Strontium causes osteomalacia in chronic renal failure rats

IRIS SCHROOTEN, WALTER CABRERA, WILLIAM G. GOODMAN, SIMONNE DAUWE, LUDWIG V. LAMBERTS, RITA MARYNISSEN, WALTER DORRINÉ, MARC E. DE BROE, and PATRICK C. D’HAESE

Departments of Nephrology and Chemistry, University of Antwerp, Belgium, and Department of Radiology Science, UCLA, Los Angeles, California, USA

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Background. We recently reported an association between increased bone strontium (Sr) levels and osteomalacia in dialysis patients.

Methods. To delineate whether or not Sr acts as a causal factor in the development of osteomalacia, we devised the following study: four groups of chronic renal failure (CRF) rats were given Sr, aluminum (Al), both of these compounds or none of the elements (controls).

Results. Administration of Sr and/or Al resulted in increased bone levels of the respective elements. Histological examination revealed impairment of mineralization in the Sr group and to a lesser extent in the Al group as compared to the control group. There was also a significant increase in osteoid area in the Sr group, but not in the Al group. No differences in bone surface or erodic perimeter were noted between the various study groups. Histochemically, Sr could be localized in calcified bone, mainly in new bone close to the osteoid/calcification front, a critical site of bone mineralization. Histochemical findings were confirmed by electron probe X-ray microanalysis.

Conclusions. These findings indicate that Sr accumulation in chronic renal failure rats resulted in the development of osteomalacic lesions, in contrast to the Al group where adynamic bone disease was induced in the present set-up. Further studies are required to define the mechanism by which way Sr causes osteomalacia in chronic renal failure rats.

Patients on chronic maintenance dialysis usually develop some type of renal osteodystrophy, either hyperparathyroidism, adynamic bone disease, osteomalacia or a mixed lesion. In addition, because of the impaired renal function, the medication and the use of contaminated parenteral fluids, trace elements may accumulate in dialysis patients. An association between bone aluminum (Al) accumulation and the development of osteomalacia and to a lesser extent adynamic bone disease has repeatedly been established [1–3]. Aside from Al, much less is known about the effects of other trace metals on bone metabolism in dialysis patients.

In a recent multicenter study, histological/chemical examination of 100 bone biopsies of dialysis patients presenting the various types of renal osteodystrophy revealed bone strontium (Sr) levels, as well as Sr/calcium (Ca) ratios to be increased in osteomalacic patients. Bone Sr levels were not only elevated compared to individuals with a normal bone histology, but also in comparison to all other types of renal osteodystrophy [4].

Like Al and Ca, Sr is a ‘bone-seeking’ element 99% of its total body burden being stored in bone [5]. Sr exhibits a number of physicochemical similarities with Ca and therefore has been used as a reliable index for Ca-absorption [6]. In previous experiments an important influence of Sr on Ca homeostasis [7] has been postulated, as well as interactions with vitamin D synthesis [8] and bone metabolism [9], two processes that are disturbed in chronic renal failure. In experimental studies in rats and mice with intact renal function a dose-dependent effect of Sr on different parameters of bone metabolism has been demonstrated. Low doses of Sr stimulate bone formation [9] and increase bone mass [10], while high Sr doses induce hypomineralization [10] and transiently inhibit bone resorption [11, 12]. Furthermore, in vitamin D3-repleted chicks it has been shown that by inhibiting both the synthesis of 1,25-dihydroxycholecalciferol and intestinal Ca absorption, dietary Sr can induce rickets, reflected by a diminished growth and an improper bone mineralization, histological features highly comparable to these of osteomalacia [8]. Recently, an epidemiological study in Turkey demonstrated a significantly higher prevalence of the clinical diagnosis of rickets in children living in a region with a high soil Sr content and where nutrition was mainly based on cereals, compared to that in children living in a low Sr region [13].

Although Rudolph, Alfrey and Smythe [14] made the interesting suggestion in 1973 that Sr could play a role in renal osteodystrophy in the dialysis patient, to the best of our knowledge we are the first to demonstrate an association between increased bone Sr levels in dialysis patients.

Key words: aluminum, bone, adynamic bone disease, dialysis, bone mineralization, osteodystrophy, hyperparathyroidism, hypomineralization.
and the presence of a particular type of renal osteodystrophy, that is, osteomalacia [4]. These epidemiological data, however, do not allow us to conclude whether Sr played a causative or contributive role in the development of the bone disease. Here we report data from a study in rats demonstrating a causal role of Sr in the development of osteomalacia in chronic renal failure.

METHODS

Experimental design

Using the remnant kidney model, chronic renal failure was induced in 32 female Wistar rats at eight weeks of age. Under sodium pentobarbital anesthesia (60 mg/kg) two branches of the renal artery of the right kidney were ligated and two weeks later the left kidney was removed. Four weeks after nephrectomy rats were divided into four groups: 8 controls, 8 rats loaded with Sr (Sr), 8 loaded with Al (Al) and 8 animals loaded with both Sr and Al (Sr + Al). In all groups except for the Sr-group, one rat died after induction of the chronic renal failure. Sr loading was done seven days/week by adding SrCl2 · 6H2O at a concentration of 10.0 g/liter (0.34% Sr) to the animals’ drinking water, as previously described [9, 10, 15]. Aluminum loading was done orally six days/week by addition of 400 mg of Al(OH)3 to 40 g of rat chow and addition of 10 mg/liter of AlCl3 to the drinking water. On day 7 a solution containing 17.9 mg AlCl3 · 6H2O and 15.6 mg citrate in 500 µl saline (0.9%), pH ± 5 was injected intraperitoneally. Control and Sr-loaded rats received on the same day an intraperitoneal injection of 15.6 mg citrate dissolved in 500 µl saline (0.9%), pH ± 5. Food and drink administered to the Sr groups was checked for the presence of Al and vice versa. Rats were pair fed and weighed weekly. Animals had free access to food and water. Water as well as food consumption were recorded daily. The loading protocol was continued over 12 weeks. The rats were sacrificed the day following the end of the loading protocol. At five and two days before sacrifice, respectively demeclocycin (30 mg/kg) and tetracycline · HCl (30 mg/kg) labeling were performed. At regular times during the loading period, blood and urine samples were taken and frozen at −80°C until analysis (Fig. 1). For urine collection the animals were housed individually in metabolic cages and 24 hour urine samples were collected. Blood was collected from the tail vein during the loading period and by cardiac puncture at sacrifice under ether anesthesia. Bone was removed at sacrifice and stored at −20°C until analysis.

Biochemical and chemical determinations

Creatinine in serum and urine was determined according to the Jaffé method. iPTH in serum was determined using the INS-PTH kit (Incstar Corp., Stillwater, MN, USA), validated for the analysis of rat serum [16].

Sr and Al in serum, urine, and bone were analyzed using a Zeeman 3030 atomic absorption spectrometer equipped with an HGA-600 graphite furnace, an AS-60 autosampler and an Anadex DP-9500B silent scribe printer, all from Perkin-Elmer (Norwalk, CT, USA). To determine Sr, serum samples were diluted fourfold in a 0.5 ml/liter Triton X-100 to 1 ml/liter HNO3 solution, whereas urine samples were diluted 1:20 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:60 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:20 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:60 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:60 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:60 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:60 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:60 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:60 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:60 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17]. Al in serum was determined after 1:3 dilution of the samples in RO-water while urine samples were diluted 1:60 with HNO3. Bone samples were digested with concentrated HNO3 in stoppered polytetrafluoroethylene (Teflon®) tubes [17].
Sr and/or Al. Material showing detectable Al (1 µg/liter or more) or Sr (3 µg/liter or more) after a five-day contact with doubly distilled water and nitric acid (10%) were discarded. The use of glassware was avoided and only doubly distilled water was used for sample preparation. Methods for the determination of Sr and Al have been described by us in detail previously [17–19]. Ca and P in serum were determined with a Vitros 750 XRC spectrophotometer in the Laboratory of Biochemistry of the University Hospital of Antwerp. Ca determinations in urine and bone were done by flame-AAS (Mod. 3110; Perkin-Elmer) after diluting the samples 1:500 in RO-water to which 1 g/liter lanthane was added to avoid ionization-interferences.

**Bone histology**

At sacrifice, femurs and tibiae were freed of soft tissue. From each rat one bone sample was, after removal of skin and muscle tissue, weighed immediately and stored at −20°C until bulk analysis with AAS. The second sample, which was used for histological examination, was fixed for 24 hours in Burkhard solution and then transferred to a 70% ethanol solution. Longitudinal sections (4 µm) of the proximal tibia were cut with a Jung K microtome and stained with toluidine blue. Histological examination and classification were done at the UCLA School of Medicine (W. Goodman). Bone histological data as well as dynamic parameters are reported using standardized nomenclature and definitions [20, 21]. The parameters measured include: total bone area [B. Ar (%); the area of trabecular bone, including both mineralized bone and osteoid, expressed as a percentage of the total tissue area], osteoid area [O. Ar (%); the measured area of osteoid expressed as a percent of the total bone area], osteoid thickness [O. Th (µm); the mean width of surface osteoid seams, calculated by dividing the measured osteoid area by the length of the osteoid seams], osteoid perimeter [O. Pm (%); perimeter occupied by osteoid, minimum width specified for the measurement of osteoid surface], eroded surface [E. Pm (%); the percentage of trabecular bone surface characterized by the presence of scalloped bone resorptive lacunae], double labeled surface [dLS (%); percentage of total endosteal surface exhibiting a double fluorescent tetracycline label], mineral apposition rate [MAR (µm/day); distance between the midpoints or between the corresponding edges of two consecutive tetracycline labels, divided by the time interval between the labeling periods], mineralization lag time [MLT (days); mean time interval between deposition and mineralization of any infinitesimal volume of matrix, averaged over the entire lifespan of the osteoid seam] and bone formation rate [BFR (µm²/mm²/day); volume of bone formed per unit of time, calculated as the product of mineral apposition rate and mineralizing surface].

**Localization of strontium**

Histochemically Sr could be demonstrated by modification of a method described by Waterhouse [22]. This method is based on a specific chelation of Sr by sodium rhodizonate under the formation of a reddish-brown color. Using this method, originally developed for the staining of Sr in insects, the element could be demonstrated on histological sections of bone provided the pH was lowered from 5.5 to 3.5, by using an acetate buffer instead of the conventional phosphate buffer and at a temperature of 0°C instead of at room temperature.

Sr was also demonstrated microanalytically by electron probe X-ray microanalysis (EPXMA). For this purpose, 100-µm thick carbon coated histological sections of rat bone samples embedded in glycol-methylmethacrylate were analyzed with a Jeol JSM 6300 (Jeol, Tokyo, Japan).

**Statistics**

All results are expressed as mean ± SD. Statistical analysis of the data was done using the one way ANOVA followed by the Bonferroni correction when more than two groups were considered. A P value < 0.05 was considered to be significant at a two-tailed level.

**RESULTS**

**Biochemical and chemical data**

The daily food and water consumption did not differ between the four groups under study, resulting in comparable weights at sacrifice. Comparison of serum creatinine levels before induction of CRF and at sacrifice using the remnant kidney model indicates that a moderate degree of renal failure was induced (Table 1). As also indicated in Table 1, urine output was comparable at start of loading and at sacrifice among the various study groups. There was a tendency for the intracellular parathyroid hormone (iPTH) to increase during the loading period in all groups, which, however, was not significant. Serum Ca and P concentrations at start of loading were within the normal range in all groups and had not changed by the time of sacrifice (Table 1). At sacrifice, urinary Ca excretion was significantly elevated in the Sr and Sr/Al group (Table 1). Sr, Al and Ca concentrations in bone of the different groups are presented in Table 2. Loading of the animals with Sr, Al or the combination of both elements led to a significant accumulation of these in the respective groups. In order to correct for bone density, we also determined the Ca concentration in bone. The bone Ca content did not differ between the groups under study. Hence, the Sr/Ca and Al/Ca ratios showed the same trend as noted for the absolute concentrations. As for bone, serum Sr and Al concentrations were also significantly higher in the groups loaded with these elements (Table 2).
Table 1. Biochemical parameters in serum and urine of the various study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>Sr</th>
<th>Al</th>
<th>Sr/Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine mg/dl</td>
<td>Pre-CRF (week 0)</td>
<td>0.98 ± 0.64</td>
<td>0.86 ± 0.42</td>
<td>0.62 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>At sacrifice</td>
<td>1.36 ± 0.27</td>
<td>1.60 ± 0.52</td>
<td>1.36 ± 0.10</td>
</tr>
<tr>
<td>Serum Ca mg/dl</td>
<td>Start loading (week 6)</td>
<td>10.35 ± 0.30</td>
<td>10.45 ± 0.69</td>
<td>10.54 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>At sacrifice</td>
<td>10.20 ± 0.43</td>
<td>10.69 ± 0.39</td>
<td>10.77 ± 0.72</td>
</tr>
<tr>
<td>Serum P mg/dl</td>
<td>Start loading (week 6)</td>
<td>6.14 ± 0.73</td>
<td>6.84 ± 1.29</td>
<td>4.70 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>At sacrifice</td>
<td>5.38 ± 0.85</td>
<td>5.61 ± 1.72</td>
<td>4.75 ± 0.67</td>
</tr>
<tr>
<td>Urinary Ca μg/mg creatinine</td>
<td>Start loading (week 6)</td>
<td>20.18 ± 12.04</td>
<td>17.54 ± 11.19</td>
<td>11.61 ± 8.04</td>
</tr>
<tr>
<td></td>
<td>At sacrifice</td>
<td>8.92 ± 4.83</td>
<td>47.31 ± 27.13</td>
<td>20.87 ± 22.39</td>
</tr>
</tbody>
</table>

Abbreviations are: Sr, strontium; Al, aluminum; Ca, calcium; P, phosphorus.

P < 0.05 vs. pre-loading; P < 0.05 vs. C

Table 2. Serum and bone Sr and Al concentrations, bone Ca concentrations and Sr/Ca and Al/Ca ratios

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>Sr</th>
<th>Al</th>
<th>Sr/Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Sr mg/g</td>
<td>0.027 ± 0.004</td>
<td>3.521 ± 0.888</td>
<td>0.032 ± 0.017</td>
<td>3.332 ± 0.954</td>
</tr>
<tr>
<td>Bone Al μg/g</td>
<td>2.10 ± 1.90</td>
<td>1.10 ± 0.40</td>
<td>26.3 ± 7.90</td>
<td>36.3 ± 13.6</td>
</tr>
<tr>
<td>Bone Ca mg/g</td>
<td>191 ± 19</td>
<td>183 ± 15</td>
<td>177 ± 8.3</td>
<td>172 ± 14</td>
</tr>
<tr>
<td>Bone Sr/Ca ×10^4</td>
<td>0.14 ± 0.01</td>
<td>22.0 ± 5.80</td>
<td>0.19 ± 0.10</td>
<td>22.7 ± 9.87</td>
</tr>
<tr>
<td>Bone Al/Ca ×10^6</td>
<td>11.0 ± 10.4</td>
<td>6.92 ± 2.51</td>
<td>156 ± 46.3</td>
<td>259 ± 134</td>
</tr>
<tr>
<td>Serum Sr mg/liter</td>
<td>Start loading (week 6)</td>
<td>0.021 ± 0.006</td>
<td>0.024 ± 0.005</td>
<td>0.018 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>At sacrifice</td>
<td>0.028 ± 0.011</td>
<td>0.032 ± 0.011</td>
<td>0.035 ± 0.008</td>
</tr>
<tr>
<td>Serum Al μg/liter</td>
<td>Start loading (week 6)</td>
<td>3.0 ± 5.6</td>
<td>1.0 ± 0.0</td>
<td>10.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>At sacrifice</td>
<td>2.0 ± 1.6</td>
<td>3.0 ± 1.2</td>
<td>14 ± 7.3</td>
</tr>
</tbody>
</table>

Abbreviations are in Table 1.

Histological data

Figure 2 shows a Goldner staining of a control (Fig. 2a), a Sr-loaded (Fig. 2b) and an Al-loaded rat (Fig. 2c). The increased amount of osteoid in the Sr-loaded animal documents osteomalacia. By contrast, Goldner staining of the rat loaded with Al shows that the amount of osteoid is comparable to that in the controls.

There was no difference in bone area between all groups (Fig. 3A). The osteoid area, however, was clearly increased in the Sr group, compared to the control animals and the group that received only Al (Fig. 3B). The mean osteoid area in the Sr/Al group was comparable to that of the Sr group, though there was substantial individual variation. A comparable trend was observed in the osteoid perimeter (Fig. 3C) and osteoid thickness (Fig. 3D). The histomorphometric parameters reflecting mineralization, namely dLS, MAR, BFR and MLT, also differed from the control group. Both dLS (Fig. 3E) and MAR (Fig. 3F) in the Sr and Sr/Al loaded animals were approximately one-fourth the values seen in the controls. Intermediate values were noted for the Al group. A similar trend was noted for the BFR (Fig. 3G). The MLT (Fig. 3H) was clearly increased in the three groups, which were loaded with Sr, Al or both of these elements. No significant differences in eroded surface were found (Fig. 3I), which indicates the absence of an effect on bone resorption from either of the two toxins.

Localization of strontium

Rhodizionate staining histochemically localized Sr in calcified bone, mainly in new formed bone in close proximity to the mineralization front and surrounding the osteoid. In a control rat staining was negative (Fig. 4). Histochemical findings were confirmed by EPXMA. By using this technique the element could also be demonstrated in calcified bone near the mineralization front (Fig. 5).

DISCUSSION

As we previously reported, in a histological/chemical survey of 100 bone biopsies from dialysis patients coming from several centers in various countries, increased Sr concentrations as well as Sr/Ca ratios were observed in the bone of patients suffering from osteomalacia as compared to all other types of renal osteodystrophy [4]. Although the prevalence of osteomalacia has decreased in the overall dialysis population, the disease is still frequently observed in less developed countries. In this context it is of interest that we only found increased bone Sr concentrations in patients living in these countries. Hence, for these individuals strontium toxicity may be a serious potential problem. To which extent
the water purification system, with or without a reverse osmosis system, the use of contaminated concentrates, or the diet play a role in the Sr accumulation in dialysis patients has not yet been shown [23]. The fact that most Sr present in the body is being eliminated by the kidney may also contribute to its accumulation in dialysis patients [24].

The experiments described in this paper were designed to assess the role of Sr in the development of osteomalacia in dialysis patients. The remnant kidney rat model was used, which is well recognized to be a suitable method to induce a moderate, stable chronic renal failure in animals. Loading of the rats was done over a relatively long period. This relatively long loading protocol resembles the actual chronic accumulation of trace elements in dialysis patients. Data from the present study for the first time demonstrate that Sr can cause osteomalacia. Based on a pilot study and other reports [9, 10, 15], relatively high amounts of Sr were used in the present experiment to correct for the relatively higher bone turnover and glomerular filtration rate in rats compared to humans, which would protect the rats against accumulation of Sr and its deleterious effects.

No differences in iPTH values were observed between the different groups nor did the iPTH significantly increase after induction of chronic renal failure. This agrees with previous reports that iPTH elevation occurs after at least 20 weeks in this model [25]. Comparison of the iPTH levels between Sr loaded animals and the control group further shows that the element seems not to influence iPTH secretion. This is a controversial point that is in agreement with some prior studies [26] and disagreement with others [27, 28]. No data are available on the effect of Sr on the synthesis of iPTH.

Although no differences in serum Ca concentrations were observed between the different groups, urinary Ca was increased in the rats loaded with Sr. This was noted in the absence of any difference in urinary volumes, excluding that the enhanced urinary Ca excretion could be due to diuresis. Since high concentrations of Sr have been described to decrease Ca absorption either directly or indirectly through the element’s interference with vitamin D biosynthesis [8], and because no differences in serum Ca levels were observed between the different groups, it is highly unlikely that the increased urinary output was due to an increased Ca absorption in the treated groups. In view of the above, we suggest that the observed increment might result from a delayed or reduced incorporation of Ca in bone secondary to Sr accumulation. Also, the incorporation of Sr in bone has been shown to be accompanied by a mild distortion of the crystal lattice, which in turn leads to an increased bone mineral solubility and thus a lower bone mineral density [29], which consequently may result in an enhanced efflux of Ca from bone and thus an increased urinary Ca excretion. Although no data exist, Sr might also inhibit tubular reabsorption of Ca.

Histological analysis clearly show an increased amount of

Fig. 2. Goldner staining of a control (a), a Sr-loaded (b) and an Al-loaded rat (c). Note the increased amounts of osteoid in the Sr-loaded animal and the absence of osteoid in the Al-loaded rat.
Fig. 3. Histological data of the various groups. *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \).
osteoid in rats of the Sr-group, as compared to the controls and Al-loaded animals. The decreased bone formation and increased MLT (the time it takes for osteoid to mineralize) document dynamically the impairment of mineralization. A defective mineralization in combination with an increased osteoid volume are two major characteristics of osteomalacia. Hence, the lesions we describe in rats loaded with Sr have histological features of typical osteomalacia [20, 30].

In rats loaded with Al the degree of mineralization was also decreased, though osteoid volume was not increased. Since in this group the amount of osteoid was comparable to that in the control animals, these rats actually developed...
an adynamic bone disease. This pathology, characterized by a decreased mineralization in the absence of an increased osteoid, suggests an effect on osteoblast activity rather than on mineralization. Our findings agree with studies performed in rats with normal renal function. In these rats Al administration led to a decrease in bone and matrix formation that was not accompanied by an increase in osteoid [31, 32]. In another study in which Al was administered to both normal rats and rats with chronic renal failure, osteomalacia was induced. In that study, however, Al was administered at a dose that was almost twofold higher than the one we used [2].

In the group of animals loaded with both Sr and Al some rats developed adynamic bone disease, whereas in others osteomalacia occurred. To which extent the development of the latter bone disease is aggravated by the concerted accumulation of both elements cannot be deduced from our data. This observation, together with the fact that Al-loaded rats in our study developed only adynamic bone disease, suggests that the so-called “Al-induced osteomalacia” in dialysis patients might not solely be due to Al, but could be the result of a multi-element effect. In this respect, epidemiological findings of our multicenter study demonstrating a good correlation between Al and Sr in the osteomalacic patients but not in those presenting the other types of renal osteodystrophy is of particular interest [4]. In previous studies, Rudolph et al [14] and Canavese et al [33] also made the suggestion that in addition to Al, Sr might play a role in the development of renal osteodystrophy in dialysis patients.

The fact that we could not observe any differences between the Sr groups and controls in the eroded surface, which is an indirect measure of bone resorption, indicates the absence of an effect of Sr on bone resorption under the conditions of the present study. Other studies in both rats and mice with normal renal function demonstrated an inhibition of bone resorption by Sr [11, 12].

Localization of Sr was done by a modification of the method of Waterhouse [22] using Na-rhodizonate, a histochemical staining technique that was initially developed to demonstrate Sr in soft tissues. Modification of this method permits identification of Sr in bone when present at high enough concentrations. Histochemical staining of Sr showed the presence of the element in mineralized bone, mainly at the region of the osteoid/calcification front, thus in newly forming bone. The presence of Sr at this site may point to a possible interference of the element with bone mineralization. By using electron probe X-ray microanalysis (EPXMA), a technique that detects X-rays generated by an electron beam, the histochemical observations could be confirmed. Scarce data in literature based on X-ray microanalysis have also demonstrated the presence of Sr in mineralized bone, mainly present in newly formed bone [15].

A preferential deposition of Sr at the osteoid/calcification front is similar to the deposition of Al in bone noted in dialysis patients with either osteomalacia or adynamic bone disease. Deposition at the osteoid/calcification front of both elements, however, is different: whereas in the case of Al usually one line is demonstrated histochemically, staining for Sr shows a series of parallel lines.

When comparing both elements, not only a different pattern of deposition is seen, but the mechanisms of Sr and Al effects on bone are also different. Aluminum seems to have a direct as well as indirect effect, through the inhibition of iPTH on the osteoblast, which results in a defective osteoblast function [34]. Our data and those of others indicate that Sr affects mineralization in the presence of an active osteoblast and does not inhibit iPTH [11, 26].

In conclusion, Sr loading can induce osteomalacia in a chronic renal failure rat model. In contrast to Sr, Al loading results in the development of adynamic bone disease. Histochemically and by means of electron microscopy, Sr is localized in mineralized bone, mainly at the osteoid/calcification front. Our data point to a causative role for Sr in the development of osteomalacia. The mechanism by which Sr induces this bone disease is not yet known.

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APPENDIX

Abbreviations used in this article are: Al, aluminum; B. Ar, total bone area; BFR, bone formation rate; Ca, calcium; CRF, chronic renal failure; d.L. Ar, double-labeled surface area; E. Pm, eroded surface; EPXMA, electron probe X-ray microanalysis; iPTH, intact parathyroid hormone; MAR, mineral apposition rate; MLT, mineralization lag time; O. Ar, osteoid area; O. Th, osteoid thickness; Sr, strontium.

REFERENCES


