Yukari Ueda et al.: Pharmacokinetic Characterization of Calcium from Three Calcium Salts (Calcium Chloride, Calcium Acetate and Calcium Ascorbate) in Mice

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Abstract: Calcium is an essential mineral, and its deficiency causes several diseases such as osteoporosis. The absolute bioavailability of calcium using modern pharmacokinetic methods has not been determined even though the relative bioavailability of calcium from various calcium salts has been examined using classic kinetics and pharmacokinetics. The serum calcium concentrations of three calcium salts, calcium chloride, calcium acetate and calcium ascorbate, were measured at various times after intravenous (i.v.) and oral administrations in mice, and the pharmacokinetic behaviors of the salts were investigated using a non-compartmental model. The degree of dissociation of the calcium salts was determined based on the extent of freezing-point depression. The pharmacokinetic parameters, MRT, Vdss, CLtot and AUC for i.v. administration of calcium at 15 and 30 mg/kg from three calcium salts indicated that all three may undergo similar mechanisms of calcium metabolism. The pharmacokinetic process was linear due to a first-order reaction. The pharmacokinetic parameters of calcium after oral administration at 150 mg/kg indicated that the calcium absorption was significantly different among the three calcium salts. The absolute calcium bioavailability of calcium ascorbate and calcium acetate was 2.6 and 1.5-fold, respectively, greater than that of calcium chloride. The mean residence time, MRTab, for absorption of calcium from calcium ascorbate was longer than those from calcium chloride and calcium acetate. Furthermore, it was estimated that calcium absorbed by passing through the intestinal membrane was the dissociated form because of higher degrees of apparent dissociation for the three salts. The calcium absorbability from calcium ascorbate via the intestinal track is significantly higher than those of calcium chloride and calcium acetate.

Keywords: Calcium bioavailability, Pharmacokinetics, Calcium chloride, Calcium acetate, Calcium ascorbate

Introduction

Calcium is an essential mineral element, acting primarily as a component in bone, as well as playing various physiological control roles in cells, even when found at low levels.²,³ It has been shown that calcium deficit causes various diseases, including osteoporosis, hypertension, hypercholesterolemia, and cancer.³,⁴ The absorption of calcium takes place through both active and passive transport from the gut lumen after food intake.¹,⁵-⁷ The calcium absorbability from diets or its elements is traditionally defined to be the quantity of calcium in the blood, urine or body compartments, particularly bone, after multiple administrations or ingestions over several days, and its extent is defined as absorption, fractional absorption, or nutrient bioavailability of calcium, measured usually by mass balance techniques.⁸,⁹ Studies have shown that calcium absorbability is as low as 20–40% after calcium salt administration,¹⁰,¹¹ and many modern diets do not provide the recommended levels of calcium (400–1,200 mg/day).¹² Thus, dietary calcium supplements have been recommended for prevention of calcium related diseases, and various calcium salts including calcium carbonate and calcium lactate have been examined as calcium supplement sources.¹³,¹⁴

Using classic kinetics for several compartmental models, it has been shown that retention of calcium in bones changes during various physiological growth periods over a life span¹³,¹⁴ or during diseases¹¹,¹² in humans, and furthermore, calcium absorption is different depending on the calcium salt.¹⁵-¹⁷ Neer et al.¹⁶ reported the analysis of the time-course of calcium concentration in serum or urine of humans after intravenous administration using a four compartmental kinetic model schema jointed in series. O’Brien et al.¹⁸ showed that net deficits in bone calcium balance occurred during pregnancy and lactation. Schulze et al.¹⁹ showed that rates of bone calcium deposition were lower than those in healthy
children, and the alterations in bone turnover contribute to reduced bone mass in girls with cystic fibrosis. Furthermore, Yergey et al. measured fractional absorption of calcium carbonate using a dual-isotope approach. Cai et al. showed that the turnover rate in the gastrointestinal tract was much slower for calcium ascorbate than calcium acetate.

As reviewed by Wagner, and Rowland and Tozer, pharmacokinetics is a kinetic method advanced from classical kinetic theory applied to study drugs and toxins, and is useful for diagnosis and therapeutic treatments. In pharmacokinetics, the kinetic processes of the drug in the body are simply divided into five steps referred to as ADME, that is, absorption (A), distribution (D), metabolism (M) and excretion (E), and assumed usually to be a linear first-order reaction. The metabolic processes for a drug are defined using pharmacokinetic parameters including the area under the plasma concentration curve (AUC), bioavailability (F), distribution of volume, and clearance (CL), based on a compartmental or non-compartmental model. When dose D of a drug is given, the pharmacokinetic characterization of calcium absorption should help to understand its therapeutic effects and its use in treatment strategies because of the following relationship:

\[ F \times D = AUC \times CL \]

AUC is defined from the increment in the plasma concentration after a drug dosing.

\[ \text{AUC}(0-\infty) = \int_0^\infty C_p(t) \, dt \]

(1)

The calcium absorption is defined to be the quantity taken into blood through the dietary track as shown in Eq. (1). Although AUC itself is a relative quantity in proportion to the administered dose, and thus is a measure of the extent of calcium absorption of various calcium salts, the absolute bioavailability (F) is defined as \( \text{AUC}_{oral} \) after an oral dose of \( D_{oral} \), normalized with \( \text{AUC}_{iv} \) after an intravenous (i.v.) dose of \( D_{iv} \) as follows:

\[ F = \left( \frac{\text{AUC}_{oral}}{\text{D}_{oral}} \right) / \left( \frac{\text{AUC}_{iv}}{\text{D}_{iv}} \right) \]

(2)

When a certain drug (B), dosed \( D_{B} \), is compared with a standard drug (A), dosed \( D_{A} \), the relative bioavailability (\( F_B \)) is the quantity indicating the equivalency between drugs A and B as follows:

\[ F_R = \left( \frac{\text{AUC}_{B}}{\text{D}_{B}} \right) / \left( \frac{\text{AUC}_{A}}{\text{D}_{A}} \right) \]

(3)

Usually the standard drug (A) has established pharmacokinetics, and is assumed to be temporarily absorbed across the digestive track, and is used to describe the bioequivalency of drug absorption. Although the absorption of a drug is usually discussed using the absolute bioavailability, the absolute bioavailability for calcium has been disregarded from classic kinetic and pharmacokinetic studies to date because of it being an endogenous substance, and the use of dual isotopic methods is required for pharmacokinetic studies. Normally AUC has been used as a relative measure for the extent of calcium absorption. Tsuchiya et al. showed that the calcium absorbability from calcium ascorbate is almost comparable with, or higher than that from calcium chloride, and is significantly higher than that from calcium carbonate. Hanzlik et al. demonstrated that after oral administration in humans, calcium formate is superior to calcium carbonate and calcium citrate for the delivery of calcium. However, no one has examined absolute calcium bioavailability using modern pharmacokinetics, even though Yergey et al. indicated theoretically that the fractional absorption using dual-isotope approaches are closely related to the concept of absolute bioavailability defined from pharmacokinetics.

To examine different calcium supplements, the absorbability of various calcium salts has been studied, and results indicated that food or its elements, like lactose, can enhance calcium absorption via a paracellular route in the intestine, but what causes the different degrees of absorption between the calcium salts is a topic of discussion. Several studies have suggested that solubility plays a crucial role in intestinal absorption, but others have suggested that solubility has little or no correlation with bioavailability. Different studies have indicated that a cationic ion group may play a specific role in the enhancement of intestinal calcium absorption. Hanes et al. noted that absorption of calcium oxalate did not require dissociation in rats, but Suzuki & Hara concluded that an increased intracellular calcium ion concentration in rat small-intestinal enterocytes due to non-digestible saccharides caused increased calcium absorption.

The aim of our study was to compare the absolute calcium bioavailability from a single dose of three water-soluble calcium salts: calcium chloride, calcium acetate and calcium ascorbate, in mice, and examine the effect of the anions on the pharmacokinetics of absorption, disposition and elimination of calcium.

**Materials and Methods**

**Chemicals**

Calcium chloride hexahydrate, calcium acetate hydrate and calcium ascorbate dihydrate were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Other reagents were purchased from commercial sources and were of the highest grade available.

**Animals and pharmacokinetic procedures**

Seven- to ten-week-old male ddY mice, weighing 20–30 g, were obtained from SLC Co., Ltd. (Shizuoka, Japan). Animals had free access to food (commercial diet, MF pellets, Oriental Animal Foods Co., Osaka, Japan) and water during the experimental period. Animals fasted for 18–20 h prior to administration of the calcium salt doses, but had free access to water. Solutions of calcium (1% w/v) were prepared for each calcium salt, and either 15 or 30 mg per kg of body weight (mg/kg) of calcium was intravenously administered to the tail vein under weak ether anesthesia. Blood (ca. 0.02 mL) was collected from the orbital sinus at 0, 5, 15, 30, 45, 60, 90, 105 and 120 min. The blood
samples were centrifuged at 6,000 rpm for 2 min at room temperature (25 ± 1°C). The plasma calcium concentration was measured spectroscopically at 620 nm using a microplate reader model 680 (Bio-Rad Laboratories Inc., Orlando, FL, USA) and a calcium diagnostic kit (code 437-58201) from Wako Pure Chemical Industries Ltd., Osaka, Japan. For oral administration, calcium (150 mg/kg) was delivered to the duodenum using a stainless steel intubation needle and a 1.0 mL syringe (29Gx1/2) under weak ether anesthesia. The plasma calcium concentration was determined using the same method as described above. All protocols conformed to the guide for the institutional animal care and use of Tokushima Bunri University, Tokushima, Japan.

**Pharmacokinetic calculations**

Calcium pharmacokinetic parameters were calculated using the MOMENT software program, for moment analysis of the increment of plasma calcium concentration-time curves after administration of calcium. The calculations were based on statistical moment theory using an iterative least-squares method. The plasma clearance (CL$_{iv}$), mean residence time (MRT$_{iv}$) and
volume of distribution (V_{dss}) at steady-state after intravenous (i.v.) administration of the dose (D_{iv}) were determined using the equations described by Yamaoka et al.\textsuperscript{24} as follows:

\[
V_{dss} = \frac{D_{iv} \cdot MRT_{iv}}{AUC_{iv}} \quad (4)
\]

\[
CL_{iv} = \frac{V_{dss}}{MRT_{iv}} \quad (5)
\]

The absolute bioavailability (F_{A}) is calculated using Eq. (2).

AUC (0- \tau) was calculated as follows:

\[
AUC (0- \tau) = AUC (0- \tau) + C_{p} (\tau)/ \lambda \quad (6)
\]

Where \tau is the time of the last plasma concentration (C_{p}) and \lambda is the apparent elimination rate constant calculated as the slope of the plasma concentration at the time \tau after semilogarithmic transformation. AUC (0-\tau) was calculated using the trapezoidal rule with linear interpolation. MRT_{iv} is the mean residence time in the body compartment following the intravenous administration and MRT_{oral} is the mean residence time in all compartments following the oral administration. The elimination kinetic constant (k_{el}) and the absorption kinetic constant (k_{ab}) in the compartment model are related to following relationships: MRT_{iv} = 1/k_{el} and MRT_{oral} = 1/k_{el} + 1/k_{ab}, respectively.\textsuperscript{24} Thus the mean residence time (MRT_{ab}) in the absorption track is as follows:

\[
MRT_{ab} = \frac{1}{k_{ab}} \quad (7)
\]

**Table 1. Pharmacokinetic parameters of Ca\textsuperscript{2+} in mice after i.v. or oral administration of saline solution of either calcium chloride, calcium acetate or calcium ascorbate.**

<table>
<thead>
<tr>
<th>Salts</th>
<th>Dose (mg/kg)</th>
<th>AUC_{iv} (µg•min/mL)</th>
<th>MRT_{iv} (min)</th>
<th>CL (mL/min/kg)</th>
<th>V_{dss} (mL/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl\textsubscript{2}</td>
<td>15</td>
<td>1484.5 ± 41.0</td>
<td>29.3 ± 1.3</td>
<td>10.1 ± 0.3</td>
<td>296.5 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2870.6 ± 90.8</td>
<td>33.0 ± 1.1</td>
<td>10.5 ± 0.3</td>
<td>345.1 ± 15.8</td>
</tr>
<tr>
<td>CaAc\textsubscript{2}</td>
<td>15</td>
<td>1507.9 ± 128.4</td>
<td>29.1 ± 2.0</td>
<td>10.0 ± 0.9</td>
<td>289.9 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2637.2 ± 121.6</td>
<td>30.0 ± 2.4</td>
<td>11.4 ± 0.5</td>
<td>340.9 ± 24.7</td>
</tr>
<tr>
<td>CaAs\textsubscript{2}</td>
<td>15</td>
<td>1193.9 ± 101.7</td>
<td>30.4 ± 1.0</td>
<td>12.6 ± 1.1</td>
<td>383.6 ± 20.9</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2711.3 ± 154.2</td>
<td>32.5 ± 1.2</td>
<td>11.0 ± 0.6</td>
<td>359.2 ± 34.7</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>30.8 ± 2.1</td>
<td>10.9 ± 1.1</td>
<td>335.9 ± 38.6</td>
<td></td>
</tr>
</tbody>
</table>

**Measurement of the degree of dissociation of the calcium salts**

To measure the degree of dissociation of the calcium salts, based on their colligative properties in dilute solution, the extent of freezing-point depression (\Delta T_{f}(\degree C)) was determined with a Beckmann thermometer, calibrated from +1 to –6\degree C. Each value was determined in triplicate. All solutions were prepared in molar
concentrations, 150, 125, 100, 50, 25 and 0 mM. The result is that in a dilute electrolyte solution, $\Delta T_f$ is directly proportional to the molality concentration ($m_B$) of the solution according to Eq. (8) below:38)

$$\Delta T_f = i \cdot K_f \cdot m_B$$  (8)

where the cryoscopic constant ($K_f$) is 1.86°C for an aqueous solution, which is dependent on the properties of the solvent. The van’t Hoff factor $i$ accounts for the practical number of individual particles (typically ions) formed by a compound in solution. Thus if $N$ is the total number of particles furnished by one molecule of the solute, for example $N = 3$ for calcium chloride in an aqueous solution, the degree of dissociation ($\alpha$) is as follows:38)

$$\alpha = \frac{(i - 1)}{(N - 1)}$$  (9)

**Statistical analysis**

Data are presented as the mean value ± SD. A parameter was considered to be significantly different when the $p$ values were $< 0.05$ using a Student’s t-test.

**Results**

**Pharmacokinetic analysis after i.v. administration**

As shown in Figure 1, the plasma calcium concentrations in mice decreased to the control level two hours after i.v. calcium administration of 15 or 30 mg/kg from the aqueous solution of calcium chloride, calcium acetate or calcium ascorbate. The pharmacokinetic parameters of calcium were calculated from the increment in the plasma calcium concentration over the mean control level of calcium (8.08 ± 0.07 mg/dL) after i.v. administration, as shown in Figure 1, and summarized in Table 1.

**Insert Figure 1 and Table 1 here**

The results indicate that the following pharmacokinetic parameters, MRT, CL and $V_{dss}$, of calcium for each calcium salt were not significantly different between the calcium doses of 15 and 30 mg/kg ($p < 0.05$). These parameters were also not significantly different among the three calcium salts. Thus the pharmacokinetic behavior of calcium in the mice was not affected by the partner anions in the calcium salts. Furthermore, the AUC value after i.v. calcium administration of 30 mg/kg was 1.96-fold (mean) higher than after i.v. calcium administration of 15 mg/kg ($p < 0.05$). This suggests that the pharmacokinetic process is linear in the calcium dosing range of 15 to 30 mg/kg.

**Pharmacokinetic analysis after oral administration**

Figure 2 shows the time-course of plasma calcium concentration after oral administration of 150 mg of calcium/kg from an aqueous solution of one of the three calcium salts.

**Insert Figure 2 here**

The pharmacokinetic parameters of calcium were calculated from the increment in the plasma calcium concentration over the mean control level of calcium after oral administration, as shown in Figure 2, and summarized in Table 1. The absolute bioavailability values ($F_a$’s) of calcium from dosing with the three calcium salts were calculated by comparing the AUC after oral administration with the AUC after i.v. administration of 30 mg of calcium per kg of body weight, as shown in Eq. (2). The results indicate that after administration of calcium from calcium chloride, calcium acetate or calcium ascorbate, the plasma calcium concentration reaches a maximum ($C_{max}$) of 94.5, 103.6, and 100.8 µg/mL at $T_{max}$, respectively. The $F_a$ value for calcium from calcium ascorbate (14.8%) was significantly higher than those for calcium chloride and calcium acetate (5.7 and 8.6%, respectively) ($p < 0.05$). The quantities absorbed into the circulating blood ($F_a \cdot D_{oral}$ = $AUC_{oral} \cdot CL_{oral}$),24) 8.9 mg/kg, 12.4 mg/kg and 21.9 mg/kg, respectively, demonstrates that calcium exhibits a linear absorption behavior in mice after oral administration of calcium chloride, calcium acetate or calcium ascorbate.

**The degree of the dissociation of calcium salts**

To confirm the effect of the degree of dissociation ($\alpha$) of calcium salts on their absorbability, we measured the extent of freezing-point depression $\Delta f$ (°C) in dilute calcium solution, and the degree of dissociation ($\alpha$) of each calcium salt was calculated using Eq. (8).
As shown in Figure 3, a change in the degree of dissociation linearly depended on the calcium concentration \( m_B \) from 25 to 150 mM in aq solution as follows,

\[
\alpha = -2.02 \times 10^3 m_B + 0.924, \quad r = 0.998 \quad (10)
\]

\[
\alpha = -0.61 \times 10^3 m_B + 0.923, \quad r = 0.934 \quad (11)
\]

\[
\alpha = -0.46 \times 10^3 m_B + 0.917, \quad r = 0.936 \quad (12)
\]

The linear functions were obtained by the least-squares calculation with high correlation coefficients \( r \) (\( n = 3 \)). Thus the degrees of intrinsic dissociation \( (\alpha_B) \) were found to be 0.92 ± 0.00, 0.92 ± 0.01 and 0.92 ± 0.01 for calcium chloride, calcium acetate and calcium ascorbate, respectively, at the calcium concentration \( m_B = 0 \) M. The values are not significantly different from each other. However, the apparent \( \alpha \) values (0.67 ± 0.00, 0.84 ± 0.01 and 0.87 ± 0.01, respectively) were different at the orally administered calcium concentration of 125 mM.

**Discussion**

The serum calcium concentration of three calcium salts, calcium chloride, calcium acetate and calcium ascorbate, were measured at various times after i.v. and oral administrations in mice, and the pharmacokinetic behaviors of the salts were investigated using a non-compartmental model, in which the \( F_A, \) AUC, \( MRT, \) \( V_{dss} \) and \( CL_{tot} \) values of calcium were calculated. The pharmacokinetic parameters, \( MRT, \) \( V_{dss} \) and \( CL_{tot} \) for i.v. administration of calcium at 15 and 30 mg/kg from the three calcium salts showed that the calcium metabolism might be physiologically similar among the three salts \( (p < 0.05) \). Furthermore, the AUC for each salt was proportional to the dose, and therefore the pharmacokinetic process may be linear due to a first-order reaction. On the other hand, the pharmacokinetic parameters of calcium after oral calcium administration of 150 mg/kg of body weight indicated that the calcium absorption was significantly different between the three calcium salts. The rank of the absolute calcium bioavailability was calcium ascorbate > calcium acetate > calcium chloride, while the values for calcium ascorbate and calcium acetate were 2.6 and 1.5-fold, respectively, greater than that of calcium chloride. The mean residence time, \( MRT_{ab} \), was 33.9 min for absorption of calcium from calcium ascorbate, significantly longer than those from calcium chloride and calcium acetate, 15.8 and 14.2 min, respectively, even though the \( C_{max} \) values of the three salts were comparable. That is, the rank order of the absolute bioavailability of calcium from the three calcium salts is consistent with the rank of the calcium absorbability determined by Tsugawa et al.\(^{20}\) and Cai et al.\(^{20}\). This may be explained by the fact that calcium may form a soluble calcium complex with ascorbate, which may prolong its transit time through the small intestine.

Although Hanes et al.\(^{36}\) concluded that absorption of calcium oxalate does not require dissociation in rats, it is not clear whether dissociated calcium ion is generally required or not during absorption of calcium in the intestine. Generally, it is believed that dietary calcium sources are absorbed as its free or ionized form from the intestine, and thus the extent of absorption depends on their solubility in aqueous solution. We found that the intestinal absorbability of dissociated calcium might be superior to calcium from non-dissociated salts because the degrees of apparent dissociation of the three salts are relatively high at 0.67, 0.84 and 0.87 for calcium chloride, calcium acetate and calcium ascorbate at 125 mM, respectively. Furthermore, Suzuki & Hara\(^{37}\) showed that an increase in intracellular calcium ion concentration due to non-digestible saccharides causes an increase in calcium absorption. Thus, further studies are needed to determine for each calcium salt whether it is absorbed from the intestine as its free or ionized form. It will be particularly important to clarify which factors are responsible for the long transit time of calcium through the small intestine.

In conclusion, as shown in this study, pharmacokinetic methods should prove to be more effective for studying acute absorption following single dosing even for endogenous nutrients like calcium, while the traditional balance method may be suitable for studying chronic absorption following multiple doses. Furthermore, pharmacokinetic methods may also be useful for the study of other nutritional elements. Thus further studies are needed to elucidate calcium transport mechanisms and their link to calcium bioavailability.

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