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Strontium Substitution for Calcium in Lithogenesis

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Abstract

Purpose—Strontium has chemical similarity to calcium, which enables the replacement of calcium by strontium in biomineralization processes. Incorporating strontium into human bone and teeth has been studied extensively but little research has been performed of the incorporation of strontium into urinary calculi. We used synchrotron based x-ray fluorescence and x-ray absorption techniques to examine the presence of strontium in different types of human kidney stones.

Materials and Methods—Multiple unique human stone samples were obtained via consecutive percutaneous nephrolithotomies/ureteroscopies. A portion of each stone was sent for standard laboratory analysis and a portion was retained for x-ray fluorescence and x-ray absorption measurements. X-ray fluorescence and x-ray absorption measurements determined the presence, spatial distribution and speciation of strontium in each stone sample.

Results—Traditional kidney stone analyses identified calcium oxalate, calcium phosphate, uric acid and cystine stones. X-ray fluorescence measurements identified strontium in all stone types except pure cystine. X-ray fluorescence elemental mapping of the samples revealed co-localization of calcium and strontium. X-ray absorption measurements of the calcium phosphate stone showed strontium predominately present as strontium apatite.

Conclusions—Advanced x-ray fluorescence imaging identified strontium in all calcium based stones, present as strontium apatite. This finding may be critical since apatite is thought to be the initial nidus for calcium stone formation. Strontium is not identified by standard laboratory stone analyses. Its substitution for calcium can be reliably identified in stones from multiple calcium based stone formers, which may offer opportunities to gain insight into early events in lithogenesis.

Keywords

kidney; urolithiasis; strontium; calcium; spectrometry; x-ray emission

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Strontium is a divalent cation that is located in the same column of the periodic table of the elements as calcium. Thus, strontium has chemical properties and interactions similar to those of calcium. Strontium exists as 4 stable isotopes (^{84}Sr, ^{86}Sr, ^{87}Sr and ^{88}Sr), of which ^{87}Sr is radioactive. These isotopes have a known geographic distribution. Thus, evaluating the ratio of the 4 isotopes present in bones or uroliths can be used to fingerprint strontium exposure. Additional unstable radioactive strontium isotopes also exist. Indeed, ^{90}Sr levels were traced in milk across the United States in the late 1950s and early 1960s during the era of nuclear weapon testing.[1] The strontium-to-calcium ratio in teeth and bone has been used to help evaluate the strontium exposure and geographic mobility of Neolithic Homo sapiens.[2] Although there is a global differential distribution of strontium, regional evaluation of trace elements present in stones in the United States have yielded no difference, which is likely attributable to the geographic mobility of the population.[3] The presence of radioactive strontium in biomineralization can serve as a marker of radioactivity exposure.[4] The radioactive isotope of strontium has been used as a marker in human calcium absorption experiments.[5–7]

The human body processes strontium in much the same way as calcium, as demonstrated in intestinal absorption and renal filtration studies.[8,9] The kidneys process strontium in a manner similar to calcium and strontium can be administered as a surrogate marker for calcium. Hypercalciuric stone formers have increased strontium absorption compared to normocalciuric patients.[10] Strontium is absorbed by the gut at a rate similar to that of calcium[11,12] and calcium absorption testing can be performed with strontium.[13] Therefore, strontium has long been used to study calcium metabolism in humans and evaluate differences in metabolism in patients with and without evidence of abnormal calcium processing, for example those with osteoporosis.[5]

Strontium substitutes for calcium in biomineralization during bone formation, which has been exploited in osteoporosis studies and in the development of osteoporosis medications. Strontium ranelate is recognized as a cost saving, effective medication for the treatment and prevention of osteoporosis related fractures[14,15] and it may prevent mortality in frail individuals at risk for fracture.[16,17] Strontium ranelate decreases bone resorption and increases bone formation.[18] Strontium is incorporated into hydroxyapatite in bone by replacing a percent of calcium ions, which causes an apparent increase in bone mineral density. This increase in density is due to an increase in bone density and higher x-ray attenuation when measuring strontium.[19] Strontium ranelate increases bone volume and bone trabecular thickness.[20] However, some studies suggest that the dimensions of the bone mineral crystals are not increased and strontium substituting for calcium in hydroxyapatite is the major change.[21]

Although extensive research has been performed on strontium incorporation into bone and strontium handling by the kidney, less research has been done on strontium incorporation in urolithiasis. Strontium was suggested to compete with calcium for incorporation in calcium based stones,[22] and strontium and other trace elements were noted in calcium based uroliths.[23,24] In vitro experiments in nanobacteria revealed strontium incorporation in early stone formation,[25] but there has been little research on strontium incorporation into uroliths in in vivo models. We used synchrotron radiation imaging techniques to characterize the

incorporation of strontium into kidney stones by evaluating the location and speciation of strontium in human stone samples. Since strontium can serve as a tagged marker, we postulate that strontium may serve as a particularly valuable tool for quantifying and monitoring calcium lithogenesis in in vitro and in vivo models.

MATERIALS AND METHODS

Human stone samples were obtained via consecutive percutaneous nephrolithotomy and ureteroscopy procedures. A portion of each stone was sent for standard FTIR laboratory analysis to 1 of 3 commercial laboratories used by our medical center. A portion of each stone was retained and evaluated with x-ray fluorescence and x-ray absorption measurements at the Advanced Light Source synchrotron radiation facility, Lawrence Berkeley National Laboratory, as previously described (S. Blaschko et al, unpublished data). A portion of the whole stones allotted for synchrotron radiation evaluation were microtome sectioned with a diamond blade and evaluated by x-ray fluorescence mapping to minimize x-ray fluorescence absorption artifacts, which may occur in thicker samples. X-ray fluorescence and x-ray absorption measurements were used to examine the presence, spatial distribution and speciation of strontium in each stone sample. Methods of stone evaluation other than synchrotron radiation based analysis cannot provide elemental mapping as well as speciation of trace elements.

RESULTS

FTIR stone analysis identified composite calcium oxalate, calcium phosphate, uric acid and cystine stone samples. FTIR analysis did not identify the presence of trace elements and could not identify strontium in any stone samples. X-ray fluorescence and x-ray absorption measurements confirmed the same stone composition identified by FTIR in each stone sample (data not shown).

X-Ray Fluorescence

X-ray fluorescence elemental mapping of each stone sample demonstrated that strontium is incorporated into uroliths with the same specificity and localization as calcium. Strontium was not found in any stones that did not contain calcium. Strontium co-localization with calcium was clearly noted in the mixed composition uric acid and calcium oxalate stone, in which strontium was only found in the calcium oxalate component (fig. 1). X-ray fluorescence measurements identified strontium in all stone types except pure cystine, which was expected based on the lack of calcium in cystine urolithiasis (data not shown). The co-localization of strontium with calcium was also clearly observed in microtome sectioned stone samples, in which the bright spots revealed a high amount of calcium corresponded to bright spots of localized strontium (fig. 2).

X-ray fluorescence was also done to determine the elements present at specific locations of interest identified by x-ray fluorescence spatial elemental mapping. This evaluation confirmed the presence of strontium in all stone samples except the cystine stone sample. The presence of iron, zinc and lead was also noted in the calcium stones (figs. 2 and 3).

X-ray Absorption Near Edge Spectroscopy

Absorption studies allowed for the determination of the speciation of the type of strontium incorporated into the uroliths by fitting the measured stone sample x-ray absorption curve against the spectra of known samples. This analysis showed that 80% of the strontium was present as strontium apatite and 20% was present as strontium carbonate (fig. 4).

DISCUSSION

Although prior studies have demonstrated the presence of trace elements in calcium based stones, information on the spatial distribution of these elements is mostly lacking.[3,22,24] In prior studies that provide the spatial distribution of strontium, resolution is fivefold lower[26] and strontium speciation is not provided.[26,27] Our evaluation of strontium in urolithiasis highlights the advantages of synchrotron radiation compared with traditional FTIR stone analysis and other methods of elemental mapping. X-ray fluorescence elemental mapping provides information about the localization of calcium and trace elements present throughout each sample (figs. 1 and 2). FTIR vaporizes each sample during processing and, thus, obfuscates elemental mapping. X-ray fluorescence spectra analysis provides the elemental composition of stones at areas of interest with specific identification of trace elements, which are not reported with FTIR analyses (fig. 3). Although FTIR can identify the primary components present in mixed composition stones, the identification and speciation of trace elements available with x-ray absorption analysis are lacking (fig. 4). Trace elements, including those demonstrated by this synchrotron radiation analysis, have a role in lithogenesis[28] and in stabilizing calcium oxalate dihydrate.[29] The presence of strontium has correlated with the presence of zinc in uroliths[26] and the study of the critical role of zinc in stone formation is promising (T. Chi et al, unpublished data). However, additional studies must be completed before recommending analysis of trace elements in 24-hour urine analyses.

X-ray fluorescence analysis clearly shows that strontium co-localizes with calcium in calcium based stone formation. In addition, the kidneys process strontium similarly to calcium.[9] The amount of strontium and apatite in kidney stones is correlated.[28] These findings validate the use of strontium as a marker for studying calcium lithogenesis in vivo and in vitro. Moreover, radioactive and nonradioactive strontium may serve as a marker depending on prior strontium exposure in in vivo models. Future studies are warranted with tagged strontium as a marker for calcium in in vitro and in vivo experiments.

Our x-ray absorption studies show that in human kidney stones 80% of strontium appears as strontium apatite and 20% appears as strontium carbonate. Since hydroxyapatite is thought to serve as the nidus for stone formation and more trace elements are found in apatite stones than in other stone types,[3] the presence of strontium apatite indicates that strontium may be useful for studying the events of lithogenesis and stone enlargement. In support of this, strontium was present during early stone formation in nanobacteria studies[25] and more strontium is found in the kidney stones of older patients than in the kidney stones of younger patients.[30]

Strontium has been demonstrated to interact in other hydroxyapatite biomineralization processes. Strontium in the form of strontium ranelate interacts with hydroxyapatite. In many situations it can increase bone mineral density and decrease fractures in high risk individuals. It is not surprising to find a similar incorporation of strontium into nephrolithiasis in calcium based urinary stones. The incorporation of strontium in calcium stone forming patients who receive strontium ranelate for osteoporosis is an area of research open to study. This population likely has de facto tagged uroliths due to regular strontium administration. We hypothesize that these patients would have a higher amount of strontium incorporation during stone formation than patients who do not receive strontium ranelate but the implication of this is yet unknown. Strontium apatite and calcium apatite have approximately the same hardness since they are categorized as apatite, level 5 on the Mohs hardness scale. Adding strontium to calcium stones may affect the brittleness of the stone structure due to differences in the atomic size of strontium and calcium, causing differences in chemical bonds. The administration of strontium at set time points in in vitro and in vivo models may be used to study the interaction of strontium apatite in early stone formation. Additionally, strontium may be added to new therapeutics to possibly aid in the targeting of developing kidney stones.

CONCLUSIONS

Strontium is present in the human diet in trace amounts that vary with geographic location. Strontium is processed by the gut and kidneys in a manner similar to calcium.[8,9] Synchrotron radiation imaging techniques show that strontium co-localizes with calcium in calcium based stones and it is present primarily as strontium apatite. This suggests that strontium hydroxyapatite, like calcium hydroxyapatite, may be a nidus in calcium based stone formation. Radioactive strontium may be administered and measured, and may serve as a valuable marker to study calcium based lithogenesis. Patients on strontium ranelate for osteoporosis may serve as a de facto population of patients with tagged kidney stones due to regular strontium administration. Knowledge of strontium incorporation into calcium based uroliths may be leveraged to develop new therapies.

Acknowledgments

Abbreviations and Acronyms

FTIR   Fourier transform infrared spectroscopy

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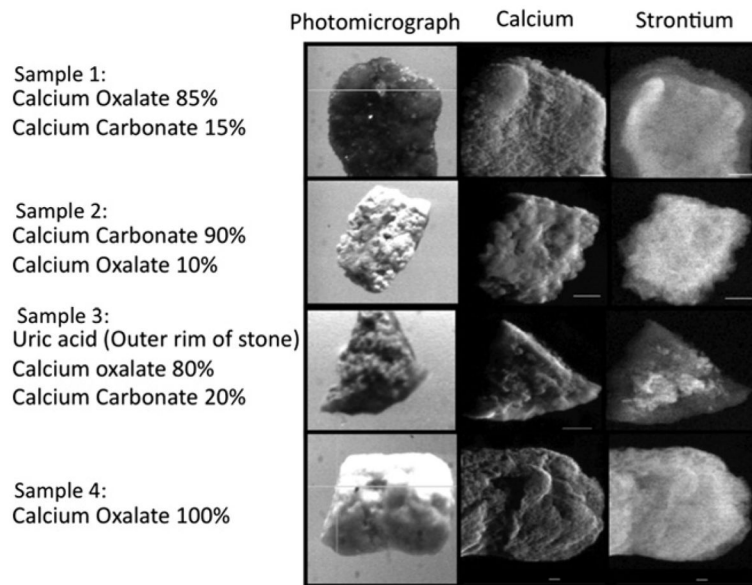
Figure 1.
X-ray fluorescence elemental mapping demonstrates co-localization of strontium and calcium in different stone types. Photomicrograph shows gross pictures of stones retrieved for analysis. Calcium and strontium distribution is similar in each calcium based stone type. X-ray fluorescence radiation from calcium is absorbed more than that of strontium so that sample surface topology influences final image. Thus, calcium images show more contrast variation.
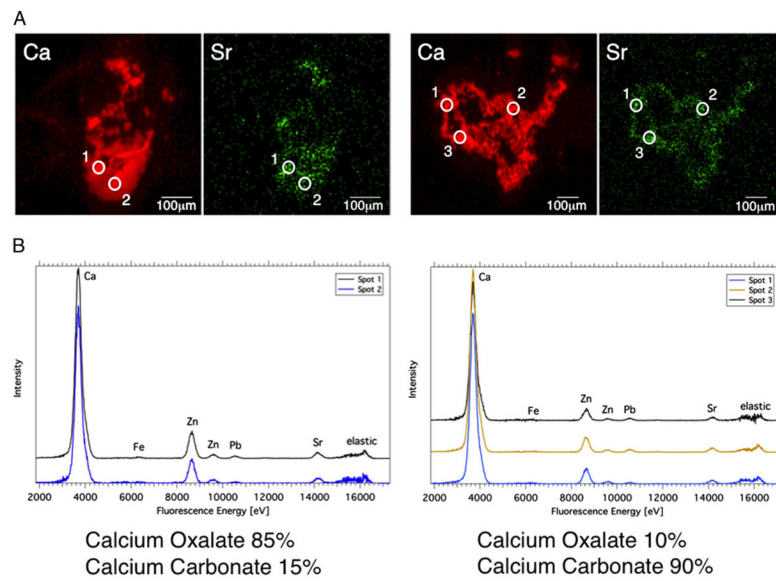
Figure 2.
A, spatial elemental mapping of 2 microtome sectioned samples shows co-localization of strontium with calcium. Circles indicate where sample x-ray fluorescence spectra were acquired. Scale bar indicates 100 *μ*m. *B*, x-ray fluorescence spectra acquired at specific locations on samples reveal small amounts of strontium, iron, zinc and lead.
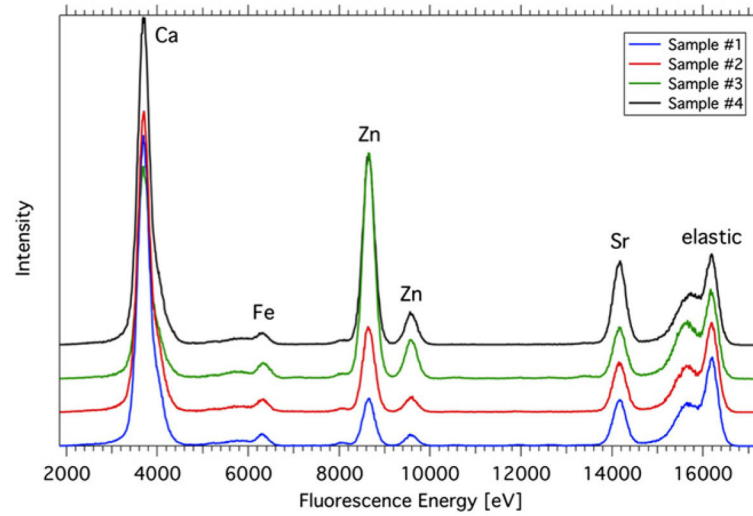
Figure 3.
X-ray fluorescence spectra of 4 calcium containing stones shows strontium, iron, zinc and lead in each sample. Low lead levels were also observed, which are not visible in this presentation.
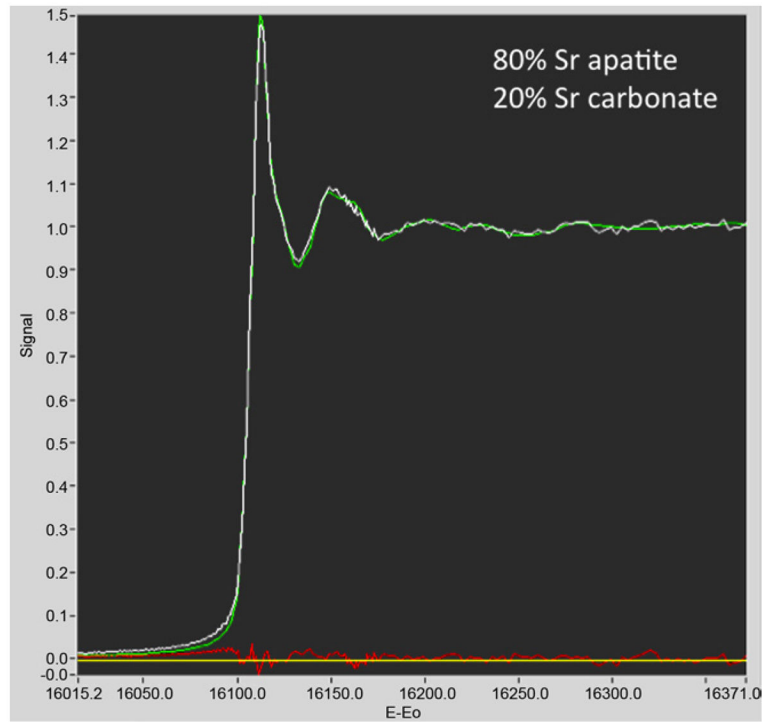
Figure 4.
X-ray absorption near edge spectroscopy of calcium oxalate stone sample 4 allowed for speciation of strontium type by fitting stone sample x-ray absorption curve against curve of known samples. Analysis revealed that strontium present was 80% strontium apatite and 20% strontium carbonate.