One-Hour Test for Estimating Intestinal Absorption of Calcium by Using Stable Strontium as a Marker

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Intestinal absorption of calcium is a relevant marker in a broad spectrum of diseases; however, its determination in clinical practice is difficult. Our aim was to design a simple test for this based on stable strontium (89Sr). The correlation between the intestinal absorption of simultaneously administered 45Ca and 89Sr was investigated in patients with various disorders of the bone and calcium metabolism. The area under the curve for the period 0–60 min after dosing (AUC0–60 in mmol·L−1·min), being a representative measure of intestinal absorption, showed a close correlation between both elements (r = 0.90, P <0.001). Moreover, the measure of agreement in classifying the patients as low, intermediate, or high absorbers was high (κ = 0.80). We conclude that a test based on measuring AUC0–60 of strontium is a fast and inexpensive way to obtain reliable information about the level of intestinal calcium absorption.

Indexing Terms: bone/atomic absorption spectrophotometry/radioassay compared

Determining the intestinal absorption of calcium is important in gaining insight into the etiology, prevention, and treatment of calcium-related diseases. However, the tests available for this have significant disadvantages. The balance study, often considered the golden standard, is very time-consuming and expensive. The application of procedures based on the use of radioactive isotopes of calcium (45Ca, 44Ca) is limited because of the long half-life of the tracer (45Ca), possible contamination of the tracer with other radioactive nuclides (1), and restricted availability related to a short half-life (47Ca). Therefore, procedures using stable isotopes (42Ca, 43Ca) were introduced. However, these isotopes are very expensive, and their quantification requires advanced techniques such as mass spectrometry (2). An appropriate absorption test for clinical use should discriminate low, normal, and high absorption; should be easy to perform with minor discomfort for the patient; and should be suitable for large-scale application and for the possibility of frequently repeated testing of one person.

Previously, strontium (Sr) was proposed as a tracer for calcium because of its chemical and physical similarities (3). In vitro studies indicated that both elements use a common carrier system, but the carrier has a lower affinity for strontium than for calcium (4). In 1987 Milsom et al. (5) introduced a test based on the use of nonradioactive strontium as an alternative for the calcium-tracer tests. However, the variable used in their study, the fractional absorption at 240 min (FC240), includes not only absorption but also features of distribution and elimination. Therefore, we studied the correlation between 45Ca and stable strontium (89Sr) after simultaneous administration to patients with various disorders of calcium and bone metabolism, in order to develop a simple clinical test based on strontium and specifically representative for intestinal absorption.

Subjects and Methods

Sixty-three patients (20 women, ages 27–60 years; 43 men, ages 18–62 years) with growth-hormone deficiency (n = 29), osteoporosis (n = 7), hypothyroidism (n = 2), or idiopathic hypercalcicuric urinary stone formation (n = 25) were tested. All procedures followed were in accordance with the Helsinki Declaration. After fasting overnight, each patient drank 200 mL of test solution containing 2.5 mmol of SrCl2 (Merck, Amsterdam, The Netherlands) and 2.5 mmol of CaCl2 enriched with 5 μCi of 45Ca (45CaCl2·2H2O, 10–40 Ci/g of calcium; Medgenix Diagnostica, Fleurus, Belgium). Blood samples (5 mL) were withdrawn via an indwelling catheter at t = 0, 5, 10, 15, 30, 60, and 90 min and 2, 3, 4, and 5 h.

The samples were collected into heparin-containing tubes and centrifuged at 1500g for 10 min. Plasma was separated and stored at −20°C until analysis. All plasma samples were analyzed for 89Sr by graphite-furnace atomic absorption spectrophotometry (6) and for 45Ca by liquid scintillation counting. Increments of the calcium concentration were back-calculated from the 45Ca content of the plasma samples by using the specific activity of the ingested calcium. We calculated the area under the plasma calcium-time curve over the first 60 min (AUC0–60, mmol·L−1·min) as a variable representative of intestinal absorption. Absorption predominates over distribution and excretion during this time interval; besides, this short time interval makes the test practical for performance in an outpatient clinic, yet allows multiple blood sampling for an accurate estimation. Moreover, for comparison with results in the literature, we determined the fractional absorption at 60 min (FC60) (7) and at 240 min (FC240) (5). The correlation between 45Ca and 89Sr was determined by linear regression after descriptive statistics had demonstrated that all variables displayed a normal distribution. To calculate the measure of agreement between the two elements for classifying low, intermediate, and high absorbing patients, we carried out a
Results

Within each patient the plasma concentration–time curves of $^{46}$Ca and $^{88}$Sr showed comparable shapes (Fig. 1). The plasma concentration of $^{46}$Ca was about double that of $^{88}$Sr, despite the equal dosage of carrier administered. Maximal plasma concentrations were reached at 123 ± 50 min for calcium and at 135 ± 54 min for strontium. Because, during the first 60 min, absorption predominates over distribution and excretion in almost all patients, we calculated $F_{Ca}$ and $AUC_{Ca}$ for both elements. In addition, because of its mention in the literature, we determined $F_{C440}$. The regression parameters and correlation coefficients for $F_{Ca}$, $AUC_{Ca}$, and $F_{C440}$ are summarized in Table 1. The $\kappa$ coefficient determined for $AUC_{Ca}$ was 0.80 ($P < 0.001$) (Fig. 2), which implies 79% agreement between the classification of absorption levels of strontium and calcium. The ranges for a low, intermediate, and high $AUC_{Ca}$ for both elements are summarized in Table 2.

Discussion

All calculated parameters demonstrated a close correlation between $^{46}$Ca and $^{88}$Sr, indicating that strontium can be used as a marker for intestinal calcium absorption. These results confirm the relation between the two elements already demonstrated in animal (9) and human (5, 10) studies. Based on the correlation coefficients and other results of linear regression, there is no marked preference for either variable. Relative to its mean value, the standard error of the estimate is comparable for all variables, indicating that the uncertainty with which the calcium values are predicted by the strontium values is of equal magnitude, regardless of type of variable (Table 1). In 1981, Marshall and Nordin (7) reported a close correlation between $F_{Ca}$ and intestinal calcium absorption determined by the balance technique. However, the use of $AUC_{Ca}$ as the variable representative for intestinal absorption has various advantages over $F_{Ca}$ and $F_{C440}$, the variables described in the literature (5, 10). The calculation of $F_{Ca}$ takes into account 15% of the body weight to determine the amount present in plasma and extracellular fluid. In cases of an abnormal body composition, e.g., obesity, anorexia, this can result in underestimation or overestimation of the intestinal absorption. Secondly, $AUC_{Ca}$ better reflects intestinal absorption than $F_{C440}$ because, in contrast to the situation at 240 min, at 60 min the distribution and excretion processes are of minor importance. Thirdly, $AUC_{Ca}$ is a more reliable variable, being based on more than one measurement, whereas $F_{Ca}$ and $F_{C440}$ are based on single measurements of the plasma concentration. Moreover, using $AUC_{Ca}$ shortens the duration of the test from 240 to 60 min, which makes the test more suitable for large-scale experiments and for testing in an outpatient clinic. $AUC_{Ca}$ and $AUC_{Sr}$ agreed well in classifying

<table>
<thead>
<tr>
<th>$AUC_{Ca}$, mmol · L${}^{-1} ·$ min</th>
<th>$AUC_{Sr}$, mmol · L${}^{-1} ·$ min</th>
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</thead>
<tbody>
<tr>
<td>$F_{Ca}$</td>
<td>2.02</td>
</tr>
<tr>
<td>$F_{C440}$</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Fig. 2. Correlation of $AUC_{Ca}$ of calcium vs strontium ($r = 0.90$, $P < 0.001$) in idiopathic hypercalciuric stone formers ($\times$) and in patients with osteoporosis ($\circ$), hypothyroidism ($\bullet$), or growth-hormone deficiency ($\oplus$).

Table 1. Regression parameters for various pharmacokinetic measures describing the correlation between calcium and strontium.

<table>
<thead>
<tr>
<th>$AUC_{Ca}$</th>
<th>Slope</th>
<th>Intercept</th>
<th>$r$</th>
<th>$S_{xy}$</th>
<th>Ca</th>
<th>Sr</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{Ca}$</td>
<td>1.79</td>
<td>0.30</td>
<td>0.90</td>
<td>0.20</td>
<td>1.31</td>
<td>0.57</td>
</tr>
<tr>
<td>$F_{C440}$</td>
<td>2.02</td>
<td>0.02</td>
<td>0.91</td>
<td>0.03</td>
<td>0.13</td>
<td>0.08</td>
</tr>
</tbody>
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Table 2. Classification intervals for $AUC_{Ca}$ of calcium and strontium based on tertiles containing equal numbers of results.

<table>
<thead>
<tr>
<th>$AUC_{Ca}$ (mmol · L${}^{-1} ·$ min)</th>
</tr>
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<tbody>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Calcium</td>
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<tr>
<td>Strontium</td>
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$n = 63$; $n = 21$ per tertile.

Fig. 1. Typical plasma concentration–time curve of calcium and strontium after simultaneous oral administration of 2.5 mmol of each mineral.
the different tertiles of absorption (κ = 0.80), indicating that \( \text{AUC}_{0-60}^{\text{Sr}} \) has as much clinical relevance as \( \text{AUC}_{0-60}^{\text{Ca}} \). However, the reference limits for low, intermediate, and high absorption have to be studied in more detail (in this study, the choice of limits was quite arbitrary). Future investigations will focus on this and on the discriminative power of the test procedure.

We conclude that a test based on the \( \text{AUC}_{0-60}^{\text{Sr}} \) of strontium is a fast, inexpensive method for obtaining reliable information about the intestinal absorption of calcium and can be applied in most clinics.

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References