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EXPLORATION OF INTESTINAL ABSORPTION AND RENAL EXCRETION OF CALCIUM BY STABLE STRONTIUM TEST IN CAMELS (*Camelus dromedarius*)

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ABSTRACT

The present study was undertaken to evaluate the intestinal calcium absorption using stable strontium (Sr) as a surrogate marker in five newborn camels. Two tests were performed with an interval of 10 days for calculating the within-animals variation of plasma Sr (CV%). The area under the concentration-time curve (AUC^{0-300}) 5 hours after an oral $SrCl_2$ load of 4.1 mmol was 10.11 ± 0.542 mmol/L^{-1}/min. The within-animals CV% of AUC^{0-300} was 11.6. In our animals, plasma calcium (Ca) and phosphorus (Pi) levels were not significantly modified by Sr load. The fractional Sr excretion 5 hours after oral load in percentage of Sr dose administered was 1.03 ± 0.22% and Sr renal clearance was 4.12 ± 0.51 mL/min. Sr test had no significant effect upon renal excretion of Ca and Pi. Our results showed that in newborn camels, Sr absorption test is simple, reproductible and may be used in exploration of intestinal Ca absorption.

Key words: Calcium intestinal absorption, dromedary, strontium test

Regulation of bone metabolism during postnatal growth involved changes in intestinal calcium (Ca) absorption (Brommage, 1989). Several factors appeared able to contribute to regulation of this metabolism by modulating the intestinal Ca absorption in camels (EL-Khasmi *et al*, 2001; El-Khasmi, 2002). However, to our knowledge, there are no reports concerning the use of markers for estimating intestinal absorption of Ca in this species. In fact, it is largely accepted that transport of strontium (Sr) ions through enteral and renal tubular cells is mediated by the same membrane carriers as used for Ca, and a highly significant correlation has been observed between Sr and Ca absorption (Hart and Spencer, 1967; Milson *et al*, 1987). Therefore, the oral administration of stable Sr is considered suitable for assessing Ca absorption and excretion in clinical practice in man (Vezzoli *et al*, 1998), rat (Corradino *et al*, 1971) and domestic ruminants (Comar and Wasserman, 1964; Gibbons *et al*, 1972; Wadhwa and Care, 2000). Thus, in the work reported here, our objective was to assess the reproductibility of the Sr absorption test and Sr renal clearance in newborn camels.

Materials and Methods

Animals and treatments

The study was carried out at the research station of Laayoune (South Morocco) on 5 newborn camels (*Camelus dromedarius*) aged 4-6 months and weighing 137 ± 12 Kg (Mean ± SEM). All animals were healthy during experimentation. The reproductibility of the Sr test was assessed by repeating the test with an interval of 10 days for calculating the within-animal variation (CV %). Animals orally received within 1 min, 360 ml

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of the test solution containing 4.1 mmol of $SrCl_2$ (Sigma, Aldrich).

At the beginning of the test (0 time), and 30, 60 and 300 min thereafter,a blood sample was withdrawn by puncture from the right jugular vein for the determination of minerals. Blood samples were centrifuged at 1500 g for 10 min. Urine was also collected during a period of 5h before the test and 5h thereafter. Urinary volume was measured. Subsequently, separated plasma and urine samples were frozen at -20°C until analysis.

Assays

In plasma and urine, Ca was measured by atomic absorption spectrophotometry and Pi colorimetrically (Phosphore monoreactif, Biotrol, 75140 Paris Cedex 03). The Sr levels were measured by atomic absorption spectrophotometry at 460.7 nm with use of an acetylene-air flame in 10-fold-diluted plasma and 50-fold-diluted urine with 20 g/L lanthanum and 100 ml / L hydrochloric acid as the diluent.

Calculations

Plasma Sr concentration measured were corrected for endogenous Sr concentrations, determined at t = 0. The area under the concentration-time curve up to time t (AUC^{0-t}), expressed as mmol L^{-1}.min, was calculated at 30, 60 and 300 min (AUC^{0-30}, AUC^{0-60} and AUC^{0-300}). Fractional Sr excretion 5 hours after oral load (FE %) in percentage of Sr dose administered (D) was: (U_{300} / D) x 100 , where U_{300} is the quantity of Sr excreted in urine during 5 h. Sr renal clearance CR_{300} (mL/min) was obtained from U_{300}/AUC^{0-300}.

Within-animal reproductibility of plasma Sr, including analytical and biological variability was calculated as the CV (Liu *et al*, 1978), from its values in test 1 (baseline, x_i1) and test 2 (replicated test, x_i2) by the equation :

$$CV\ \% = 100.\frac{\sqrt{\frac{1}{2n}\sum (x_i1 - x_i2)}}{\frac{\sum (x_i1 \,.\, x_i2)}{2n}}$$

[n = the number of animals (5)].

The CV for the AUC was 19.3, 18.7 and 11.6%, respectively after 30, 60 and 300 min. The CV of Sr urine excretion was 31.8%. Results are given as means ± SEM. One way analysis of variance was used to compare values at 0 time with those measured after Sr loading.

Results and Discussion

The profile of Sr test showed that plasma concentration of this ion was greatest 300 min after the oral Sr load (Fig 1) and Sr absorption (AUC^{0-300}) was 10.11 ± 0.542 mmol L^{-1} min (table I). In our animals, the kinetics of appearance of Sr in blood (figure 1) is similar to that observed in other animals (Gibbons *et al*, 1972; Sips *et al*, 1997). The available data show that intestinal transfer of Sr during the first hour depended more on duodenal and jejunal absorption efficiency (Leeuwenkamp *et al*, 1990; Sips *et al*, 1994). According to Dumont *et al* (1960), Sr transport is passive only and regulated by biohumoral factors similar to those regulating Ca metabolism. Others concluded for an active intestinal Sr absorption and indicated a common transport mechanism for Ca and Sr (Hendrix *et al*, 1963; Wasserman, 1988). Whereas, Sips *et al* (1997) suggested that Sr transport across the intestinal wall is active and vitamin D dependant. In fact, we have already estabilished in newborn camels that 1,25-dihydroxyvitamin D_3 potentialised the intestinal absorption of Sr (El-Khasmi, 2002).

In mammals, Sr is present as a bivalent ion and a trace element and taking no part in the biological cycle (Spencer *et al*, 1960; Leeuwenkamp *et al*, 1990). A comparison of Sr test with the single-isotope radio-Ca absorption test in the same group of patients showed a close correlation between the fractional absorption rates of the two elements (Reid *et al*, 1986). Thus, intestinal Sr absorption is becoming accepted as a clinical and diagnostic tool for assessing intestinal Ca absorption in human (Dijkgraaf-Ten Bolscher *et al*, 2000) and animal (Gibbons *et al*, 1972; Sips *et al*, 1997).

Table 1. Data of Sr oral load test in five newborn camels (Mean ± SEM).

AUC^{0-300} mmol L^{-1} min	U_{300} µmoles	CR_{300} mL/min	FE%
10.11 ± 0.542	42.13 ± 3.71	4.12 ± 0.51	1.03 ± 0.22

AUC^{0-300} : area under the concentration-time curve up to time 300 min; U_{300} : quantity of Sr excreted in urine during 300 min; CR_{300} : Sr renal clearance (U_{300}/AUC^{0-300}); FE % : fractional Sr excretion 5 h after oral load in percentage of Sr dose administered [(U_{300}/D)x100]. D : dose administered (4.1 mmoles).

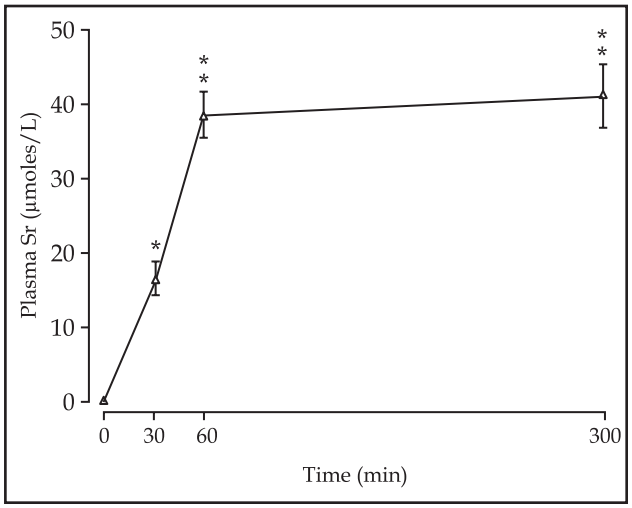

Fig 1. Mean plasma concentration of strontium (Sr) after an oral load of 4.1 mmoles of $SrCl_2$ in five newborn camels. (M ± SEM, *p<0.05, **p<0.01, comparison with values measured at 0 time).

In this investigation, 1.03% of the Sr load are excreted in urine and renal clearance of this element was 4.12 ± 0.51 mL/min (Table I). In fact, Sr ions are more quickly excreted in urine and in enteral lumen with digestive secretions (Comar *et al*, 1957; Spencer *et al*, 1960; Hart and Spencer, 1967). These suggestions could be attributed to a lower cellular carrier affinity for Sr (Varecka and Carafoli, 1982; Vezzoli *et al*, 1995). Furthermore, others findings showed that Sr ions are extensively reabsorbed by the renal tubule (Milson *et al*, 1987; Leeuwenkamp *et al*, 1990). In camels, high renal reabsorption of Sr (table 1) may be explicated by a particular adaptation to desert conditions. This adaptation is also argumented by the stable levels of urinary levels of Ca and Pi during our test (Fig 2).

In camels, Sr like Ca may be absorbed, and because of its good reproducebility, the Sr absorption is a sensitive test, and may provide an appropriate measure for intestinal Ca absorption and modulating effects of several factors. We have used the concentration-time curve to assess a possible changes in the absorption profile of Ca.

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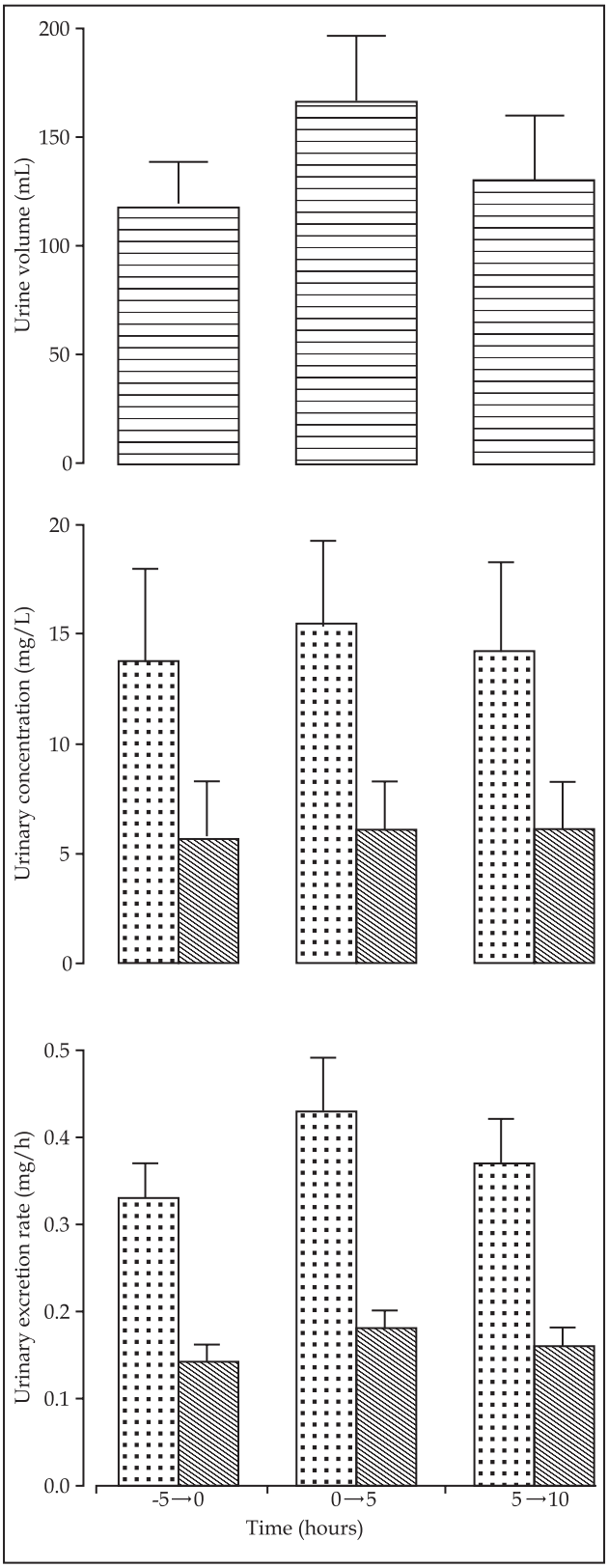

Fig 2. Urine volume and urinary concentration and excretion of calcium (▨) and phosphorus (▨) in 5 new born camels orally treated with 4.1 mmol of $SrCl_2$ solution (means ± SEM, oral load was given during the first min of 0 - 5 hours period).

References

Brommage R (1989). Measurement of calcium and phosphorus fluxes during lactation in the rat. Journal of Nutrition 119:428-438.

Comar CL and Wasserman RH (1964). Strontium. In : Comar CL, Bronner F (eds) Mineral Metabolism. Vol. 2, Part A. Academic Press, New York. pp 523-571.

Comar CL, Wasserman RH, Ulberg S and Andrews GA (1957). Strontium metabolism and strontium-calcium determination in man. Proc. Soc. Exp. Biol. Med. 95:386-391.

Corradino RA, Ebel JW, Craig PH, Taylor AN and Wasserman RH (1971). Calcium absorption and the vitamin D_3 dependent calcium-binding protein, 1. Inhibition by dietary strontium. Calcif. Tissue Res. 7:81-92.

Dijkraaf-Ten-Bolscher M, Netelenbos JC, Barto R and Van Der Vijgh WJF (2000). Strontium as a marker for intestinal calcium absorption: The stimulatory effect of calcitriol. Clinical Chemistry 46:248-251.

Dumont PA, Curran PF and Solomon AK (1960). Calcium and strontium in rats small intestine their fluxes and their effect on Na flux. Journal of Genetics and Physiology 43:1119-1136.

EL-Khasmi M (2002). Régulation endocrinienne du métabolisme phosphocalcique chez la chamelle en lactation et son nouveau-né. Thèse de Doctorat d'Etat ès Sciences, Université Hassan II-Mohammedia, Faculté des Sciences Ben Msik, Casablanca, Maroc. pp 171.

EL-Khasmi M, Riad F, Safwate A, Hidane K, Faye B, Davicco MJ, Coxam V and Barlet JP (2001). Postpartum evolution of mammary secretion of minerals and 25-hydroxyvitamin D in lactating camels (*Camelus dromedarius*). Journal of Camel Practice and Research 8:131-135.

Gibbons RA, Sansom BF and Sellword R (1972). The passage of calcium and strontium across the gut of anesthetised goat. Journal of Physiology 222:397-406.

Hart H and Spencer H (1967). Rate of initial entry of 47Ca and 85Sr from the intestine into the vascular space. Proc. Soc. Exp. Biol. Med. 126:365-371.

Hendrix JZ, Alcock NW and Archibald RM (1963). Competition between calcium, strontium and magnesium for absorption in the isolated rat intestine. Clinical Chemistry 9:734-744.

Leeuwenkamp OR, Van der Vijgh WJ, Husken BC, Lips P and Netelenbos JC (1990). Human pharmacokinetics of orally administered strontium. Calcif. Tissue Int. 47:136-141.

Liu W, Stamler J, Dyer A and McKeever P (1978). Statistical methods to assess and minimise the role of intra-individual variability in observing the relationship between dietary lipids and serum cholesterol. J. Chronic Dis. 31:399-418.

Milson S, Iibbertson K, Hannan S, Shaw D and Pybus J (1987). Simple test of intestinal Ca absorption measured by stable strontium. British Medical Journal 295:231-234.

Reid IR, Pybus J, Lim TM, Hannon S and Ibbertson HK (1986). The assessment of intestinal calcium absorption using stable strontium. Calcif. Tissue Int. 38:303-305.

Sips AJAM, Barto R, Netelenbos JC and Van Der Vijgh WJ (1997). Preclinical screening of the applicability of strontium as a marker for intestinal calcium absorption. American Journal of Physiology 272(3 Pt 1) : E422-E428.

Sips AJAM, Netelenbos JC, Barto R, Lips P and Van der Vijgh WJF (1994). One-hour test for estimating intestinal absorption of calcium by using stable strontium as a marker. Clinical Chemistry 40:257-259.

Spencer H, Laszlo D and Brother M (1957). Strontium-85 and calcium-45 metabolism in man. Journal of Clinical Investigation 36:680-687.

Spencer H, Li M, Samachson J and Laszlo D (1960). Metabolism of strontium-85 and calcium-45 in man. Metaboilsm 9:916-925.

Varecka L and Carafoli E (1982). Vanadate-induced movements of Ca and K in human red blood cells. J. Biol. Chem. 257:7414-7421.

Vezzoli G, Baragetti I, Zerbi S, Caumo A, Soldati L, Bellinzoni P, Centemero A, Rubinacci A, Moro GL and Bianchi G (1998). Strontium absorption and excretion in normocalciuric subjects: Relation to calcium metabolism. Clinical Chemistry 44:586-590.

Vezzoli G, Reina MC, Zerbi S, Spaventa R, Soldati L, Cusi D and Bianchi G (1995). (Ca+Mg) ATPase, calcium influx in erythrocytes of patients with idiopathic hypercalciuria. Biochem. Biophys. Res. Commun. 217: 1099-1104.

Wadhwa DR and Care AD (2000). Effects of strontium on the absorption of calcium, magnesium and phosphorus ions from the ovine reticulo-rumen. Journal of Comparative Physiology [B] 170:225-229.

Wasserman RH (1988). Strontium as a tracer for calcium in biological and clinical research. Clinical Chemistry 44: 437-439.