Estimation of Bioavailability of Salmon Calcitonin from the Hypocalcemic Effect in Rats (I): Pharmacokinetic-Pharmacodynamic Modeling Based on the Endogenous Ca Regulatory System

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Summary: The hypocalcemic effect of salmon calcitonin (sCT) after intravenous administration was explained on the basis of an integrated pharmacokinetic-pharmacodynamic (PK-PD) model with the endogenous Ca regulatory system in the rat. The pharmacokinetics of sCT described by a conventional two-compartment model showed the extremely rapid elimination of sCT from plasma (MRT; 6.86 min). The hypocalcemic effect of sCT reached a peak from 0.5 to 1.5 hrs after administration, and the peak time tended to prolong with increasing doses. This delay in pharmacological effect of sCT against plasma concentration may be a result of a summation of multiple actions of the endogenous Ca regulatory system including feedback control. The plasma Ca regulation system in the rat was investigated by i.v. bolus administration of calcium gluconate and/or endogenous (rat) calcitonin (rCT). Since non-linearity in the relationship between Ca and rCT concentrations in plasma was observed, we assumed that rCT was secreted in accordance with the plasma Ca level via an exponential function. The pharmacokinetics of rCT was represented as a linear one-compartment model. To link the rCT level with plasma Ca level, an additional effect compartment was required to explain the delay in onset and decline of the pharmacological effect. This Ca regulation model explained the observed Ca and rCT profiles in plasma after administration of Ca and/or rCT. The plasma Ca levels after administration of sCT could be well described by the present integrated model. This suggested the potential for prediction of plasma sCT concentration only from the hypocalcemic effect after extravascular administration of sCT, using this PK-PD model.

Key words: salmon calcitonin; PK-PD model; hypocalcemic effect; calcium regulation; feedback control

Introduction

Calcitonin (CT), an endogenous polypeptide hormone, is therapeutically used for the treatment of Paget’s disease, osteoporosis and hypercalcemic conditions. Since oral bioavailability is extremely low, CT is often administered intranasally to overcome the proteolytic degradation in the gastrointestinal tract and the first-pass effect in the liver. However, the bioavailability is still low even if CT was intranasally administered with absorption enhancers. One of the reasons for the low bioavailability is the presence of various proteolytic enzymes (ex. trypsin, aminopeptidase) in the nasal mucosa.

In our previous report, we evaluated the nasal absorption of salmon CT (sCT) coadministered with protease inhibitors to characterize the enzymatic barrier of the nasal mucosa. In that report, we also assessed the bioavailability following i.n. administration of sCT using the pharmacological availability (PA) calculated only from the hypocalcemic effect, as a substitute of the extent of bioavailability. Although PA has often been used to assess the absorption of sCT, the hypocalcemic effect of sCT is a result of summation of multiple actions of sCT, endogenous CT, parathyroid hormone (PTH), 1,25-dihydroxy vitamin D3 (1,25-(OH)₂D₃) and other calcitropic factors. These endogenous factors compose the Ca regulatory system and coordinate to...
keep constant Ca levels in plasma. Therefore, there is no conclusive proof that plasma concentrations of sCT are always proportional to the hypocalcemic effects, and the use of PA in evaluating drug absorption might lead to erroneous conclusions.\(^{15}\) Therefore, determination of plasma concentrations of sCT is indispensable for the accurate evaluation of bioavailability. For the analytical methods of sCT, high-performance liquid chromatography (HPLC) and radioimmunoassay have been widely used;\(^{10,14,15}\) however, these assay methods generally have many practical difficulties such as poor specificity, poor sensitivity, and requirement of large sample volume, for determination of low concentrations of sCT, especially after the nasal administration. To evaluate the bioavailability of sCT after extra-vascularly administration using the hypocalcemic effect, this chapter demonstrated the construction of a pharmacokinetic-pharmacodynamic (PK-PD) model for the hypocalcemic effect of sCT, after intravenous administration in rats. In this model, the endogenous Ca regulation system was also characterized based on plasma Ca and endogenous CT (rCT) levels.

**Materials and Methods**

**Chemicals:** Synthesized salmon calcitonin (sCT) was kindly supplied by Teikoku Hormone Mfg. Co. (Tokyo, Japan). Rat calcitonin (rCT) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO). Calcium gluconate was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). 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 tap water. On the day before the experiment, the rats were lightly anesthetized with ethyl ether, and were implanted surgically with a combination of silastic (Dow Corning Corp., Midland, MI) and PE50 (Clay Adams, Parsippany, NJ) in a catheter, which was inserted into the femoral vein to allow for drug administration and blood sampling. For the infusion study, a combination catheter of PE10 (Clay Adams, Parsippany, NJ) and PE50 was inserted into the femoral artery for drug infusion. The catheters were externalized through the back of the neck region and secured. The animals were allowed at least 15 hr under fasting to recover from catheterization before the experiments. All of these methods had previously been approved by the Animal Experimentation Committee of Osaka University of Pharmaceutical Sciences.

**Animal experiments:** Pharmacokinetics and pharmacodynamic of sCT: On the day of the experiment, the rats were housed in individual metabolic cages. Synthesized sCT was dissolved in saline containing 0.1% w/v BSA and injected into the femoral veins via the catheters at doses ranging from 0.075 to 0.6 IU/kg. In the infusion study, 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. In the pharmacokinetic study, the blood samples were transferred into tubes containing EDTA-2Na, and then centrifuged (10,000 rpm for 3 min). The isolated plasma was frozen and stored at −20°C until analysis. The plasma sCT levels were determined by radioimmunoassay (Peninsula Laboratories, Inc., San Carlos, CA) using the rabbit anti-salmon calcitonin serum. In the pharmacodynamic study, the obtained blood samples were transferred into tubes containing heparin (1 IU), and then centrifuged. The plasma Ca concentrations were determined using the o-cresolphthalein complexone method (Calcium C Test Wako®, Wako Pure Chemical Industries, Osaka, Japan). Investigation of the Ca regulatory system: Calcium gluconate was dissolved in saline, and was injected into the femoral veins at doses of 10 and 20 mg calcium/kg. The rCT solution, dissolved in saline and 0.1 mol/l sodium acetate-HCl (pH 4.0) containing 0.1% w/v BSA, was given via the femoral vein catheter at doses of 0.5 and 1 μg/kg. The sCT solution (0.15 and 0.225 IU/kg) was also injected intravenously as mentioned above. Blood samples were withdrawn and centrifuged to isolate plasma. The plasma rCT levels were determined by radioimmunoassay (Peninsula Laboratories, Inc., San Carlos, CA) using the rabbit anti-rat calcitonin serum. Ca levels in the plasma were also determined as described above.

**Data analysis:** The concentration-time data was analyzed by a nonlinear regression program, FKDM\(^{16}\) 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 above the effect-time curve (AAEs) following the i.v. administration of sCT, were calculated by the linear trapezoidal method.

**Theoretical**

Figure 1 represents the PK-PD model for the hypocalcemic effect after administration of sCT. This
Fig. 1. Diagrammatic Representation of the PK-PD Model for the Hypocalcemic Effect of sCT.
The pharmacokinetics is linked to the Ca regulation system (gray area) through the pharmacodynamic model.

The model consists of endogenous regulation system of Ca concentration in rat plasma. In the i.a. infusion study, the pharmacokinetics of sCT in plasma was described by a two-compartment open model, as follows.

\[
\frac{dsCT_1}{dt} = K_0 - (k_{12} + k_{10}) \cdot sCT_1 + k_{21} \cdot sCT_2 \quad \text{Eq. 1}
\]

\[
\frac{dsCT_2}{dt} = k_{12} \cdot sCT_1 - k_{21} \cdot sCT_2 \quad \text{Eq. 2}
\]

\[
sCT_{1C} = \frac{sCT_1}{V_c} \quad \text{Eq. 3}
\]

Where \( sCT_1 \) is the amount of sCT in the central (plasma) compartment, \( sCT_2 \) is the amount of sCT in the peripheral compartment (mIU). \( K_0 \) is the infusion rate (mIU/min), \( k_{10}, k_{12}, \) and \( k_2 \) are first-order constants (min\(^{-1}\)). \( V_c \) is the distribution volume (mL) of the central compartment, and \( sCT_{1C} \) is the plasma concentration (mIU/mL) of sCT. In the i.v. bolus administration study, the following equation is substituted for Eq. 1.

\[
\frac{dsCT_1}{dt} = -(k_{12} + k_{10}) \cdot sCT_1 + k_{21} \cdot sCT_2 \quad \text{Eq. 4}
\]

A regulation system for blood Ca concentration has been studied physiologically, and reported by some investigators.\(^{17-20}\) As the final purpose of this study is concerned, it is not necessary to construct a complicated model including several factors for the regulation system because of an increase in information required for the modeling. Then we focused on the relationship between plasma Ca and rCT. Briefly, CT plays a major role in the Ca homeostasis; the secretion of endogenous CT is dependent on increasing in the blood Ca level, and thus causes suppression of blood Ca concentrations through inhibition of bone resorption. In the present study, we then assumed as follows; a) The plasma Ca is generated by a zero-order kinetic process (\( K_{Ca0} \) mg/dL/min), and eliminated by a first-order kinetic process. b) The endogenous CT (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. The differential equations of the model are as follows.

\[
\frac{dCa}{dt} = K_{Ca0} - \beta \cdot rCT_1 - k_{Ca10} \cdot Ca \quad \text{Eq. 5}
\]

\[
\frac{drCT}{dt} = a - k_{rCT10} \cdot rCT \quad \text{Eq. 6}
\]

\[
\frac{drCT_1}{dt} = \tau_{rCT} \cdot rCT - \tau_{rCT1} \cdot rCT_1 \quad \text{Eq. 7}
\]

\[
\frac{drCT_2}{dt} = \tau_{rCT} \cdot rCT_1 - \tau_{rCT1} \cdot rCT_2 \quad \text{Eq. 8}
\]

Where Ca is the plasma Ca concentration (mg/dL). rCT, rCT1, and rCT2 are the rCT concentrations (ng/dL) in the plasma and the effect compartments, respectively. The parameter \( k_{Ca10} \) and \( k_{rCT10} \) are the first-order kinetic constants (min\(^{-1}\)) for the Ca and rCT, respectively. \( \tau_{rCT} \) is the first-order kinetic constant (min\(^{-1}\)) for the effect compartments of rCT. \( \beta \) is the proportional constant for the effect of rCT on the plasma Ca concentration, and \( \phi_1 \) and \( \phi_2 \) are proportional constants for the secretion of rCT.

To describe the effect of sCT, a similar PK-PD model to the effect of rCT was applied, as follows.

\[
\frac{dE_1}{dt} = \tau_{sCT} \cdot sCT_1 - \tau_{sCT1} \cdot E_1 \quad \text{Eq. 9}
\]

\[
\frac{dE_2}{dt} = \tau_{sCT} \cdot E_1 - \tau_{sCT2} \cdot E_2 \quad \text{Eq. 10}
\]

Where \( E_1 \) and \( E_2 \) are the amount (mIU) of sCT in the effect compartments, and \( \tau_{sCT} \) is the first-order kinetic constant (min\(^{-1}\)). The hypocalcemic effect of sCT was described by a Langmuir type equation. The differential equation for the Ca concentration after sCT administration (Eq. 5) was rearranged to
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\[ \frac{dCa}{dt} = K_{Ca} - \beta \cdot rCT - \gamma \cdot k_{Ca} \cdot Ca \]  
Eq. 11

where

\[ \gamma = \frac{E_{max}}{Q + E_2} \]

\( E_{max} \) is the maximum effect of sCT (mg/dL/min). Q is the affinity constant of sCT for the receptor.

**Results**

**Pharmacodynamics and pharmacokinetics of sCT:**

Figure 2A shows changes in plasma Ca concentrations after i.v. bolus administration of sCT (0.075–0.6 IU/kg). The hypocalcemic effect appeared to reach a peak from 0.5 to 1.5 hrs after administration, but the time of appearance of the peak tended to prolong with increasing doses. After the peak, the significant hypocalcemic effect lasted at least 1 hr. The total amount of hypocalcemic effect of evaluated by AAE, increased linearly as the logarithm of the doses, as shown in Fig. 2B.

In the preliminary study, we found that the elimination of sCT after i.v. bolus administration is too fast to be determined precisely. Then, sCT (5 IU/kg/hr) was infused for 75 min to reach a steady-state concentration (Fig. 3A). A two-exponential decline in plasma sCT levels was observed after infusion stopped (plotted points). These data were fitted to the two-compartment model shown in Fig. 1. The value of the Akaike Information Criterion (AIC) for the result of modeling was −238 comparing with −199 for the 1-compartment modeling. 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 1. The short MRT (6.86 min) indicated the fast elimination of sCT from the plasma. The pharmacokinetics of sCT following i.v. bolus administration of sCT was simulated according to Eqs. 2–4 and the parameters in Table 1. Figure 3B shows the model-predicted plasma sCT level after bolus administration of sCT (0.15 IU/kg). The relationship between the plasma sCT concentration and the plasma Ca level after i.v. administration showed a typical counter-clockwise hysteresis (data not shown).

**Modeling of the Ca regulation system:** Changes in plasma Ca and rCT levels following i.v. bolus administration of Ca gluconate (10 and 20 mg/kg) are shown in Fig. 4A. The hypocalcemic effect of rCT was observed for at least 1 hr after rCT administration (Fig. 5A), while plasma rCT concentration recovered to its baseline level within 30 min after administration (Fig. 5B). This indicates the presence of a significant delay in hypocalcemic effect against plasma rCT concentration.

To describe the endogenous Ca regulation system, a model shown in Fig. 1 was constructed and Ca and rCT concentrations in plasma were fitted. Solid lines shown...
Fig. 3. Pharmacokinetic Profiles of sCT. Panel A: Time Courses of Plasma sCT Level during and after i.a. Infusion of sCT (5 IU/kg/hr). Panel B: The Simulated Time Course of Plasma sCT Level after i.v. Bolus Administration of sCT (0.15 IU/kg).
Each symbol indicates an individual animal. The line represents the theoretical value.

in Fig. 4 and Fig. 5 represent the result of the least-squares regression fitting, and the estimated physiological parameters are summarized in Table 2. In the Ca loading studies, the regression curves for the plasma Ca concentration well fitted to the observed data (Fig. 4A), and the curves for the plasma rCT level also fitted the observed data (Fig. 4B). In the rCT administration studies, the fitted curves for the plasma Ca concentrations explained the observed data well (Fig. 5A);

Table 1. Pharmacokinetic Parameters for sCT in Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimates ± S.D.</th>
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<tbody>
<tr>
<td>$k_{12}$ (min$^{-1}$)</td>
<td>0.677 ± 0.50</td>
</tr>
<tr>
<td>$k_{21}$ (min$^{-1}$)</td>
<td>0.309 ± 0.093</td>
</tr>
<tr>
<td>$k_{10}$ (min$^{-1}$)</td>
<td>0.466 ± 0.18</td>
</tr>
<tr>
<td>$V_c$ (mL)</td>
<td>69.6 ± 21</td>
</tr>
<tr>
<td>$V_t$ (mL)</td>
<td>153 ± 46</td>
</tr>
<tr>
<td>$V_{ss}$ (mL)</td>
<td>222 ± 67</td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>32.4 ± 9.7</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>6.86</td>
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</table>

Vt: the distribution volumes in the central and tissue compartment, Vss: the distribution volume in the steady-state, CL: the total clearance, MRT: the mean residence time ($V_{ss}$/CL).

however, the curve for the plasma rCT level described the observed data only at the lower dose (0.5 µg/kg, Fig. 5B).

PK-PD analysis of the hypocalcemic effect of sCT: Using the model for the Ca regulation system, the PK-PD analysis for the hypocalcemic effect of sCT after i.v. administration was carried out according to Eqs. 2–4 and Eqs. 6–11. Since there was a typical counter-clockwise hysteresis between plasma sCT levels and plasma Ca concentrations, an additional effect compartment were introduced between the PK model and the Ca regulation model. The solid lines shown in Fig. 6, represent the results of the fitting of the plasma Ca level to the model. The regression curves fitted the observed data well. The estimated PD parameters are listed in Table 2.

Discussion

Pharmacokinetics of sCT: In the infusion study, the pharmacokinetics of sCT was described using a conventional linear two-compartment open model. Although the time-concentration profile of plasma sCT after i.v. bolus administration could not be obtained for the lack of analytical sensitivity, the model-predicted profile showed the extremely rapid elimination of sCT from plasma; the plasma sCT concentration 30 min after i.v. bolus administration was less than 1% of the initial concentration (Fig. 3B, Table 1). The elimination half-life in the rat was shorter than that in human study, and this may be attributable to the different activity of the enzymatic degradation. Despite of rapid elimination, the hypocalcemic effect of sCT appeared to reach the peak more than 30 min after administration, and the peak time tended to prolong with increasing doses (Fig. 2A). These results suggests that neither distribution rate to the active site nor metabolic rate alone seems sufficient to explain the significant delay in onset and elimination for the hypocalcemic effect. Since hypocalcemic effect of sCT is known to be a result of a summation of multiple actions of the endogenous Ca regulation system, characterization of the plasma Ca regulation system is indispensable for the correlation of
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Fig. 4. Responses of the Endogenous Ca Regulation System to Ca Loading in Rats. Panel A: Time Courses of Plasma Ca Concentration after i.v. Bolus Administration of Ca Gluconate. Panel B: Time Courses of Plasma rCT (Endogenous Calcitonin) Concentration after i.v. Bolus Administration of Ca Gluconate. Each value represents the mean ± S.E. of 3–5 experiments. The dashed and solid lines represent the theoretical values at doses of 10 and 20 mg/kg Ca gluconate, respectively.

Fig. 5. Responses of the endogenous Ca regulation system to rCT (endogenous calcitonin) in Rats. Panel A: Time Courses of Plasma Ca Concentration after i.v. Bolus Administration of rCT. Panel B: Time Courses of Plasma rCT Concentration after i.v. Bolus Administration of rCT. Each value represents the mean ± S.E. of 4 experiments. The dashed and solid lines represent the theoretical values at doses of 0.5 and 1.0 μg/kg rCT, respectively.

plasma sCT with its biological effect, quantitatively.

Using the PK-PD model, we explained the pharmacological effect after i.v. administration of sCT. However, pharmacokinetic parameters of sCT were based on higher plasma concentrations in the infusion study. There have been no reports concerning the dose-relative pharmacokinetics of sCT at these low 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 parameters can not be decide for lack of the observed concentrations of sCT. Therefore, a linear pharmacokinetics of sCT was assumed in the investigated dose range.

Modeling of the endogenous Ca regulatory system: The acute regulation of blood Ca level is known to be accomplished by a feedback action of endogenous CT in
Table 2. Physiological Parameters for Endogenous Ca Regulation System and Pharmacodynamic Parameters for the Hypocalcemic Effect of sCT

<table>
<thead>
<tr>
<th>Physiological parameters</th>
<th>Estimates ± S.D.</th>
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<tbody>
<tr>
<td>( k_{Ca} ) (mg/dL/min)</td>
<td>3.92 ± 0.14</td>
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<tr>
<td>( k_{rCT} ) (min(^{-1}))</td>
<td>0.450 ± 0.0083</td>
</tr>
<tr>
<td>( \Phi_{i} ) (ng/dL/min)</td>
<td>0.0687 ± 0.012</td>
</tr>
<tr>
<td>( \Phi_{e} ) (dL/mg)</td>
<td>0.302 ± 0.022</td>
</tr>
<tr>
<td>( k_{CTH} ) (min(^{-1}))</td>
<td>0.183 ± 0.0077</td>
</tr>
<tr>
<td>( t_{1/2} (\text{min}^{-1}) )</td>
<td>0.0538 ± 0.017</td>
</tr>
<tr>
<td>( \beta ) (× 10(^{6}) min(^{-1}))</td>
<td>0.0225 ± 0.014</td>
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<tr>
<th>Pharmacodynamic parameters</th>
<th>Estimates ± S.D.</th>
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<tr>
<td>( E_{max} ) (mg/dL/min)</td>
<td>1.02 ± 0.10</td>
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<tr>
<td>1/Q (mIU)</td>
<td>0.00265 ± 0.0013</td>
</tr>
<tr>
<td>( t_{ct} (\text{min}^{-1}) )</td>
<td>0.0368 ± 0.0048</td>
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Fig. 6. Comparison of Calculated Plasma Ca Concentration with Observed Data after i.v. Bolus Administration of sCT in Rats. Each value represents the mean ± S.E. of 4 experiments and the solid lines represent the fitted curves.

In summary, the pharmacokinetics of sCT was confirmed to be a nonlinear relationship between plasma Ca levels and plasma rCT concentrations was observed, and the onset processes of pharmacological effect of sCT is fundamentally independent of dose and dosing route. Therefore, plasma Ca levels after i.n. administration of sCT could be described using this quantified model for the Ca regulation system. Moreover, plasma sCT concentration-time profiles following i.n. administration of sCT could be predicted from the hypocalcemic effect using the model. The potential for application of this PK-PD model to the i.n. administration study will be explored in the succeeding article.
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References


