
Makoto MIYAZAKI,a Shin-ichi SAWADA,a Takayoshi NISHIDE,a Kazunori IWANAGA,a
Kazuo MORIMOTO,b and Masawo KAKEMI,a,∗

Department of Pharmaceutics, Osaka University of Pharmaceutical Sciences, 4–20–1 Nasahara, Takatsuki, Osaka 569–1094, Japan and Department of Pharmaceutics, Hokkaido College of Pharmacy, 7–1 Katsuraoaka-cho, Otaru, Hokkaido 047–0264, Japan. Received July 30, 1999; accepted September 17, 1999

A novel method of assessing the extent of oral bioavailability of arginine-vasopressin (AVP) from pharmacological data was presented. After intravascular administration (i.v. bolus or short-term infusion) of AVP to rats, the relationship between blood concentrations and its effect on both mean arterial pressure (hemodynamic effect) and urinary sodium concentration (anti-diuretic effect) was described on the basis of an integrated pharmacokinetic–pharmacodynamic (PK–PD) model. A direct model was used for the hemodynamic response, while an indirect response model, rather than a hypothetical link model was used for the anti-diuretic response. A sigmoid Emax model was applied to describe the drug–receptor interaction. Pharmacological responses after intravascular administration of AVP were reasonably described by the PK–PD model. However, PD parameters estimated by the PK–PD analysis suggested that apparent receptor affinity rather than efficacy in i.v. bolus study was significantly higher than that in the short-term infusion study. This fact indicated that PK–PD relationship was influenced by the intravascular input rate of AVP. We then investigated the relationship between plasma concentration and amount of AVP bound to the V1 receptors in the kidney. The result indicated that the amount of AVP bound to the receptors after i.v. bolus injection was always greater than that after short-term infusion. Since the PK–PD relationship after oral administration was almost identical with that after short-term infusion, the PK–PD model obtained in the short-term infusion study was used to assess the extent of oral bioavailability (EBA). The EBA values, estimated from pharmacological effects (hemodynamic effect and anti-diuretic effect) after oral administration of 5 μg/kg of AVP were 0.68% to 0.93% and were almost identical with the actual EBA value (0.81%). From these results, we concluded that oral bioavailability of AVP was reasonably predicted by the PK–PD model, provided that appropriate pharmacological effects and appropriate intravascular dosing rate as a reference formulation are available. The method may be an alternative to methods based on plasma concentrations, when drug concentration cannot be measured and when appropriate pharmacological data are available.

Key words bioavailability; pharmacological data; pharmacokinetics; pharmacodynamics; vasopressin; arginine-vasopressin (AVP)

The extent of bioavailability (EBA) is defined as the fraction or percent of an administered dose reaching the systemic circulation. It is one of the most important indexes for the evaluation of drug formulations. Most methods available for determining EBA use plasma concentration level after oral administration, with reference to similar data after intravenous administration. However, there are several reasons to use pharmacological response instead of plasma concentrations. For example, drug levels in plasma may not be available for lack of sensitivity, precision or accuracy in an analytical method, while high quality pharmacological response data are available. Recently, many endogenous peptides, cytokines and their analogues have been developed for clinical use. These drugs have potent pharmacological effects even at a very low dose; however, they are often eliminated rapidly from the systemic circulation. To maintain a constant plasma concentration, and usually to maintain a relatively constant response, various drug delivery system (DDS) technologies, particularly controlled-release formulations, have been applied to these drugs. Although distinct pharmacological responses were observed after administration of these formulations, assessment of accurate EBA was often difficult, because of problems in measurement of blood concentrations. Instead of using the area under the plasma concentration–time curve (AUC), the area under the pharmacological effect–time curve (AUE) was often used to estimate bioavailability; however, AUE is not always proportional to AUC, and therefore EBA could not be estimated directly from AUE. The first approach for assessment of bioavailability from pharmacological data was the report of Smolen. He presented both theory and various applications, and his method has been widely accepted; however, the applicability was limited because a large amount of pharmacodynamic data were required. Recently Gillespie and his coworkers provided a theoretical framework for a new, generalized method of assessing bioavailability from pharmacological data. Their method was based on deconvolution and was highly generalized, but it has not been widely accepted because a mechanism-based pharmacodynamic model could not be incorporated into that model, while mechanisms of drug action and the relationship between drug concentration and receptor binding have recently been identified in many drugs. The purpose of this study was to propose a method of assessing EBA from pharmacological responses. Vasopressin ([Arg9]-vasopressin: AVP) was used as a model peptide drug. AVP is a nonapeptide-hormone and is often used clinically for hypophyseal diabetes insipidus. It has vasopressor activity by constricting the vascular smooth muscle and also has antidiuretic activity by changing water permeability in the collecting duct of the kidney. In the present study, we constructed a mechanism-based pharmacokinetic (PK)–pharmacodynamic (PD) model for two different pharmacological ef-
fects of AVP, and assessed the extent of oral bioavailability (EBA_{av}) from pharmacological data, using the PK-PD model.

MATERIALS AND METHODS

Chemicals AVP was purchased from Sigma Chemical Co. (St. Louis, MO). Camostat mesilate (FOY-305) was supplied by Ono Pharmaceutical Co., Ltd. (Osaka, Japan). Saline and lactated Ringer's solution were from Otsuka Pharmaceuticals, Ltd. (Tokyo, Japan). [Tyrosyl-3,5-(n)-H]vasopressin [Arg^8] (7.4 MBq/ml) was purchased from Amersham Pharmacia Biotech AB (Uppsala, Sweden). All other reagents and solvents were of reagent grade and were obtained commercially (Wako Pure Chemical Industries, Osaka).

Animals and Surgery Male Wistar rats (Japan SLC, Inc., Shizuoka, Japan) weighing 240 to 280 g were used in this study. The rats were housed in constant environmental facilities (temperature: 24±1°C, humidity: 55±10%) exposed to 12:12 h light–dark cycles and maintained on a standard diet, and tap water was given ad libitum for more than 1 week. On the day prior to the experiment, rats were lightly anesthetized with ethyl ether, and were implanted surgically with a combination of a silastic (Dow Corning Corp., Midland, MI) and PE50 (Clay Adams, Parsippany, NJ) catheters into the right jugular vein for bolus injection and blood sampling. In the intravenous infusion study, rats were also implanted with a combination of PE10 and PE50 catheters into the right femoral vein and into the right femoral artery, for drug administration and measurement of the hemodynamic effect, respectively. In the oral administration study, rats were also implanted surgically a combination of PE10 and PE50 catheters into the esophagus. For collecting urine, another PE10 catheter was implanted into the bladder of the animals. All catheters, except that for the urine sampling, were externalized through the back in the neck region and secured. After closure, animals were housed individually and allowed to recover. Unless otherwise specified, all animal experiments were carried out under non-restraint, non-anesthesia and a fasting state. These animal experiments had all previously been approved by the Animal Experimentation Committee of Osaka University of Pharmaceutical Sciences.

Determination of AVP Concentration in Plasma AVP was dissolved in lactated Ringer's solution, and was injected into the jugular vein through the catheter at the dose levels of 25–250 ng/kg (administration volume: 0.1 ml). Blood samples were withdrawn from the jugular vein at predose (blank plasma), and at designated times up to 60 min postdose. The blood samples were transferred to test tubes containing disodium EDTA (1 mg/ml blood), and then centrifuged. Four hundred microliters of isolated plasma was stored frozen at −20°C until assay. Plasma AVP concentrations were determined by the radioimmunoassay (AVP-RIA kit, Mitsubishi Petrochemical Co., Ltd., Tokyo).

Determination of the Hemodynamic Effect of AVP Each rat was housed in an individual metabolic cage and was left at least for 1 h. Then, blood pressure was measured via a strain gauge pressure transducer (P10EZ, Vigo-Spectramed Japan Co., Ltd., Tokyo) connected with the femoral artery catheter. Hemodynamic measurements (systolic pressure, diastolic pressure, and heart rate) before and after AVP administration were recorded every 30 s with a polygraph (Poly-Graph 366 system, NEC San-ei Instruments, Co., Ltd., Tokyo) and a digital oscilloscope (System 400, Nicolet Japan Corporation, Tokyo), connected with a microcomputer (Dyna-Book J-3100SS, Toshiba Corporation, Tokyo). AVP was given by i.v. bolus injection (1–5000 ng/kg, volume: 0.1 ml/rat), by short-term (10 min) infusion (1–5000 ng/kg, 4.0 ml/h) or by oral administration (5, 50 μg/kg, volume: 0.5 ml/rat). In the oral administration study, FOY-305 (2.5 mg/rat) was suspended in the AVP solution and was co-administered. Mean arterial pressure (MAP) was calculated from systolic blood pressure (SBP) and diastolic blood pressure (DBP) via the following equation:

\[
MAP = \frac{(SBP - DBP)}{3} + DBP \text{ (mmHg)}
\]

Measurement of Sodium Concentration in Urine Each rat was housed in a Bollman's cage and was infused with lactated Ringer's solution via the femoral vein catheter, at a rate of 7.4 ml/h for 2.5 h, and the urine flow rate was recorded. AVP (1–500 ng/kg) was then administered by bolus injection (administration volume: 0.1 ml) via the jugular vein or by short-term (10 min) infusion (7.4 ml/h) via the femoral vein. For the oral administration study, AVP was given with FOY-305 (2.5 mg/rat) at the dose levels of 1–50 ng/kg (administration volume: 0.5 ml) via the esophagus cannula. Urine samples were collected every 10 or 20 min after AVP administration for measurement of the urine flow rate and determination of urinary sodium concentration. The sodium concentration was analyzed by an atomic absorption spectrophotometer (AA-670, Shimadzu Corp., Tokyo). Since the decrease in urine flow rate after AVP administration was directly proportional to the increase of sodium concentration in the urine (data not shown), the effect of AVP on the urinary sodium concentration was considered a surrogate marker of the anti-diuretic effect of AVP.

AVP Binding Receptor Assay The binding of [3H]AVP to the V1 receptor of the kidney was determined by the acid-wash method reported by Sato et al.10,11 Briefly, under pento-barbital Na anesthesia, [3H]AVP was administered by constant infusion at the dose levels of 1–200 ng/ml (4 ml/h) for 60 min. At the end of the infusion, PE10 and PE50 catheters were implanted surgically into the renal artery and renal vein, respectively. The ureter was also cannulated. Krebs-Ringer bicarbonate buffer pH 7.4 (KRB: 120 mmol/l NaCl, 4.5 mmol/l KCl, 1 mmol/l MgSO4, 1.8 mmol/l Na2HPO4, 0.2 mmol/l NaH2PO4, 25 mmol/l NaHCO3, 1.25 mmol/l CaCl2, 28 mmol/l Na-glucose) was perfused at the flow rate of 1.9 ml/min for 10 min through the renal artery, and then the perfusate was switched to an acid buffer (pH 3.0). The outflow from the renal vein was collected and the radioactivity of [3H]AVP was determined by a liquid scintillation counter. Assuming that the unbound [3H]AVP was completely washed out by the 10 min KRBB, perfusion, the amount of bound AVP was calculated from the difference between the total amount of [3H]AVP in the acid buffer outflow and the amount of [3H]AVP in the KRBB. outflow for 30 min. Although [3H]AVP was also filtered by the glomerulus during the perfusion, the amount of [3H]AVP recovered in the urine was almost constant compared with the total amount recov-
erated from the renal vein and the ureter. Therefore, $[{^3H}]$AVP filtered by the glomerulus was excluded in the total binding amount.

**Data Analysis** The concentration–time data was analyzed by a nonlinear regression program FKDM$^{12}$ using a MicroVAX II computer (DEC, Maynard, MS) or a PC/AT compatible personal computer. The differential equations were solved by Runge-Kutta-Gill method. Unless otherwise specified, $AUC$ was calculated by the linear trapezoidal method. $EBA_{p,o}$ was calculated by the following equation:

$$EBA_{p,o} = \frac{AUC_{p,o}}{\frac{D_{p,o}}{AUC_{o,v}} + D_{i,v}}$$

where $D_{p,o}$ and $D_{i,v}$ are the oral and intravenous dose of AVP, respectively.

**THEORETICAL**

To determine the relationship between plasma concentration and pharmacological effects after intravenous administration of AVP, PK–PD models were constructed under the following assumptions: (1) the disposition of AVP is described by a conventional two compartment model, (2) the endogenous vasopressin is secreted into the central compartment by a zero-order rate process, (3) the distribution and elimination of AVP are described by first-order kinetics, and (4) the site of action is in the central compartment.

The model shown in Fig. 1A represents the PK–PD model for the hemodynamic effect of AVP after i.v. bolus injection, short-term infusion, and/or oral administration in the rat. For the i.v. bolus study, the differential equations of the model are,

$$\frac{dA_1}{dt} = R-(k_{12} + k_{10})A_1 + k_{12}A_2$$

(1)

$$\frac{dA_2}{dt} = k_{12}A_1 - k_{12}A_2$$

(2)

$$C = \frac{A_1}{V}$$

(3)

where $A_1$ is the amount of AVP in the central compartment, $A_2$ is the amount of AVP in the peripheral compartment, $R$ is the zero-order production rate of endogenous vasopressin (pg/min), $k_{12}$, $k_{21}$ and $k_{10}$ are the first-order rate constant (min$^{-1}$) between each compartment, $C$ is the plasma concentration of AVP, and $V$ is the distribution volume (ml/kg) of the central compartment. At $t=0$, $A_1 = D + C_{00}V$, where $D$ is the bolus dose (pg/kg) of AVP and $C_{00}$ is the baseline concentration of endogenous vasopressin in plasma.

During the constant infusion study, the differential equation for the amount of AVP in the central compartment (Eq. 1) is replaced by Eq. 4:

$$\frac{dA_1}{dt} = k_o + R-(k_{12} + k_{10})A_1 + k_{12}A_2$$

(4)

where $k_o$ is the zero-order infusion rate (pg/min) of AVP.

For oral administration study, the differential equations are,

$$\frac{dA_{p,o}}{dt} = -k_{a_{p,o}}A_{p,o} - k_{c_{p,o}}A_{p,o}$$

(5)

$$\frac{dA_i}{dt} = R + k_{w_{i,p,o}}A_{p,o} - (k_{12} + k_{10})A_1 + k_{12}A_2$$

(6)

$$k_{w_{i,p,o}} = \frac{E_{max,k_{w_{i,p,o}}} \cdot ED_{50}}{ED_{50} + D_{p,o}}$$

(7)

where $A_{p,o}$ is the amount of AVP in the administration site (in the gastrointestinal tract) and $k_{c_{p,o}}$ is the first-order rate constant (min$^{-1}$) for the elimination and/or degradation from the gastrointestinal (GI) tract. Since AVP absorption from the GI tract showed a typical saturation kinetics (data not shown), it was assumed that $k_{c_{p,o}}$ value in Eq. 5 varies with dose, according to the Langmuir type equation as shown in Eq. 7, where $E_{max,k_{w_{i,p,o}}}$ is the maximum value of absorption rate constant (min$^{-1}$), $ED_{50}$ is the dose when the absorption rate constant gives 50% of $E_{max,k_{w_{i,p,o}}}$ and $D_{p,o}$ is the oral dose of AVP.

Since there was no apparent counter-clockwise or clockwise hysteresis between the plasma concentration and the hemodynamic effect of AVP, a direct link model was applied. A
sigmoid $E_{\text{max}}$ model with a baseline effect was used to describe the relationship between plasma concentrations $C_1$ (pg/ml) and MAP (mmHg),

$$\text{MAP} = \text{MAP}_0 + \left( \frac{\text{MAP}_{\text{max}} - \text{MAP}_0}{C_1 + \text{MAP}_{\text{max}}} \right) e^{\gamma_{(\text{MAP})} \text{MAP}}$$

(8)

where $\text{MAP}_0$ (mmHg) is the baseline level of mean arterial pressure, $\text{MAP}_{\text{max}}$ (mmHg) is the maximum hemodynamic effect of AVP, $EC_{50(MAP)}$ (pg/ml) is the plasma concentration of AVP that produces 50% of the $\text{MAP}_{\text{max}}$, and $\gamma_{(\text{MAP})}$ is Hill's constant.

The model shown in Fig. 1B represents the PK–PD link model for the anti-diuretic response to AVP. The PK model for the disposition of AVP was identical to the model described above. Since there was typical counter-clockwise hysteresis between the plasma AVP concentration and the anti-diuretic effect, an indirect link model was introduced. It is well known that principal cells in the renal collecting duct have $V_2$ receptors on their basolateral membranes and that the anti-diuretic effect of AVP is mediated by the $V_2$ receptors by increasing the water permeability. From the facts, it is assumed that the central (plasma) compartment of AVP also involved the site of action; however, the mechanism of the anti-diuretic action requires time, resulting in a lag between concentration–time and effect–time relationship. As shown in Fig. 1B, we assumed two consecutive compartments, $U_{\text{Na}}$ and $U_{\text{Na}}$, representing sodium concentrations (mmol/l) in the collecting duct. In the present model, inflow of sodium to the collecting duct was described by a zero-order rate process and efflux of sodium from the collecting duct was described by first-order processes. A sigmoid $E_{\text{max}}$ model was also applied to explain the relationship between the plasma concentration of AVP and sodium influx in the $U_{\text{Na}}$ compartment.

The differential equations of the model are,

$$\frac{dU_{\text{Na}}}{dt} = \frac{U_{\text{NaMAX}} C_1^{(\text{NO})}}{EC_{50(\text{NO})} + C_1} + k_{\text{in}} - k_{\text{out}} U_{\text{Na}}$$

(9)

$$\frac{dU_{\text{Na}}}{dt} = k_{\text{in}} U_{\text{Na}} - k_{\text{out}} U_{\text{Na}}$$

(10)

where $k_{\text{in}}$ is a zero-order influx rate (mmol/l/min) of sodium in the $U_{\text{Na}}$ compartment, $U_{\text{NaMAX}}$ is the maximum effect (mmol/l/min) of AVP on sodium influx, and $EC_{50(\text{NO})}$ is the AVP concentration (pg/ml) at 50% of $U_{\text{NaMAX}}$, $k_{\text{in}}$ and $k_{\text{out}}$ are first-order rate constants (min$^{-1}$), and $\gamma_{(\text{NO})}$ is Hill's constant.

RESULTS

PK–PD Relationship Plasma vasopressin concentration–time profiles after i.v. bolus injection of AVP are shown in Fig. 2 as plotting points. Although there was a baseline value due to the endogenous vasopressin, a two-exponential decline of plasma concentration was observed. These data were fitted to the model shown in Fig. 1A, and the PK parameters were estimated. The solid lines shown in Fig. 2 represent the results of least squares fit to the model and the estimated PK parameters are listed in Table 1. Since PK parameters were not different among the doses examined (data not shown), the disposition of AVP in the rat was assumed to be linear.

![Fig. 2. Pharmacokinetic Profiles of AVP in Plasma after i.v. Bolus Injection of AVP at Doses of 25 (○), 50 (△) and 250 (□) ng/kg in Rats](image)

Plotted points represent the mean±S.E. of observations ($n=3$) and the solid lines represent theoretical curves.

| Table 1. PK and PD Parameters after i.v. Bolus Injection and Short-Term Infusion of AVP in Rats |
|---|---|---|
| Symbol | Bolus | Short-term infusion |
| $k_{12}$ | 0.0944 ± 0.0200 | min$^{-1}$ |
| $k_{21}$ | 0.111 ± 0.0360 | min$^{-1}$ |
| $k_{30}$ | 0.274 ± 0.0210 | min$^{-1}$ |
| $V$ | 483.04 ± 44.52 | ml/kg |
| $R$ | 0.382 | pg/ml/min |
| $T_{1/2}$ | 2.532 ± 0.194 | min |
| $MAP_0$ | 113.025 | 113.025 | mmHg |
| $MAP_{\text{max}}$ | 53.589 ± 4.405 | 53.069 ± 18.871 | mmHg |
| $EC_{50(\text{NO})}$ | 19.137 ± 4.344 | 43.657 ± 18.396 | pg/ml |
| $\gamma_{(\text{NO})}$ | 1.591 ± 0.391 | 1.637 ± 0.241 |
| $U_{\text{NaMAX}}$ | 61.324 ± 24.204 | 42.072 ± 10.559 | mmol/l |
| $EC_{50(\text{NO})}$ | 2.312 ± 0.339 | 8.390 ± 8.298 | pg/ml |
| $\gamma_{(\text{NO})}$ | 3.036 ± 0.607 | 1 |
| $k_{\text{in}}$ | 0 | 3.351 ± 2.895 | mmol/l/min |
| $k_{\text{out}}$ | 0.272 ± 0.0414 | 0.246 ± 0.0568 | min$^{-1}$ |
| $k_{\text{out}}$ | 0.195 ± 0.0912 | 0.0958 ± 0.0373 | min$^{-1}$ |

Data are expressed as the mean±S.D.

Time courses of hemodynamic effects following i.v. bolus injection or short-term infusion of AVP are shown in Fig. 3 as plotting points. The hemodynamic effect reached a peak and then dissipated. This effect induced by AVP was dose-dependent and the time to reach the peak was consistently observed at just after administration of the drug. There was no symptom to reaching the maximum response even at the highest dose. These facts imply there is a direct relationship between plasma concentrations and the hemodynamic response to AVP. Time courses of anti-diuretic effects following i.v. bolus injection or short-term infusion of AVP are shown in Fig. 4 as plotting points. The anti-diuretic effect induced by AVP was also dose-dependent, however, the time to reach the peak effect increased with dose. No further increase in urinary sodium concentration at doses higher than 50 ng/kg was observed (data not shown). These facts imply there is no direct relationship between plasma concentrations and the anti-diuretic response to AVP.
The PK–PD analysis was carried out according to Eqs. 1—3 and 8, using the hemodynamic effects of AVP, after i.v. bolus injection (25, 50 ng/kg) or short-term infusion (25, 50, 100 ng/kg). The fitted lines shown in Fig. 3 described the pharmacological effects adequately. The estimated PD parameters for the hemodynamic effect are listed in Table 1. Although the MAP_max and \( \gamma_{\text{MAP}} \) values were not different between the two dosing studies, the EC_{50(MAP)} value in the i.v. bolus study was about half of that in the short-term infusion study.

The PK–PD analysis for the anti-diuretic response to AVP was also carried out according to Eqs. 1—3, 9 and 10, after bolus injection (2.5, 5, 25, 50 ng/kg), or short-term infusion (1, 5, 10, 50 ng/kg). The fitted lines shown in Fig. 4 well described the anti-diuretic effect. The estimated PD parameters for the anti-diuretic effect are also listed in Table 1. The estimated \( U_{\text{max}} \) value was comparable between the two dosing studies; however, the EC_{50(Na)} value in the i.v. bolus study was about 1/4 that in the short-term infusion study. These facts indicate that the pharmacological responses to AVP after bolus injection were much more sensitive than that after short-term infusion.

Prediction of Plasma AVP Concentration after Oral Administration Since oral bioavailability of AVP is known to be extremely poor due to its first-pass metabolism, FOY-305, a trypsin and aminopeptidase inhibitor, was co-administered with AVP to prevent rapid destruction in the GI tract. The plotted points shown in Fig. 5A and B represent the time course of the hemodynamic response to AVP after oral administration (5 and 50 \( \mu \)g/kg) in the rat. Without measuring the plasma concentration of AVP, these pharmacological data were then fitted to Eqs. 2, 3 and 5—8, and the absorption PK parameters, namely, \( k_{p,i} \), \( V_{\text{max},i} \), and \( ED_{50} \), were estimated. All other systemic PK parameters such as first-order rate constants, the volume of distribution and so on were shared among oral and i.v. routes of administration. Since PD parameter values, especially \( EC_{50(MAP)} \) values, were significantly different between short-term infusion study and i.v. bolus study, we estimated the absorption parameters separately. The solid lines shown in Fig. 5A represent the results of least squares fit using the PD parameters of the i.v. bolus study, while the solid lines shown in Fig. 5B represent the results of least squares fit using the PD parameters of short-term infusion study. The fitted values in both cases well traced the observed data and the estimated absorption PK parameters are summarized in Table 2.

The plotted points shown in Fig. 5C and D represent the time course of the anti-diuretic response to AVP after oral administration (5 and 50 \( \mu \)g/kg) in the rat. The peak effect after 50 \( \mu \)g/kg dose was almost identical with that after 5 \( \mu \)g/kg dose, and duration of the effect after higher dose was much longer than that after lower dose. These facts indicated that the maximum anti-diuretic response might be attained after oral administration of 50 \( \mu \)g/kg of AVP. These pharmacological data were also fitted to Eqs. 2, 3, 5, 6, 9, and 10, and the absorption PK parameters were estimated as described above. Although an identical absorption kinetic model, described by Eq. 7, was also applied to analyze the anti-diuretic response to AVP, the absorption PK parameters
Fig. 5. Comparison of Calculated Hemodynamic (A and B) and Anti-diuretic (C and D) Effects with Observations after Oral Administration of AVP at Doses of 5 (○) and 50 (▲) μg/kg in Rats. Plotted points represent the mean±S.E. of observations (n=3—4). The solid lines are simulated curves using the PD parameters of i.v. bolus injection (A and C) and short-term infusion (B and D).

Table 2. Absorption PK Parameters Estimated Using the Hemodynamic and the Anti-diuretic Data According to the PD Parameters of i.v. Bolus Injection and Short-Term Infusion in Rats

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Hormodynamic effect</th>
<th>Bolus</th>
<th>Short-term infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_{max}$ (min⁻¹)</td>
<td>26.315 ± 0.363</td>
<td>56.338 ± 0.502</td>
</tr>
<tr>
<td></td>
<td>$ED_{50}$ (μg/kg)</td>
<td>1.91 ± 0.0720</td>
<td>2.213 ± 1.910</td>
</tr>
<tr>
<td></td>
<td>$k_{ap}$ (×10⁻³ min⁻¹)</td>
<td>4.27 ± 0.243</td>
<td>3.558 ± 0.195</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Anti-diuretic effect</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>(μg/kg)</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>$k_{ap}$ (×10⁻³ min⁻¹)</td>
<td>7.490 ± 1.423</td>
<td>0.741 ± 0.0905</td>
<td>53.494 ± 8.003</td>
</tr>
<tr>
<td>$k_{ap}$ (×10⁻³ min⁻¹)</td>
<td>2.857 ± 0.614</td>
<td>0.830 ± 0.124</td>
<td>9.639 ± 1.186</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±S.D.

did not converge between two doses. Therefore, we were obliged to estimate the $k_{ap}$ value as the first-order rate constant for each dose. The solid lines shown in Fig. 5C represent the results of least squares fit using the PD parameters obtained by the i.v. bolus study, while the solid lines shown in Fig. 5D represent the results of least squares fit using the PD parameters of short-term infusion. The estimated absorption PK parameters for each dose are summarized in Table 2. The $k_{ap}$ value showed a distinct nonlinear characteristic among doses, however, the $k_{ap}$ value also showed a nonlinear characteristic.

Prediction of Oral Bioavailability by Pharmacological Effects  The solid lines shown in Fig. 7 represent the simulation curves of the plasma concentration–time profiles after oral administration of AVP. Integrating the simulated plasma concentration–time curve from $t=0$ to $\infty$ numerically, $EBA_{p,o}$ was calculated (Table 3). The $EBA_{p,o}$ values predicted by the pharmacological effects were 0.5 to 0.9% in the 5 μg/kg dose study and 0.07 to 0.17% in the 50 μg/kg dose study. These data indicated definitely that AVP was poorly absorbed from the GI tract and that PD parameters used in the PK–PD model analysis significantly affected the prediction values of $EBA_{p,o}$. In order to assess the accuracy of the prediction values of $EBA_{p,o}$, the time course of actual plasma concentration after oral administration of AVP (5 and 50 μg/kg) was determined. As shown in Table 3, this actual value was 0.81% in the 5 μg/kg dose study, and was 0.25% in the 50 μg/kg dose study. These results indicated that the $EBA_{p,o}$ values estimated by the pharmacological effects in short-term infusion studies well predicted the actual value, however, the $EBA_{p,o}$ value estimated by i.v. bolus injection study significantly underestimated the actual value, especially at the higher dose.

Drug Receptor Binding  As mentioned above, the pharmacological response to AVP in the rat was highly dependent on the input (administration) rate. This might be one of the reasons for the poor prediction of $EBA_{p,o}$ by PK–PD analysis,
especially in the i.v. bolus injection studies. If the relationship between plasma drug concentration and the receptor occupancy differs with dosing rate, the discrepancy of the EC₅₀MAP values between i.v. bolus study and short-term infusion study might be explained. The interrelationship between the plasma concentration and the receptor occupancy of AVP, as well as that between the receptor occupancy and the pharmacological response was thus investigated under the steady-state condition. Since the anti-diuretic effect was mediated by the interaction of AVP with V₂ receptors in the kidney, we focused our attention only on the V₂ receptors.

The plotted points shown in Fig. 7A represent the relationship in the rat between the amount of AVP bound to the V₂ receptors in the kidney and the steady-state plasma concentrations of AVP under the constant infusion of [³H]AVP for 60 min. As shown, the amount of AVP bound to the receptors increased linearly as the logarithm of the steady-state plasma concentrations of AVP increased. The anti-diuretic effect increased linearly as the logarithm of the amount of AVP bound to the receptors increased (Fig. 7B). These relationships can be described mathematically by the following log-linear equations,

\[ B_{N_0}^{SS} = s_1 \log C_{N_0}^{SS} - I_1 \]  
\[ C_{N_0}^{SS} = s_2 \log B_{N_0}^{SS} + I_2 \]

where \( C_{1}^{SS} \) (pg/ml) is the steady-state concentration of AVP in plasma, and \( C_{N_0}^{SS} \) (mmol/l) is the sodium concentration in urine, \( B_{N_0}^{SS} \) is the amount of AVP (pg/kidney) bound to the V₂ receptors at steady-state, \( s_1 \) (ml/kidney) and \( s_2 \) (kidney-mmol/l/pg) are coefficients, and \( I_1 \) (pg/kidney) and \( I_2 \) (mmol/l) are constants. Then, the data shown in Fig. 7A and B were fitted to the Eqs. 11 and 12, respectively, and the coefficients and constants were estimated. The straight lines shown in Fig. 7A and B represent the least squares fit to the data, and the estimated parameters are listed in Table 4.
Since those PD relationships were obtained under the steady-state condition (plasma AVP concentration was kept constant), we could not incorporate those relationships into the previous PK–PD model directly. If the drug–receptor interaction was instantaneous, and the transduction of receptor occupancy into the primary pharmacological response at the site of action (CNA) was much faster than the secondary post-receptor events, the relationships obtained at steady-state (Eqs. 11, 12) should still hold under non-steady-state condition too:

\[ B_{\text{w}} = s_1 \cdot \log C_N - I_1 \]  

(3)

\[ C_{\text{na}} = s_2 \cdot \log B_{\text{na}} + I_2 \]  

(4)

The time lag between the primary pharmacological response (CNA) and the anti-diuretic response (Uw/o) to AVP under a non-steady-state condition was handled by inserting a transit compartment, as follows:

\[ \frac{dU_{\text{w/o}}}{dt} = \frac{(C_{\text{na}} - U_{\text{w/o}})}{\tau} \]  

(5)

where \( \tau \) is a transfer time parameter.

The overall PK–PD model for the anti-diuretic response to AVP, including the receptor-binding process, the transduction process and the post-receptor processes is summarized in Fig. 1C. The anti-diuretic effect of AVP, from the start to the end of the constant infusion, can be described by Eqs. 2–4 and 13–15, while the anti-diuretic effect after i.v. bolus injection can be described by Eqs. 1–3 and 13–15. The anti-diuretic effect–time profiles in the constant infusion, in the short-term infusion and in the bolus injection were re-fitted to the overall PK–PD model, and the PD parameters shown in Eqs. 13 through 15 were estimated. The parameters \( s_1 \) and \( I_1 \) provide the drug receptor binding at the site of action. Parameters \( s_2 \) and \( I_2 \) provide the transduction processes, and parameter \( \tau \) provides the post-receptor processes. If there is any difference in value of the PD parameters among i.v. bolus and infusion studies, the difference might be the direct cause of the input-rate-dependent pharmacological effect of AVP. The results are summarized in Fig. 8 and Table 4. The estimated \( E_0 \), \( s_2 \), \( I_1 \) and \( \tau \) values were comparable among these dosing studies; however, \( s_1 \) value in the i.v. bolus study (213 ml/kidney) was much greater than those in the infusion studies (150 ml/kidney). This result indicates that the amount of AVP bound to the V2 receptors after i.v. bolus injection is much greater than that in the infusion study, even at the same plasma concentration.

The anti-diuretic effect after oral administration of AVP was also fitted to the overall PK–PD model (Eqs. 2, 3, 5–7 and 13–15), and the PD parameters were estimated. (All of the PK parameter values, including the absorption parameters, were fixed to the previous values.) The estimated PD parameters including \( s_1 \) value in the oral study were almost identical to those in the infusion studies. This fact suggests that the amount of AVP bound to the V2 receptors after oral administration is always smaller than that expected from the i.v. bolus study. Consequently, oral bioavailability calculated by the PK–PD model of i.v. bolus administration always underestimates the actual value.

DISCUSSION

Pharmacokinetics of AVP The pharmacokinetic analysis of AVP in the rat was carried out using a conventional linear two-compartment model with a baseline value. An extremely short half-life in plasma and a relatively small volume of distribution were observed, and these PK parameters were fundamentally consistent with the report of Janáky et al. In the oral administration study, we used FOY-305 to prevent rapid degradation of AVP in the GI tract, for our pre-
liminary investigation indicated that without FOY-305, no pharmacological response to AVP was observed in the present dose range. In spite of co-administration of FOY-305, AVP was still degraded rapidly by a first-order process in the GI tract, as shown in Table 1. The first-order degradation rate constant $k_{d,p}$ estimated by the PK–PD model of the hemodynamic response, was consistent with the in vitro studies reported.

After oral administration of AVP, a non-linear (dose-dependent) absorption kinetic model was assumed in the present study. Result of PK–PD analysis of hemodynamic effects suggested that the rate of GI absorption was easily saturated as dose increased. Vilhardt and Lundin reported their in vitro study that 1-deamino-8-d-arginine vasopressin (dDAVP), an analogue of vasopressin, was transported through the intestinal wall by passive transport. However, the in vivo absorption process of AVP involves not only permeation through intestinal membrane but also degradation and metabolism in the membrane. The precise mechanism for the present non-linear absorption of AVP is still unclear and further investigation is needed.

Pharmacodynamics of AVP
The pharmacological response to AVP is known to be mediated by at least two types of receptors on the cell surface, namely $V_1$ and $V_2$ receptors. $V_1$ type receptors are present on vascular smooth muscle cells and platelets and elevate blood pressure, whereas $V_2$ type receptors exist on the epithelial cells of the distal tubules and the collecting ducts of the kidney, and increase the reabsorption of water, NaCl and urea. $V_2$ receptor-mediated effects of vasopressin occur at concentrations far lower than are required to engage the $V_1$ receptor-mediated effects. This differential sensitivity may be due to difference in receptor affinities. The dissociation constants ($K_d$) for $V_1$ and $V_2$ receptors were reported to be about 6000 and 390 pmol/L, respectively. This is the reason that the dose range of AVP used in the hemodynamic studies was 10 times higher than that used in the anti-diuretic studies. Using the $K_d$ value for the $V_2$ receptors, we calculated the fraction of $V_2$ receptor occupancy of AVP in the rat, and found that only 23% of the $V_2$ receptors were occupied by AVP even at the highest dose (1000 ng/kg/h). In the present study, we used the log-linear model (Eq. 13) rather than the sigmoid $E_{max}$ model to describe the relationship between the plasma concentration and the amount of AVP bound to $V_2$ receptors. Therefore, use of the log-linear model was shown to be appropriate.

Assessment of $EBA_{p,a}$ Estimated by the Pharmacological Effects
As shown in Table 3, $EBA_{p,a}$ values estimated by the pharmacological effects of AVP in short-term infusion studies well predicted the actual $EBA_{p,a}$. The $K_d$ values for $V_1$ and $V_2$ receptors suggest that for the $EBA_{p,a}$ of high dose range, hemodynamic effects ($V_1$ receptors) should be used, while for the $EBA_{p,a}$ of low dose range, anti-diuretic effect ($V_2$ receptors) should be used. Thus, we confirmed that the present method might be useful for the assessment of bioavailability of high potency drugs, where plasma concentrations were not available due to lack of sensitivity.

The $EBA_{p,a}$ value obtained by the anti-diuretic effect of i.v. bolus study significantly underestimated the actual $EBA_{p,a}$, especially at the higher dose. As shown in Fig. 5C and D, after 50 μg/kg oral administration, maximum anti-diuretic response to AVP might be attained. In that situation, the one-to-one correspondence between plasma concentrations and pharmacological effects does not hold, and therefore estimation of plasma concentrations from the pharmacological effect is extremely difficult. Consequently, poor prediction of $EBA_{p,a}$ by anti-diuretic response in the higher dose range resulted. To obtain a reasonable estimate of $EBA_{p,a}$, the PD effect of oral administration must be in the range from 20 to 80% of the maximum effect.

As mentioned above, the amount of AVP bound to the $V_2$ receptors after i.v. bolus injection was much greater than that in the oral study, even at the same plasma concentration. This is the direct cause of the poor prediction of $EBA_{p,a}$; however, the precise mechanism for the input rate dependent receptor-binding is still unclear. Here, we speculate on this mechanism as follows. In the case of bolus injection, plasma concentrations of AVP increase and reach the maximum concentration just after the administration and then decrease rapidly. The receptors contact AVP in high concentrations, but in a short period of time, while in the case of constant infusion or oral administration, plasma concentrations increase gradually to reach the maximum value. If the receptors contact the drug for a long period of time, the receptors may internalize and cause desensitization. Since there have been several reports of internalization, down-regulation and desensitization of AVP receptors, the difference between the amount of AVP bound to the $V_2$ receptors after i.v. bolus injection and that after oral administration might be explained, in part, by the receptor-desensitization.

The purpose of this study was to assess the $EBA_{p,a}$ values estimated by the pharmacological effects of AVP. The accuracy of $EBA_{p,a}$ is solely dependent on how accurate we predict the plasma concentrations for oral administration (and therefore $AUC_{p,a}$) from the PD data following standard (intravascular) administration of AVP. Therefore, PD processes including the receptor-binding process, the transduction process and the post-receptor processes in the oral administration study should be identical with those in the standard (intravascular) administration study. Since PD processes of the short-term infusion study were identical with those of the oral study, plasma concentrations of AVP after oral administration were predicted accurately by the pharmacological effects of short-term infusion, rather than by the i.v. bolus study.

In conclusion, a novel method of assessing the extent of oral bioavailability of AVP from pharmacological data has been presented, and the results demonstrate that the bioavailability is reasonably predicted by a PK–PD model, provided appropriate pharmacological effects and appropriate intravenous dosing rates are available. The method may be an alternative to methods based on plasma concentrations, when drug concentration cannot be measured and when appropriate pharmacological data are available.

REFERENCES