

The Effects of Sevelamer Hydrochloride and Calcium Carbonate on Kidney Calcification in Uremic Rats

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Abstract. The control of serum phosphorus (P) and calcium-phosphate (Ca × P) product is critical to the prevention of ectopic calcification in chronic renal failure (CRF). Whereas calcium (Ca) salts, the most commonly used phosphate binders, markedly increase serum Ca and positive Ca balance, the new calcium- and aluminum-free phosphate binder, sevelamer hydrochloride (RenaGel), reduces serum P without altering serum Ca in hemodialysis patients. Using an experimental model of CRF, these studies compare sevelamer and calcium carbonate (CaCO₃) in the control of serum P, secondary hyperparathyroidism (SH), and ectopic calcifications. 5/6 nephrectomized rats underwent one of the following treatments for 3 mo: uremic + high-P diet (U-HP); UHP + 3% CaCO₃ (U-HP+C); UHP + 3% sevelamer (U-HP+S). Sevelamer treatment controlled serum P independent of increases in serum Ca, thus reducing serum Ca × P product and further deterioration of renal function, as indicated by the highest creatinine clearances. Sevelamer was as effective as CaCO₃ in the control of high-P-induced SH, as shown by similar serum PTH levels, parathyroid (PT) gland weight, and markers of PT hyperplasia.

Also, both P binders elicited similar efficacy in reducing the myocardial and hepatic calcifications induced by uremia. However, sevelamer caused a dramatic reduction of renal Ca deposition (29.8 ± 8.6 μg/g wet tissue) compared with both U-HP (175.5 ± 45.7 μg/g wet tissue, *P* < 0.01) and the U-HP+C (58.9 ± 13.7 μg/g wet tissue, *P* < 0.04). Histochemical analyses using Von Kossa and Alizarin red S staining of kidney sections confirmed these findings. The high number of foci of calcification in the kidney of uremic controls (108 ± 25) was reduced to 33.0 ± 11.3 by CaCO₃ and decreased even further with sevelamer (16.4 ± 8.9, *P* < 0.02 versus CaCO₃). Importantly, the degree of tubulointerstitial fibrosis was also markedly lower in U-HP+S (5%) compared with either U-HP+C (30%) or U-HP (50%). It is concluded that in experimental CRF in rats, despite a similar control of serum P and SH, sevelamer is more effective than CaCO₃ in preventing renal Ca deposition and tubulointerstitial fibrosis, including better preservation of renal function. These findings cannot be extrapolated to human disease, and further studies in patients are necessary to determine the benefits of either P binder.

In end-stage renal disease, hyperphosphatemia and elevated calcium-phosphate (Ca × P) product associate with ectopic calcifications and increased risk of calciphylaxis, resulting in higher prevalence of morbidity and mortality from cardiovascular events (1–6). High serum phosphorus (P) levels worsen uremia-induced secondary hyperparathyroidism by enhancing parathyroid hyperplasia and parathyroid hormone (PTH) synthesis and secretion (7–8). Elevated PTH levels cause ectopic calcification not only by enhancing serum P and Ca × P product through inducing high bone turnover, but also by increasing both serum and intracellular calcium (Ca) (9–10). The control of serum P in patients with chronic renal failure is therefore important to the prevention of increases in Ca × P

product, secondary hyperparathyroidism, and ectopic calcifications (9).

Dietary P restriction, dialysis treatment, and administration of phosphate-binders are the current therapies for hyperphosphatemia in chronic renal failure. The most commonly used phosphate-binders contain aluminum salts, calcium carbonate (CaCO₃), or calcium acetate. Calcium salts increase serum Ca and could worsen soft tissue calcifications, especially in patients on vitamin D therapy. Administration of 1,25(OH)₂D₃, while suppressing PTH synthesis, increases intestinal Ca absorption and calcium-phosphate mobilization from bone (9–10).

The role of P in the progression of renal failure (11) and the protective effects of P restriction on renal function (12) have been known for more than 20 yr. Chronic renal failure causes a reduction in nephron mass and in P excretion. Phosphate retention not only induces secondary hyperparathyroidism but also accelerates renal failure by promoting renal calcification (13). Gimenez *et al.* (14) showed a correlation between renal Ca deposition, hyperphosphatemia, and the progression of renal failure in 246 renal biopsies. Patients with serum creatinine levels above 1.5 mg/dl had higher serum Ca × P product, renal Ca content, and histologic Ca deposition (14).

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Ibels *et al.* (15) showed that dietary restriction of P prevents the progression of renal failure in nephrectomized rats. In contrast, high dietary P induced a rapid deterioration of renal function (16). Phosphorus toxicity associates with renal calcium-phosphate precipitation and tubulointerstitial damage, resulting in acceleration of nephrocalcinosis (17).

In 1980, Walser (18) described the association between CaCO_3 administration in uremic patients and increases in serum creatinine concentration after 2 to 4 wk of treatment. At the time, he concluded that “. . . an increase in the serum $\text{Ca} \times \text{P}$ product may accelerate progression of renal failure and suggest caution in the use of calcium supplements for this reason.”

To reduce the side effects of the commonly used calcium salts, a new aluminum- and calcium-free phosphate-binder was developed. Poly-allylamine hydrochloride (sevelamer hydrochloride, RenaGel; GelTex Pharmaceuticals, Inc, Waltham, MA) controls serum P levels with no hypercalcemia. Furthermore, sevelamer reduces LDL cholesterol by 30% and increases HDL cholesterol by 18% (19).

The present studies compare sevelamer and CaCO_3 in the control of serum P, prevention of secondary hyperparathyroidism, and reduction of renal calcifications in an experimental model of chronic renal failure. Sevelamer and CaCO_3 are equally effective in reducing serum P levels and in preventing secondary hyperparathyroidism. Importantly, sevelamer is more effective than CaCO_3 in preventing increases in serum $\text{Ca} \times \text{P}$ product and in reducing renal Ca deposition, including better preservation of renal function.

Materials and Methods

Experimental Protocol

Uremic (5/6-nephrectomized) female Sprague-Dawley rats aged 5 to 6 wk and weighing 200 to 225 g were studied. For 5/6-nephrectomy, several branches of the left renal artery were ligated and the right kidney excised. After 7 d of uremia, blood was taken to control serum creatinine, Ca, and P, and rats were allocated to three groups with similar renal function and serum $\text{Ca} \times \text{P}$ product. Uremic rats then underwent one of the following dietary regimens for 12 wk: (a) high-P diet (0.9% P; 0.6% Ca) (U-HP); (b) high-P diet + 3% CaCO_3 (U-HP+C); and (c) high-P diet + 3% sevelamer (U-HP+S). Powdered diets were purchased from Dyets, Inc. (Bethlehem, PA). Both CaCO_3 (Sigma Chemical, St. Louis, MO) and sevelamer hydrochloride (RenaGel) were added to the high-P diet daily.

All experimental protocols were approved by the Animal Study Committee at Washington University School of Medicine.

Analytical Determinations

Rats were weighed monthly, and blood was drawn (tail vein) at 1, 4, and 8 wk to monitor serum creatinine, Ca, P, and $\text{Ca} \times \text{P}$ product. On the last 5 d of treatment, rats were placed in metabolic cages. Twenty-four-hour urine were collected, and daily dietary intake was monitored. Results were taken from the last 3 d of treatment. After 12 wk, rats were anesthetized and sacrificed by exsanguination. Arterial blood (aortic puncture) was drawn for analytical determinations. Urine samples were acidified, and each 24-h urine sample was analyzed for creatinine, calcium, and phosphorus. Plasma and urinary phosphate, and serum and urinary creatinine were determined using an

autoanalyzer (COBAS-MIRA Plus, Branchburg, NJ). Total serum and urinary calcium were measured by atomic absorption spectrophotometry using a Perkin-Elmer 1100B spectrophotometer (Perkin-Elmer, Norwalk, CT). Creatinine clearance measurements were calculated using the standard formula: $C_{Cr} = (U_{Cr} \times V_u)/S_{Cr}$. Urinary excretion is expressed as milligrams of total calcium or phosphorus excreted in 24 h. Intact PTH levels were measured by an immunoradiometric assay specific for intact rat PTH (Immunotopics, San Clemente, CA). Parathyroid glands were surgically removed and weighed on a CAHN-31 microbalance (Cahn Instruments, Inc. Cerritos, CA). $1,25(\text{OH})_2\text{D}_3$ levels were measured in plasma samples using the solid phase extraction procedure and radioreceptor assay by Hollis *et al.* (20).

Immunohistochemical Analyses of Parathyroid Glands

Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) and transforming growth factor- α (TGF- α) was performed on sections of 10% neutral buffered formalin-fixed overnight at 4°C and switched to 70% ethanol, paraffin embedded parathyroid glands following protocols described in previous studies (21). Specificity of the primary antibodies was tested by immunohistochemical staining of rat parathyroid tissue replacing the primary antibody with mouse IgG1. For TGF- α immunostaining, parathyroid tissue was pretreated with 0.05% saponin for 30 min at room temperature. Tissue was then blocked with 10% preimmune goat serum and incubated with primary antibody (1.13 $\mu\text{g}/\text{ml}$ for PCNA; 10 $\mu\text{g}/\text{ml}$ for TGF- α) for 12 h at room temperature. Twenty-four consecutive sections of tissue were cut for each parathyroid gland. Immunohistochemical staining was evaluated independently by three different blinded individuals. Ten different tissue sections were analyzed per rat for each experimental condition.

Immunohistochemical staining of PCNA protein was quantitated using a Nikon Diaphot-TMD microscope coupled to a camera and an image analysis system. Images of stained tissue sections were acquired using a DAGE-330 color camera and captured with a Pentium P-166 IBM compatible computer. The digitized images were converted to a gray scale and analyzed using Image-Pro plus software (Media Cybernetics) according to Mize's study (22) as described before (21). To eliminate variation, the microscope light source intensity used during image capture was kept constant for all sections stained on a given day.

Quantification of Calcium Deposition in Kidney, Myocardium, and Liver

Calcium content in kidney, myocardium, and liver was measured as described by Jono *et al.* (23). Tissue (three samples for each remnant kidney, myocardium, or liver) was weighed on a CAHN-31 microbalance (Cahn Instruments, Inc) and decalcified with 0.6 N HCl for 24 h. The calcium content of HCl supernatants was determined by atomic absorption spectrophotometry using a Perkin-Elmer 1100B spectrophotometer. Calcium content in each sample was corrected by wet tissue weight and expressed as μg Ca/g wet tissue.

Morphologic Analysis of Kidney Calcification

After sacrifice, the remnant kidney was removed and cleaned of fascia and adipose tissue. Sagittal sections of renal tissue were fixed in buffered formalin. Five-micrometer sections were stained with hematoxylin-eosin and with periodic acid-Schiff (PAS) and then processed for light microscopic evaluation.

The entire tissue section was evaluated for calcium deposition by von Kossa and Alizarin red S stains as follows. For von Kossa stain,

slides were deparaffinized and hydrated to water. Five-percent silver nitrate solution (S-01334, Sigma) was placed on the slides and incubated for 1 h. Slides were rinsed four times in distilled water, placed in thiosulfate solution for 5 min, and counterstained in nuclear fast red solution for 5 min. Slides were then rinsed in tap water, dehydrated, cleared in 95% ethylalcohol, 100% ethylalcohol, and xylene, and cover slips were mounted. For Alizarin red S stain, slides were deparaffinized, hydrated, and placed in Alizarin red S solution (Alizarin sodium monosulfonate from A-3757, Sigma). When red-orange color appeared, excess stain was taken off. Slides were counterstained, cleared, and mounted as previously reported (24). For Alizarin red S stain, the tissue was viewed under polarized light. Semiquantitative counts of calcifications were performed as follows. The entire kidney section was examined, and all the foci of calcification were counted (four kidney sections per animal, for a total of five rats per group). Histologic features were quantified by three different individuals blinded to treatment of the rats.

Statistical Analyses

ANOVA was employed to assess statistical differences between all experimental groups tested. Multiple comparisons using the stringent Bonferroni test measured the statistical significance of the differences between every possible two-group comparison. Unpaired two-tailed *t* test was used to compare baseline and uremia 3-mo time points within experimental groups.

Results

The efficacy of sevelamer and CaCO₃ in preventing high P-induced secondary hyperparathyroidism and renal calcifica-

tions was determined 3 mo after induction of renal insufficiency by 5/6-nephrectomy, in rats.

Serum Chemistry

Table 1 shows serum chemistries in uremic rats for all the experimental conditions tested at the beginning of the study and after the 3 mo of treatment. Serum creatinine increased in uremic control animals fed the high-P diet from a basal of 1.5 ± 0.1 to 2.3 ± 0.2 mg/dl ($P < 0.01$) after 3 mo of uremia. The increase in serum creatinine levels was prevented in uremic rats fed the same high-P diet by treatment with either sevelamer (1.4 ± 0.2 mg/dl; $P < 0.05$) or CaCO₃ (1.7 ± 0.2 mg/dl; $P < 0.05$).

Serum P levels decreased in both sevelamer (6.5 ± 0.9 mg/dl; $P < 0.01$) and CaCO₃ (7.5 ± 0.5 mg/dl; $P < 0.01$) groups compared with uremic controls (11.9 ± 0.7 mg/dl). At the doses tested, there were no significant differences in serum P between sevelamer- and CaCO₃-treated animals.

As expected, serum total Ca was higher in the uremic rats treated with CaCO₃ (10.6 ± 0.1 mg/dl) compared with those receiving sevelamer (9.5 ± 0.1 mg/dl; $P < 0.05$) or uremic controls (8.6 ± 0.5 mg/dl; $P < 0.01$).

Serum Ca \times P product was reduced in both sevelamer-treated uremic rats (110 ± 6.8 to 61 ± 8.3 mg²/dl²; $P < 0.01$) and the CaCO₃ group (109 ± 3.8 to 80 ± 5.3 mg²/dl²; $P < 0.01$). Importantly, only in the sevelamer-treated group, the serum Ca \times P product differed significantly from that in

Table 1. Serum chemistry^a

	U-HP (<i>n</i> = 7)	U-HP+S (<i>n</i> = 7)	U-HP+C (<i>n</i> = 7)
Creatinine (mg/dl)			
baseline	1.5 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
uremia (3 mo)	2.3 ± 0.2^e	1.4 ± 0.2^c	1.7 ± 0.2^c
Phosphorus (mg/dl)			
baseline	9.2 ± 0.3	10.5 ± 0.3	10.4 ± 0.3
uremia (3 mo)	11.9 ± 0.7^e	6.5 ± 0.9^{be}	7.5 ± 0.5^{be}
Calcium (mg/dl)			
baseline	10.1 ± 0.1	10.5 ± 0.3	10.5 ± 0.3
uremia (3 mo)	8.6 ± 0.5^f	9.5 ± 0.1^f	10.6 ± 0.1^{bd}
Ca \times P product (mg ² /dl ²)			
baseline	92 ± 3.8	110 ± 6.8	109 ± 3.8
uremia (3 mo)	101 ± 4.5	61 ± 8.3^{be}	80 ± 5.3^e
pH			
baseline	7.39 ± 0.01	7.43 ± 0.02	7.46 ± 0.02
uremia (3 mo)	7.30 ± 0.03^e	7.37 ± 0.01^{ce}	7.39 ± 0.01^{ce}

^a Uremic (5/6-nephrectomized) rats underwent one of the following experimental protocols for 3 mo: 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 \pm SEM; *n* = number of rats.

^b $P < 0.01$ versus U-HP from Bonferroni analysis.

^c $P < 0.05$ versus U-HP from Bonferroni analysis.

^d $P < 0.05$ versus U-HP+S from Bonferroni analysis.

^e $P < 0.01$ comparing baseline and uremia (3 mo) time points, comparing unpaired two tailed *t* test.

^f $P < 0.05$ comparing baseline and uremia (3 mo) time points, comparing unpaired two tailed *t* test.

uremic-untreated animals ($61 \pm 8.3 \text{ mg}^2/\text{dl}^2$ versus $101 \pm 4.5 \text{ mg}^2/\text{dl}^2$; $P < 0.05$).

Although serum pH decreased with the progression of renal failure in all experimental groups, both sevelamer and CaCO_3 prevented the drop in pH below the physiologic range that occurred in uremic controls.

Serum $1,25(\text{OH})_2\text{D}_3$ levels did not differ significantly between uremic controls ($20.2 \pm 3.8 \text{ pg/ml}$) and sevelamer-treated animals ($17.0 \pm 4.5 \text{ pg/ml}$) but were reduced in the CaCO_3 group ($10.3 \pm 4.3 \text{ pg/ml}$).

Creatinine Clearance, Urinary Calcium, and Urinary Phosphorus

As shown in Table 2, the reduction in creatinine clearance in the U-HP ($0.30 \pm 0.05 \text{ ml/min}$) group was prevented only by sevelamer treatment ($0.60 \pm 0.14 \text{ ml/min}$; $P < 0.01$), whereas CaCO_3 had no effect ($0.36 \pm 0.04 \text{ ml/min}$).

Treatment with CaCO_3 increased 24-h urinary Ca excretion from $8.9 \pm 0.8 \text{ mg/24 h}$ in uremic controls to $18.6 \pm 3.1 \text{ mg/24 h}$ ($P < 0.01$), whereas sevelamer induced a modest increase to $13.2 \pm 1.9 \text{ mg/24 h}$ ($P < 0.05$), a value significantly lower than that observed with CaCO_3 treatment ($P < 0.05$).

Table 2 shows that 24-h urinary phosphorus decreased from $201 \pm 13 \text{ mg/24 h}$ in the uremic control group to $150 \pm 15 \text{ mg/24 h}$ with sevelamer treatment and to $137 \pm 16 \text{ mg/24 h}$ with CaCO_3 . As with serum phosphorus levels, the decrease in urinary phosphate was not different between sevelamer and CaCO_3 -treated rats.

Effects of Sevelamer and CaCO_3 on Serum PTH and Parathyroid Gland Growth

Figure 1A depicts serum PTH levels in each experimental condition. In the untreated uremic rats fed the high-P diet, serum PTH ($1808 \pm 150 \text{ pg/ml}$) levels were much higher than in the CaCO_3 - or sevelamer-treated rats fed the same diet. Both sevelamer ($387 \pm 48 \text{ pg/ml}$; $P < 0.01$) and CaCO_3 ($356 \pm 50 \text{ pg/ml}$; $P < 0.01$) prevented the increase in serum PTH induced by high dietary P.

Figure 1B shows the effects of the sevelamer and CaCO_3 treatment on parathyroid gland weight. In untreated uremic rats fed high-P diet, the weight of the parathyroid glands was

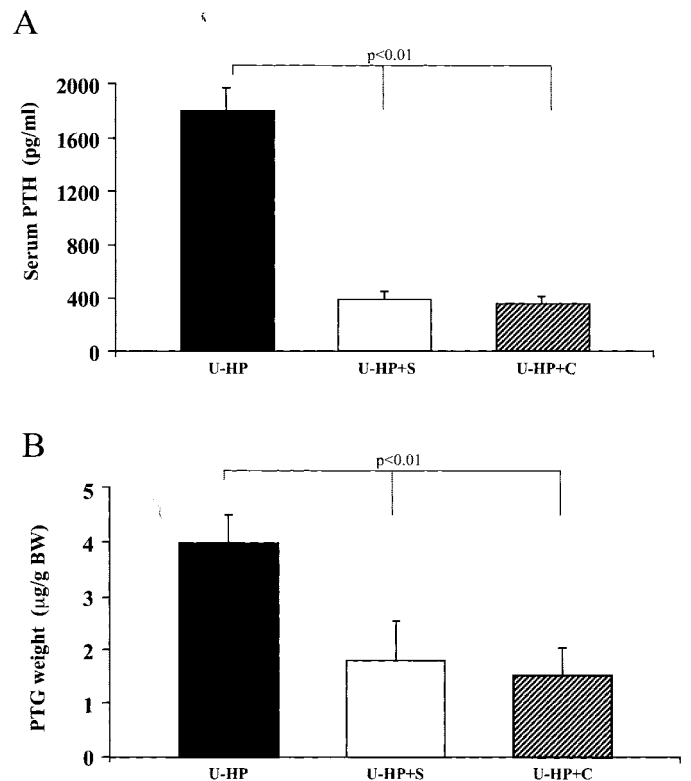


Figure 1. Effects of sevelamer and CaCO_3 on serum parathyroid hormone (PTH) and parathyroid gland growth. Serum PTH (A) and parathyroid gland weight (B) in uremic (5/6-nephrectomized) rats undergoing one of the following experimental protocols for 3 mo: uremic control + high-phosphorus diet (U-HP) (closed bars); uremic + HP diet + 3% sevelamer (U-HP+S) (open bars); uremic + HP diet + 3% calcium carbonate (U-HP+C) (hatched bars). Results represent the mean and SEM from seven rats per group. P values were obtained by ANOVA and Bonferroni tests.

higher ($3.99 \pm 0.44 \text{ µg/g body wt}$) than in treated animals fed the same diet. Both sevelamer ($1.81 \pm 0.75 \text{ µg/g body wt}$; $P < 0.01$) and CaCO_3 ($1.52 \pm 0.42 \text{ µg/g body wt}$; $P < 0.01$) prevented the enhancement of parathyroid gland growth observed in uremic rats fed a high P diet.

Table 2. Creatinine clearance, urinary phosphorus, and urinary calcium^a

	U-HP (n = 7)	U-HP+S (n = 7)	U-HP+C (n = 7)
Creatinine clearance (ml/min)	0.30 ± 0.05	$0.60 \pm 0.14^{\text{bc}}$	0.36 ± 0.04
Calcium (mg/24 h)	8.9 ± 0.8	$13.2 \pm 1.9^{\text{de}}$	$18.6 \pm 3.1^{\text{b}}$
Phosphorus (mg/24 h)	201 ± 13	$150 \pm 15^{\text{d}}$	$137 \pm 16^{\text{b}}$

^a Uremic (5/6-nephrectomized) rats underwent one of the following experimental protocols for 3 mo: 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 \pm SEM from three different 24-h urine collections. n = number of rats.

^b $P < 0.01$ versus U-HP.

^c $P < 0.01$ versus U-HP+C.

^d $P < 0.05$ versus U-HP.

^e $P < 0.05$ versus U-HP+C from Bonferroni analysis.

To directly measure parathyroid cell proliferation rates, we examined immunohistochemical expression of PCNA, a marker of mitotic activity, and TGF- α , a marker associated with uremia- and high P-induced parathyroid hyperplasia. Figure 2 (upper panels) shows higher levels of PCNA and TGF- α in a parathyroid gland from uremic rats fed a high-P diet compared with CaCO₃- (middle panels) or sevelamer-treated

(lower panels) groups. Parathyroid PCNA expression after 3 mo of uremia was lower (40% reduction) in rats fed the high-P diet when treated with either sevelamer or CaCO₃.

These findings demonstrate that sevelamer is as effective as CaCO₃ in reducing both parathyroid hormone secretion and parathyroid-cell growth induced by uremia and high dietary phosphorus.

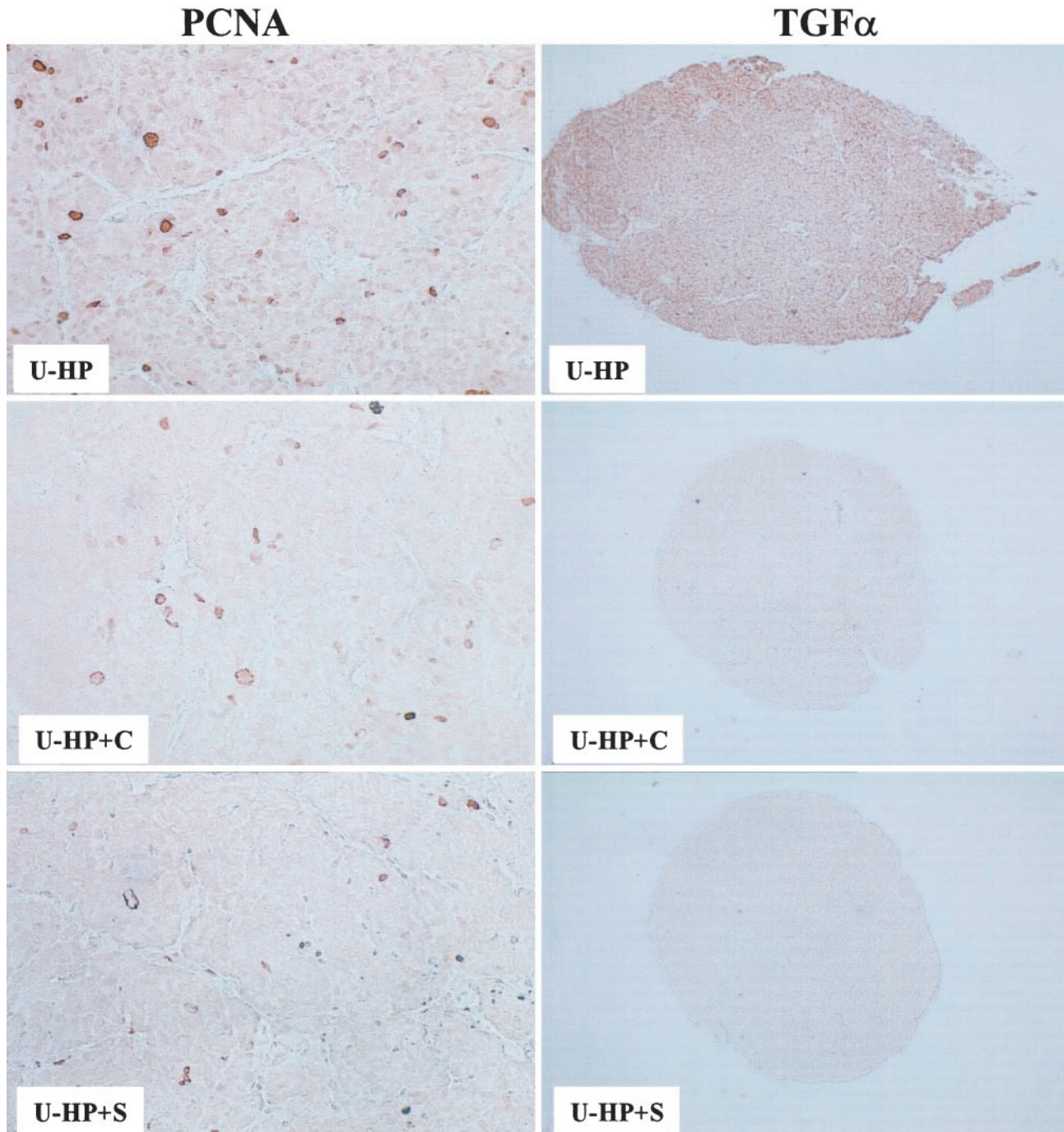


Figure 2. Effects of sevelamer and CaCO₃ on parathyroid proliferating cell nuclear antigen (PCNA) and transforming growth factor- α (TGF- α) expression. Representative photomicrographs of immunohistochemical staining of PCNA and TGF- α expression in rat parathyroid tissue from 5/6-nephrectomized controls (top panels) or rats treated with either calcium carbonate (middle panels) or sevelamer (bottom panels). Magnifications: $\times 400$ for PCNA staining; $\times 100$ for TGF- α staining.

Effects of Sevelamer and CaCO_3 on Calcium Deposition in Myocardium and Liver

Chronic renal failure increased Ca content in rat myocardium and liver compared with animals with normal renal function fed the same high-P diet (13.1 ± 8.5 versus 3.5 ± 1.1 $\mu\text{g/g}$ wet myocardial tissue; 6.1 ± 2.8 versus 2.9 ± 0.6 $\mu\text{g/g}$ wet liver tissue). Both sevelamer and CaCO_3 reduced Ca deposition in myocardium and liver. The decrease in Ca content at 3 mo did not differ with either phosphate binder.

Effects of Sevelamer and CaCO_3 on Renal Calcium Deposition

Figure 3 shows kidney Ca content in all experimental groups. Uremia markedly increased kidney Ca content compared with rats with normal renal function fed the same high-P diet (175.5 ± 45.7 versus 5.8 ± 0.8 $\mu\text{g/g}$ wet tissue; $P < 0.01$). Most importantly, a dramatic reduction of renal Ca deposition was observed in the sevelamer group (29.8 ± 8.6 $\mu\text{g/g}$ wet tissue) compared with both uremic controls (175.5 ± 45.7 $\mu\text{g/g}$ wet tissue; $P < 0.01$) and the CaCO_3 group (58.9 ± 13.7 $\mu\text{g/g}$ wet tissue; $P < 0.04$).

Effects of Sevelamer and CaCO_3 on Calcification of Kidney Tissue

Calcifications seen on kidney hematoxylin-eosin-stained sections were highlighted with von Kossa and Alizarin red S staining. Representative kidney sections from each experimental group depicting different staining are shown in Figure 4. An apparent significant decrease in kidney calcifications in the

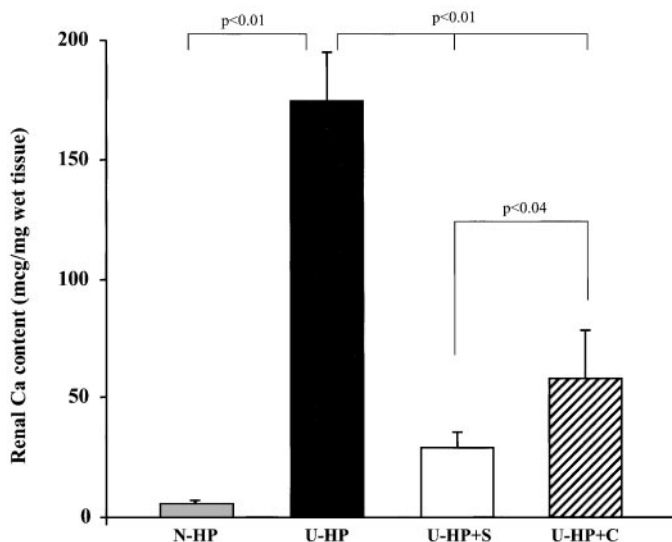


Figure 3. Effects of sevelamer and CaCO_3 on renal calcium content. Renal calcium deposition in normal and uremic (5/6-nephrectomized) rats undergoing one of the following experimental protocols for 3 mo: normal + high-phosphorus diet (N-HP) (gray bar); uremic control + HP diet (U-HP) (closed bar); uremic + HP diet + 3% sevelamer (U-HP+S) (open bar); uremic + HP diet + 3% calcium carbonate (U-HP+C) (dashed bar). Bars and errors bars represent the mean and SEM from seven rats. P values were obtained by ANOVA and Bonferroni tests.

sevelamer-treated group is shown in Figure 4 (lower panels) in comparison with uremic controls (upper panels) or rats treated with CaCO_3 (middle panels). In addition, the higher number of foci of calcification found in uremic controls (108 ± 25), measured by semiquantitative analysis of kidney calcifications, was reduced by CaCO_3 (33.0 ± 11.3) and even further by sevelamer (16.4 ± 8.9 ; $p < 0.02$ versus CaCO_3) (Figure 5).

Furthermore, in uremic rats fed high-P diet, the remnant kidney sections showed severe interstitial fibrosis and tubular dilatation, occupying approximately 50% of the kidney surface area. Significant acute and chronic inflammation were detected as well. Periglomerular fibrosis and increased number of globally sclerosed glomeruli were present. In contrast, the remnant kidneys in CaCO_3 -treated rats showed less interstitial inflammation, fibrosis and tubular atrophy, occupying about 30% of the kidney surface area. Most importantly, the renal histology in sevelamer-treated rats was almost free of inflammation, interstitial fibrosis, and tubular dilatation (5% of the kidney surface area). Figure 6 depicts the described histologic findings in the corresponding animals.

Discussion

These studies demonstrate in rats with chronic renal failure that, despite the similar efficacy of sevelamer hydrochloride and CaCO_3 in controlling serum phosphorus and secondary hyperparathyroidism, sevelamer better prevents renal calcium deposition, preserving renal function.

Hyperphosphatemia due to decreased P excretion (25,26) worsens secondary hyperparathyroidism, which is commonly present in patients with chronic renal failure. High serum P directly enhances parathyroid cell proliferation and PTH synthesis and secretion (6,7). High P also enhances parathyroid function indirectly by decreasing calcitriol synthesis and serum ionized Ca levels, which further elevates circulating PTH (27,28). High serum PTH induces osteitis fibrosa and bone loss, thus increasing serum Ca \times P product (29,30) and ectopic calcification (3,31). In addition to the described effects regarding bone resorption, high PTH may also cause metastatic microcalcifications through elevations in cytosolic Ca (9,10). In rats, Borle *et al.* (16) showed that high P-induced hyperparathyroidism caused nephrocalcinosis. Elevated levels of serum PTH induced intracellular Ca accumulation and Ca-P deposition in renal tissue (16).

Conversely, P restriction counteracts the mitogenic signals for parathyroid hyperplasia triggered by renal failure, thus preventing parathyroid gland enlargement (7). Furthermore, in an experimental model of established secondary hyperparathyroidism, the switch from high P intake to P restriction normalized serum PTH levels within 1 wk (32). The molecular mechanisms by which phosphate restrictions effectively suppress hyperparathyroidism are incompletely understood. However, it is clear that the control of serum P is critical for effective treatment in renal failure patients. Because of difficulties with patients' compliance to a P restricted diet, the current treatment of hyperphosphatemia demands phosphate binders. One obvious limitation of calcium-based phosphate binders, increased Ca load and serum Ca in patients with end-stage renal disease

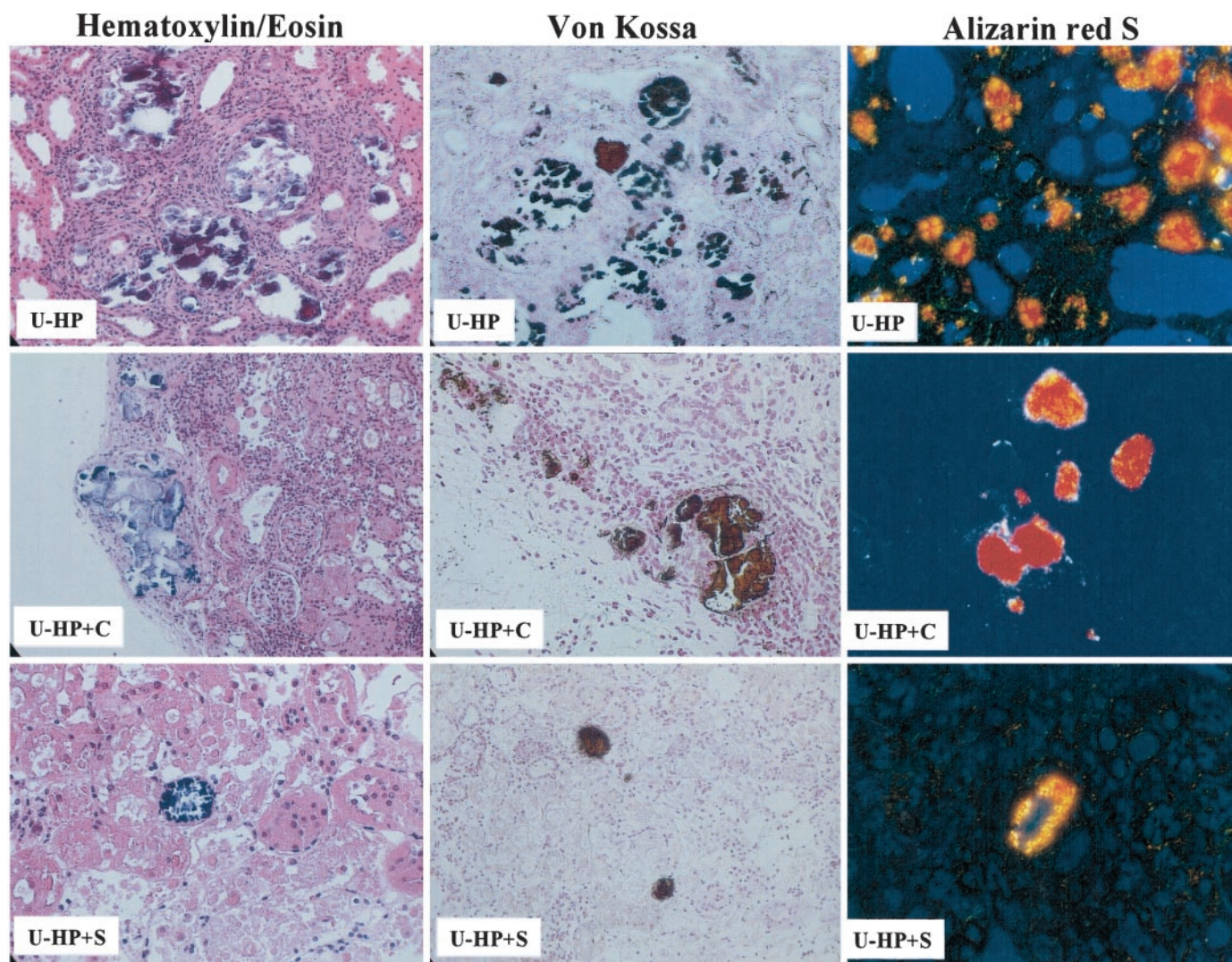


Figure 4. Effects of sevelamer and CaCO_3 on kidney calcification. Representative photomicrographs of hematoxylin-eosin, von Kossa, and Alizarin red S staining in remnant kidney tissue of 5/6-nephrectomized rats undergoing one of the following experimental protocols for 3 mo: uremic control + high-phosphorus diet (U-HP) (upper panels); uremic + HP diet + 3% calcium carbonate (U-HP+C) (middle panels); uremic + HP diet + 3% sevelamer (U-HP+S) (lower panels). Alizarin red S staining was used polarized microscopy.

(33–35), led to the development of a new phosphate binder, sevelamer. In dialysis patients, this calcium- and aluminum-free compound reduces serum phosphorus and PTH levels with no hypercalcemia (19,36,37).

In the present studies of chronic renal failure in rats, sevelamer treatment reduced serum P independently of increases in serum Ca levels, leading to a lower serum $\text{Ca} \times \text{P}$ product when compared with uremic controls. Sevelamer reduction of serum P appears to mediate its efficacy to control both parathyroid hyperplasia and PTH secretion; serum $1,25(\text{OH})_2\text{D}_3$ levels were similar between uremic controls and the sevelamer-treated rats.

Although sevelamer and CaCO_3 were equally effective in controlling serum P levels and secondary hyperparathyroidism, no difference in $\text{Ca} \times \text{P}$ product was evident between CaCO_3 -treated rats and uremic controls. It is clear that an additional factor, such as sevelamer's improved control of the $\text{Ca} \times \text{P}$

product, mediated the higher efficacy of sevelamer in reducing renal Ca deposition. In fact, Ahmed *et al.* (38) showed an association between hyperphosphatemia, elevated serum $\text{Ca} \times \text{P}$ product, and calciphylaxis in dialysis patients. Although nephrocalcinosis is not a common factor in the progression of renal failure, Gimenez *et al.* (17) reported a significant positive correlation between renal Ca content and serum creatinine in patients with impaired renal failure. Biopsied patients with serum creatinine higher than 1.5 mg/dl had higher levels of serum P, serum $\text{Ca} \times \text{P}$ product, and renal Ca content (17). Recent studies by Goodman (2) and Guerin (34) have implicated the dose of calcium-based P binders as a risk for coronary artery calcification in end-stage renal failure patients.

In our uremic rat model, there was no evidence of high P-induced aortic calcifications 3 mo after the onset of renal failure. However, Ca content in rat myocardium and liver was higher than in normal controls. Both sevelamer and CaCO_3

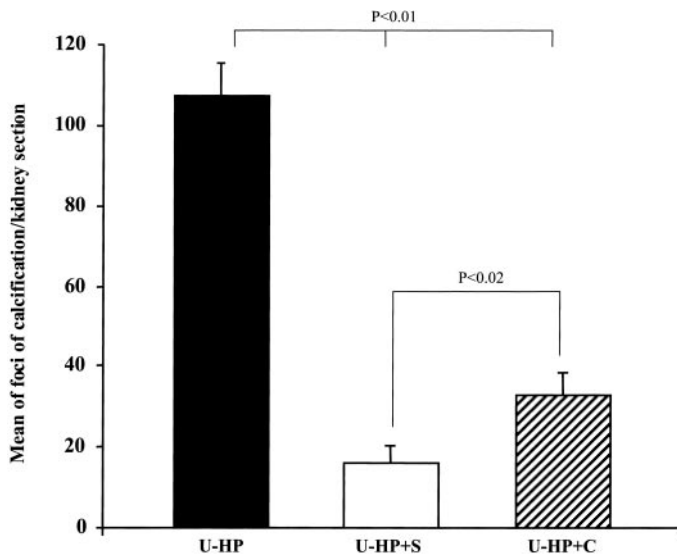


Figure 5. Effects of sevelamer and CaCO_3 on kidney foci of calcification. Mean of foci of calcification in remnant kidney tissue uremic (5/6-nephrectomized) rats undergoing one of the following experimental protocols for 3 mo: uremic control + high-phosphorus diet (U-HP) (closed bar); uremic + HP diet + 3% sevelamer (U-HP+S) (open bar); uremic + HP diet + 3% calcium carbonate (U-HP+C) (dashed bar). Results represent the mean and SEM from four sections/rat in five rats per group. *P* values were obtained by ANOVA and Bonferroni tests. Magnification, $\times 20$.

were equally effective in reducing Ca deposition in these tissues.

Importantly, despite the similarities of sevelamer and CaCO_3 in controlling myocardial Ca deposition in rats after 3 mo of uremia and high-Pe diet, differences were evident when Ca deposition was measured in the kidneys. These findings suggest a tissue-specific and time-dependent sensitivity for $\text{Ca} \times \text{P}$ product induction of calcification (Figure 4). In fact, there was a significant reduction of renal Ca content in sevelamer-treated rats compared with the CaCO_3 group, also evident in histologic studies using von Kossa and Alizarin red S staining

of kidney sections. Further validation came from the demonstration that sevelamer was more effective than CaCO_3 in preventing elevations in the number of foci of calcification compared with uremic controls (Figure 5). These findings suggest that the significant reduction of renal Ca deposition found in the uremic rats treated with sevelamer may be in association with the lower serum $\text{Ca} \times \text{P}$ product compared with uremic controls and CaCO_3 -treated animals. Moreover, renal function deterioration, assessed by measurements of creatinine clearance, was prevented only in sevelamer-treated rats. No differences in creatinine clearance were observed between the uremic and the CaCO_3 -treated animals. In fact, the severe tubulointerstitial fibrosis, present in remnant kidneys of uremic rats fed high dietary P (50% of the kidney surface area), was reduced to 30% of the kidney surface area by treatment with CaCO_3 and almost abolished in sevelamer-treated rats (5% of the kidney surface area) (Figure 6). These data support the existing evidence on the role of hyperphosphatemia in the deterioration of renal failure in rats and the efficacy of sevelamer in better ameliorating its progression compared with CaCO_3 .

In 5/6-nephrectomized rats, low dietary P prevented increases in serum creatinine levels, improved kidney histology, and decreased renal Ca content (14). Moreover, in uremic rats fed a normal-P diet (0.5% P), renal histology presented extensive tubulointerstitial lesions and nephrocalcinosis compared with a low-P diet (0.2% P) (39). Furthermore, 5/6-nephrectomized rats placed on high-P (1.0% or 2.0%) diets developed higher renal Ca content and more histologic damage compared with animals on a normal-P (0.5%) diet (11). The demonstration in our experimental model in the rat that both P binders were equally effective in controlling serum P indicates that the lower $\text{Ca} \times \text{P}$ product in the sevelamer group may be the main determinant of its advantage in a better preservation of renal function.

In conclusion, sevelamer is an effective agent in reducing $\text{Ca} \times \text{P}$ product, preventing kidney calcification, and preserving renal function in uremic rats. These findings cannot be extrapolated to human disease, and further studies in patients are

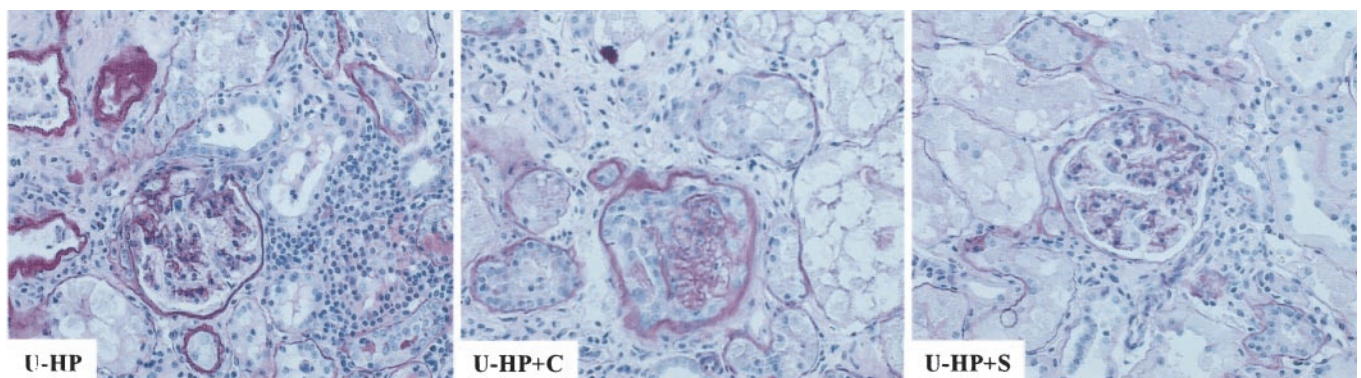


Figure 6. Effects of sevelamer and CaCO_3 on kidney histology. Representative photomicrographs of periodic acid-Schiff (PAS) staining in remnant kidney tissue of 5/6-nephrectomized rats undergoing one of the following experimental protocols for 3 mo: Uremic control + high-phosphorus diet (U-HP) (right panel); uremic + HP diet + 3% calcium carbonate (U-HP+C) (middle panel); and uremic + HP diet + 3% sevelamer (U-HP+S) (left panel). Magnification, $\times 400$.

necessary to determine the benefits of either (CaCO_3 versus sevelamer) P binder.

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