PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR PRALMORELIN HYDROCHLORIDE IN RATS

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ABSTRACT:

Pralmorelin hydrochloride (pralmorelin), consisting of six amino acid residues, is a growth hormone-releasing peptide. The aim of this study is to analyze the pharmacokinetics of pralmorelin after intravenous bolus administration to rats, and to develop a physiologically based pharmacokinetic (PB-PK) model to describe and predict the concentrations of pralmorelin in blood and tissues. Pralmorelin (3 mg/kg) was administered intravenously to 24 Sprague-Dawley rats. Groups of three rats were sacrificed by decapitation at each designated time point (up to 4 h), and plasma and tissues (brain, lung, heart, liver, kidney, small intestine, muscle, adipose, and skin) were collected. Bile was also pooled until decapitation. The concentration of pralmorelin in samples was determined by liquid chromatography-tandem mass spectrometry. Plasma concentrations of pralmorelin declined rapidly in a biexponential manner. Biliary excretion of pralmorelin was so rapid that 80% of the dose was recovered unchanged in the bile within 1 h after administration. The distribution parameters in each tissue were obtained by using a hybrid model and an integration plot. They revealed that the distribution of pralmorelin into liver was blood flow-limited, and its distribution was permeability-limited in all other tissues. The PB-PK model developed in this study well described the time courses of pralmorelin concentration in the blood and tissues of rats.

Pralmorelin hydrochloride (GHRP-2), a synthetic growth hormone-releasing peptide consisting of six amino acid residues, was developed by C. Y. Bowers of Tulane University (New Orleans, LA) and Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan) (Bowers, 1993). It acts through both the growth hormone secretagogue receptor, which is distinct from hypothalamic growth hormone-releasing hormone receptor, and the ghrelin receptor, and it has been developed as both a diagnostic and a therapeutic agent for growth hormone deficiency. Pralmorelin is rapidly excreted unchanged into the bile from the systemic circulation after intravenous administration to rats.

Recently, various biologically active peptides and their analogs have been considered as drug candidates, including somatostatin analogs (Bauer et al., 1982), endothelin antagonist (Nirei et al., 1993; Nishikibe et al., 1993), and renin inhibitor (Ondetti and Cushman, 1981). These small peptides, consisting of 5 to 10 amino acid residues, have common characteristics, in that they are rapidly taken up by the liver and most of the dose is subsequently excreted unchanged into the bile (Berelowitz et al., 1978; Greenfield et al., 1989; Cathapermal et al., 1991). Both in vitro and in vivo studies have demonstrated that active transport systems are involved in the hepatic uptake and biliary excretion of these peptides (Yamada et al., 1997; Akhteruzzaman et al., 1999; Kato et al., 1999). Therefore, pralmorelin may also be excreted by active transport systems. However, few studies have been conducted to elucidate the in vivo tissue distribution properties of small peptides.

Physiologically based pharmacokinetic (PB-PK) models provide a systematic understanding of the pharmacokinetic behavior of drugs based on physiological parameters. PB-PK models are useful for drug development because they are helpful in describing the distribution, excretion, and biotransformation of drugs, can provide interspecies scale-up, and enable us to predict the drug concentration profile for any dose and route of administration under various physiological conditions, such as impairment of liver or kidney (Sato et al., 1987; Hosseini-Yeganeh and McLachlan, 2002).

The aim of this study is to analyze the distribution of pralmorelin and to establish a PB-PK model well describing the profiles of pralmorelin concentration in the blood and tissues of rats.

Materials and Methods

Animals. Adult male Sprague-Dawley rats weighing 208 to 289 g were purchased from Charles River Japan, Inc. (Kanagawa, Japan) and housed in a temperature-controlled room on a normal 12-h light/dark cycle, with free access to food and water. All experimental procedures were consistent with those stipulated in the Kaken Pharmaceutical Co., Ltd. Guide for the Care and Use of Experimental Animals.

Materials. Pralmorelin hydrochloride (D-alanyl-3-(2-naphthyl)-D-alanyl-L-alanyl-L-tryptophyl-D-phenylalanyl-L-lysaminide dihydrochloride) was synthe-
FIG. 1. Chemical structure of pralmorelin hydrochloride.

FIG. 2. Schematic representation of a one-organ model for a noneliminating organ. Indicated are the blood flow (Q), the arterial blood concentration (C_a), the concentration in the capillary bed (C_b), the tissue concentration (C_t), the tissue weight (V_wt), the membrane diffusion intrinsic clearance (PS · f_b), the backflux rate constant (k_2), and the distribution volume that consists of blood space and the space in which the drug concentration rapidly equilibrates with blood (K_{p_0} · V_{wt}).
compartment model, which consists of a compartment in rapid equilibrium with blood (“rapid compartment”) and a deep compartment in slow equilibrium with blood. Figure 2 is the schematic representation of a one-organ model for a noneliminating organ, which describes the distribution of a drug (Sato et al., 1987). Each tissue consists of the capillary bed and two distinct compartments. The first is a blood flow-limited compartment (the rapid compartment) that is in rapid equilibrium with the drug concentration in the blood, i.e., the concentration \( [C]_c \) in the capillary bed. In addition, the sum of the distribution volumes of the blood flow-limited compartment and the volume of the capillary bed (i.e., the distribution volume rapid compartment) can be described as the product \( (K_{po} \cdot V_{ct}) \) of \( K_{po} \) and the tissue weight \( V_{ct} \). The other is the compartment in which the distribution is membrane permeability-limited (the deep compartment). In the latter compartment, the membrane permeation clearance from the rapid compartment into the deep compartment and the backflux rate constant from the deep compartment into the rapid compartment are represented as \( PS \cdot f_k \) and \( k_s \), respectively.

We defined \( K_{po} \) and \( K_{p0} \) as the tissue-to-blood concentration ratio attributable to the drug in the deep compartment and that attributable to the rapid compartment, respectively. Because the \( K_{po} \) values in three early points (5, 15, and 30 min) were constant, the average of these three points was regarded as \( K_{po} \).

In addition, a hybrid model (Fig. 2) was fitted to tissue concentrations using the arterial blood concentration-time profile to determine \( PS \cdot f_k \) and \( k_s \).

The hepatic uptake of pralmorelin was so rapid that we could not estimate the \( PS \cdot f_k \) and \( k_s \) values. Therefore, we designed another in vivo hepatic uptake study, as described in the following section.

To investigate the nature of rapid compartment, we attempted to compare the estimated \( K_{po} \) value with the \( K_{p0} \) value of inulin, a marker of extravascular space. Unfortunately, the \( K_{p0} \) value of inulin was not available, so that we compared the tissue-to-plasma concentration ratio \( (K_{p,plasma}) \) of inulin at 5 min with that of pralmorelin instead. Furthermore, we regarded the \( K_{p,plasma} \) value at 5 min as the distribution into the rapid compartment. We measured the \( K_{p,plasma} \) values at 5 min after intravenous administration of pralmorelin at a dose of 3 mg/kg and \([\text{H}]\text{Pralmorelin} \) at a dose of 100 \( \mu \)g/kg and compared with that of inulin previously reported by Yamada et al. (1997).

Analysis of Initial Hepatic Uptake after Administration of [3H]Pralmorelin. Under pentobarbital sodium (50 mg/kg i.p.) anesthesia, the femoral vein and artery were cannulated. [3H]Pralmorelin solution (3.23 \( \mu \)Ci, 100 \( \mu \)g/kg) was administered through the femoral vein, and blood samples were collected at intervals of 10 to 30 s, prior to and at the time of sacrifice. At designated times 30 s to 3 min after administration, the rats were sacrificed, the liver was excised, and a portion of the tissue was weighed and counted for radioactivity. When a tracer amount of pralmorelin was given intravenously and liver uptake was measured within a period short enough to disregard the backflux and biliary excretion of the parent drug and metabolites from the liver, the liver uptake rate of pralmorelin can be described by the following differential equation,

\[
\frac{dX_t}{dt} = K_1 \cdot C_a
\]  
*(2)*

Integration of eq. 2 and division by \( C_a \) gives the following, eq. 3.

\[
K_{f_k}(t) = \frac{K_1 \cdot AUC(t)}{C_a}
\]  
*(3)*

The uptake clearance of the tissue, \( K_{f_k} \), was obtained from the initial slope of \( K_{po} \) (mL/g tissue) versus AUC(t)/C(t) (min) (Yamazaki et al., 1993).

The uptake clearance of the tissue (\( K_1 \)) was a hybrid parameter of \( PS \cdot f_k \) (the membrane permeation clearance) and blood flow (\( Q_b \)). In the liver, we assumed that: 1) each compartment constituting a whole organ is well stirred (“well stirred” model), 2) only unbound pralmorelin can diffuse across the membrane into each tissue, 3) only unbound pralmorelin is subject to metabolism and elimination, 4) binding equilibrium of pralmorelin and the distribution into blood cells are rapid enough so that the processes of binding to and dissociation from blood cells are not rate-determining. Based on the above assumptions, the relationship between the uptake clearance of the tissue (\( K_1 \); mL/min/g tissue) and the membrane permeation clearance \( (PS \cdot f_k; \text{mL/min}) \) is given by eq. 4.

\[
K_1 \cdot V_{ct} = \frac{Q_b \cdot f_k \cdot PS}{Q_b + f_k \cdot PS}
\]  
*(4)*

Therefore, \( PS \cdot f_k \) is expressed as follows.

\[
PS \cdot f_k = \frac{K_1 \cdot V_{ct} \cdot Q_b}{Q_b - K_1 \cdot V_{ct}}
\]  
*(5)*

Development of PB-PK Model. Figure 3 represents the developed PB-PK model for the distribution and excretion of pralmorelin in rats. The model consists of nine tissues and blood compartments that reflect real organs or anatomic tissues in rats. These tissues are connected in parallel between the arterial and venous circulations in this PB-PK model. The blood flows from the venous pool via the pulmonary artery into the lung and then out via the pulmonary vein into the arterial pool. Except for the liver and lung, all tissues are supplied from the arterial circulation, and blood coming out of these tissues flows directly into the venous circulation. The liver receives its blood supply from both the hepatic artery and portal vein. In this model, pralmorelin was assumed to be eliminated only by the liver and kidney, but not by other tissues. In the liver, the distribution of pralmorelin was rapid and is assumed to be blood flow-limited.

The mass balance equations for the PB-PK model are shown in the Appendix. The equations were integrated numerically and simultaneously using a Macintosh G4 (Apple Computer, Cupertino, CA) with MLAB (Civilized Software, Bethesda, MD).

Results

Total and Hepatic Clearance of Pralmorelin. Plasma concentrations of pralmorelin declined quickly in a biexponential manner after intravenous administration. Pralmorelin was excreted into the bile very rapidly, and 80% of the dose was recovered in the bile within 1 h.
The distribution volumes of pralmorelin were small for the skin, adipose, muscle, brain, and heart, and large for the liver and kidney. For analysis, all plasma data were converted to blood concentrations by means of eq. 1.

The total blood clearance of pralmorelin was 419 ml/h. Since previous studies have reported that almost 80% of the dose of pralmorelin is excreted unchanged into the bile, and 8% of the dose is excreted into urine within 24 h after intravenous administration, we assumed that 8% of the dose was eliminated by the kidney and the rest was assigned to the liver in this study. Consequently, the hepatic and renal blood clearances were calculated as 382 and 36.9 ml/h, respectively. Renal blood clearance is almost equal to the product of the glomerular filtration rate (245 ml/h; Yamada et al., 1997) and the PS value in the liver was determined by curve-fitting using a hybrid model, with the arterial blood concentration-time profile as the input function (Hosseini-Yeganeh and McLachlan, 2002) and the estimated parameters are listed in Tables 1 and 2.

Analysis of Initial Hepatic Uptake after Administration of [3H]Pralmorelin. The time profiles of [3H]pralmorelin concentrations in the blood and liver within short period after administration were investigated to estimate the initial hepatic uptake. The $K_p$, $K_{pH}$, $PS \cdot f_b$, and $k_2$ are presented in Table 1.

To determine whether the estimated $K_{p0}$ value is consistent with the vascular volume in tissue, the tissue-to-plasma concentration ratio ($K_{p,plasma}$) of pralmorelin at 5 min after intravenous administration was compared with that of inulin. Figure 4 shows that the relationship between the $K_{p,plasma}$ of inulin and pralmorelin (100 µg/kg and 3 mg/kg) at 5 min after intravenous bolus administration in rats was linear ($y = 1.20x; r = 0.775$).

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volume and blood flow for each tissue that were taken from Hosseini-Yeganeh and
McLachlan (2002). The tissue volume was converted to tissue weight based on the assumption
of the tissue gravity of 1 g/ml.

### Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue Weight ($V_{\text{wt}}$)</th>
<th>Tissue Blood Flow*</th>
<th>$\text{Cl}_{\text{int}}$b</th>
<th>$K_{p,\text{plasma}}$c</th>
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<td>Arterial blood</td>
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<td></td>
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<tr>
<td>Venous blood</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
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<td>234</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
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<tr>
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<td>2394</td>
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<tr>
<td>Adipose</td>
<td>10</td>
<td>24</td>
<td></td>
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</tr>
</tbody>
</table>

* The volume and blood flow for each tissue were taken from Hosseini-Yeganeh and McLachlan (2002). The tissue volume was converted to tissue weight based on the assumption of the tissue gravity of 1 g/ml.

b $\text{Cl}_{\text{int}}$ is estimated using blood concentration and the renal blood clearance is calculated from the urinary excretion. The extrarenal clearance is assumed as the hepatic blood clearance and calculated using the blood flow. Renal blood clearance is assumed to be equal to the glomerular filtration rate.

c Estimated from a hybrid model using the blood concentration vs. time curve as the input function.

### Discussion

Pralmorelin is rapidly eliminated from the body via the hepatobiliary route in rats. To clarify the determinant factors of the pharmacokinetics of pralmorelin, we analyzed the tissue distribution profile of pralmorelin using a PB-PK model, which enabled us to predict the profile of the drug concentration in each tissue from that in the blood.

The $K_{p,\text{plasma}}$ value represents the tissue-to-blood concentration ratio attributable to the drug in the rapid compartment (distribution volume that consists of the blood space and the volume in rapid equilibrium with blood). The $K_{p,\text{plasma}}$ value often coincides with a fraction of the capillary bed volume in the tissue. However, $K_{p,\text{plasma}} \cdot V_{\text{wt}}$ values in this study were significantly higher than the capillary bed volumes, suggesting that pralmorelin rapidly distributes into the space other than the capillary bed (Fig. 2). Therefore, we compared the $K_{p,\text{plasma}}$ values of pralmorelin at 5 min after intravenous administration with those of inulin, a marker for extracellular space, because the $K_{p,\text{plasma}}$ value at 5 min is considered to reflect the rapid compartment (Fig. 4). We found that the $K_{p,\text{plasma}}$ values of pralmorelin are linearly correlated with those of inulin for several noneliminating tissues. Thus, the $K_{p,\text{plasma}} \cdot V_{\text{wt}}$ reflects the distribution to the capillary bed and extracellular space.

Furthermore, the $K_{p,\text{plasma}}$ (3.91) of pralmorelin for the liver was much higher than that of inulin (0.17), whereas the $K_{p,\text{plasma}}$ (1.82) of pralmorelin for the kidney was lower than that of inulin (9.20; Yamada et al., 1997). The membrane permeation clearance ($PS \cdot f_{pl}$; 99.7 ml/min) into the liver, calculated from the $K_{p,\text{plasma}}$ value based on the well-stirred model, was far larger than $Q_{H}$ (11.8 ml/min). Taking into consideration that the distribution of pralmorelin into all tissues except for the liver was membrane permeability-limited, pralmorelin may be taken up into hepatocytes by active transport systems.

On the other hand, the relatively poor distribution of pralmorelin into the kidney may be explained by the difference in the protein binding ratio in blood between pralmorelin and inulin. The unbounded fraction ($f_{ub}$) of inulin in blood is almost 1.0, whereas that of pralmorelin is very low (0.216). Therefore, the glomerular filtration rate of pralmorelin is much smaller than that of inulin. However, the $K_{p,\text{plasma}}$ (1.82) of pralmorelin for the kidney is still higher than 1. This accumulation in the kidney may be attributable to the binding of pralmorelin, the retention of filtered pralmorelin in the renal tubules, or both (Sato et al., 1987).

In this study, we separately examined the initial hepatic uptake by using [3H]pralmorelin, because the hepatic uptake of pralmorelin was so remarkably rapid, to develop the PB-PK model for the liver. As shown in Figs. 6 and 7, the concentration of pralmorelin estimated by the PB-PK model agreed well with the observed data. For drugs that are subject to rapid hepatobiliary excretion, the uptake clearance may not be accurately estimated by use of the sampling schedule for other organs. In such a case, it is essential to estimate accurately the hepatic uptake clearance for developing the PB-PK model.

However, the PB-PK model underestimated the liver in a terminal elimination phase. It may be due to the adsorption of pralmorelin to the endothelium and/or the parenchymal cell in the liver. Cationic macromolecules tend to bind electrostatically to the surface of hepatocytes in a nonspecific manner (Nishida et al., 1991). Since pralmorelin has positive charge at physiological pH, it may be adsorbed electrostatically on the surface of hepatocytes. Indeed, our preliminary experiment using isolated rat hepatocytes indicates that pralmorelin highly binds to the surface of hepatocytes (data not shown). Therefore, the underestimation of pralmorelin concentrations in the liver is conceivably attributable to the adsorption, which provides a compartment with rapid binding and slow dissociation, not described by the present model for the liver with the assumption of rapid equilibrium with blood.

Another possible explanation is the sequestration of pralmorelin in the liver.

![FIG. 6. Concentration-time profiles for pralmorelin in rat blood and liver after administration of pralmorelin at a dose of 3 mg/kg (i.v.). The symbols represent experimentally observed concentrations and the solid lines are the simulated concentration profiles using the PB-PK model ($n = 3$, mean ± S.D.). Concentration-time profiles in semilogarithmic form are shown in insets.](image-url)
into the liver. Since the membrane permeation clearance into the liver was much larger than hepatic blood flow, hepatic distribution of pralmorelin is conceivably attributable to the active transport system, such as transporter or endocytosis, and its passive influx and efflux may be quite limited. Therefore, although pralmorelin taken up into the liver is excreted unchanged into the bile, it may be partially subject to hepatic sequestration.

The concentrations of pralmorelin at 30 min in most tissues were higher than model simulates. However, the blood concentrations were also slightly increased at the same time. Because the time course of blood concentration was obtained by a one-point-per-animal method, the blood concentration at 30 min might be accidentally increased. Therefore, the aforementioned failure in the simulation of tissue concentration may not be due to the flaw of the model.

In conclusion, we have developed a PB-PK model for pralmorelin in rats. The model agreed well with the observed data. The present
study also demonstrated that a blood flow-limited compartment and a membrane permeability-limited compartment can account for the distribution of pralmorelin in all tissues except for the liver, and the former represents the distribution to the capillary bed and interstitial fluid. The uptake of pralmorelin into the liver was very rapid, suggesting the existence of active transport systems for pralmorelin in the liver.

Appendix: Differential Mass Balance Equations for the PB-PK Model

\( Q_t \) and \( V_{wt} \) represent the blood flow rate and the tissue weight, respectively. \( C_v, C_{a}, C_q, \) and \( C_b \) are the pralmorelin concentrations in the tissue, arterial blood, venous blood, and capillary bed, respectively. \( K_{ps} \) and \( f_b \) represent the tissue-to-blood partition coefficient and the unbound fraction of pralmorelin. LU, HE, MU, SK, H, GU, R, BR, and AD represent lung, heart, muscle, skin, liver, small intestine, kidney, brain and adipose tissue, respectively. \( CL_{in} \) is the intrinsic clearance.

**Arterial Blood**

\[
\frac{dC_v}{dt} = \frac{Q_{oa}}{V_{LU}} \cdot (Ch_{LU} - C_v) \tag{A1}
\]

**Venous Blood**

\[
\frac{dC_a}{dt} = \sum (Q_{Hi} \cdot C_{Hi} + Q_{LU} \cdot C_{LU} + Q_{PS} \cdot K_{PS} - Q_{oa} \cdot C_v) \tag{A2}
\]

Pralmorelin Concentrations in Tissues with Blood Flow-Limited Uptake

(i) Eliminating tissue (Liver)

\[
V_{WT} \frac{dC_{Hi}}{dt} = (Q_{Hi} - Q_{LU}) \cdot C_v + Q_{LU} \cdot C_{LU} - Q_{oa} \cdot C_v - C_{Hi}
\tag{A3}
\]

where \( Q_{Hi} \) is the sum of the hepatic artery and portal vein blood flow rates.

Pralmorelin Concentrations in Tissues with Permeability-Limited Uptake

(i) Lung

\[
(\frac{K_{PS} \cdot V_{WT} \cdot LU}{dt} = \frac{Q_{oa} \cdot (C_v - C_{LU})}{V_{LU}} - PS_{LU} \cdot f_b \cdot C_{LU} + k_q \cdot C_{Hi} \cdot V_{WT} \tag{A4}
\]

\[
\frac{dC_{LU}}{dt} = PS_{LU} \cdot f_b \cdot C_{LU} - k_q \cdot C_{Hi} \cdot V_{WT} \tag{A5}
\]

(ii) Noneliminating tissues

\[
(\frac{K_{PS} \cdot V_{WT} \cdot LU}{dt} \cdot \frac{dC_{LU}}{dt} = \frac{Q_{oa} \cdot (C_v - C_{LU})}{V_{LU}} - PS_{LU} \cdot f_b \cdot C_{LU} + k_q \cdot C_{Hi} \cdot V_{WT} \tag{A6}
\]

(iii) Eliminating tissue (Kidney)

\[
V_{WT} \frac{dC_b}{dt} = P_{SR} \cdot f_b \cdot C_{bR} + k_q \cdot C_{Hi} \cdot V_{WT} \tag{A11}
\]

\[
\frac{dC_{bR}}{dt} = \frac{Q_{oa} \cdot (C_v - C_{bR})}{V_{bR}} - (PS_{bR} + GFR) \cdot f_b \cdot C_{bR} + k_q \cdot C_{Hi} \cdot V_{WT} \tag{A12}
\]

where \( K_{ps} \) and \( V_{wt} \) represents the distribution volume that consists of the blood space and the volume in rapid equilibrium with blood, the concentration of which is equal to \( C_r \). The concentration in the whole tissue \( (C_v) \) is given as the sum of the concentration in the intracellular space \( (C_i) \) and \( C_b \). (eqs. A6, A9, A12).

**References**


