Intravenous strontium gluconate as a kinetic marker for calcium in healthy volunteers

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- 1 We have studied the pharmacokinetics of stable strontium in 10 healthy male volunteers. We gave each volunteer 5 mmol strontium gluconate by intravenous infusion over 1 h and measured strontium concentrations in plasma and urine samples for 20 days. The plasma strontium concentration *vs* time data for each volunteer were fitted by a triexponential function using NONLIN. Compartmental model-dependent and model-independent pharmacokinetic variables were then calculated.
- 2 The mean half-life we report (5.4 days) is longer than that previously reported (about 2 days), since we continued sampling for 20 days. However, the rates of clearance (CL 9.4 ml min⁻¹, CL_R 5.4 ml min⁻¹, and CL_{NR} 4.0 ml min⁻¹) are similar to those previously reported, and the apparent volume of distribution at steady state (64 l) is similar to the values previously reported for the size of the exchangeable pool of both strontium and calcium.
- 3 The similarities in the pharmacokinetic behaviour of strontium and calcium suggest that the *in vivo* disposition of strontium may be used as a marker of calcium disposition and for studying the effects of drugs such as the calcium antagonists.

Keywords strontium pharmacokinetics calcium disposition

Introduction

A great deal of useful information about calcium metabolism in man has been obtained using strontium, whose distribution in the body is similar to that of calcium. Radioactive isotopes of calcium (⁴⁵Ca, ⁴⁷Ca) and of strontium (⁸⁵Sr) have been most commonly used to study calcium metabolism in both man (Bauer & Ray, 1958; Dow & Stanbury, 1960; Fraser *et al.*, 1960; Reeve *et al.*, 1976, 1983; Spencer *et al.*, 1957, 1960) and animals (Comar *et al.*, 1956; MacDonald, 1958; Walser & Robinson, 1962). These studies have been complemented by X-radiological and metabolic balance studies (Charkes *et al.*, 1966; Warren & Spencer, 1976).

Although the pharmacokinetics of stable strontium have been used as a tool for studying calcium disposition, previous studies in man have been poorly reported. This paucity of information has resulted primarily from two factors—firstly, the relative shortness of duration of previous studies (Eisenberg & Gordan, 1961; Root *et al.*, 1966) and secondly problems in developing a sufficiently sensitive and specific method for the measurement of strontium concentrations in biological samples. Therefore, in our studies we have continued sampling for 20 days and have devised a sensitive and specific method for measuring strontium concentrations in

plasma and urine. These data have previously been presented elsewhere in abbreviated form (Moraes *et al.*, 1989a).

Methods

Subjects

Ten healthy male volunteers agreed to participate. Their mean (s.d.) age was 33.1 (9.5) years (range 19–55 years), and their mean (s.d.) body weight was 76.4 (8.7) kg (range 69–100 kg). None of the subjects regularly took any drugs and all were non-smokers. No restrictions were placed on their activities during the study period. The study protocol was approved by the Central Oxford Research Ethics Committee and all the subjects gave their informed consent before participating.

Sample collection

The study started after the collection of a control plasma sample and a single 24 h urine sample for the measure-

ment of endogenous concentrations of strontium. Each volunteer was given a single dose of 5 mmol of strontium gluconate, diluted with physiological saline to a final volume of 120 ml, and injected intravenously at a constant rate over 1 h through a cannula in a forearm vein. Eighteen 10 ml blood samples were taken at 1, 2, 3, 4, 5, 6, and 8 h and at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, and 20 days after the beginning of the strontium infusion. All of the blood samples were collected into heparinized tubes and centrifuged at 1200 g for 15 min. The plasma was separated and stored at -20°C until analysis. Every day during the study 24 h urine samples were collected. The urine volumes were measured and 20 ml aliquots were stored at 4°C until analysis.

Strontium analysis

Plasma and urinary strontium concentrations were measured in duplicate by atomic absorption spectrophotometry using a graphite furnace. Because there are detectable concentrations of strontium in both the plasma and the urine of healthy volunteers, we used the method of additions (David, 1962).

The inter- and intra-assay coefficients of variation were respectively 6.0% and 5.0% for plasma and 3.4% and 3.9% for urine. In these experiments 10 samples were analysed repeatedly and the strontium concentrations were $0.4\ \mu\text{mol l}^{-1}$ in plasma and $2.4\ \mu\text{mol l}^{-1}$ in urine. The limits of detection of strontium were $15\ \text{nmol l}^{-1}$ in plasma and $6\ \text{nmol l}^{-1}$ in urine.

Pharmacokinetic analysis

After subtraction of the endogenous concentrations, the plasma strontium concentration vs time data for each volunteer were plotted semilogarithmically. The curve was stripped graphically into three exponential components by the method of least squares in order to obtain initial estimates of the slopes and intercepts of the components (Gibaldi & Perrier, 1982). Using these initial estimates the data were fitted by a triexponential function using unweighted non-linear regression (NONLIN; Metzler *et al.*, 1974).

In order to evaluate the goodness of the non-linear fitting process, analysis of the residuals was performed. In choosing a triexponential function we fitted the data by functions involving two, three, and four exponentials, and used the Akaike Information Criterion (Yamaoka *et al.*, 1978a) to select the polyexponential with the minimum number of exponential terms necessary to describe the data adequately.

The exponents and coefficients of the triexponential function were then used to calculate the following pharmacokinetic variables: AUC and AUMC (both measured to infinity), MRT, $t_{1/2}$, CL, V_{ss} (calculated from AUMC), and $V_{ss(j)}$ (V_{ss} calculated from the model). These compartmental model-dependent and model-independent variables were calculated using methods described by Nagashima *et al.* (1968) and Yamaoka *et al.* (1978b).

The curve of the rate of urinary excretion of strontium vs time became mono-exponential after the first 24 h following the intravenous administration of strontium. A first-order elimination rate constant was obtained from the terminal slope of the urinary excretion rate vs

time curve and this was used to calculate the half-life ($t_{1/2\text{ ur}}$).

Renal clearance (CL_R) was determined from the slope of a Cartesian plot of the urinary excretion rate vs the plasma strontium concentration in the plasma at the time corresponding to the mid-point of the urine collection period, with slope equal to renal clearance. Non-renal clearance (CL_{NR}) was obtained as the difference between total clearance and renal clearance.

The data are given throughout as mean (s.d.).

Results and discussion

Endogenous plasma concentrations of strontium were $0.45\ (0.13)\ \mu\text{mol l}^{-1}$ and baseline urinary strontium concentrations were $5.0\ (3.7)\ \mu\text{mol l}^{-1}$.

The plasma strontium concentration-time profiles after intravenous administration for the two subjects with the longest and shortest half-lives are shown in Figure 1. The analyses of the residuals are shown in the insets.

Half-life of strontium

The three phases of the disappearance of strontium from the plasma had mean half-lives of 1.2 (0.9) h, 21 (14) h, and 5.4 (2.7) days (range 2.5 to 9.3 days). The apparent

Figure 1 Plasma strontium concentration-time curves after the intravenous administration of 5 mmol of stable strontium to the two volunteers with a) the shortest and b) the longest terminal elimination half-lives (2.5 days and 9.1 days respectively). The squares represent the experimental data and the line the curve of the best non-linear fit. The insets show the residuals plotted against the dependent variable.

Table 1 The pharmacokinetics of stable strontium obtained from compartmental model-independent analysis, model-dependent analysis, and analysis of the urinary data after the intravenous administration of 5 mmol of stable strontium gluconate to 10 healthy volunteers. The results are shown as mean (s.d.)

<i>Compartmental model-independent analysis</i>	
$t_{1/2,z}$ (days)	5.4 (2.7)
MRT (days)	5.1 (2.1)
CL (ml min ⁻¹)	9.4 (3.0)
V_{ss} (l)	64 (22)
<i>Model-dependent analysis</i>	
$V_{ss(ij)}$ (l)	71 (25)
V_1 (l)	10 (5)
V_2 (l)	18 (9)
V_3 (l)	43 (16)
<i>Urinary data analysis</i>	
$t_{1/2,ur}$ (days)	4.1 (1.1)
CL _R (ml min ⁻¹)	5.4 (1.6)
CL _{NR} (ml min ⁻¹)	4.0 (1.7)

terminal elimination half-life of strontium (Table 1) showed large interindividual variability, with a mean value of about 2 to 3 times longer than that previously reported by Eisenberg & Gordan (1961) (2.2 days), Root *et al.* (1966) (1.8 days), and Bauer & Ray (1958) (2.7 days). Both the mean residence time and the half-life obtained from the urinary excretion data were similar to the half-life obtained from the first-order elimination rate constant (Table 1).

This large difference between the results of our study and those of previous workers is due to the fact that we continued our study for 20 days, and this allowed us to identify a long terminal elimination phase. Of course, we cannot rule out the possibility that sampling for a longer time would have revealed a still longer terminal phase, but after 20 days plasma strontium concentrations were near the limit of assay detection with the dose we used.

For comparison, we analysed our strontium concentration-time data for the five concentrations measured at 24, 48, 72, 96, and 144 h, in order to mimic the way in which Eisenberg & Gordan (1961) carried out their study. The concentration-time profile between 24 and 144 h was linear and a single exponential function described the data adequately (not shown). The half-life obtained by calculating the first-order elimination rate constant had a mean value of 2.3 (0.5) days (range 1.6 to 2.9 days). These results are in good agreement with those of Eisenberg & Gordan (1961) and confirm that previous reports of a shorter half-life of strontium than we have shown here arose from shorter sampling times. Harrison *et al.* (1955) reported a half-life of 4.5 days after the i.v. administration of strontium to two subjects studied over 15 to 21 days.

Compartmental model-independent analysis

In Table 1 are shown the results of the compartmental model-independent analysis of the plasma concentration vs time curves (mean residence time, apparent volume of distribution at steady state, and plasma clearance) and the results of the analysis of the urinary data (half-life, renal clearance, and non-renal clearance).

Table 2 The volume of the exchangeable pool in healthy subjects after the intravenous administration of calcium or strontium

Author(s)	Subjects (n)	Tracer	Exchangeable pool (l)
Heaney & Whedon (1958)	1	⁴⁵ Ca	65
Nordin (1959)	7	⁴⁷ Ca	47
Lafferty <i>et al.</i> (1964)	2	⁴⁷ Ca	63
Cohn <i>et al.</i> (1966)*	7	⁴⁷ Ca	48
Neer <i>et al.</i> (1967)	10	⁴⁷ Ca	63
Mean	27		55
Bauer & Ray (1958)	5	⁸⁵ Sr	67
Fraser <i>et al.</i> (1960)	15	⁸⁸ Sr	40
Eisenberg & Gordan (1961)	25	⁸⁸ Sr	43
Ray <i>et al.</i> (1965)	10	⁸⁵ Sr	55
Eisenberg (1966)	10	⁸⁸ Sr	46
Cohn <i>et al.</i> (1966)*	7	⁸⁵ Sr	46
Root <i>et al.</i> (1966)	17	⁸⁸ Sr	57
Mean	89		49

The data are shown as mean values. ⁸⁸Sr is stable strontium.

*Cohn *et al.* (1966) gave ⁴⁷Ca and ⁸⁵Sr simultaneously.

Apparent volume of distribution Previous workers have assumed that the apparent volume of distribution of calcium and strontium at steady state represents the total exchangeable pool of calcium, and have estimated the size of that pool at between 43 and 72 l. These data are summarized in Table 2, and they are consistent with the mean steady-state apparent volume of distribution of strontium we have found here (64 l). Table 2 also shows the similarity between previous estimates of the sizes of the exchangeable pools of calcium and strontium. These results suggest that the apparent volume of distribution of strontium, measured in the way we have described, can be used to estimate the size of the exchangeable pool of calcium.

Clearance The mean clearance of strontium was 9.4 (3.0) ml min⁻¹, a value which is similar to that reported by others. Bauer & Ray (1958) using intravenous ⁸⁵Sr reported a mean clearance of 11.7 ml min⁻¹, Eisenberg & Gordan (1961) found a mean clearance of 9.4 ml min⁻¹ in a 6 day study with intravenous stable strontium, and under the same conditions Eisenberg (1966) found a mean clearance of 10.0 ml min⁻¹.

Comparative studies of strontium and calcium have shown that both are eliminated mainly via the kidneys (Samachson & Spencer-Laszlo, 1962; Spencer *et al.*, 1957, 1960; Warren & Spencer, 1976). In our study, the renal clearance of strontium was 57.5% of the total clearance, in agreement with the results of previous workers. The fact that the renal clearance of strontium (5.4 ml min⁻¹) is much smaller than glomerular filtration rate (120 ml min⁻¹) indicates that there is extensive tubular reabsorption of strontium, as there is for calcium (Robertson, 1976).

We did not measure two other routes of strontium excretion—sweat and faeces. While some authors (Johnson *et al.*, 1950) have suggested that sweat is normally an important route of calcium loss, Eisenberg & Gordan (1961) showed that measurements of sweat calcium and strontium concentrations during physical exercise, even at maximal rates, accounted for only a

small portion of calcium or strontium loss. Harrison *et al.* (1966) reported a 10 day cumulative faecal excretion of 15% of an intravenous dose of strontium. Spencer *et al.* (1960) reported approximately 9% in 6 days, and 8 to 17% (mean 13.9%) during a 12 day cumulative excretion study after the simultaneous administration of ^{85}Sr and ^{45}Ca . However, the cumulative faecal excretion of ^{45}Ca was of similar magnitude (10.5%), in agreement with the results of Dow & Stanbury (1960), who showed little difference in faecal losses between the two tracers, and concluded that the major route of excretion is through the kidney.

Compartmental-model analysis

The mean apparent volume of distribution at steady state obtained from the rate constants of the three-compartment open mamillary model ($V_{ss(ij)}$) is shown in Table 1. It was similar to the value found by the compartmental model-independent method.

In picking a three-compartment model we have assumed that strontium is distributed in a central compartment, from which elimination occurs, and that this compartment is reversibly connected to two peripheral compartments. The respective volumes of these compartments were 10, 18, and 43 l (Table 1).

Compartments 2 and 3 can clearly be taken to represent exchangeable pools of strontium, which may be identified with similar pools of calcium. The volumes of these compartments are compatible with the identification of these pools as the intracellular space of soft tissues (the smaller pool) and the superficial zone of bony tissue (the larger pool), as has been previously suggested by others.

This model for the analysis of strontium kinetics is similar to that for the analysis of calcium proposed by Cohn *et al.* (1965, 1966). In their study, compartments 1 and 2 were lumped together and were taken to represent a small exchangeable pool containing plasma, extracellular fluid, and the intracellular space of soft tissues.

Compartment 3 was taken to represent a large exchangeable pool of bony tissue. They also included a compartment to represent the non-exchangeable pool of calcium in the deep skeletal tissue, and they assumed that there was no movement from this compartment into the central compartment during the period studied. This pool is not represented in our model.

The work of Nordin *et al.* (1962), Dow & Stanbury (1960), and Cohn *et al.* (1966), as well as the data summarized in Table 2, suggests that the strontium and calcium exchangeable pools are of the same size, implying that the same tissues are involved in their disposition. Bauer & Ray (1958) drew the same conclusion from their work. In the present model the volume of the larger exchangeable pool of strontium (compartment 3) was 43 l. The mean volume of the exchangeable strontium pool was measured by Eisenberg & Gordan (1961); they found it to be about 43 l.

Conclusions

These results suggest that calcium and strontium share similar pharmacokinetic properties, and that the *in vivo* disposition of strontium may therefore be used to study the disposition of calcium. It follows that the *in vivo* disposition of strontium may also be used to study the effects of drugs, such as calcium antagonists, on the disposition of calcium, and we have elsewhere briefly reported that nifedipine does indeed alter the disposition of strontium (Moraes *et al.*, 1989b).

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