FUNDAMENTAL PHARMACOKINETIC PROPERTIES OF BIPERIDEN: TISSUE DISTRIBUTION AND ELIMINATION IN RABBITS

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(Received September 26, 1985)

The pharmacokinetics of biperiden in rabbits were examined at three doses (0.2, 0.8, and 3.2 mg/kg i.v.). The data were interpreted in terms of a three-compartment open model with a linear excretion rate. The serum unbound fraction and the blood-to-plasma concentration ratio were determined as 0.39 and 1.2, respectively, over a wide concentration range (25–10000 ng/ml). Rapid and complete absorption from the injection site in muscle to the systemic circulation was observed. The bioavailability of muscular injection was unity. The hepatic extraction ratio was 0.94, and the high plasma clearance could be explained in terms of hepatic blood flow rate-limited elimination. The major tissues in which biperiden was distributed were fat and muscle. The highest tissue-to-plasma partition coefficient in the steady-state was obtained for the lung. These three tissues comprised 56% of the total distribution volume.

Keywords — biperiden; pharmacokinetics; tissue distribution; tissue-to-plasma partition coefficient (Kp); blood-to-plasma concentration ratio; protein binding; elimination; rabbit

INTRODUCTION

Biperiden (BP) is an antiparkinsonian drug with a recognized anticholinergic activity in man. Its pharmacokinetic behaviour has not yet been satisfactorily characterized despite the fact that it is a widely used therapeutic drug. Specific and sensitive gas chromatographic methods have become available only recently,1,2) and this explains, in part, the scarcity of pharmacokinetic data on the drug in humans1,3) and rabbits.2) The reported characteristics of BP pharmacokinetics are great distribution volume and large plasma clearance.1–3) Although it is assumed that the high plasma clearance is due to metabolism,4) the influence of various factors affecting the clearance, such as plasma protein binding, blood-to-plasma partition coefficient, plasma flow rate and blood flow rate, have not been satisfactorily clarified. Moreover, no information on the tissue distribution of the drug has yet been obtained.

Because of limited human data on the pharmacokinetic and pharmacodynamic characteristics of BP, animal data must be discussed in order to clarify and support the human findings.

The purposes of this study were to elucidate the metabolism and tissue distribution behaviour of BP in rabbits. The present paper also discusses the most important factors causing the large plasma clearance that exceeds liver plasma flow rate and the great distribution volume of the drug.

MATERIALS AND METHODS

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

Determination of Serum Protein Binding — The extent of binding of BP to rabbit serum protein was measured by the equilibrium dialysis technique using two compartment plates with 0.8 ml sample volume. The equilibrium concentrations of BP in both serum and buffer compartments were measured at 37 °C. The free BP fraction was obtained by calculating the ratio between the concentrations of the drug in the buffer and in the serum compartments. In this study, the volume shifts in the compartments were within 0.05 ml.

Determination of Blood-to-Plasma Concentration Ratio — A conventional in vitro method was performed as follows. After administration of heparin at a dose of 0.1 ml/100 g body weight (100 units), whole blood was collected via the femoral artery. Aliquots (0.1 ml) of isotonic

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buffer solution containing various amounts of BP were added to 5 ml of whole blood. The samples were incubated with shaking (very slowly) for 30 min at 37 °C. The concentrations of BP in the plasma after separation by centrifugation and in whole blood were then assayed.

Animal Experiments — Adult male albino rabbits weighing 2.1±0.2 kg (mean ± S.D.) were fasted for 16 h prior to the experiment with free access to water. The procedure employed was essentially the same as that described previously.\(^5\) Under light ether anesthesia the femoral artery was cannulated with polyethylene tubing. BP was administered to the rabbit via the marginal auricular vein. Cannulated rabbits were kept in a supine position on restraining plates.

BP, which was dissolved in saline, was injected over 2 min either through the marginal vein or intramuscularly. For plasma concentration determination, blood samples (ca. 2 ml) were withdrawn from the femoral artery through the cannula at designated time intervals after drug administration and collected in heparinized tubes. The blood was centrifuged at 3000 rpm and the plasma was separated. The plasma samples were kept at 4 °C until they were assayed.

To determine the renal and hepatic clearances, intravenous infusions were performed after intravenous bolus injection of the priming dose. The femoral artery, femoral vein, ureters and bile duct were cannulated. BP solutions were prepared with saline and infused through the femoral vein using a priming dose for each drug. After a steady-state level of BP plasma concentration was achieved, urine and bile were collected and blood was withdrawn through the femoral artery at the midpoint of the collection period.

The procedure used to measure the hepatic extraction ratio of BP was essentially the same as that described previously.\(^5\) At steady state, the plasma concentrations of BP in the femoral artery and hepatic vein were determined.

To determine the tissue-to-plasma partition coefficient in the steady-state, infusion studies were performed at three different constant rates. For tissue sampling, rabbits were sacrificed at specified times after drug administration. The tissues were quickly excised, rinsed well with ice-cold saline, blotted, and weighed. The procedure for obtaining tissue homogenates was essentially the same as described previously.\(^5\)

**Analytical Procedures** — Concentrations of BP in plasma and urine were determined by a gas chromatographic assay as described in the preceding paper.\(^3\) To determine the concentrations in tissues, the same method was applied to the tissue homogenized solutions. 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 were analyzed using model-independent moment analysis.\(^6\) The area under the plasma concentration versus time curve was estimated by means of the trapezoidal rule. The time of infusion started and the initial BP concentration were set to 0. The plasma concentration at 2 min was used as determined by the three-compartment analysis. The last determined plasma concentration was extrapolated to infinite time by using the terminal slope of the log plasma concentration-time curve.

The data-fitting to the three-compartment pharmacokinetic model was analyzed using the NONLIN program\(^7\) with the aid of a FACOM-170F digital computer at the Data Processing Center, Kanazawa University.

**RESULTS**

**Serum Protein Binding of BP and Determination of Blood-to-Plasma Concentration Ratio in the Rabbit**

At first, the linearity of serum protein binding and the blood-to-plasma concentration ratio (RBP) were examined. These two parameters

![Graph showing protein binding profiles for BP as a function of the post-dialysis bound and unbound concentrations at 37°C. The points represent the experimental values, and the solid line is the mean of the experimental values.](image-url)
have been shown to be important in establishing pharmacokinetic models of drugs.\(^8,9\) The results of the binding experiments are shown in Fig. 1. Apparently, BP binding to serum protein was linear over a wide BP concentration range (25—10000 ng/ml). The value of the serum unbound fraction \((f_u)\) was 0.39 ± 0.04 (mean ± S.D.). The RBP in the rabbit was also linear over a wide concentration range of BP (25—10000 ng/ml) as shown in Fig. 2. The value was 1.17 ± 0.05 (mean ± S.D.).

**Intravenous Bolus Injection Studies**

The time courses of BP in rabbit plasma are shown in Fig. 3. The pharmacokinetic data presented in Fig. 3 indicate that the distribution and elimination of BP after intravenous administration of 0.2 to 3.2 mg/kg can be interpreted in terms of a three-compartment open model in ac-

![Graph](image1.png)

**FIG. 2. Distribution of BP into Red Blood Cells**

The points represent the experimental values, and the solid line is the mean of the experimental values.

![Graph](image2.png)

**FIG. 4. Plasma Concentration—Time Courses of BP after Termination of Infusion in Rabbits**

Infusion was carried out at a rate of 4.2 µg/min over 24 h.

![Graph](image3.png)

**FIG. 3. Plasma Concentration—Time Courses of BP Following an Intravenous Injection in Rabbits**

At least three rabbits were used to determine the dose level per time-point. The average body weight of rabbits was 2.1 kg. Each point represents the mean ± S.D. The detailed pharmacokinetic parameters are cited in the text. The curves are model-predicted concentrations based on nonlinear least-squares fitting of data to Eq. 1. Key: ●, 0.2 mg/kg; △, 0.8 mg/kg; ○, 3.2 mg/kg.

![Graph](image4.png)

**FIG. 5. Plasma Concentration—Time Course of BP Following an 0.8 mg/kg Intramuscular Injection in Rabbits**

At least three rabbits were used to determine the dose level per time-point. The average body weight of rabbits was 2.1 kg. Each point represents the mean ± S.D. The detailed pharmacokinetic parameters are cited in the text. The curves are model-predicted concentrations based on nonlinear least-squares fitting of data to Eq. 1.
cordance with:

\[ C_p = D \cdot (A \exp(-\alpha t) + B \exp(-\beta t) + C \exp(-\gamma t)) \]  

(1)

where \( C_p \) and \( D \) are the plasma concentration and administered dose (mg/kg body weight), respectively. \( A, B, C, \alpha, \beta, \) and \( \gamma \) correspond to the pharmacokinetic parameters representing the plasma concentration of BP. Iterative nonlinear least-squares analysis of all the data in Fig. 3 to be fitted to three simultaneous Eq. 1 of different doses, using the NONLIN computer program\(^7\) provided the following parameters. \( A = 781 \pm 112 \text{ ng/ml, } B = 669 \pm 4.1 \text{ ng/ml, } C = 10.7 \pm 0.9 \text{ ng/ml, } \alpha = 0.323 \pm 0.029 \text{ min}^{-1}, \beta = 0.0134 \pm 0.0009 \text{ min}^{-1}, \) and \( \gamma = 0.00198 \pm 0.00011 \text{ min}^{-1}. \) During the fitting procedure, the concentration was weighted by the reciprocal of its square.

**Intravenous Infusion and Intramuscular Injection Studies**

The time course of BP in rabbit plasma after termination of long-term infusion is shown in Fig. 4. The elimination rate in the postdistributive phase was determined as \( 0.00190 \pm 0.00011 \text{ min}^{-1} \) by a nonlinear least squares method. The time course of BP in plasma after intramuscular administration is shown in Fig. 5. Iterative nonlinear least-squares analysis of the data in Fig. 5 was run as described above. The values of the parameters were \( A = 286.3 \pm 32.1 \text{ ng/ml, } B = 110.4 \pm 21.4 \text{ ng/ml, } C = 17.8 \pm 2.8 \text{ ng/ml, } \alpha = 0.121 \pm 0.017 \text{ min}^{-1}, \beta = 0.0285 \pm 0.0043 \text{ min}^{-1}, \) and \( \gamma = 0.00274 \pm 0.00047 \text{ min}^{-1}. \) Table I lists the results of the model-independent moment analysis, the area under the plasma concentration versus time curve (AUC), mean residence time (MRT), volume of distribution at steady state per body weight (\( Vdss/BW \)) and total body clearance per body weight (\( Cl_{tot}/BW \)) of BP in rabbit with a comparison between intravenous injection and intramuscular injection.

**Extraction of BP from the Circulation by Liver and Kidney**

The plasma concentration of BP and the excreted rates were measured and are listed in

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**TABLE I.**  **Pharmacokinetic Parameters of Biperiden in Rabbits**\(^a\)

<table>
<thead>
<tr>
<th>Parameters(^b)</th>
<th>Administration route and dose</th>
<th>Intravenous</th>
<th>Intramuscular</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.2 mg/kg</td>
<td>0.8 mg/kg</td>
<td>0.2 mg/kg</td>
</tr>
<tr>
<td>( AUC^{c} \text{ ng-min/ml} )</td>
<td>40700 ± 1200</td>
<td>9970 ± 440</td>
<td>2600 ± 170</td>
</tr>
<tr>
<td>MRT (^d) \text{ min}</td>
<td>251 ± 18</td>
<td>233 ± 28</td>
<td>252 ± 46</td>
</tr>
<tr>
<td>( Vdss/BW^{c} \text{ l/kg} )</td>
<td>19.8 ± 1.8</td>
<td>18.7 ± 2.6</td>
<td>19.4 ± 4.2</td>
</tr>
<tr>
<td>( Cl_{tot}/BW^{c} \text{ ml/min/kg} )</td>
<td>78.7 ± 2.4</td>
<td>80.3 ± 3.5</td>
<td>77.0 ± 5.1</td>
</tr>
</tbody>
</table>

\( ^a \) Determined by model-independent moment analysis. \( ^b \) Data are presented as the mean ± S.D. \( ^c \) Calculated from a set of mean plasma concentration and its S.D. at each time after drug administration.

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**TABLE II.**  **Urinary and Biliary Recoveries of Intact Biperiden at Various Steady-State Plasma Concentrations**

<table>
<thead>
<tr>
<th>Plasma(^d) \text{ concentration} (ng/ml)</th>
<th>Excretion rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bile (ng/min)</td>
</tr>
<tr>
<td>10.2</td>
<td>- (^b)</td>
</tr>
<tr>
<td>26.6</td>
<td>- (^b)</td>
</tr>
<tr>
<td>34.0</td>
<td>- (^b)</td>
</tr>
<tr>
<td>109</td>
<td>2.17</td>
</tr>
<tr>
<td>149</td>
<td>27.2</td>
</tr>
<tr>
<td>722</td>
<td>64.0</td>
</tr>
</tbody>
</table>

\( ^a \) The blood sampling was carried out through the femoral artery. \( ^b \) Undetectable.
Table II. The biliary recoveries of intact BP were almost negligible over a wide plasma concentration range (10.2–722 ng/ml). The urinary recoveries were also very small and the urinary clearance of intact BP was determined as 0.695 ± 0.429 ml/min/kg BW (mean ± S.D., n = 6). In order to obtain the hepatic extraction ratio, the concentration of BP in both the arterial and hepatic venous plasma were measured at steady state conditions. The hepatic extraction ratio (ER) was determined as 0.94 ± 0.04 (mean ± S.D., n = 6).

**Tissue-to-Plasma Partition Coefficient**

The tissue-to-plasma partition coefficient in the steady-state was calculated and the values are listed in Table III.

The volume of distribution at steady state, $V_d_{ss}$, is given by:

$$V_d_{ss} = V_b RBP + \sum K_{p, non} V_{t, non} + \sum K_{p, dis} V_{t, dis}$$

where $V_b$, $V_{t, non}$, and $V_{t, dis}$ are the blood volume, the volume of the non-disposing organ and that of the disposing organ (e.g., liver and kidney), respectively. $K_{p, non}$ and $K_{p, dis}$ are the tissue-to-plasma partition coefficients of BP for the non-disposing organ and the disposing organ, respectively.

**DISCUSSION**

There has been no information on BP serum protein binding in man and experimental animals. In the present study, over the 25–10000 ng/ml range of BP serum concentration, the fraction of BP bound was approximately 60 percent with equilibrium dialysis. No distinct dissociation constant of BP for serum protein was determined, although it was indicated that the value was greater than 10000 ng/ml (ca. 30 μM) and/or that the serum protein binding is of nonspecific type. The blood-to-plasma concentration ratio was also independent of the initial drug concentration below 10000 ng/ml. It is widely assumed that a blood-to-plasma concentration ratio which exceeds the value of the plasma space in blood is a reflection of good drug permeation into the red blood cells and a large propensity for binding to the cell component.

In our preliminary experiment, BP had a higher lipophilicity than thiopental, which is well distributed throughout adipose tissue. Accordingly, BP can penetrate through the plasma membrane of normal cells because of such high lipophilicity. By considering the values of hematocrit (≈ 0.35) and RBP (≈ 1.17), the fraction of BP bound to erythrocytes was calculated as 73 percent. Although the main ligand of BP in erythrocytes was not surveyed in the present study, it is notable that the large binding observed in erythrocytes is the same as that in the plasma.

To simplify the calculation of the pharmacokinetics, it is reasonable to regard the protein binding and blood-to-plasma concentration ratio as being linear.

The time course of BP in the plasma after injection via the femoral vein in rabbits was well fitted to a three-compartment open model. Hollmann analyzed the pharmacokinetics of BP in

**TABLE III. Tissue-to-Plasma Partition Coefficients ($K_p$) of Biperiden for Various Tissues of Rabbit**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$V_t$ (l/kg) $^a)$</th>
<th>$K_p$ $^b)$</th>
<th>$K_p V_t$ (l/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.0792</td>
<td>1.17 ± 0.05</td>
<td>0.093 ± 0.003</td>
</tr>
<tr>
<td>Lung</td>
<td>0.0073</td>
<td>131 ± 6</td>
<td>0.956 ± 0.042</td>
</tr>
<tr>
<td>Brain</td>
<td>0.0026</td>
<td>25.7 ± 8.8</td>
<td>0.067 ± 0.023</td>
</tr>
<tr>
<td>Heart</td>
<td>0.0026</td>
<td>34.3 ± 8.7</td>
<td>0.089 ± 0.023</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.0064</td>
<td>31.3 ± 4.6</td>
<td>0.200 ± 0.029</td>
</tr>
<tr>
<td>Gut</td>
<td>0.0515</td>
<td>22.5 ± 8.4</td>
<td>1.16 ± 0.43</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.480</td>
<td>8.48 ± 2.46</td>
<td>4.07 ± 1.18</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0515</td>
<td>120 ± 16</td>
<td>6.15 ± 0.83</td>
</tr>
<tr>
<td>Skin</td>
<td>0.100</td>
<td>9.91 ± 1.96</td>
<td>0.991 ± 0.196</td>
</tr>
<tr>
<td>Bone</td>
<td>0.0884</td>
<td>5.19 ± 0.59</td>
<td>0.459 ± 0.052</td>
</tr>
</tbody>
</table>

$^a)$ From ref. 16 of the references listed herein. $^b)$ The value of $K_p$ is the ratio of tissue concentration to plasma concentration at steady state. Data are presented as the mean ± S.D.
man after oral administration using a two-compartment open model with elimination constants of 1.11 and 0.03 h\(^{-1}\) (elimination half-life 0.62 and 23 h). In our experiment, plasma concentration of BP in rabbit decreased rapidly in the first 5 min after intravenous injection, followed by a moderate decrease with a half-life of 0.5 h. The terminal elimination half-life was longer than 5.8 h. Since the rapid distribution phase was usually diminished in the case of oral administration, the second and terminal phases in rabbit correspond to the terms of the two-compartment model in man. The terminal elimination rate after the termination of long intravenous infusion was coincident with the three-compartment analyzed parameter. So it is reasonable to say that the elimination rate is linear with a wide plasma concentration range (0.9—100 ng/ml).

Rapid and complete absorption from the injection site in muscle to the systemic circulation was observed (Fig. 5). The pharmacokinetics of BP after intravenous injection and intramuscular administration were almost equal. The mean absorption time (MAT) was nearly equal to zero, and the bioavailability of intramuscular injection was unity (AUC \(_{i.m.}/AUC \_i.v. = 1\)).

The value of \(C_{\text{tot}}/\text{BW}\) was in good agreement between intravenous and intramuscular administration (Table I). The mean value of \(C_{\text{tot}}/\text{BW}\) was 78.7 ml/min/kg. It is reported that total clearance of BP is high and that it is essentially due to metabolism, for no unchanged BP is excreted \(\text{via}\) the kidney.\(^{41}\) In our study, urinary recovery of intact BP was observed in rabbit, but the urinary clearance was less than 1% of the total body clearance. By considering the values of glomerular filtration rate (GFR, = 3—5 ml/min/kg BW)\(^{19}\) and \(f_u (=0.39)\), and ignoring the renal tubular secretion, the reabsorption fraction in kidney is calculated to be about 0.4—0.6. It is a reasonable value because of the high lipophilicity of BP. Biliary recovery of intact BP was also very small and the hepatic extraction ratio was almost unity. In spite of the high lipophilicity, no appearance of BP in the plasma was observed after oral administration in the rabbit (data not shown). These results suggest that the metabolism of BP in the liver was very fast and that the extraction was almost complete. The hepatic plasma flow rate (\(Q_p\)) and hepatic blood flow rate (\(Q_b\)) of the rabbit are reported to be 44\(^{14}\) and 68 ml/min/kg BW,\(^{15}\) respectively. The total clearance of BP exceeded not only the hepatic plasma flow rate but also the hepatic blood flow rate. The plasma clearance of BP in the liver was calculated as below.

Since BP removal rates will be equal no matter whether clearance is expressed in terms of blood or plasma, it follows that

\[
C_{b} = \frac{C_{p} \cdot (1 - \text{Hct} + (C_{RBC}/C_{p}) \cdot \text{Hct})}{(C_{ap} - C_{vp})/C_{ap}}
\]

where \(C_b\) is the blood clearance based on blood concentrations. Blood concentration \(C_b\) is related to plasma concentration by the relationship

\[
Q_{b} = \frac{C_{ab} - C_{vb}}{C_{ab}}
\]

where \(Q_b\) is the hepatic blood flow rate, \(C_{ab}\) and \(C_{vb}\) are the arterial and venous blood concentrations of the drug. The extraction ratio (\(C_{ap} - C_{vp})/C_{ap}\) are equal if the red blood cell-to-plasma concentration ratio is constant on both sides of the artery and vein.

\[
C_{b} = \frac{Q_{b} \cdot (1 - \text{Hct} + (C_{RBC}/C_{p}) \cdot \text{Hct})}{(C_{ap} - C_{vp})/C_{ap}}
\]

and from Eq. 3,

\[
Q_{b} = \frac{Q_{b} \cdot (C_{ap} - C_{vp})}{(C_{ap} - C_{vp})/C_{ap}}
\]

If rapid equilibrium of BP between red blood cells and plasma occurs,

\[
C_{p} = Q_{b} \cdot \text{ER} \cdot \text{RBP}
\]

By considering the values of \(Q_{b} (= 68 \text{ ml/min/kg BW}), \text{ER} = 0.94, \) and \(\text{RBP} = 1.17\), the hepatic clearance of BP was calculated to be 76.5 ml/min/kg. This value occupied above 97% of \(C_{\text{tot}}\) which was obtained from the moment
Pharmacokinetics of Biperiden

analysis. It is reasonable to say that the metabolism of BP occurs in the liver and that metabolism is the major process of elimination.

There has been a report concerning the large distribution volume of BP in humans.\(^{1,2}\) By assuming that most of the metabolism occurs in the liver, the distribution volume of each of ten non-disposing organs was calculated from Eq. 2 and the values are listed in Table III. These ten organs comprise 87% of the total actual body weight.\(^{16}\) In this study, the major tissues in which BP was distributed were fat and muscle. These two tissues comprise 53% of the total distribution volume. Although the weight of fat was less than 5% of total body weight, its role of distribution of the drug was more than 30%. This was due to the high \(K_P\) value. Although muscle occupies 21% of the total distribution volume, the \(K_P\) value is not so high. If the mass of muscle were to decrease, there would be no remarkable change in distribution volume. For example, a 5% increase in muscle against total body weight would produce only a 2% increase in the total distribution volume. By contrast, if a 5% increase against total body weight occurred in fat, a 30% increase would be observed in the total distribution volume. The highest \(K_P\) value was observed in lung. With the addition of BP distribution to lung, these three tissues comprised 56% of the total distribution volume.

In conclusion, the total body clearance of BP was considered to be due to hepatic metabolism and to be almost blood flow-dependent. The two major organs in which the drug was distributed were defined as fat and muscle.

These characteristic properties of BP are interesting, since they imply that the dosage regimen of BP must be changed with regard to changes in hepatic blood flow rate and the mass of fat and/or muscle in the diseased state, especially changes of fat volume. Further detailed pharmacokinetic studies are now under way in our laboratory from the standpoint of clinical pharmacy.

Acknowledgements

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

REFERENCES


