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Sevelamer hydrochloride attenuates kidney and cardiovascular calcifications in long-term experimental uremia

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Sevelamer hydrochloride attenuates kidney and cardiovascular calcifications in long-term experimental uremia.

Background. In chronic renal failure (CRF), hyperphosphatemia and an elevated calcium-phosphate product are associated with vascular calcification and increased cardiovascular morbidity and mortality. Previous data have demonstrated that 3-month treatment of uremic rats with sevelamer was associated with less nephrocalcinosis compared to calcium carbonate ($CaCO_3$), despite similar control of serum phosphorus, calcium-phosphorus product (Ca x P product), and secondary hyperparathyroidism. There was no evidence of aortic calcification after 3 months of uremia (*J Am Soc Nephrol* 13:2299–2308, 2002). The present studies explore the influence of sevelamer and $CaCO_3$ on cardiovascular and kidney calcifications in long-term experimental uremia over 6 months.

Methods. Normal and 5/6 nephrectomized rats (U) were fed a high phosphorus (HP) diet for 6 months. Two phosphate binders, $CaCO_3$ and sevelamer, were administered and their influence on hyperphosphatemia, secondary hyperparathyroidism, kidney/myocardial/aortic calcification, and renal function was compared.

Results. All uremic rats began the study with the same degree of renal failure. Sevelamer was as effective as $CaCO_3$ in reducing serum phosphorus, Ca x P product, and attenuating secondary hyperparathyroidism. Despite similar serum cholesterol levels, rats in the U-HP + sevelamer group had markedly lower calcium deposition in the myocardium and aorta (myocardium, 72 ± 4 μg/g wet tissue; aorta, 736 ± 156 μg/g wet tissue) compared to rats in either the U-HP + $CaCO_3$ group (myocardium, 179 ± 48, $P < 0.05$; aorta, 1308 ± 343, $P < 0.05$) or the U-HP group (myocardium, 98 ± 10, NS; aorta, 2150 ± 447, $P < 0.05$). Dual immunohistochemical analysis for calcium and endothelial cell markers demonstrated that myocardial calcium deposition was intravascular within capillaries. Furthermore, calcium deposition in the kidney of uremic rats treated with sevelamer (582 ± 111 μg/g wet tissue) was lower than that found in uremic rats treated with $CaCO_3$ (1196 ± 180 μg/g wet tissue). Sevelamer-treated rats had less deterioration in renal function with an associated lower serum creatinine, higher creatinine clearance, and less proteinuria. There was no difference in overall mortality between the three experimental groups.

Conclusion. In long-term experimental CRF, in addition to controlling serum phosphorus and secondary hyperparathyroidism as efficiently as $CaCO_3$, treatment with the phosphate-binder sevelamer attenuates vascular and kidney calcification.

Key words: vascular calcification, phosphate, renal failure.

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A higher incidence of coronary artery and cardiac valve calcification occurs in dialysis patients compared to the general population [1–4]. Ectopic or vascular calcification in chronic renal failure (CRF) is associated with elevated levels of serum phosphorus and calcium-phosphate (Ca × P) product and is likely an important contributor to increased cardiovascular morbidity and mortality [5–8].

Vascular calcification in renal failure patients was previously considered a passive process resulting from deposition of calcium-phosphate crystals in the setting of an elevated Ca x P product and secondary hyperparathyroidism. Recent studies, however, suggest that vascular calcification is instead an active, cell-mediated process in which vascular smooth muscle cells develop bone-forming functions [9–12]. The mechanisms mediating and the treatment of this ectopic calcification remain incompletely defined [6]. In vitro studies by Jono et al [13] have demonstrated that high inorganic phosphate levels, greater than 1.4 mmol/L, increase human smooth muscle cell calcification rates and increase the expression of the osteoblastic differentiation markers, osteocalcin and Osf2/Cbfa-1, via a sodium-phosphate cotransporter called Pit-1. Additional in vitro studies by Chen et al [14] have shown that phosphorus and other factors in uremic serum increase osteopontin expression and calcification in bovine vascular smooth muscle cells [14]. Finally, a recent clinical trial published by Chertow, Burke, and Raggi [15] revealed that sevelamer, when compared to calcium-based phosphate binders, attenuated the progression of coronary and aortic calcification in hemodial-

ysis patients as identified by electron beam computed tomography. Thus, the control of hyperphosphatemia with phosphate binders in patients with CRF may not only prevent a worsening Ca × P product and secondary hyperparathyroidism, but also influence the development of the active, cell-mediated process of vascular calcification.

In previous years, the most commonly used phosphate binders contained either aluminum or calcium salts, such as calcium carbonate ($CaCO_3$) or acetate. Whereas aluminum exposure may cause neurologic, skeletal, and hematologic toxicity [16–17], calcium-based phosphate binders can lead to hypercalcemia and potentially worsen the risk of soft tissue and cardiovascular calcifications [18–20]. To avoid these side effects, an aluminum- and calcium-free phosphate binder, sevelamer hydrochloride, has been developed. Sevelamer is effective in controlling hyperphosphatemia and reducing serum Ca × P product levels in dialysis patients [21–23].

Our group recently published the results of a study comparing the effects of the two phosphate binders $CaCO_3$ and sevelamer in a 3-month experimental model of uremia. Despite an equal control of hyperphosphatemia and secondary hyperparathyroidism, uremic rats on a high phosphate diet and treated with sevelamer were noted to have less nephrocalcinosis compared to uremic rats treated with $CaCO_3$. Uremic control rats fed a high phosphate diet without a phosphate binder had the greatest degree of kidney calcification. Furthermore, uremic rats treated with either of these phosphate binders were found to have decreased calcification in the myocardium and liver compared to uremic control rats. Uremic rats on sevelamer also had better renal function after 3 months compared to uremic controls and those rats on $CaCO_3$. There was no evidence of aortic vascular calcification after 3 months of uremia in either uremic controls or those rats treated with phosphate binders [24].

Based on these findings, the present studies were conducted in uremic rats fed a high phosphate diet to address any differences between 6-month treatment with sevelamer and $CaCO_3$ on vascular calcification, kidney calcification, and renal function. Our findings indicate that sevelamer attenuated kidney and cardiovascular calcification more adequately than $CaCO_3$, despite a similar control of serum phosphorus, Ca × P product, parathyroid hormone (PTH), and parathyroid gland enlargement.

METHODS

Experimental design

Renal insufficiency was induced by 5/6 nephrectomy in 5 to 6 weeks-old female Sprague-Dawley rats, weighing 200 to 225 g. In this procedure, several branches of the left renal artery were ligated and the right kidney excised. After 7 days of uremia, blood was taken to determine serum creatinine, calcium, and phosphorus. Rats were subsequently divided into three experimental groups, each with similar serum creatinine and serum Ca × P product values. The three groups of uremic rats were fed a high phosphorus diet (0.9% phosphorus; 0.6% calcium, and 20% protein) for 6 months. Group 1 (38 rats), uremic 5/6 nephrectomized rats (U) fed a high phosphorus diet (HP) (U-HP), received no phosphate binder and served as the uremic control for the deleterious effects of prolonged high phosphorus intake. Two phosphate binders were compared. Group 2 (30 rats), U-HP + $CaCO_3$ (U-HP + C), received 3% $CaCO_3$ as the phosphate binder, whereas group 3 (29 rats), U-HP + sevelamer (U-HP + S), received 3% sevelamer. An additional group of rats with normal kidney function was fed a similar high phosphorus diet and served as a nonuremic control group. Powdered diets were purchased from Dyets, Inc. (Bethlehem, PA, USA). $CaCO_3$ (Sigma Chemical Co., St. Louis, MO, USA) and sevelamer hydrochloride (RenaGel®) (GelTex Pharmaceuticals, Inc., Waltham, MA, USA) were added to the powdered, high phosphorus diet. The dose (3%) of sevelamer and $CaCO_3$ was defined in pilot studies which demonstrated that 3% sevelamer and $CaCO_3$ were equally effective in reducing serum phosphorus in uremic rats fed the same high phosphorus diet utilized in the present studies. On average, rats consumed approximately 20 g of powdered diet per day.

Following 5/6 nephrectomy, rats were weighed monthly and then placed into metabolic cages during the last 5 days of the 6-month treatment. Twenty-four–hour urine samples were collected and daily dietary intake monitored. Average 24-hour urinary excretion of calcium, phosphorus, and protein was measured during the last 3 days of treatment. After 6 months, rats were killed and blood was drawn from the dorsal aorta for analytic determinations. Parathyroid glands were microsurgically removed and weighed using a microbalance (CAHN-31) (Orion Instruments, Inc., Boston, MA, USA).

The remnant kidney and the entire myocardium were removed from rats at the time of the sacrifice, weighed on a microbalance, and cut into three pieces perpendicular to the longitudinal axis. One piece from each tissue was rinsed in phosphate buffered saline (PBS) and embedded in paraffin. The two remaining pieces were carefully snap-frozen for calcium deposition analysis.

Aortas were dissected and three (0.5 cm) pieces from the descending thoracic aorta to the iliac branch were removed per rat (at approximately the same anatomic level). Each sample was then cut into two pieces. One piece was weighed on a microbalance and retained for measurements of calcium deposition; the other was rinsed in PBS and embedded in paraffin.

All experimental protocols were approved by the Ani-

mal Studies Committee at Washington University School of Medicine.

Analytic determinations

Urine samples were acidified and analyzed for 24-hour excretion of creatinine, total protein, calcium, and phosphorus. Serum and urinary levels of phosphate and creatinine, as well as urinary protein, were determined using an autoanalyzer (COBAS-MIRA Plus, Branchburg, NJ, USA). Total serum and urinary calcium were measured by atomic absorption spectrophotometry using a Perkin-Elmer 1100B spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). Creatinine clearances were calculated using the standard formula:

$$C_{Cr} = U_{Cr}\text{ (mg/dL)} \times V_u \text{ (mL in 24 hours)/[1440 min} \times S_{Cr}\text{ (mg/dL)]}$$

where C_{Cr} is creatinine clearance, U_{Cr} is urinary clearance, V_u is urinary volume, and S_{Cr} is serum creatinine. Urinary calcium, phosphorus, and protein were expressed as milligrams excreted in 24 hours. Intact PTH levels were measured by an immunoradiometric assay specific for intact rat PTH (Immutopics, San Clemente, CA, USA).

Quantification of calcium deposition in kidney, myocardium, and aorta

Calcium content in remnant kidney, myocardium, and aorta was measured as previously described by Jono et al [13]. Tissue (three samples from each remnant kidney, myocardium, and aorta) was weighed and decalcified with 0.6 N HCl for 24 hours. Calcium content in HCl supernatants was determined by atomic absorption spectrophotometry. Results were corrected by wet tissue weight and expressed as micrograms calcium/gram wet tissue.

Kidney, myocardial, and aortic morphology

Five-micrometer tissue sections were stained with hematoxylin-eosin and processed for light microscopy. Sections were examined for calcium deposition using von Kossa staining. Briefly, slides were deparaffinized, hydrated with water, incubated in 5% silver nitrate solution (Sigma S-01334) (Sigma Chemical Co.) for 1 hour, rinsed four times with distilled water, followed by a thiosulphate solution for 5 minutes, and counterstained with nuclear fast red solution for 5 minutes. Slides were then rinsed in tap water, dehydrated, cleared in 95% ethyl alcohol, 100% ethyl alcohol, and xylene, and cover slips were mounted. Semiquantitative assessment of calcification was performed as follows. The entire kidney, myocardium, and aortic sections were examined and all foci of calcification were counted (four sections per animal and a total of six rats per group). Precise localization of calcium deposits within the myocardium was aided by immunohistochemical staining of endothelial cells with antibody to factor VIII. Briefly, slides were deparaffinized, hydrated, immersed in citrate buffer (pH = 6.0) for 10 minutes (×2), and stained with polyclonal antibody to human factor VIII (1:1000) overnight (Sigma Chemical Co.). Slides were then rinsed, incubated with universal Link antibody (Biocare, CA, USA), and developed with diabenzidine (DAB). Endothelial cells lining arterioles and capillaries were highlighted with this stain. Histologic features were quantified by three different individuals blinded to treatment.

Statistical analyses

Analysis of variance (ANOVA) was employed to assess statistical differences between all experimental groups, except for mortality rates, in which a chi-square test was performed. Multiple comparisons using the stringent Bonferroni test measured the statistical significance of the differences between every possible two-group comparison. An unpaired, two-tailed *t* test was used to compare time points at baseline and 6 months within experimental groups.

RESULTS

Serum biochemistries (i.e., calcium, phosphorus, Ca × P product, creatinine, and cholesterol), renal function, markers of secondary hyperparathyroidism, and calcium deposition in the kidney, myocardium, and aorta were compared between the three groups of rats (U-HP, U-HP + C, and U-HP + S).

Effects of sevelamer and $CaCO_3$ on serum biochemistries

After 6 months of uremia, serum creatinine increased from baseline in U-HP and U-HP + C rats, but not in U-HP + S rats (Table 1). Serum phosphorus and Ca × P product were significantly lower in both U-HP + S and U-HP + C rats compared to U-HP control rats. Although there was a trend toward lower levels in U-HP + S rats, no statistical difference was found in serum phosphorus and Ca × P product between U-HP + S and U-HP + C rats ($P = 0.29$ comparing phosphorus levels). Serum calcium levels were also similar among the three uremic groups after 6 months of uremia. Acid-base balance (serum bicarbonate and pH) and cholesterol levels were not statistically different among the three uremic groups.

Effects of sevelamer and $CaCO_3$ on body weight

Baseline mean body weights for U-HP, U-HP + S, and U-HP + C groups, respectively, were 217 ± 21 g, 215 ± 18 g, and 209 ± 9 g. Those rats surviving to 6 months of uremia in U-HP, U-HP + S, and U-HP + C groups had the following mean body weights: 298 ± 10 g,

Table 1. Serum chemistries

	N-HP ($N = 6$)	U-HP ($N = 6$)	U-HP + S ($N = 6$)	U-HP + C ($N = 6$)
Creatinine *mg/dL*				
Baseline	0.70 ± 0.05	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
6 months	0.88 ± 0.03	2.3 ± 0.3	1.5 ± 0.1[b]	2.0 ± 0.3
Phosphorus *mg/dL*				
Baseline	4.5 ± 0.4	9.6 ± 0.4	9.4 ± 0.5	9.6 ± 0.3
6 months	4.5 ± 0.5	10.9 ± 1.3	5.9 ± 0.6[a,c]	6.7 ± 1.1[a,c]
Calcium *mg/dL*				
Baseline	9.4 ± 0.3	9.3 ± 0.2	9.4 ± 0.3	9.3 ± 0.3
6 months	9.2 ± 0.2	9.3 ± 0.2	9.2 ± 0.3	9.3 ± 0.3
Ca × P product mg^2/dL^2				
Baseline	42 ± 4	87 ± 7	87 ± 8	89 ± 0.8
6 months	42 ± 4	101 ± 14	54 ± 5[a,c]	61 ± 8[b,c]
pH				
6 months	7.35 ± 0.01	7.25 ± 0.01	7.31 ± 0.02	7.26 ± 0.04
HCO_3 *mEq/L*				
6 months	24.8 ± 0.9	20.5 ± 1.1	21.5 ± 0.7	21.5 ± 1.2
Cholesterol *mg/dL*				
6 months	98 ± 6	204 ± 11	203 ± 26	163 ± 24

Uremic (5/6 nephrectomized) rats underwent one of the following experimental protocols for 6 months: uremic control + high phosphorus diet (U-HP), uremic + HP diet + 3% sevelamer (U-HP + S), uremic + HP diet + 3% calcium carbonate (U-HP + C). Values represent the Mean ± SEM; N = number of rats.
[a] $P < 0.01$ vs. U-HP from analysis of variance (ANOVA) and Bonferroni analysis
[b] $P < 0.05$ vs. U-HP from ANOVA and Bonferroni analysis
[c] $P < 0.01$ comparing baseline and uremia 6 month time-points, using two-tailed *t* test

Table 2. Creatinine clearance, urinary protein, phosphorus, and calcium

	U-HP ($N = 6$)	U-HP + S ($N = 6$)	U-HP + C ($N = 6$)
Creatinine clearance *mL/min*	0.46 ± 0.08	0.74 ± 0.09[b,c]	0.48 ± 0.08
Urinary protein *mg/24 hours*	259 ± 47	160 ± 12[a,b]	268 ± 28
Urinary calcium *mg/24 hours*	1.83 ± 0.1	3.17 ± 0.6	3.77 ± 0.6[b]
Urinary phosphorus *mg/24 hours*	30.8 ± 2.5	37.9 ± 1.7[c]	26.6 ± 3.2

Uremic (5/6 nephrectomized) rats underwent one of the following experimental protocols for 6 months: uremic control + high phosphorus diet (U-HP), uremic + HP diet + 3% sevelamer (U-HP + S), Uremic + HP diet + 3% calcium carbonate (U-HP + C). Values represent the Mean ± SEM; N = number of rats.
[a] $P < 0.01$ vs. U-HP + C; [b] $P < 0.05$ vs. U-HP; [c] $P < 0.05$ vs. U-HP + C, from analysis of variance (ANOVA) and Bonferroni analysis

332 ± 11 g, and 294 ± 9 g, respectively. U-HP + S rats had significantly higher body weights compared to U-HP and U-HP + C rats after 6 months of uremia.

Effects of sevalemer and $CaCO_3$ on mortality

Mortality rates at six months were high among all uremic groups: U-HP 84%, U-HP + S 79%, and U-HP + C 80%. These values did not differ significantly between groups ($P = 0.64$).

Effects of sevelamer and $CaCO_3$ on creatinine clearance, urinary protein, calcium, and phosphorus excretion

As shown in Table 2, creatinine clearance was equally impaired in $CaCO_3$-treated rats and uremic controls. Sevelamer-treated rats had higher creatinine clearances and lower urinary protein excretion compared to the other two groups of uremic rats. Treatment with $CaCO_3$ was associated with higher urinary calcium excretion compared to uremic controls, but did not differ with that of sevelamer treatment.

Effects of sevelamer and $CaCO_3$ on secondary hyperparathyroidism

As noted in Figure 1A, the average serum PTH in untreated uremic rats fed a high phosphate diet was 2085 ± 616 pg/mL with a 95% confidence interval (95% CI) ranging from 503 to 3667 pg/mL. The elevation in serum PTH induced by high dietary phosphorus in uremic controls was markedly attenuated by treatment either with sevelamer (528 ± 158 pg/mL, $P < 0.01$; 95% CI 122 to 934) or $CaCO_3$ (708 ± 285 pg/mL, $P < 0.01$; 95% CI 0 to 1439). Mean serum PTH levels did not differ significantly according to treatment with either sevelamer or $CaCO_3$ ($P = 0.30$). Mean serum PTH levels in nonuremic rats fed a high phosphorus diet were 27.1 ± 7.0 pg/mL; 95% CI 9.2 to 44.8 (data not shown in Fig. 1A). Figure 1B shows that untreated uremic rats developed a marked increase in parathyroid gland weight (6.33 ± 1.52 μg/g body weight) compared to uremic rats treated with phosphate binders. Treatment with both sevelamer (2.77 ± 0.27 μg/g body weight, $P < 0.01$) and $CaCO_3$ (2.99 ± 0.39 μg/g body weight, $P < 0.01$) was associated with

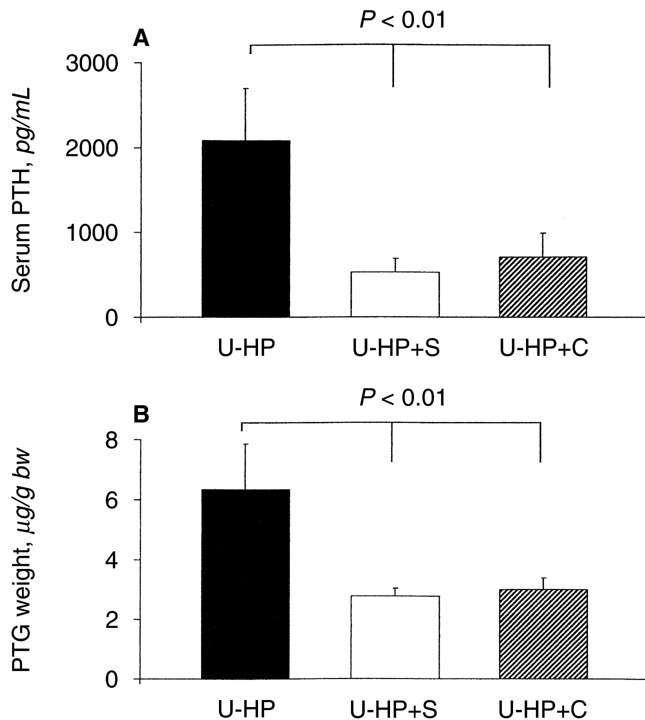

Fig. 1. Effects of sevelamer and calcium carbonate ($CaCO_3$) on serum parathyroid hormone (PTH) and parathyroid gland growth. Serum PTH (*A*) and parathyroid gland weight (*B*) in uremic (5/6 nephrectomized) rats undergoing one of the following experimental protocols for 6 months: (■) uremic control + high phosphorus diet (U-HP); (□) uremic + high phosphorus diet + 3% sevelamer (U-HP + S); (▨) uremic + high phosphorus diet + 3% $CaCO_3$ (U-HP + C). Results represent the mean ± SEM from six rats per group. *P* values were obtained by analysis of variance (ANOVA) and Bonferroni tests.

reduced enlargement of parathyroid glands compared to uremic controls.

Effects of sevelamer and $CaCO_3$ on calcium deposition in kidney, myocardium, and aorta

Table 3 shows calcium content in kidney, myocardium, and aorta in all experimental groups. All three groups of uremic rats had markedly increased calcium deposition in the kidney compared to normal, nonuremic rats fed the same high phosphate diet. However, a dramatic reduction of renal calcium content was observed in the sevelamer group compared to both uremic controls and the $CaCO_3$ group. Interestingly, myocardial calcium content was even higher in $CaCO_3$ group than in uremic controls. In contrast, sevelamer-treated rats developed less myocardial calcium deposition compared to $CaCO_3$-treated rats. Untreated uremic rats had increased calcium content in the aorta compared to normal rats. Sevelamer treatment greatly reduced aortic calcium deposition compared to both uremic controls and $CaCO_3$-treated rats.

Histologic effects of sevelamer and $CaCO_3$ on kidney calcifications

Hematoxylin-eosin and von Kossa staining of representative kidney sections from each experimental group are shown in Figure 2. Kidney calcification was greater in untreated uremic (Fig. 2 B and F) and $CaCO_3$-treated rats (Fig. 2 D and H) compared to normal animals (Fig. 2 A and E) fed the same high phosphate diet. Kidney calcification was markedly lower in uremic rats treated with sevelamer (Fig. 2 C and G). In addition, semiquantitative analysis of kidney calcification (Fig. 3A) showed an association between chemical measurement of calcium content and the number of foci of calcification, being lower in the sevelamer group than in uremic controls and the $CaCO_3$ group.

Histologic effects of sevelamer and $CaCO_3$ on myocardium and aortic calcifications

Figures 4 and 5 show representative myocardial and aortic sections, respectively, from each experimental group. Both hematoxylin-eosin and von Kossa staining demonstrate the efficacy of sevelamer in attenuating calcification of the myocardium and aorta. Myocardial (Fig. 4) and aortic (Fig. 5) sections from untreated (Fig. 4 B and F and Fig. 5 B and F) and $CaCO_3$-treated (Fig. 4 D and H and Fig. 5 D and H) uremic rats showed more calcification than normal animals (Fig. 4 A and E and Fig. 5 A and E) and sevelamer-treated rats (Fig. 4 C and G and Fig. 5 C and G). Calcifications in the myocardium were specifically localized within capillaries, as demonstrated in heart sections stained with an antibody to the endothelial marker factor VIII (Fig. 6). No calcifications were observed within the cardiac interstitium. Calcifications in the aorta were localized within the intimal and medial layers. Figure 3 B and C also depict a higher number of foci of calcification in the myocardium and aorta of uremic controls and $CaCO_3$-treated rats, which parallels the higher calcium content in these tissues measured biochemically. Again, sevelamer-treated rats had markedly reduced foci of calcification in myocardium and aorta compared to the other two uremic groups.

DISCUSSION

These studies provide a long-term, experimental model of the influence of uremia and the phosphate binders, sevelamer and $CaCO_3$, on vascular and kidney calcification in rats with CRF. Aortic calcification was evident after 6 months of uremia, unlike our previous 3-month study [24]. Furthermore, despite a similar efficacy in controlling serum phosphorus, Ca × P product, and secondary hyperparathyroidism, those uremic rats treated with sevelamer developed less vascular calcification in the myocardium and aorta and less nephrocalcinosis compared to uremic rats treated with $CaCO_3$. Interestingly,

Table 3. Calcium content in kidney, myocardium, and aorta

	N-HP	U-HP	U-HP + S	U-HP + C
Kidney calcium *μg/g wet tissue*	112 ± 9	1650 ± 286	582 ± 111[a,b]	1196 ± 180
Myocardial calcium *μg/g wet tissue*	53 ± 5	98 ± 10	72 ± 4[b]	179 ± 48
Aortic calcium *μg/g wet tissue*	390 ± 63	2150 ± 447	636 ± 96[b,c]	1308 ± 343

Renal calcium deposition in normal and uremic (5/6 nephrectomized) rats undergoing one of the following experimental protocols for 6 months: normal + high phosphorus diet (N-HP), uremic control + HP diet (U-HP), uremic + HP diet + 3% sevelamer (U-HP + S), uremic + HP diet + 3% calcium carbonate (U-HP + C). Values represent the mean ± SEM from 12 different tissue samples of kidney, myocardium, and aorta.
[a]$P < 0.01$ vs. U-HP, [b]$P < 0.05$ vs. U-HP + C, [c]$P < 0.05$ vs. U-HP from analysis of variance (ANOVA) and Bonferroni analysis

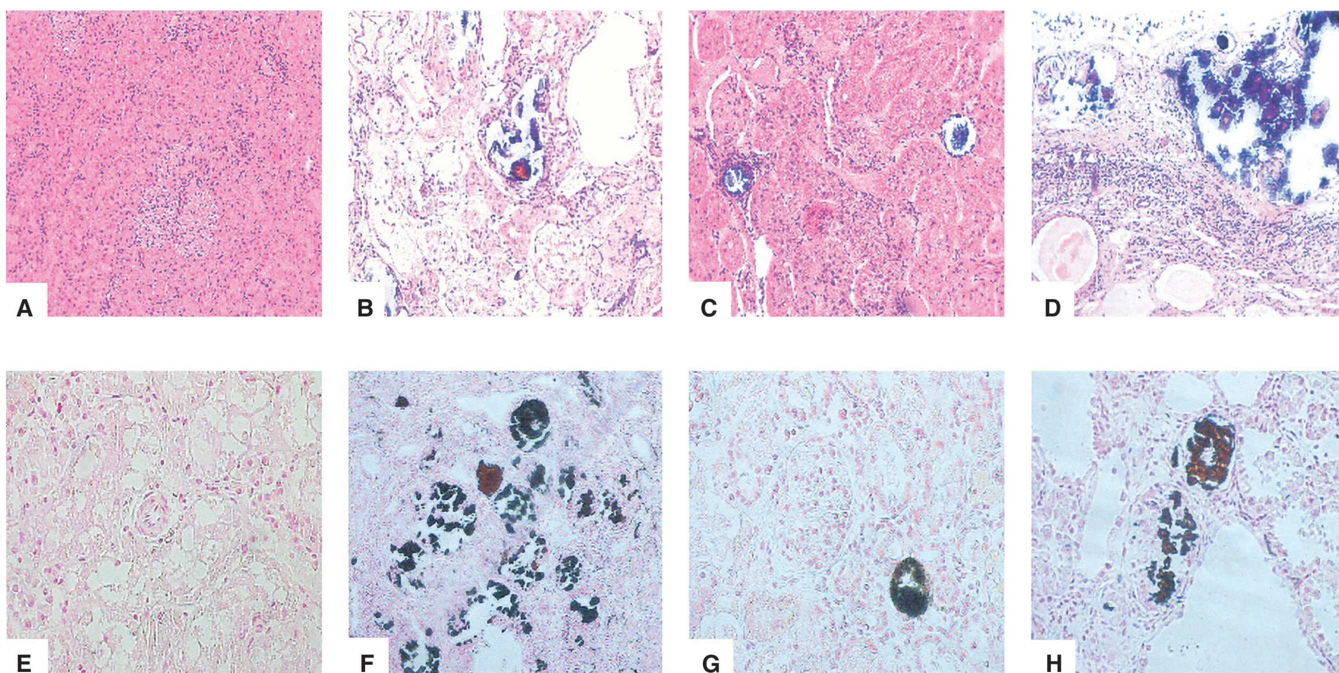

Fig. 2. Effects of sevelamer and calcium carbonate ($CaCO_3$) on kidney calcification. Representative photomicrographs of hematoxylin-eosin (*A* to *D*) and von Kossa (*E* to *H*) staining in remnant kidney tissue of normal and 5/6 nephrectomized rats undergoing one of the following experimental protocols for 6 months: normal + high phosphorus diet (N-HP) (A and E), uremic control + high phosphorus diet (U-HP) (B and F), uremic + high phosphorus diet + 3% sevelamer (U-HP + S) (C and G), uremic + high phosphorus diet + 3% $CaCO_3$ (U-HP + C) (D and H). Magnification 400×.

in those rats surviving to 6 months, treatment with sevelamer also attenuated the deterioration in renal function seen in uremic controls and $CaCO_3$-treated rats. Treatment with either phosphate binder had no influence on mortality compared to uremic controls.

In recent years, vascular calcification in patients with CRF and end-stage renal disease (ESRD) has been increasingly recognized as an important contributor to overall cardiovascular morbidity and mortality. The exact factors and mechanisms regulating vascular calcification remain incompletely defined, yet there is increasing evidence that this is an active, cell-mediated process [10–11, 13–14, 25].

Many factors, such as high phosphorus, calcium, and vitamin D therapy, may contribute to the development of vascular calcification [25]. Various in vitro studies have demonstrated that phosphorus and unidentified factors in uremic serum play a potential role in the pathogenesis of vascular calcification [13–14]. Such studies have demonstrated an increased expression of osteoblast-specific proteins and calcification by vascular smooth muscle cells in response to high levels of serum phosphorus and uremic serum. Hyperphosphatemia itself has been identified as an independent risk factor for increased cardiovascular mortality in patients with ESRD [25]. Consequently, the control of hyperphosphatemia with phosphate binders in patients with CRF and ESRD has been appropriately emphasized.

Phosphate binders currently used to manage hyperphosphatemia in ESRD patients include sevelamer and the calcium-containing binders, $CaCO_3$, and calcium acetate. Sevelamer is an aluminum- and calcium-free phosphate binder, which does not promote hypercalcemia and has favorable effects on the lipid profile, with an associated reduction in low-density lipoprotein (LDL) and increase in high-density lipoprotein (HDL) choles-

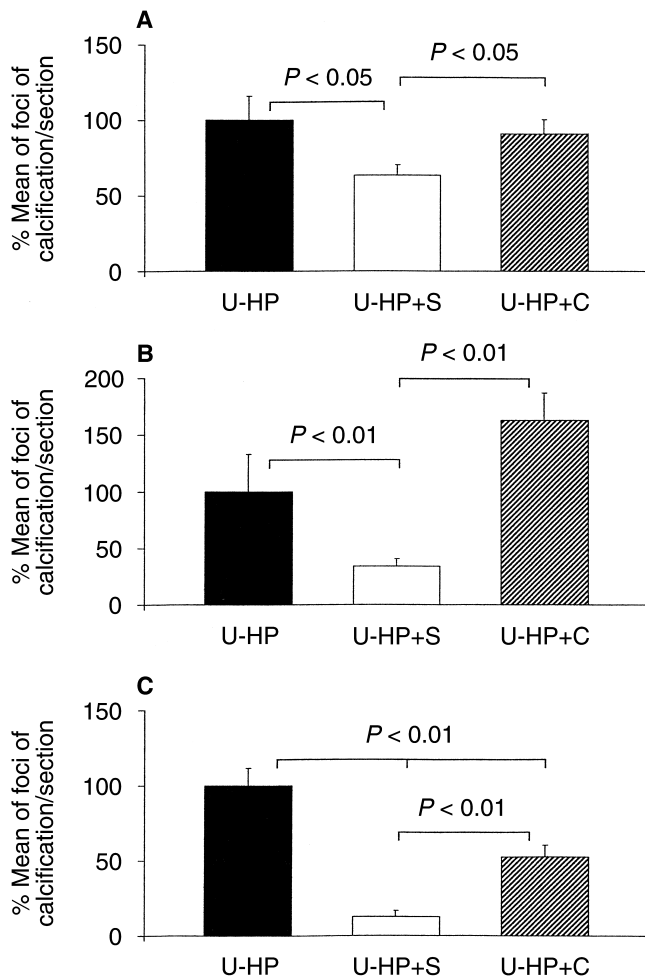

Fig. 3. Semiquantitative analysis of foci of calcifications in kidney, myocardium, and aorta. Mean of foci of calcification in kidney (*A*), myocardium (*B*), and aorta (*C*) in uremic (5/6 nephrectomized) rats undergoing one of the following experimental protocols for 6 months: (■) uremic control + high phosphorus diet (U-HP), (□) uremic + high phosphorus diet + 3% sevelamer (U-HP + S), (▨) uremic + high phosphorus diet + 3% calcium carbonate ($CaCO_3$) (U-HP + C). Normal rats do not have any calcification. Results represent the Mean ± SEM for six rats per group. *P* values were obtained by analysis of variance (ANOVA) and Bonferroni tests.

terol [21]. A potential concern with the use of calcium-containing phosphate binders has been suggested over the past few years. A study of young hemodialysis patients by Goodman et al [1] noted a correlation between coronary artery calcification detected by electron beam computed tomography and duration of dialysis, serum phosphorus levels, serum Ca × P product, and daily intake of calcium. Another study in 200 hemodialysis patients by Chertow, Burke, and Raggi [15] showed that sevelamer attenuated the progression of coronary and aortic calcification better than calcium-based phosphate binders after 1 year. Subjects treated with sevelamer had lower serum calcium, total cholesterol, and LDL levels compared to subjects treated with calcium-based phosphate binders. Thus, sevelamer's effects on vascular calcification may have been secondary to its lower calcium load versus lipid-lowering properties. Furthermore, the influence of type of the phosphate binder on cardiovascular morbidity and mortality has yet to be prospectively studied in humans.

Our study in long-term experimental uremia shows that treatment with sevelamer, when compared to $CaCO_3$, is associated with less vascular calcification within the myocardium, aorta, and kidney. One potential explanation for the increased vascular calcification in $CaCO_3$-treated rats could be the greater severity of renal dysfunction in this group compared to sevelamer-treated rats. A recent in vitro study by Chen et al [14] demonstrated that there are unidentified factors in uremic serum that potentiate vascular calcification. In support of this concept, uremic control rats in our study also had worse renal function and excessive aortic calcification compared to sevelamer-treated rats.

Hypothetically, a difference in lipid metabolism is yet another potential contributing factor for the increased vascular calicification noted in $CaCO_3$-treated rats compared to sevelamer-treated rats. In vitro studies have shown that acetylated LDL stimulates [26] while HDL inhibits [27] vascular smooth muscle cell calcification. In our study, total cholesterol levels of $CaCO_3$-treated rats trended lower than sevelamer-treated rats, a finding which could be explained by reduced dietary intake in the setting of worse renal function. LDL and HDL levels were not evaluated in our study. In human studies, sevelamer has been shown to consistently reduce LDL and frequently increase HDL levels. Such an improved lipid profile could potentially play a role in the lower degree of vascular calcification seen in sevelamer-treated rats. However, as already noted, the $CaCO_3$-treated rats had lower total cholesterol levels and LDL levels typically parallel such levels.

In addition to a lower degree of vascular calcification, treatment with sevelamer for 6 months was associated with less kidney calcification compared to $CaCO_3$. These results are similar to those published by our group involving a 3-month experimental model of uremia. Interestingly, as noted above, renal function, as measured by serum creatinine, creatinine clearance, and proteinuria, was preserved in those rats treated with sevelamer and surviving to 6 months. The reason for this is unclear and remains speculative. Nephrocalcinosis may have been a contributing factor, as suggested by our previous studies [24]. Uremic rats in each treatment group were fed a diet with similar phosphorus and protein content, thus minimizing the effect of other potential confounding factors known to influence the progression of renal failure. It is important to note that, despite the preserved renal function and lower vascular calcification in sevelamer-treated rats, there were high mortality rates in all uremic groups, which did not differ significantly.

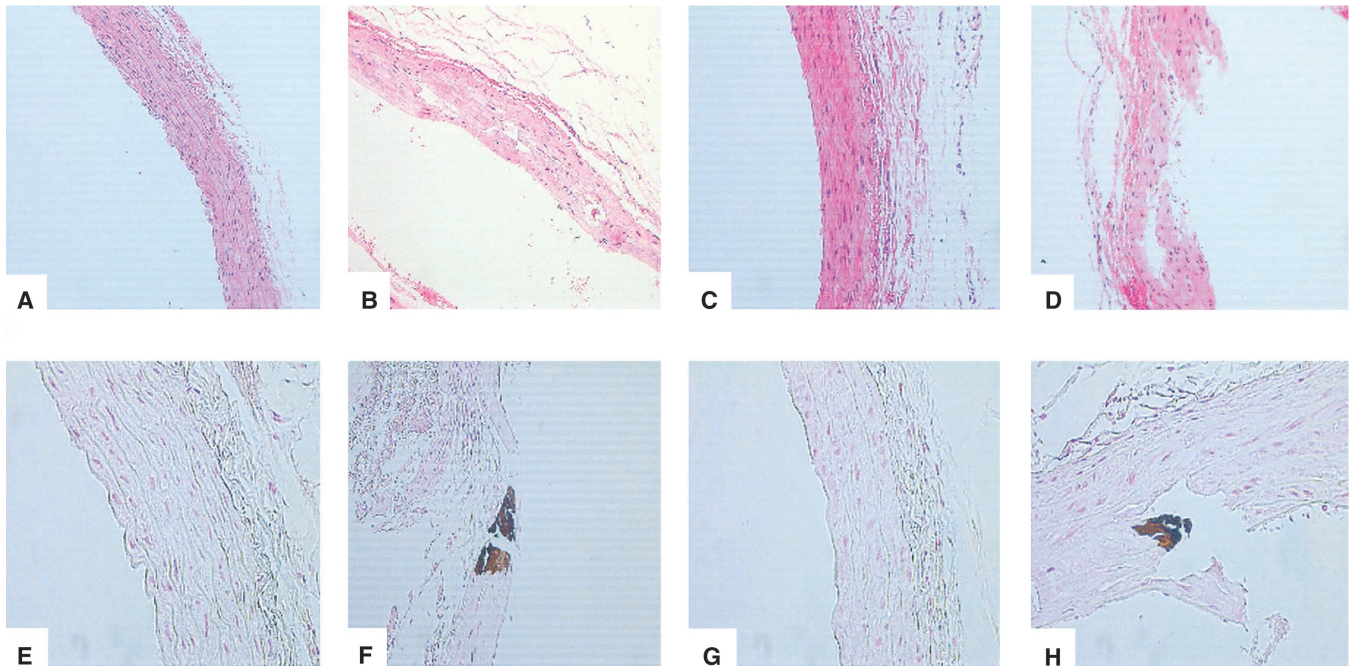

Fig. 4. Effects of sevelamer and calcium carbonate ($CaCO_3$) on myocardial calcification. Representative photomicrographs of hematoxylin-eosin (*A* to *D*) and von Kossa (*E* to *H*) staining in myocardial tissue of normal and 5/6 nephrectomized rats undergoing one of the following experimental protocols for 6 months: normal + high phosphorus diet (N-HP) (A and E), uremic control + high phosphorus diet (U-HP) (B and F), uremic + high phosphorus diet + 3% sevelamer (U-HP + S) (C and G), uremic + high phosphorus diet + 3% $CaCO_3$ (U-HP + C) (D and H). Magnification 400×.

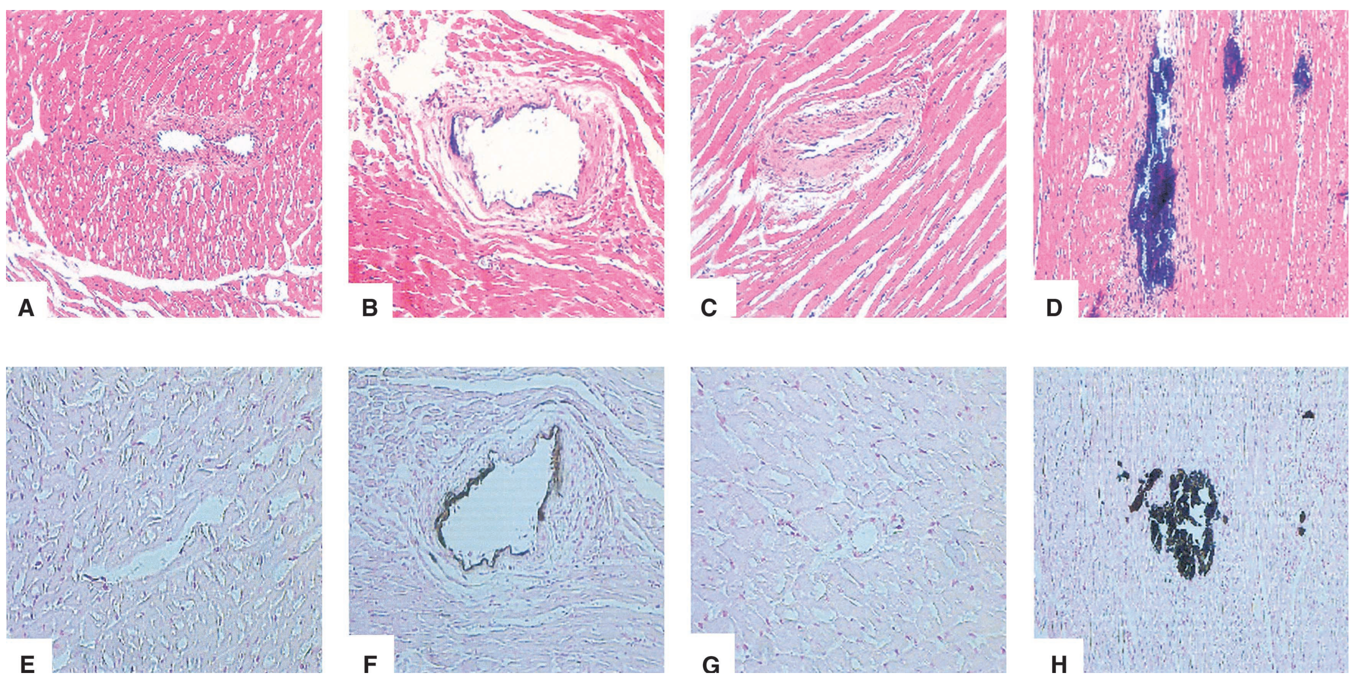

Fig. 5. Effects of sevelamer and calcium carbonate ($CaCO_3$) on aortic calcification. Representative photomicrographs of hematoxylin-eosin (*A* to *D*) and von Kossa (*E* to *H*) staining in aortic tissue of normal and 5/6 nephrectomized rats undergoing one of the following experimental protocols for 6 months: normal + high phosphorus diet (N-HP) (A and E), uremic control + high phosphorus diet (U-HP) (B and F), uremic + high phosphorus diet + 3% sevelamer (U-HP + S) (C and G), uremic + high phosphorus diet + 3% $CaCO_3$ (U-HP + C) (D and H). Magnification 400×.

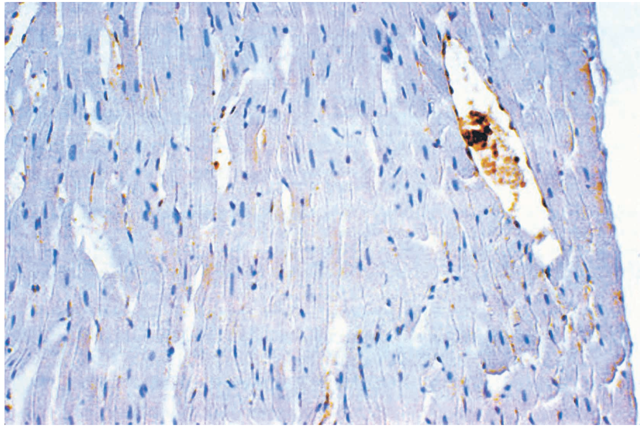

Fig. 6. Intravascular localization of myocardial calcification. Representative photomicrograph demonstrating calcifications within myocardial capillaries. Calcifications and factor VIII (a specific endothelial cell marker) stain brown (magnification 800×).

CONCLUSION

These studies of long-term experimental uremia suggest that, despite similar control of hyperphosphatemia and other markers of secondary hyperparathyroidism, sevelamer may be more effective than $CaCO_3$ in attenuating cardiovascular and kidney calcification. Potential mechanisms mediating these differences are only in the early stages of elucidation.

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REFERENCES

1. Goodman WG, Goldin J, Kuizon BD, *et al*: Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 342:1478–1483, 2000
2. Schwartz U, Buzzello M, Ritz E, *et al*: Morphology of coronary atherosclerotic lesions in patients with end-stage renal failure. *Nephrol Dial Transplant* 15:218–223, 2000
3. London GM, Pannier B, Marchais SJ, Guerin AP: Calcification of the aortic valve in the dialyzed patient. *J Am Soc Nephrol* 11: 778–783, 2000
4. Raggi P, Boulay A, Chasan-Taber S, *et al*: Cardiac calcification in adult hemodialysis patients. A link between end-stage renal disease and cardiovascular disease? *J Am Coll Cardiol* 39:695–701, 2002
5. Block GA, Hulbert-Shearon TE, Levin NW, Port FK: Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: A national study. *Am J Kidney Dis* 31:607–617, 1998
6. Cozzolino M, Dusso A, Slatopolsky E: Role of calcium x phosphate product and bone associated proteins on vascular calcification in renal failure. *J Am Soc Nephrol* 12:2511–2516, 2001
7. Slatopolsky E, Brown A, Dusso A: Role of phosphorus in the pathogenesis of secondary hyperparathyroidism. *Am J Kidney Dis* 37:S54–S57, 2001
8. Massry SG: The toxic effects of parathyroid hormone in uremia. *Semin Nephrol* 3:306–328, 1983
9. Wallin R, Wajih N, Greenwood GT, Sane DC: Arterial calcification: a review of mechanisms, animal models, and the prospects for therapy. *Med Res Rev* 21:274–301, 2001
10. Schinke T, McKee MD, Kiviranta R, Karsenty G: Molecular mechanisms of arterial calcification. *Ann Med* 30:538–541, 1998
11. Bostrom K: Insight into the mechanisms of vascular calcification. *Am J Cardiol* 88(Suppl 1):20–22, 2001
12. Proudfoot D, Shanahan CM: Biology of calcification in vascular cells: Intima versus media. *Herz* 26:245–251, 2001
13. Jono S, McKee MD, Murry CE, *et al*: Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 87:e10–e17, 2000
14. Chen N, O'Neill K, Duan D, Moe S: Phosphorus and uremic serum up-regulate osteopontin expression in vascular smooth muscle cells. *Kidney Int* 62:1724–1731, 2002
15. Chertow GM, Burke SK, Raggi P: Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. *Kidney Int* 62:245–252, 2002
16. Cannata-Andia JB: Reconsidering the importance of long-term low-level aluminum exposure in renal failure patients. *Semin Dial* 14:5–7, 2001
17. Kausz AT, Antonsen JE, Hercz G, *et al*: Screening plasma aluminum levels in relation to aluminum bone disease among asymptomatic dialysis patients. *Am J Kidney Dis* 34:688–693, 1999
18. Block GA, Port FK: Re-evaluation of risks associated with hyperphosphatemia and hyperparathyroidism in dialysis patients: Recommendations for a change in management. *Am J Kidney Dis* 35: 1226–1237, 2000
19. Guerin AP, London GM, Marchais SJ, Metivier F: Arterial stiffening and vascular calcifications in end-stage renal disease. *Nephrol Dial Transplant* 15:1014–1021, 2000
20. Hsu CH: Are we mismanaging calcium and phosphate metabolism in renal failure? *Am J Kidney Dis* 29:641–649, 1997
21. Chertow GM, Burke SK, Dillon MA, Slatopolsky E: Long-term effects of sevelamer hydrochloride on the calcium x phosphate product and lipid profile of hemodialysis patients. *Nephrol Dial Transplant* 14:2907–2914, 1999
22. Bleyer AJ, Burke SK, Dillon M, *et al*: A comparison of the calcium-free phosphate binder sevelamer hydrochloride with calcium acetate in the treatment of hyperphosphatemia in hemodialysis patients. *Am J Kidney Dis* 33:694–701, 1999
23. Slatopolsky EA, Burke SK, Dillon MA: RenaGel, a non-absorbed calcium- and aluminum-free phosphate binder, lowers serum phosphorus and parathyroid hormone. *Kidney Int* 55:299–307, 1999
24. Cozzolino M, Dusso A, Liapis H, *et al*: Effects of sevelamer hydrochloride and calcium carbonate on kidney calcification in uremic rats. *J Am Soc Nephrol* 13:2299–2308, 2002
25. Qunibi W, Nolan C, Ayus J: Cardiovascular calcification in patients with end-stage renal disease: A century-old phenomenon. *Kidney Int* 62(Suppl 82):S73–S80, 2002
26. Proudfoot D, Davies J, Skepper J, *et al*: Acetylated low-density lipoprotein stimulates human vascular smooth muscle cell calcification by promoting osteoblastic differentiation and inhibiting phagocytosis. *Circulation* 106:3044–3050, 2002
27. Parhami F, Basseri B, Hwang J, *et al*: High-density lipoprotein regulates calcification of vascular cells. *Circ Res* 91:570–576, 2002