinsic clearance of unbound drug concentration and the tissue-to-plasma unbound concentration ratios. Protein-binding parameters and blood-to-plasma concentration ratios were determined, and linear parameters were obtained in beagles and humans over a wide concentration range. The hepatic intrinsic clearance of humans was predicted from the animal data. The coincidence of each tissue-to-plasma unbound concentration ratio between rats and rabbits was confirmed in the steady state, and the mean tissue-to-plasma unbound concentration ratios were used for the prediction of the plasma concentration–time courses of beagles and humans. The predicted lines fitted the observed plasma concentrations of beagles and a patient well after a single intravenous injection and repeated intramuscular administrations, respectively.

Keywords—biperiden; physiological pharmacokinetics; animal scale up; rat; rabbit; beagle; human; tissue-to-plasma unbound concentration ratio; hepatic intrinsic clearance

Introduction

Biperiden (BP) is an anticholinergic drug applied in the treatment of parkinsonian syndrome.1 In contrast to the extensive clinical use of BP, knowledge of its pharmacokinetic behavior, metabolism and body distribution in man is still limited. Only two reports of BP serum concentration in man after single oral administration have been made to date,2,3 while no data are available on serum concentration after repeated parenteral administration of BP in man.

The characteristics of BP pharmacokinetics were thought to be large distribution volume and high plasma clearance in rabbits.4,5 In order to predict the plasma concentration in humans from limited data, it is important to use animal data to establish a suitable pharmacokinetic model of BP.

Physiologically based pharmacokinetics have the advantage of interspecies scalability. For the prediction of the intrinsic clearance of an unbound drug in humans, the function of body size of animals was reported to be useful.6 Moreover, it has been claimed that the tissue-to-plasma unbound concentration ratios in animals are important to predict the concentrations of several drugs in human plasma.7,8

The purpose of this study was to establish the physiologically based pharmacokinetics of BP by using the hepatic intrinsic clearance of unbound drug and the tissue-to-plasma unbound concentrations. To predict the human plasma concentrations after repeated administrations of BP, the animal data were examined, and the possibilities of animal scale-up are discussed.
Materials and Methods

Materials—Biperiden was used as supplied by Dainippon Pharmaceutical Co., Osaka, Japan. Diazepam, which was chosen as the internal reference standard, was kindly supplied by Takeda Pharmaceutical Co., Osaka, Japan. All other chemicals were of reagent grade and were used without further purification.

Determination of Serum Protein Binding—Venous blood (60 ml) was taken from three normal subjects into disposable polyethylene syringes. Each sample was allowed to stand for 30 min at room temperature and then centrifuged at 1300 g for 10 min; the serum was stored at 4 °C, and the experiment was finished within 12 h after blood collection. The extent of binding of BP to serum proteins in beagles and humans was measured by the equilibrium dialysis technique using two compartment plates with a 0.8 ml sample volume as described previously.5) The equilibrium concentration of BP in both the serum and buffer compartments was measured after more than 8 h at 37 °C. The free BP fraction was obtained by calculating the ratio between the concentrations of the drug in the buffer and serum compartments. In this study, since the volume shift between the two compartments was within 6%, it was assumed to be negligible.

Animals and Patient—Male Wistar rats (290 ± 4 g; mean ± S.D.), adult male albino rabbits (2.1 ± 0.2 kg; mean ± S.D.) and beagles (9.0—13.0 kg) were utilized. A patient (42.3 kg), who was being concomitantly treated with BP and haloperidol, participated in the study.

The pharmacokinetic studies were undertaken as follows. BP was infused over precisely 2 min with an infusion pump via the antecubital vein of beagles. Blood samples were withdrawn through a cannula via the opposite antecubital vein at specified time intervals after the infusion, and collected in heparinized tubes, and plasma samples were separated. In the case of the patient, 3.88 mg of BP was administered intramuscularly every 8 h. Blood was drawn from the antecubital vein of the patient, allowed to clot for several h and spun to obtain the plasma. The sample was stored in a frozen state at —30 °C until assay.

Tissue Distribution—To determine the tissue-to-plasma partition coefficients at the steady state (Kt), infusion was performed at the rate of 0.765 ml/h (5.32 mg/ml BP saline solution) after intravenous bolus injection of the priming dose (3.2 mg/kg BP saline solution) to rats. At 16 h after the commencement of the infusion studies, the rats were sacrificed for tissue sampling. The tissues were quickly excised, rinsed well with ice-cold saline, blotted, and weighed. The procedure for obtaining tissue homogenate was essentially the same as that described previously.9)

Determination of Blood-to-Plasma Concentration Ratio (RBP)—A conventional in vitro method was performed as follows. The venous blood was collected from the beagles and humans via the antecubital vein into heparinized syringes. Aliquots (0.1 ml) of isotonic buffer solution containing various amounts of BP were added to 5 ml of whole blood. The samples were incubated with slow shaking for 30 min at 37 °C. The concentrations of BP in the plasma after separation by centrifugation and in whole blood were then assayed.

Analytical Procedures—Drug concentrations of BP in plasma were determined by gas liquid chromatography (GLC) assay as described in the preceding paper.4) In order to detect the low concentration of BP in plasma, 3-fold more plasma was used in the GLC assay. The detection limit of the method was 0.3 ng/ml, and the coefficient of variation was less than 10%. To determine the concentrations in tissues, the same method was applied to the tissue homogenates. Calibration curves were obtained by the same method for each biological sample. The detection limit of the method was 20 ng/ml for the homogenized samples.

Data Analysis—The BP data in beagles were analyzed according to the model-independent moment analysis procedure, as described previously.5) The data for the patient after repeated administrations were fitted to a three-compartment pharmacokinetic model by using the MULTI computer program.10) The model prediction by using differential equations was performed using a previously described program7,9,11) with an appropriate modification according to the equations in “Appendix.” A FACOM M360AP digital computer at the Data Processing Center, Kanazawa University, was used.

The measure of the fit between the observed (Cobs) and the predicted (Cpred) concentrations of BP was based on the coefficient of determination, r², calculated from the equation: \( r^2 = 1 - \Sigma^{dev^2} / Sy^2 \), where \( Sy^2 = \Sigma Y_{obs}^2 - (\Sigma Y_{obs})^2 / N \), \( dev^2 = (Y_{obs} - Y_{pred})^2 \), and N represents the number of determinations. In this calculation, the logarithmic values of Cobs and Cpred were employed as Yobs and Ypred.12)

Results

Table I shows the volume of distribution in the steady state per body weight (Vdss/BW) and total body plasma clearance per body weight (CLtot/BW) in animals. The values of Vdss/BW in animals were in the range of 9.5 to 19.3 l/kg, which indicate that extensive tissue distribution of BP is a common characteristic among the tested animals. The value of Vdss/BW of beagles was about half that of rabbits. The value of CLtot/BW in the patient was considerably lower than those of rats, rabbits, and beagles. The rank order of CLtot/BW was:
rabbits > rats > beagles > man. Although the values of $CL_{\text{bol}}/BW$ in rats and rabbits could be well explained in terms of hepatic blood flow rate-limited elimination, those of beagles and human were about half of the respective blood flow rate.

Constant serum protein binding and RBP were observed for BP in beagles and humans over a wide BP concentration range (25—10000 ng/ml). All parameters are given in Table II, together with the reported values in rats\textsuperscript{[13]} and rabbits.\textsuperscript{[5]} Similar RBP values were obtained in animals and in humans. The values of the plasma unbound fraction ($f_p$) in beagles and humans were nearly equal to that of rats, rather than that of rabbits.

The hepatic intrinsic clearance ($CL_{\text{uint},H}$) of BP was calculated by

$$CL_{\text{uint},H} = \frac{RBP \cdot Q_1 \cdot ER}{f_p \cdot (1 - ER)}$$

where $Q_1$ and $ER$ are hepatic blood flow rate and hepatic extraction ratio, respectively. The calculated values for rats and rabbits are listed in Table II.

By assuming that the elimination of BP was occurring only in the liver, the hepatic clearance of BP in beagles was calculated by means of the following equation without the determination of $ER$.

$$CL_{\text{uint},H} = \frac{RBP \cdot Q_1 \cdot CL_{\text{bol}}}{f_p \cdot (RBP \cdot Q_1 - CL_{\text{bol}})}$$

The calculated value of $CL_{\text{uint},H}$ in beagles is listed in Table II. In our previous\textsuperscript{[5]} and present experiments on the oral administration of BP in animals, BP was detected in the plasma only in the case of beagles. This may be due to the fact that $CL_{\text{uint},H}$ of beagles was the smallest among the tested animals.

The tissue-to-plasma concentration ratios ($K_p$) of BP in rabbits were reported pre-

<table>
<thead>
<tr>
<th>TABLE I. Pharmacokinetic Parameters of Biperiden</th>
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<tr>
<td>Parameters</td>
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<tr>
<td>$Vd_{\text{bol}}/BW$ (l/kg)</td>
</tr>
<tr>
<td>$CL_{\text{bol}}/BW$ (ml/min/kg)</td>
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</table>

\textsuperscript{[a]} Ref. 13. \textsuperscript{[b]} Ref. 5. \textsuperscript{[c]} Determined by model-independent moment analysis. Data are presented as the mean $\pm$ S.D. Calculated from a set of mean plasma concentrations and S.D. at each time after drug administration. \textsuperscript{[d]} Determined by three-compartment model analysis of the plasma concentration time course after repeated administration of BP.

| TABLE II. Physiological and Pharmacokinetic Parameters of Biperiden |
|-------------------------|----------------|--------------|-------------|----------|
| Parameters               | Rats           | Rabbits      | Beagles     | Humans   |
| $f_p$                   | 0.110 $\pm$ 0.041\textsuperscript{[e]} | 0.393 $\pm$ 0.04\textsuperscript{[f]} | 0.096 $\pm$ 0.005 | 0.097 $\pm$ 0.032 |
| RBP\textsuperscript{[g]} | 1.15 $\pm$ 0.06\textsuperscript{[d]} | 1.17 $\pm$ 0.05\textsuperscript{[d]} | 0.97 $\pm$ 0.05 | 0.95 $\pm$ 0.03 |
| ER\textsuperscript{[h]}  | 0.910 $\pm$ 0.037\textsuperscript{[e]} | 0.940 $\pm$ 0.037\textsuperscript{[e]} | —           | —         |
| $CL_{\text{uint},H}/BW$ (ml/min/kg) | 6220\textsuperscript{[e]} | 3170\textsuperscript{[e]} | 943\textsuperscript{[e]} | 176\textsuperscript{[h]} |

\textsuperscript{[a]} $f_p$ represents the unbound fraction of BP in plasma. \textsuperscript{[b]} RBP represents the blood-to-plasma concentration ratio of BP. \textsuperscript{[c]} ER represents the hepatic extraction ratio. Each value represents the mean $\pm$ S.D. The number of experiments is given in parentheses. \textsuperscript{[d]} Ref. 13. \textsuperscript{[e]} The value of the hepatic intrinsic clearance of unbound drug ($CL_{\text{uint},H}$) was calculated by using Eq. 1. \textsuperscript{[f]} Ref. 5. \textsuperscript{[g]} The value of the hepatic intrinsic clearance of unbound drug ($CL_{\text{uint},H}$) was calculated by using Eq. 2. \textsuperscript{[h]} The value of the hepatic intrinsic clearance of unbound drug ($CL_{\text{uint},H}$) of humans was predicted by using those in animals. See the text for details.
In order to determine the tissue-to-plasma concentration ratio of BP in rats, infusion studies were performed. The values thus obtained for rabbits and rats are listed in Table III. The tissue-to-plasma unbound concentration ratios ($K_{pu}$) were obtained by dividing the tissue-to-plasma concentration ratio by the value of the serum unbound fraction. The relationship between the $K_{pu}$ values of BP in rats and rabbits is shown in Fig. 1. Although the $f_p$ value of BP in rats was about one-fourth of that of rabbits, similar $K_{pu}$ values for each tissue were obtained between rats and rabbits. High $K_{pu}$ values were obtained in lung and adipose tissue. The $K_{pu}$ values of some viscera, such as the brain, heart, kidney, and gut, were in the range from 50 to 100.

Figure 2 shows the physiological pharmacokinetic model utilized here for calculation. This model was built on the basis of the following assumptions: (1) each tissue acts as a well-stirred compartment; (2) BP distribution is blood-flow limited; (3) elimination of BP occurs only from the liver in a linear manner; and (4) the $K_{pu}$ of BP is independent of the drug concentration. The mass balance equations developed according to the flow diagram in Fig. 2 are given in Appendix II. The mean values of $K_{pu}$ between rats and rabbits were used to

![Fig. 1. The Relationship between the Tissue-to-Plasma Unbound Concentration Ratios ($K_{pu}$) of Biperiden in Rabbits and Rats](image)

The line shows a positive correlation. $r = 0.933$.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$K_p$&lt;sup&gt;a&lt;/sup&gt;</th>
<th>$K_{pu}$&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rabbit&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Rat</td>
</tr>
<tr>
<td>Blood</td>
<td>1.17 ± 0.05</td>
<td>1.16 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lung</td>
<td>131.0 ± 6.0</td>
<td>60.9 ± 25.1</td>
</tr>
<tr>
<td>Brain</td>
<td>25.7 ± 8.8</td>
<td>7.0 ± 3.0</td>
</tr>
<tr>
<td>Heart</td>
<td>34.3 ± 8.7</td>
<td>7.0 ± 4.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>31.3 ± 4.6</td>
<td>11.0 ± 2.9</td>
</tr>
<tr>
<td>Gut</td>
<td>22.5 ± 8.4</td>
<td>11.0 ± 7.7</td>
</tr>
<tr>
<td>Muscle</td>
<td>8.5 ± 2.5</td>
<td>3.05 ± 1.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adipose</td>
<td>120.0 ± 16.0</td>
<td>58.0 ± 6.1&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Skin</td>
<td>9.9 ± 2.0</td>
<td>4.0 ± 2.5</td>
</tr>
<tr>
<td>Bone</td>
<td>5.2 ± 0.6</td>
<td>2.0 ± 0.99</td>
</tr>
</tbody>
</table>

<sup>a</sup> The value of $K_p$ is the tissue-to-plasma concentration ratio at the steady state. Data are presented as the mean ± S.D.  
<sup>b</sup> The value of $K_{pu}$ is the tissue-to-plasma unbound concentration ratio at the steady state. 
<sup>c</sup> Ref. 5.  
<sup>d</sup> Ref. 13.
simulate beagles and humans. As a substitute value of $K_{pu}$ in the liver and spleen, the mean value of viscera was used for the calculation.

Figure 3 shows the calculated variations of BP concentration in the plasma of 2.1-kg rabbits as a function of time. The initial dosage was 6.7 mg. The observed plasma concentration of BP agreed well with the simulated levels ($r = 0.940, p < 0.001$).

The results for plasma concentrations in beagles after intravenous administration of 3.2 mg/kg of BP are shown in Fig. 4. The observed plasma concentrations of BP coincided well with the simulated levels ($r = 0.985, p < 0.001$).

In order to predict the $CL_{uint,H}$ in humans, a regression analysis was adopted using the function of body size of animals as reported. In Fig. 5, we show a log-log plot of $CL_{uint} \times MLP$ vs. body weight in rats, rabbits, and beagles. MLP is the maximum life-span potential in years. From the regression of the BP data in Fig. 5, the following equation is obtained:
where $BW$ is the standard body weight of animals in kg, and $CL_{\text{uint}}$ is the intrinsic clearance of unbound drug in one year. In this analyzing procedure, the values of $MLP$ as reported by Boxenbaum were used. The correlation coefficient was high and statistically significant ($r = 0.999, p < 0.05$). According to the regression parameters, $CL_{\text{int,H}}$ in humans was calculated as listed in Table II. The values of $CL_{\text{uint,H}}$ of rats and rabbits were more than 10 times greater than that of humans.

Figure 6 shows the predicted and observed plasma concentration profile after intramuscular administrations of BP to the patient. The administration was repeated every 8 h. A previous study carried out at this laboratory showed that the absorption from the injection site in the muscle into the systemic circulation was rapid and complete. Moreover, the pharmacokinetics of BP after intravenous injection and intramuscular administration were almost equal in rabbits. Thus, the input function in humans was assumed to be equivalent to rapid infusion into the venous plasma. The predicted line agreed well with the observed plasma concentration in the patient. The correlation coefficient was high and statistically significant ($r = 0.980, p < 0.001$).

**Discussion**

This study demonstrates that a physiologically based pharmacokinetic model based on the hepatic intrinsic clearance of unbound drug and the tissue-to-plasma unbound concentration ratios was applicable to BP.

In our previous studies using the rabbit, we have already shown that the high plasma clearance of BP could be well explained in terms of hepatic blood flow rate-limited elimination. Although the same explanation could be applied to rats, the value of $CL_{\text{int}}/BW$ in beagles and humans seemed to be smaller than the hepatic blood flow rate. By using the
estimated value of $CL_{\text{uint}, H}$ in humans in Table II, the value of $CL_{\text{tot}}/BW$ was calculated as 10 ml/min/kg. This value is similar to the observed $CL_{\text{tot}}/BW$ (= 15.3 ml/min/kg) in humans (Table III). It was reported that no unchanged BP is excreted via the kidney in man.\textsuperscript{2} In our experiments, the urinary recovery of BP in beagles was almost negligible. (= 0.017% of the administered dose) within 48 h. Thus, it is reasonable to conclude that the route of elimination of intact BP was mainly hepatic elimination, not only in rats and rabbits, but also in beagles and humans. As shown in Table II, humans have a lower capacity for hepatic metabolism of BP. Man’s lesser quantitative ability to metabolize appears to apply to many drugs.\textsuperscript{6} Several regression parameters of the allometric exponentials have been reported, \textit{i.e.}, 1.09, 1.23, and 0.922 for antipyrine, phenytoin, and clonazepam, respectively.\textsuperscript{6} The parameter for BP coincides well with these values.

In order to construct a physiologically based pharmacokinetic model using $K_{pu}$, the coincidence of the $K_{pu}$ value of each tissue in several animals must be confirmed. We attempted to establish this most important postulate by using rats and rabbits. This is because the $f_p$ value of rabbits was the largest among the animals tested, and rats were suitable and convenient animals for determining the $K_{pu}$ values. The values of each tissue were in good agreement between rats and rabbits. Recently, Sawada \textit{et al.}\textsuperscript{8} reported that, in general, there is little difference between the unbound volume of distribution of various basic drugs in tissues of animals and humans. From the good coincidence in tissue distribution characteristic between rats and rabbits, as shown in Fig. 1, this generality was confirmed to be applicable to the BP tissue distribution in rats and rabbits. However, the value of $Vd_{ss}/BW/f_p$ in rats was somewhat larger than that of rabbits. We examined the correlation between $Vd_{ss}$ and $f_p$ in animals and humans according to the reported prediction rule of basic drugs.\textsuperscript{14} The correlation was high, but not significant ($r = 0.867$). An explanation for this is not yet available.

It is reasonable to assume that the equilibrium conditions in each tissue phase are approached in a simple, linear manner, with characteristic terms determined by tissue volumes and blood flow rates. The assumption of perfusion-limited transport is applicable to lipid-soluble drugs, for which diffusion and movement across lipoid membranes should be relatively rapid. The physiological parameters for a 70-kg human were used as described before.\textsuperscript{11} On this basis, the calculated result is in fair agreement with the observation in the patient given repeated intramuscular administrations.

In conclusion, the physiologically based pharmacokinetic model derived by using the plasma unbound concentration was useful to predict the human plasma concentration. Moreover, the hepatic intrinsic clearance of humans was well explained by the animal data. In the future, in order to clarify the pharmacodynamic action of BP, we can take into account the simulated target tissue concentrations in various disease states by using the present model.

Appendix

I: Nomenclature

General

$C =$ concentration in plasma or tissue, ng/ml
$C_u =$ unbound concentration in plasma or tissue, ng/ml
$CL_{\text{uint}, H} =$ hepatic intrinsic clearance of unbound drug, ml/min
$f_p =$ unbound fraction in plasma
$K_{pu} =$ tissue-to-plasma unbound concentration ratio
$RBP =$ blood-to-plasma partition coefficient
$Q =$ blood flow rate through tissue, ml/min
$V =$ volume of plasma or tissue, ml

Subscripts
\( b = \text{blood}; \ a = \text{arterial plasma}; \ l = \text{liver}; \ k = \text{kidney}; \ gi = \text{gastrointestinal tract}; \ lu = \text{lung}; \ h = \text{heart}; \ m = \text{muscle}; \ br = \text{brain}; \ f = \text{fat}; \ bo = \text{bone}; \ v = \text{venous}; \ s = \text{skin}; \ t = \text{tissue}. \)

**II. Model Equations for BP**

**Noneliminating organ or tissue**

\[
V_i \cdot \frac{dC_i}{dt} = RBP \cdot Q_i \cdot (C_a - C_i/(fp \cdot K_{pu,i}))
\]

**Venous blood compartment**

\[
V_b \cdot \frac{dC_v}{dt} = Q_i \cdot C_i/(fp \cdot K_{pu,i}) - Q_b \cdot C_v + IJ/RBP
\]

\[
Q_b = Q_{lu} + Q_1 + Q_h + Q_m + Q_f + Q_{bo} + Q_s
\]

**Arterial blood**

\[
V_a \cdot \frac{dC_a}{dt} = Q_{lu} (C_{lu}/(fp \cdot K_{pu,lu}) - C_a)
\]

**Lung**

\[
V_{lu} \cdot \frac{dC_{lu}}{dt} = RBP \cdot Q_{lu} (C_v - C_{lu}/(fp \cdot K_{pu,lu}))
\]

**Liver**

\[
V_1 \cdot \frac{dC_1}{dt} = RBP ((Q_1 - Q_{gi} - Q_{sp}) C_a + Q_{gi} \cdot C_{gi}/(fp \cdot K_{pu,gi}) + Q_{sp} \cdot C_{sp}/(fp \cdot K_{pu,sp}) - Q_1 \cdot C_1/(fp \cdot K_{pu,1}))
\]

\[-CL_{int,1} \cdot C_1/K_{pu,1}\]

\( IJ \) is the injection functions given by

\[
IJ = D/2
\]

(D is the dose; this function was used only within 2 min)

**Acknowledgements**

The authors are grateful to Dainippon Pharmaceutical Co., and Takeda Pharmaceutical Co., for supplying the drugs.

**References**