Estimation of Bioavailability of Salmon Calcitonin from the Hypocalcemic Effect in Rats (II): Effect of Protease Inhibitor on the Pharmacokinetic-Pharmacodynamic Relationship after Intranasal Administration

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Summary: Assessment of the extent of bioavailability (EBA) of salmon calcitonin (sCT) from hypocalcemic effects after intranasal administration was presented in rats. An integrated pharmacokinetic-pharmacodynamic (PK-PD) model with the endogenous Ca regulation system was applied. The influence of camostat mesilate, a protease inhibitor, on absorption of sCT was also estimated. Camostat, coadministered intravascularly, delayed the elimination of sCT. Although the hypocalcemic effect of sCT after i.v. administration was accelerated when camostat was coadministered intravenously, the enhanced effect could not be expressed only by pharmacokinetic change of sCT, and then the pharmacological data in the presence of camostat were analyzed to obtain optimal PD parameters. For the absorption of sCT after i.n. administration, a saturable absorptive process and a zero-order kinetic clearance from the nasal cavity were introduced to the model. The regression curves fitted the observed data, and camostat caused both an increase in maximum absorption rate and a decrease in the clearance parameter compared with the control. According to this modified PK-PD relationship, plasma sCT concentrations following i.n. administration of sCT with camostat were predicted well using its pharmacological effects. The EBA of sCT calculated from the simulated concentrations increased more than 4-folds compared with the control study. These results indicate the potential for prediction of plasma sCT concentration from the hypocalcemic effect.

Key words: salmon calcitonin; PK-PD model; hypocalcemic effect; protease inhibitor; intranasal administration; extent of bioavailability

Introduction

The intranasal absorption of salmon calcitonin (sCT) coadministered with camostat mesilate, a synthetic protease inhibitor, has been evaluated to characterize the enzymatic barrier of the nasal mucosa.1) An assessment of the bioavailability was made using the "pharmacological availability (PA)" which was calculated from the hypocalcemic effect, as a substitute of the extent of bioavailability. However, there is no conclusive proof that plasma concentrations are always proportional to the pharmacological effects, and the use of PA in evaluating drug formulations might lead to erroneous conclusions.2) Therefore, the concentration of sCT must be determined practically for the accurate assessment of bioavailability, though the available analytical method of sCT lacks adequate sensitivity for the determination of low plasma concentrations especially after intranasal administration.

In the precedent report,3) we quantified the pharmacokinetic-pharmacodynamic (PK-PD) relationship after i.v. bolus administration of sCT in rats. The result indicated the potential for predicting of plasma sCT concentrations from the hypocalcemic effect using a PK-PD model (Fig. 1). Briefly, the model consists of the hypocalcemic effect of sCT and the endogenous Ca regulation system associated with rat CT (rCT). A pharmacokinetic equation for sCT with a two-compartment...
Fig. 1. Diagrammatic Representation of the PK-PD Model for Hypocalcemic Effects of sCT.

The pharmacokinetics is linked to the Ca regulation system (gray area) through the pharmacodynamic model. sCT1 and sCT2: amounts of sCT in the central and peripheral compartment; K0: the infusion rate; k12, k21, and k10: first-order constants; V: the distribution volume of the central compartment; sCT1 and sCT2: plasma sCT concentrations; Ca: plasma Ca concentrations; rCT, rCT1, and rCT2: rCT concentrations in the plasma and the effect compartments; kCa10 and krCT10: the first-order kinetic constants for the Ca and rCT; \( \tau_{eCT} \): the first-order kinetic constant for the effect compartments of rCT; \( \gamma \): the exponential function \( (\alpha \cdot \phi \cdot e^{q2 \cdot Ca}) \); \( F_1 \) and \( F_2 \): proportional constants for the secretion of rCT; \( \beta \): the proportional constant for the effect of rCT on the plasma Ca level; E1 and E2: amounts of sCT in the effect compartments; \( \delta \): the first-order kinetic constant; \( E_{max} \): the maximum effect of sCT; \( 1/Q \): the affinity constant of sCT to the receptor (see Ref. 3).

Bioavailability Estimation of CT from Hypocalcemic Effect

The pharmacokinetic model was linked to an \( E_{max} \) model as suppression of plasma Ca generation through two effect compartments. The rCT, expressed by a one-compartment pharmacokinetic model, effects linearly suppression of plasma Ca concentration, however the generation of plasma rCT is also involved in the Ca level exponentially.

The objective of this study was to propose a method of evaluating the bioavailability of sCT through plasma concentrations predicted from the hypocalcemic effect using the PK-PD model. As a model study, the previous results after i.n. administration of sCT with or without camostat mesilate\(^1\) was reanalyzed.

Materials and Methods

Chemicals: Synthesized salmon calcitonin (sCT) was kindly supplied by Teikoku Hormone Mfg. Co. (Tokyo, Japan). Camostat mesilate (FOY-305) was supplied by Ono Pharm. Co., Ltd. (Osaka, Japan). Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO). Saline was obtained from Otsuka Pharmaceuticals (Tokyo, Japan). All other reagents and solvents were commercial products of reagent grade (Wako Pure Chemical Industries, Osaka, Japan).

Animal surgery: Male Wistar rats (Japan SLC Inc., Shizuoka, Japan), weighing 200 to 240 g, were used. The rats were housed in environmentally regulated facilities (temperature: 24 ± 1°C, humidity: 55 ± 10%), exposed to alternative 12 hr cycles (6:00 and 18:00) of light and dark for over 1 week, and allowed free access to a standard diet and tap water. The cannulas inserted into the femoral artery, femoral vein and jugular vein of each animal were exteriorized via the neck, as reported previously.\(^3\) After surgery, the animals were left at least 15 hrs under fasting to recover. All of these methods had previously been approved by the Animal Experimentation Committee of Osaka University of Pharmaceutical Sciences.

Administration: On the day of the experiment, the rats were housed in individual metabolic cages. sCT was dissolved in saline containing 0.1% BSA. Camostat mesilate was dissolved in the sCT solution at 5 mmol/L (dose: 0.5 μmol/rat). In the bolus administration study to assess the pharmacological effect, the sCT preparation was injected into the femoral veins via the catheters at doses ranging from 0.075 to 0.225 IU/kg. In the preliminary PK study, we found that the elimination of sCT after i.v. bolus administration is too fast to be
determined precisely. Then, sCT was injected continuously (5 IU/kg/hr) for 75 min into the femoral artery with an infusion pump (STS-523: Terumo corporation, Tokyo, Japan). Blood samples were withdrawn from the femoral vein predose (blank plasma), and at designated postdose intervals. Plasma sCT and Ca concentrations were determined as described previously. The i.n. administration study was demonstrated in the previous paper.

**Data analysis:** The concentration-time data was analyzed by a nonlinear regression program, FKDM using a Micro VAX II computer (DEC, Maynard, MS) or a personal computer (NEC Corporation, Tokyo, Japan). The differential equations were solved by the Runge-Kutta-Gill method. The areas under the concentration-time curve (AUC) following administration of sCT, from 0 to 5 hr, were calculated by the linear trapezoidal method. The extent of bioavailability (F) was calculated by the following equation:

\[
F = \frac{AUC_{i.n.}(0–5\,\text{h})}{AUC_{i.v.}(0–5\,\text{h})}
\]

where \(D_{i.n.}\) and \(D_{i.v.}\) are the intranasal and intravenous doses of sCT, respectively.

**Results**

**Extent of bioavailability after i.n. administration of sCT:** The time course of plasma Ca concentrations after i.n. administration of sCT (10 IU/kg) without camostat mesilate was shown in Fig. 2A (plotted points). Compared with the control group, a significant hypocalcemic effect was observed. To explain a pharmacokinetic property of the nasal absorption, we introduced an absorption process to the previous PK-PD model as shown in Fig. 1. In the modeling, we assumed as follows; a) the plasma Ca is generated by a zero-order kinetic process \((K_{Ca0})\), and eliminated by a first-order kinetic process. b) The rCT is secreted in accordance with the plasma Ca concentration by an exponential function. c) If plasma Ca level is above the normal level, rCT affects the bone resorption to maintain the normal level of plasma Ca in a feedback manner. d) The rCT exerts its pharmacological effect via an additional effect compartment. In the i.n. administration study, we assumed that sCT is absorbed from the nasal mucosa according to the Michaelis-Menten type kinetics and that sCT is eliminated from the nasal cavity by a zero-order kinetic process. The differential equations of the amount of sCT in the nasal cavity are,

\[
\frac{dsCT_n}{dt} = - k_x \cdot sCT_n - k_{ab} \quad \text{Eq. 4a}
\]

and the following equation 4b is substituted for Eq. 4b in the previous paper:

\[
\frac{dsCT_1}{dt} = k_x \cdot sCT_n - (k_{12} + k_{10}) \cdot sCT_1 + k_{21} \cdot sCT_2
\]

**Fig. 2.** Hypocalcemic Effect (A) and Model-estimated Plasma Concentration (B) of sCT after i.n. Administration of sCT (10 IU/kg) without and with camostat mesilate (0.1 and 1 μmol/rat). Each value represents the mean ± S.E. of 4 experiments (Reproduced from Ref. 1). The solid lines represent theoretical curves.

Where

\[
k_x = \frac{V_{max}}{K_m + sCT_n}
\]

\(V_{max}\) and \(K_m\) are the maximum rate of absorption and the Michaelis constant, respectively. \(K_{ab}\) is the zero-order constant and \(sCT_n\) is the amount of sCT in the...
Table 1. Absorption parameters of sCT after i.n. administration with and without camostat mesilate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>without Camostat (Control)</th>
<th>with Camostat</th>
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<tr>
<td></td>
<td>1 mmol/L</td>
<td>10 mmol/L</td>
</tr>
<tr>
<td>$K_{m}$ (mIU)</td>
<td>$1.17 \pm 64$</td>
<td>$1.17^{\circ}$</td>
</tr>
<tr>
<td>$V_{max}$ (mIU/min)</td>
<td>$200 \pm 78$</td>
<td>$371 \pm 77$</td>
</tr>
<tr>
<td>$K_{e}$ (mIU/min)</td>
<td>$21.3 \pm 10.2$</td>
<td>$9.11 \pm 4.14$</td>
</tr>
<tr>
<td>$AUC_{i,n}$ (mIU/mL min)</td>
<td>$0.570$</td>
<td>$2.35$</td>
</tr>
<tr>
<td>$F$ (%)</td>
<td>$0.870$</td>
<td>$3.58$</td>
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</table>

Data represent the computer-fitted value ± S.D. $K_{m}$, $V_{max}$, the Michaelis-Menten constants, $K_{e}$, the elimination constant from the nasal cavity, $AUC_{i,n}$; the area under the concentration-time curve after i.n. administration, $F$: the extent of bioavailability, $\circ$: value obtained from the control study.

nasal cavity. The solid line, shown in Fig. 2A, represents the response curve obtained by fitting the PK-PD model to the observed data. All of the pharmacokinetic, pharmacodynamic and physiological parameters, except absorption parameters, were identical with the previous report. The estimated absorption parameters are listed in Table 1. The solid line shown in Fig. 2B represents the model-predicted plasma sCT concentration, and reached a steady-state at about 6 mIU/mL over 0.5–1.5 hrs after administration. AUC was calculated and, thus, the extent of bioavailability ($F$) was estimated, as shown in Table 1. Although a significant hypocalcemic effect was observed after i.n. administration of sCT, extremely low bioavailability (0.87%) was indicated.

Effects of camostat mesilate on the extent of bioavailability of sCT: The effect of camostat mesilate on the pharmacokinetics of sCT was investigated. The sCT (5 IU/kg/hr) solution, including 0.5 mmol/rat camostat mesilate, was infused for 75 min, and plasma sCT concentrations were determined (Fig. 3A; plotted points). Then these observed plasma concentration data were fitted to a two-compartment open model. The solid line shown in Fig. 3A represents the result of the least-squares regression fitting and the estimated PK parameters are listed in Table 2. The distribution volumes $V_c$ and $V_t$ were significantly influenced by camostat mesilate. Figure 3B shows the model-predicted sCT concentration in plasma after i.v. bolus administration with camostat mesilate. Camostat mesilate deceased the elimination of sCT. Using these PK parameters, the hypocalcemic effect following i.v. bolus administration with camostat mesilate (0.5 mmol/rat) was predicted; however, the predicted curves did not describe the observed data (data not shown). This result indicates that camostat mesilate influenced not only PK of sCT but also pharmacological processes of sCT.
These changes may account for prolongation and 4-fold with camostat coadministration, indicating that the hypocalcemic effect was increased and prolonged as doses of camostat mesilate increased. To elucidate the influence of camostat mesilate on the absorption of sCT from the nasal mucosa, the pharmacological data were analyzed by the modified PK-PD model, using identical PK and PD parameter values for camostat coadministration shown in Table 2. The solid lines shown in Fig. 2A traced the observed data well. This indicates that the modified PK-PD model explains the PK-PD relationship after i.n. administration of sCT with camostat mesilate. The estimated absorption parameters were summarized in Table 1. Because the estimated $K_m$ value was almost identical with the control study (Fig. 2), the $K_m$ value was fixed to the control value (1.17 mIU). The estimated values of $V_{max}$ were increased while the $K_m$ values were decreased; however, these changes were depend on the doses of camostat mesilate.

**Prediction of bioavailability after i.n. administration of sCT with camostat mesilate:** Figure 2B represents the predicted plasma sCT concentrations after i.n. administration of sCT (10 IU/kg) in the presence of camostat mesilate. The solid lines show the concentration-time profiles after coadministration of camostat mesilate (0.1 and 1 μmol/rat) and the dashed line shows the profile after administration of sCT alone. As doses of camostat mesilate increased, the maximum levels of sCT were markedly enhanced and high plasma levels were prolonged. Then, AUC and the extent of bioavailability (F) were calculated and summarized in Table 1. Compared with the control, F increased more than 4-folds with camostat mesilate coadministration.

**Discussion**

Application of PK-PD model to the i.n. administration study: In order to elucidate the applicability of the PK-PD model of sCT, the hypocalcemic effect versus time data following i.n. administration was analyzed. In the present model, we assumed that the absorption process of sCT from the nasal mucosa conforms to the Michaelis-Menten type kinetics, because a linear absorptive model could not explain the observed data (data not shown). The non-linear model is often used to represent a saturable flux of substrates. The endocytotic transport of CT and elcatonin, a calcitonin derivative, from the nasal mucosa was reported. Although, this process may be related to, in part, the saturable absorption observed in the present study, the estimated parameter values are an outcome of

<table>
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<th>Pharmacokinetic parameters</th>
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<tr>
<td>$k_{12}$ (min$^{-1}$)</td>
<td>0.677 ± 0.50</td>
<td>2.97 ± 0.9</td>
</tr>
<tr>
<td>$k_{21}$ (min$^{-1}$)</td>
<td>0.309 ± 0.093</td>
<td>0.250 ± 0.12</td>
</tr>
<tr>
<td>$k_{30}$ (min$^{-1}$)</td>
<td>0.466 ± 0.18</td>
<td>1.56 ± 2.3</td>
</tr>
<tr>
<td>$V_c$ (mL)</td>
<td>69.6 ± 21</td>
<td>18.8 ± 6.6*</td>
</tr>
<tr>
<td>$V_t$ (mL)</td>
<td>153 ± 46</td>
<td>22.3 ± 7.8*</td>
</tr>
<tr>
<td>$V_{ss}$ (mL)</td>
<td>222 ± 67</td>
<td>241 ± 85</td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>32.4 ± 9.7</td>
<td>29.3 ± 10</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>6.86</td>
<td>8.25</td>
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Data represent the computer-fitted value ± S.D. Vc; the distribution volume in the central and tissue compartment. Vss; the distribution volume in the steady state, CL; the total clearance, MRT; the mean residence time (Vss/CL). *; Significantly different from control (p<0.05, t-test).

**Table 2. Effects of camostat Mesilate on the pharmacokinetic and pharmacodynamic parameters of sCT**

In order to reveal the effect of camostat mesilate on the pharmacodynamics of sCT, plasma Ca concentrations after i.v. coadministration of camostat mesilate were fitted to the PK-PD model (Fig. 4). The solid lines shown in Fig. 4 represent the theoretical values, and the estimated PD parameters are listed in Table 2. The regression curves fitted the observed data well. As shown in Table 2, the $E_{max}$ and 1/Q increased 2-fold to 4-fold with camostat coadministration, indicating that these changes may account for prolongation and enhancement in the pharmacological effect of sCT by camostat mesilate.

The plotted points shown in Fig. 2A represent the changes in plasma Ca levels following i.n. administration of sCT (10 IU/kg) with camostat mesilate (0.1 and 1 μmol/rat), which were reported by Morimoto et al. The hypocalcemic effect was increased and prolonged as doses of camostat mesilate increased. To elucidate the influence of camostat mesilate on the absorption of sCT from the nasal mucosa, the pharmacological data were analyzed by the modified PK-PD model, using identical PK and PD parameter values for camostat coadministration shown in Table 2. The solid lines shown in Fig. 2A traced the observed data well. This indicates that the modified PK-PD model explains the PK-PD relationship after i.n. administration of sCT with camostat mesilate. The estimated absorption parameters were summarized in Table 1. Because the estimated $K_m$ value was almost identical with the control study (Fig. 2), the $K_m$ value was fixed to the control value (1.17 mIU). The estimated values of $V_{max}$ were increased while the $K_m$ values were decreased; however, these changes were depend on the doses of camostat mesilate.

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This parameter. Gonda and Gipps demonstrated a mathematical model describing the rate processes for the disposition of drugs in the human nasal cavity. The model contained some parallel and sequential processes representing the drug transport by fluid flow and mucociliary clearance, drug release and absorption, and so on. In our model, these respective processes were not expressed individually as different compartments. If an enzymatic hydrolysis rate and mucociliary clearance of sCT are determined separately, the absorption profile would be simulated more precisely.

Plasma sCT profiles were predicted from the pharmacological data after i.n. administration of sCT. However, pharmacokinetic parameters of sCT were based on higher plasma concentrations compared with the predicted profile in the i.n. study. There have been no reports concerning the dose-relative pharmacokinetics of sCT at this range of doses, because disposition of sCT is extremely fast and sensitivity of assay method may be insufficient like our case. Even if a non-linear pharmacokinetics is presumed in our model, these model parameters can not be decide for lack of the observed concentrations of sCT.

Effects of camostat mesilate on the PK-PD relationship of sCT: Intranasally administered camostat mesilate caused significant decrease in the distribution volume ($V_d$) of the central compartment for sCT, resulting in a delay of the disposition of sCT (Table 2, Fig. 3). In the previous report, we demonstrated that camostat mesilate inhibits not only trypsin but leucine aminopeptidase (LAP) in nasal mucosa. Camostat mesilate also showed inhibitory effect to these proteases in plasma. It is known that trypsin and LAP distribute widely over various tissues and organs. Although camostat mesilate is quickly hydrolyzed by esterase in plasma, its metabolite, FOY-251, has inhibitory effect equal to the intact drug. Therefore, change in the distribution volume may be involved in a wide inhibitory effect of camostat mesilate and/or its metabolite. This modified pharmacokinetic property was also applied to explain plasma sCT concentrations after i.n. coadministration with camostat mesilate (Fig. 2B). In order to influence the pharmacokinetics of sCT, intranasally administered camostat mesilate has to be absorbed from nasal membrane and reach to blood flow. In spite of poorer permeability of the intestinal mucosa comparing with the nasal mucosa, total excretion of FOY-251 and main metabolite during 24 hrs in human urine was about 21% of the dose administered orally.

While, the estimated plasma Ca profile was quite different from the observed data, when the control set of PK and PD parameters was used to explain the results of i.n. study. Therefore, it is appropriate that we used the modified PK parameters in relation to camostat mesilate in the analysis of i.n. study. However, camostat mesilate might be overdosed in the i.v. study judging from the intranasal permeability.

Plasma Ca levels after i.v. bolus coadministration of sCT and camostat mesilate could not be described well by the integrated model with the following set of PK, PD and physiological parameters: the PK parameters obtained in the presence of camostat mesilate, the PD parameters obtained in the absence of camostat mesilate and the physiological parameters of Ca regulation model. Then the pharmacological data was reanalyzed to estimate the optimal PD parameters. The physiological parameters for the Ca regulation system were used without any change because these parameters were unlikely to be affected by camostat mesilate. The estimated values of $E_{max}$ and $I/Q$ were higher than those of the control condition without camostat mesilate. This effect of camostat mesilate is probably related with the inhibitory activity to enzymes such as trypsin in plasma and tissues. However, the actual mechanism is unclear and further investigation is needed.

Simulation of plasma sCT concentrations after i.n. administration of sCT with camostat mesilate: Hypocalcemic effects after i.n. administration of sCT with camostat mesilate could be well described using the PK-PD model with the Ca regulation system. Camostat mesilate also influenced the nasal absorptive properties of sCT, causing an increase in the maximum absorption rate ($V_{max}$) and a decrease in the nasal clearance ($K_{in}$), depended on the dose of camostat mesilate. These facts clearly indicate that camostat mesilate protects sCT from hydrolysis of proteases. Furthermore, the change in these parameters might reflect an improvement of permeability caused by the functional alteration of the nasal mucosa associated with protease activities. The absolute bioavailabilities, calculated from model-predicted plasma sCT concentrations, increased to 3.6 - 4.9% from the control of 0.87%, when camostat mesilate was coadministered (at doses of 0.1 - 1 μmol/ rat). Sinswat and Tengarnrungs reported that bioavailability of sCT based on observed plasma concentrations was 1.22% after i.n. administration of sCT (10 IU/kg) in rats. This fact is consistent with our result, and the bioavailability estimated from the phar-
macological effects of sCT using the PK-PD model is reasonable. In contrast, the PA of sCT, after i.n. administration of sCT with camostat mesilate, increased to 4.2–5.1% from 3.16% (data not published), showing the possibility to overestimate bioavailabilities of sCT.

In conclusion, a method of assessing the extent of bioavailability of sCT from hypocalcemic data has been presented, and we demonstrates that the bioavailability is reasonably predicted using a PK-PD model. Furthermore, this result indicates that the pharmacological availability has a possibility overestimating the bioavailability of sCT. Thus, one PK and PD parameters are established for sCT and the endogenous Ca regulation system, the extent of bioavailability and plasma sCT profile will be easily and routinely predicted without determination of plasma sCT concentrations, for example, in a development phase of novel formulations.

References