

Sevelamer hydrochloride and coronary artery calcification in chronic hemodialysis patients: a new mechanism of action

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Background

The non-calcium-based phosphate binder sevelamer hydrochloride was developed to provide chronic kidney disease patients with a polymer capable of managing hyperphosphatemia without an increase in the calcium load. These beneficial effects were postulated as the mechanism of decreased progression of vascular calcification observed with such compounds. Our objective was to investigate the effect of low-dose sevelamer hydrochloride against calcium carbonate as phosphate binders on the coronary artery calcification score (CCS) and the fibroblast growth factor 23 (FGF23) level in patients receiving regular hemodialysis for more than 1 year, in a trial to find out a novel mechanism for the decreased vascular calcification observed during sevelamer use.

Patients and methods

A total of 80 hemodialysis patients were allocated into two groups each of 40 patients. The first group received sevelamer hydrochloride 2400 mg/day (group 1), whereas the second continued on calcium carbonate 1500 mg/day (group 2). For each patient, coronary artery calcification was estimated twice, once before admission to the study and again at the end of the study period using noncontrast computed tomography. Serum calcium, phosphorus, intact parathyroid hormone (PTH), lipids, and FGF23 were also assessed in these two situations.

Results

Beside the significant decrease in serum calcium and phosphorus levels after the use of sevelamer for 6 months, there was a significant decrease in levels of FGF23 and the rate of CCS progress in group 1. Serum levels of total and low-density lipoprotein cholesterol decreased significantly in group 1. The serum PTH level did not show a significant change in either group. CCS showed a significant positive correlation with FGF23, but there was no significant correlation with serum calcium, serum phosphorus, or serum PTH in both groups.

Conclusion

Sevelamer hydrochloride suppressed the progression of coronary artery calcification, and decreased the FGF23 level significantly. The significant correlation between the serum FGF23 level and the CCS in the absence of any significant correlation between the latter on the one hand and the serum calcium, the serum phosphorus, or the serum PTH on the other might highlight a novel mechanism of action of sevelamer on the CCS.

Keywords:

calcium carbonate, coronary artery calcification, fibroblast growth factor 23, parathyroid hormone, sevelamer

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Introduction

Arterial calcifications are independent predictors of all-cause and cardiovascular disease (CVD) mortality [1]. Hyperphosphatemia and an elevated calcium–phosphorus (Ca × P) product are associated with an increased risk of arterial calcification [2]. Although calcium-based phosphate binders reduce serum phosphorus and parathyroid hormone (PTH) concentrations effectively, these agents can lead to hypercalcemia associated with increased vascular calcification [3]. The non-calcium-based phosphate binder sevelamer hydrochloride (HCl) was developed to provide chronic kidney disease patients with a polymer capable of managing hyperphosphatemia

without an increase the in calcium load [4]. These beneficial effects were postulated as the mechanism of decreased progression of vascular calcification observed with such compounds [5,6].

Dialysis patients have 100–1000 times higher serum fibroblast growth factor 23 (FGF23) levels than the general population [7]. The serum level of FGF23 is associated positively with the serum phosphate

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level [7]. There is an established relationship between high levels of FGF23 and vascular calcifications in hemodialysis (HD) patients [8,9]. This established relation might explain the increased mortality associated with high FGF23 levels encountered among HD patients [10,11].

In this study, we compared the effect of sevelamer HCl versus calcium carbonate (CaCO_3) as phosphate binders on serum phosphorus, serum calcium, PTH, FGF23, and vascular calcifications in chronic HD patients. We further investigated the correlations of coronary artery calcification (CAC) progression with FGF23, calcium, phosphorus, and PTH levels; the correlations of changes in FGF23 levels with PTH, calcium, and phosphorus in HD patients were also investigated.

Patients and methods

Patients

A total of 80 patients, receiving regular HD in the Nephrology Department of Kasr Al-Aini University Hospital for at least 6 months, were included in the study after approval of our protocol by the local ethical committee. Their mean age was 52 years (the minimum was 33 years and the maximum 65 years). Patients were divided equally into two groups on the basis of the treatment given: group 1 was treated with sevelamer HCl (patients dialyzed on Saturday, Monday, and Wednesday) and group 2 was treated with CaCO_3 (patients dialyzed on Sunday, Tuesday, and Thursday). Following is the detailed treatment regimen of the two studied groups:

- (1) Group 1 (the sevelamer HCl group): 40 nondiabetic patients with chronic renal failure on regular HD for at least 6 months were shifted to sevelamer HCl (800 mg) three times per day for 6 months (without the washout period from the previous treatment with CaCO_3).
- (2) Group 2 (the CaCO_3 group): the remaining 40 nondiabetic patients with chronic renal failure on regular HD for at least 6 months continued their treatment with 1500 mg CaCO_3 /day (600 mg elemental calcium) for 6 months (without the washout period).

HD with low-flux polysulfone hollow-fiber dialyzers with a surface area between 1.5 and 1.7 m^2 was performed three times weekly each for 4 h. The potassium concentration of the dialysate was 2.0 mEq/l, whereas the calcium concentration was 1.75 mEq/l. The blood flow rate was 300–350 ml/min, and the dialysate flow rate was 500 ml/min throughout the study.

Patients fulfilling the following criteria were included in this study:

- (i) Both sexes with end-stage renal disease on regular HD for at least 6 months,
- (ii) Patients requiring treatment with phosphate-binding agents, and
- (iii) Patients not receiving active vitamin D.

Patients with the following conditions were excluded from this study:

- (i) Diabetes,
- (ii) A history of ischemic heart disease or active liver disease,
- (iii) A history of parathyroidectomy or parathyroid ablation,
- (iv) Noncompliance to either sevelamer HCl or CaCO_3 ,
- (v) Receiving statin treatment,
- (vi) Hemodiafiltration (HDF) treatment,
- (vii) Receiving cinacalcet, or
- (viii) On a dose of more than 1500 mg CaCO_3 /day.

The medical history of all patients was obtained, and they were evaluated by thorough clinical examination. Levels of serum creatinine, blood urea, serum calcium, phosphorus, cholesterol, albumin, low-density lipoprotein (LDL), high-density lipoprotein (HDL), FGF23, and intact PTH were measured. Coronary artery calcification score (CCS) was assessed by multislice computed tomography (CT) coronary angiography to calculate CCS. All tests were repeated 6 months later at the end of the study.

The parathyroid hormone test

Serum samples were prepared promptly from whole blood and stored temporarily on ice or refrigerated at 4°C. PTH-EASIA (Roche Diagnostics, Indianapolis, Indiana, USA) is a solid-phase enzyme-amplified sensitivity immunoassay performed on a microtiter plate for the determination of intact human PTH from serum or plasma. PTH-EASIA was performed according to the manufacturer's protocol [9].

The fibroblast growth factor 23 enzyme-linked immunosorbent assay

As the intact FGF23 molecule was highly unstable (resulting in a decreased immunoreactivity over time), specimen collection and assay or storage procedures were conducted expeditiously. As recommended, samples were collected in the morning from patients fasting for 12 h. The collected samples were centrifuged, and the plasma or the media were separated from the cells. They were assayed immediately or stored at -70°C or below.

Serum levels of intact FGF23 molecules were measured using the two-site (NH₂-terminal/C-terminal) enzyme-linked immunosorbent assay (Immutopics Inc., San Clemente, California, USA). To assay the sample in duplicate, 300 µl of plasma or culture media were collected. The Human Intact FGF23 enzyme-linked immunosorbent assay (Immutopics Inc.) was performed according to the manufacturer's protocol. Intact PTH levels were determined by the enzyme-amplified sensitivity immunoassay (Roche Diagnostics) [9].

The coronary artery calcification score

Coronary vascular calcification was quantified by noncontrast CT examination of the heart. The technique was performed to detect and quantify coronary calcifications in the 80 patients included in this study. All CCS examinations were performed on a Light Speed four-detector row CT scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA). Acquisition parameters were ECG gated at 75% of the RR interval, 500 ms gantry rotation, 4×2.5 mm collimation, 80 mA, and 120 kV. To minimize the total effective patient radiation dose, the scanning was conducted with a relatively low tube current. Reconstructed axial images at different points of the cardiac cycles were sent to an off-line workstation (Advanced Workstation 4.0; General Electric Medical Systems). A dedicated cardiac reconstruction software was used to evaluate coronary arteries (Smart Score; General Electric Medical Systems). Total calcium scores of all patients were calculated with the dedicated software and expressed as Agatston scores. Using sequential axial images, any tissue above the 130 HU occupying a minimum of 0.5 mm² identified along the anatomical course of a coronary artery was considered as coronary calcification. Such tissues were highlighted and analyzed using the software. The Agatston score is a commonly used scoring method that calculates the total amount of calcium on the basis of the

number, areas, and peak Hounsfield units of the detected calcified lesions.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Statistical analyses

Data were analyzed using the statistical package of social science (SPSS) program (version 15.0; SPSS Inc., Chicago, Illinois, USA). Data were summarized as the median. We performed the *t*-test for analyzing two quantitative data and the nonparametric test (the Mann–Whitney *U*-test) when data were not distributed symmetrically. Pearson's correlation was considered weak for $r < 0.25$, mild for $0.5 > R \geq 0.25$, moderate for $0.75 > R \geq 0.5$, and strong for $R \geq 0.75$. *P*-values less than or equal to 0.05 were considered significant. As our study included a small number of patients, we used the median test.

Results

Tables 1 and 2 present the median of different parameters studied and the comparative analysis of the percent of changes for group 1 and group 2 at the beginning and at the end of the study, respectively; the serum calcium level decreased significantly in group 1 (9.4 vs. 9 mg/dl, respectively, $P = 0.001$), whereas increased significantly in group 2 (9 vs. 9.8 mg/dl, respectively, $P = 0.005$) when comparing the levels before and at the end of the study period. Beside the significant reduction in serum phosphorus in both groups, this reduction was more significant in group 1

Table 1 Medians of different parameters, including coronary artery calcification score, at the beginning and the end of the study in both groups

Parameters	Group 1			Group 2		
	Initiation	End	<i>P</i> -value	Initiation	End	<i>P</i> -value
Median of serum calcium (mg/dl)	9.4	9.0	0.001	9.0	9.8	0.005
Median of serum phosphate (mg/dl)	7.0	6.0	<0.001	7.2	6.8	0.005
Median of serum PTH (pg/dl)	268.0	272.1	0.9	294.5	309.0	0.80
Median of serum FGF23 (pg/dl)	4669.5	3011.8	<0.001	4872.0	7154.0	0.005
Median of serum cholesterol (mg/dl)	157.5	142	<0.001	147.0	179.5	0.005
Median of serum HDL (mg/dl)	42.0	58.0	<0.001	42.0	35.0	0.014
Median of serum LDL (mg/dl)	107.0	70.0	<0.001	89.5	125.5	0.005
Median CCS (AU)	200.0	203.0	<0.001	198.0	285.5	0.005

CCS, coronary artery calcification score; FGF23, fibroblast growth factor 23; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone.

Table 2 The percentage of change in different parameters, including coronary artery calcification score, in both studied groups 1 and 2

Parameters (%change)	Group	Minimum	Maximum	Median	P-value
Calcium	1	-5.0	8.0	3.9	<0.001
	2	-20.0	-5.6	-11.3	
Phosphorus	1	2.9	26.3	13.4	0.050
	2	4.1	15.7	8.4	
PTH	1	-2.7	4.1	0.5	0.7
	2	-3.7	2.4	3.5	
FGF23	1	0.0	76.2	34.7	<0.001
	2	-191.1	-26.1	-46.9	
Percentage of cholesterol change	1	1.3	20.7	11.3	<0.001
	2	-33.8	-5.5	-22.7	
Percentage of HDL change	1	-159.3	-9.1	-35.1	<0.001
	2	-10.4	25.0	14.3	
Percentage of LDL change	1	10.4	53.3	31.4	<0.001
	2	-74.6	-11.7	-21.9	
Percentage of CCS change	1	-12.0	2.9	-2.6	<0.001
	2	-117.1	-19.0	-43.5	

CCS, coronary artery calcification score; FGF23, fibroblast growth factor 23; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone.

when compared with group 2 at the end of the study (7 vs. 6 mg/dl and 7.2 vs. 6.8 mg/dl in groups 1 and 2, $P < 0.001$ and 0.005 , respectively). It is worth mentioning that the serum intact PTH did not show a significant change in either of the two groups. There was a significant decrease in the serum FGF23 in group 1 by the end of the study compared with its basal level (4669.5 vs. 3011.8 pg/ml, respectively, $P < 0.001$). These levels increased significantly in group 2 patients (4872 vs. 7154 pg/ml, respectively, $P = 0.005$). Serum total and LDL-cholesterol decreased significantly in group 1, whereas increased significantly in group 2. HDL-cholesterol increased significantly in group 1 and decreased significantly in group 2. A significant increase in the CCS was observed in both groups of patients at the end of the study; however, group 2 showed a much higher increase (200–203, $P < 0.001$, and 198–285.5, $P = 0.005$, respectively). Table 3 presents the correlations of FGF23 and CCS with other parameters at the end of the study for both groups. We found a highly significant positive correlation between the CCS and the level of FGF23 ($R = 0.7$, $P = 0.001$) and a significant negative correlation between CCS and the level of HDL ($R = -0.5$, $P = 0.03$).

Discussion

Elevated phosphate and $\text{Ca} \times \text{P}$ have been associated with vascular calcification in patients with end-stage renal disease on maintenance dialysis [4]. In the TTG and the RIND studies, a progressive increase in CAC has been observed while using calcium-containing phosphate binders [6,12–14]. Such an increase has been ameliorated with the use of non-calcium-containing

Table 3 Correlation between fibroblast growth factor 23 and coronary artery calcification score with different parameters at the end of the study in group 1

Parameters	Correlation coefficient	FGF23	CCS
FGF23	R	–	0.7
	P -value	–	0.001**
CCS	R	0.7	–
	P -value	0.001*	–
Calcium (mg/dl)	R	0.2	0.1
	P -value	0.4	0.7
Phosphorus (mg/dl)	R	-0.2	-0.03
	P -value	0.5	0.9
PTH (pg/dl)	R	-0.2	0.02
	P -value	0.5	0.9
Cholesterol (mg/dl)	R	-0.03	-0.04
	P -value	0.9	0.9
HDL (mg/dl)	R	-0.2	-0.5
	P -value	0.3	0.03*
LDL (mg/dl)	R	0.1	0.1
	P -value	0.6	0.8

CCS, coronary artery calcification score; FGF23, fibroblast growth factor 23; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; *Significant, **Highly significant.

phosphate binders [15]. The Bone Remodeling and Coronary Calcification (BRIC) study, investigating impact of phosphate binders on bone and coronary calcification, showed that calcium binders were more likely to lead to low-turnover bone disease (as seen on bone biopsy) than sevelamer. Furthermore, the low-turnover bone disease was more likely to be associated with an increased CAC [16].

CVD is the leading cause of death in patients with chronic kidney disease (CKD) [17]. The association between excess FGF23 and mortality is likely mediated by an increased cardiovascular risk. Increased

FGF23 is associated independently with an increased left ventricular mass index [18]. In a previous study conducted by our group, increased FGF23 was associated independently with aortic calcification among HD patients [9]. In the present study, we investigated the effect of sevelamer HCl against CaCO_3 on the FGF23 level and the progression of coronary calcification. In group 1, significantly lower levels of calcium, phosphate, FGF23, LDL-cholesterol, and total cholesterol were observed at the end of the study beside statistically higher levels of HDL-cholesterol. In group 2, we observed statistically higher levels of calcium, total cholesterol, LDL-cholesterol, FGF23, and CCS. These results indicate that sevelamer, but not CaCO_3 , reduces FGF23 significantly. This novel action of sevelamer HCl on FGF23 was further reported by other study groups [19,20].

Overall, CaCO_3 controlled the phosphorus level adequately. However, its effectiveness may be limited by hypercalcemia. In contrast, sevelamer-treated patients (group 1) had significantly lower levels of FGF23 compared with patients receiving CaCO_3 (group 2) as phosphate binders. FGF23 had been identified as an inducer of vascular calcification and CVD in CKD. Our data confirm that FGF23 levels are massively elevated in HD patients [9]. Therefore, the use of calcium-free phosphate binders may reduce the risk of CVD not only by reducing the calcium load, but also by reducing both serum phosphorus and FGF23 levels [20].

Similarly, there was relatively less progression of CAC in 127 incident HD patients assigned randomly to sevelamer against calcium-based phosphorus binders [13]. Our study showed a positive and significant correlation between CCS and FGF23 in HD patients, which is in agreement with a similar study [19,21]

Mirza *et al.* [22] found that a higher serum FGF23 level was associated with increased arterial stiffness exclusively in patients with an age-adjusted impaired renal function. A recent study showed that sevelamer treatment is associated with increased absorption of alimentary calcium [23]. This finding might cast doubt on the negative calcemic action of sevelamer. Besides our results, such a challenge can reinforce our hypothesis that the vascular protective effect of sevelamer is due to its effect on HDL and FGF23 rather than its effect on the $\text{Ca} \times \text{P}$ product.

Lastly we would like to emphasize the findings of Lin *et al.* [24], that the decrease in FGF23 in HD patients after the long-term use of sevelamer is associated with an increase in the serum klotho level. Klotho is the receptor cofactor mediating the action of FGF23.

Increased klotho will decrease the resistance to FGF23, and thus would result in the observed significant decrease in the serum FGF23 level [24].

Our study had some limitations. As the drug was expensive for most of our patients, we could not increase the dose of sevelamer to control the phosphorus level adequately. However, despite the relatively low dose of the drug, we succeeded in retarding vascular calcification, lowering the LDL-cholesterol, and lowering the FGF23, while increasing the HDL-cholesterol. We neither studied sex differences between CCS in both groups nor had longer follow-up data for our patients.

On the basis of the above findings, we conclude that administering sevelamer HCl at a relatively low dose in HD patients can lead to sustained reductions in serum phosphorus. In addition, we also observed sustained, favorable changes in the FGF23 and the lipid profile; the progression of CAC was also retarded. Sevelamer HCl, even at a low dose, represents a step forward in the management and the treatment of CAC.

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Conflicts of interest

There are no conflicts of interest.

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