Cinacalcet Effectively Reduces Parathyroid Hormone Secretion and Gland Volume Regardless of Pretreatment Gland Size in Patients with Secondary Hyperparathyroidism

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Background and objectives: Cinacalcet is effective in reducing serum parathyroid hormone (PTH) in patients with secondary hyperparathyroidism. However, it has not been proven whether parathyroid gland size predicts response to therapy and whether cinacalcet is capable of inducing a reduction in parathyroid volume.

Design, setting, participants, & measurements: This 52-week, multicenter, open-label study enrolled hemodialysis patients with moderate to severe secondary hyperparathyroidism (intact PTH >300 pg/ml). Doses of cinacalcet were adjusted between 25 and 100 mg to achieve intact PTH <180 pg/ml. Ultrasonography was performed to measure the parathyroid gland size at baseline, week 26, and week 52. Findings were also compared with those of historical controls.

Results: Of the 81 subjects enrolled, 56 had parathyroid glands smaller than 500 mm^3 (group S) and 25 had at least one enlarged gland larger than 500 mm^3 (group L). Treatment with cinacalcet effectively decreased intact PTH by 55% from baseline in group S and by 58% in group L. A slightly greater proportion of patients in group S *versus* group L achieved an intact PTH <180 pg/ml (46 *versus* 32%) and a >30% reduction from baseline (88 *versus* 78%), but this was not statistically significant. Cinacalcet therapy also resulted in a significant reduction in parathyroid gland volume regardless of pretreatment size, which was in sharp contrast to historical controls (n = 87) where parathyroid gland volume progressively increased with traditional therapy alone.

Conclusions: Cinacalcet effectively decreases serum PTH levels and concomitantly reduces parathyroid gland volume, even in patients with marked parathyroid hyperplasia.

Clin J Am Soc Nephrol 5: 2305–2314, 2010. doi: 10.2215/CJN.02110310

Secondary hyperparathyroidism (SHPT) is a common complication of chronic kidney disease, characterized by parathyroid hyperplasia and persistently elevated levels of parathyroid hormone (PTH) (1,2). Parathyroid hyperplasia can be divided into two types with different morphologic features: diffuse and nodular hyperplasia (3). Nodular hyperplasia is a more advanced type of hyperplasia and is associated with more marked proliferation and greater resistance to medical therapy (4).

Until recently, calcitriol and other vitamin D analogs have been the cornerstone of secretory PTH suppression in patients with chronic kidney disease; however, these agents also enhance the intestinal absorption of calcium and phosphate and result in elevations in serum calcium and phosphate concentrations (5). Moreover, the response to vitamin D sterols is substantially reduced once parathyroid hyperplasia has progressed to the advanced nodular form, presumably because of the reduced expression of calcium-sensing receptors (CaSR) and vitamin D receptors (VDR) (6–10). Several clinical studies suggest that parathyroid gland volume in excess of 500 mm^3, as evaluated by ultrasonography, appears to be a useful indicator of responsiveness to vitamin D therapy (11–13). This concept is supported by the observation that surgically removed parathyroid glands weighing >500 mg usually exhibit nodular formations (3). Interestingly, vitamin D therapy may also induce regression of parathyroid hyperplasia (14), but such an effect may be less likely in more advanced stages (15).

Cinacalcet hydrochloride, a calcimimetic agent that acts as an allosteric modulator of the CaSR, is a new option for the ther-

Received March 7, 2010. Accepted July 16, 2010.

Published online ahead of print. Publication date available at www.cjasn.org.

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ISSN: 1555-9041/512–2305

apeutic control of SHPT. A large number of clinical trials have shown that treatment with cinacalcet effectively reduces PTH levels in patients with SHPT that is refractory to vitamin D therapy (16–21). More recent studies suggest that combined therapy with cinacalcet and low doses of vitamin D sterols could be an effective strategy to treat SHPT while adequately maintaining acceptable levels of calcium and phosphorus (22–24). However, there is controversy as to whether cinacalcet is capable of controlling parathyroid hyperfunction in patients with marked parathyroid hyperplasia (25,26). In addition, although experimental studies suggest that regression of parathyroid hyperplasia could be induced by calcimimetics (27,28), this possibility has not been adequately explored in the clinical setting (26,29).

Therefore, the purpose of this study was twofold: (1) to elucidate whether parathyroid gland size could be used as an indicator of response to cinacalcet therapy and (2) to examine whether cinacalcet reduces parathyroid gland volume in patients with moderate to severe SHPT.

Materials and Methods

Study Population

Patients were considered for the study if they were 18 years of age or older and had required maintenance dialysis for at least 16 weeks. The main eligibility criteria were serum intact PTH >300 pg/ml, confirmed within a 30-day screening period, and serum calcium >9.0 mg/dl. Exclusion criteria included a history of parathyroidectomy or an unstable medical condition during the previous 30 days. The study was conducted in accordance with the principles of the Declaration of Helsinki, and all patients provided written informed consent. The study protocol was reviewed and approved by the institutional review board at each study site. This study is registered with the Cochrane Renal Group Registry, no. CRG020800134.

Study Design

This was a multicenter, open-label study conducted at eight centers in Japan between January 2008 and August 2009. The study period consisted of a 30-day screening period, a 12-week dose-titration phase, a 26-week maintenance phase, and a 14-week efficacy-assessment phase. Patients were divided into two groups according to the parathyroid gland volume at baseline: group S with all glands smaller than 500 mm^3 and group L with one or more enlarged glands larger than 500 mm^3. All study subjects received cinacalcet hydrochloride (Regpara; Kyowa Hakko Kirin, Co., Ltd., Tokyo, Japan) in an attempt to achieve target ranges specified in the Japanese Society for Dialysis Therapy (JSDT) guideline (calcium, 8.4 to 10.0 mg/dl; phosphorus, 3.5 to 6.0 mg/dl; intact PTH, 60 to 180 pg/ml) (30).

The initial dose of cinacalcet was 25 mg, given orally, once daily. The doses were increased to the next sequential daily dose in the series 25, 50, 75, and 100 mg after at least a 3-week interval if their intact PTH level was >180 pg/ml at the previous study visit, unless serum calcium was <8.4 mg/dl. If a patient's intact PTH level dropped below 60 pg/ml or serum calcium dropped below 7.5 mg/dl, the dose was reduced to the next lowest dose level. The dose of vitamin D sterols were increased only if the serum calcium remained below 8.4 mg/dl or if hypocalcemic symptoms persisted despite an increase in the dose of calcium carbonate to 3000 mg daily. Reductions in the doses of vitamin D sterols were permitted in cases in which the serum calcium was >10.0 mg/dl. No restrictions were imposed on the dose or type of phosphate binders used.

Biochemical Determinations

Blood samples were collected at the start of the dialysis session following the longest interdialytic period. Serum levels of intact PTH, calcium, and phosphorus were measured at local laboratories. Serum intact PTH levels were determined using an electrochemiluminescence immunoassay (Elecsys PTH; Roche Diagnostics, Mannheim, Germany). Serum calcium levels were corrected for albumin concentration using the modified Payne method (31).

Parathyroid Gland Imaging

High-resolution ultrasonography was performed by experienced technicians in each center at baseline, week 26, and week 52, according to a standardized protocol using a 7.5 or 10 MHz linear array transducer. Parathyroid gland volume was estimated using the ellipsoid formula ($\pi/6 \times a \times b \times c$, where a, b, and c represent the diameters of the gland in three dimensions).

Study End Points

The primary study end point was the proportion of patients with a mean intact PTH level <180 pg/ml during the efficacy-assessment phase. Secondary end points included the proportion of patients with a >30% reduction from baseline in intact PTH levels, the percent change from baseline in intact PTH, calcium, and phosphorus concentrations, and the change from baseline in parathyroid gland volume, as evaluated by ultrasonography. Safety was evaluated by reports of adverse events.

Historical Controls

The results of this prospective trial were also compared with those of historical controls who had been treated with traditional therapy alone. Patients who met the same eligibility criteria as trial participants and had undergone ultrasonography twice at an interval of 1 year between 2000 and 2008 were recruited as historical controls through a retrospective chart review.

Statistical Analysis

All values are expressed as mean ± SD or mean ± SEM, as indicated. Baseline laboratory and parathyroid gland size measurements were obtained and patient characteristics were recorded during the screening period. A comparison of baseline data between groups was performed with χ^2 tests and one-way ANOVA followed by the Bonferroni *post hoc* test. Mean values for intact PTH, calcium, and phosphorus during the efficacy-assessment phase were used to evaluate efficacy. Patients who withdrew from the study during the dose-titration phase were considered to not have met either the primary or the secondary end points. For patients who did not have any values measured during the efficacy-assessment phase, the last-observation-carried-forward method was used. Comparisons of proportions between groups were performed with the Fisher exact test. For comparison of paired proportions, the McNemar test was used. Logistic regression analysis was used to examine whether the presence of at least one enlarged gland was a predictor of response to cinacalcet therapy. Differences in parathyroid gland volume at baseline and at week 26 and week 52 were analyzed on the basis of the as-treated analysis, using repeated measures ANOVA followed by the Bonferroni *post hoc* test. Absolute changes in parathyroid gland volume from baseline to week 52 in cinacalcet trial participants were compared with those in historical controls using t test. The likelihood of achieving a >30% reduction from baseline in total parathyroid volume was compared between the cinacalcet group and historical controls using logistic regression analyses, stratified by age, sex, duration of dialysis, baseline biochemical vari-

ables, the presence of enlarged glands, or the use of intravenous vitamin D. Pearson correlation coefficient analyses were used to examine the relationships between each parameter. A 10-μg dose of maxacalcitol was considered to be equal to 1.5 μg of calcitriol, and all results for injectable vitamin D sterol dosages are presented as calcitriol equivalents. $P < 0.05$ was considered statistically significant. All analyses were performed using Dr. SPSS II for Windows, version 11.01 J (SPSS Japan, Tokyo, Japan).

Results

Study Population

A total of 81 patients were enrolled in the prospective trial: 56 had parathyroid glands smaller than 500 mm^3 (group S) and 25 had at least one enlarged gland larger than 500 mm^3 (group L). For the historical control group, 87 patients were identified from a chart review: 65 had small glands only (group HS) and 22 had one or more enlarged glands (group HL). Demographic characteristics and baseline laboratory values according to parathyroid gland size are presented in Table 1. The baseline demographics and renal history did not differ significantly between groups. Nearly all patients were receiving vitamin D sterols and phosphate binders, with little difference between groups in the types of agents used. At baseline, patients in group L and group HL had significantly higher calcium and intact PTH levels compared with those in group S and group HS, respectively. The proportion of patients achieving the target calcium level was not significantly greater in group S than in group L (75 *versus* 56%; $P = 0.08$), whereas that of phosphorus did not differ between the two groups (54 *versus* 52%; $P = 0.54$).

Ninety-six percent of patients in group S and 92% of those in group L completed the 12-week dose-titration phase; 91 and 80%, respectively, completed the 26-week maintenance phase; and 88 and 76%, respectively, completed the 14-week efficacy-assessment phase. Reasons for early discontinuation for patients in group S *versus* those in group L included the following: adverse events (4 *versus* 12%), administrative decisions (4 *versus* 8%), death (4 *versus* 0%), and loss to follow-up (2 *versus* 4%). None of the deaths were considered related to treatment.

Table 1. Demographics, baseline laboratory values, and parathyroid gland size

Characteristic	Cinacalcet Trial Participants		Historical Controls		*P*
	Group S ($n = 56$)	Group L ($n = 25$)	Group HS ($n = 65$)	Group HL ($n = 22$)	
Age (years)	62 ± 11	66 ± 12	61 ± 10	60 ± 7	0.11
Gender (%)					
men	64	60	63	64	0.99
women	36	40	37	36	
Duration of dialysis (months)	194 ± 108	162 ± 79	204 ± 117	229 ± 89	0.18
Primary cause of renal failure (%)					
glomerulonephritis	63	80	60	86	0.35
diabetes	18	8	11	0	
hypertension	2	4	3	5	
pyelonephritis	4	4	0	0	
polycystic kidney disease	4	0	3	0	
others	7	4	17	9	
unknown	4	0	6	0	
Use of vitamin D sterols (%)	93	84	94	100	0.19
maxacalcitol	48	52	45	68	0.29
calcitriol	23	20	8	0	0.014
oral vitamin D	21	12	42	32	0.018
Use of phosphate binders (%)	91	96	88	86	0.62
calcium carbonate	59	40	82	64	0.001
sevelamer hydrochloride	68	72	49	36	0.015
Dialysate calcium (%)					
2.5 mEq/L	62	52	23	5	<0.001
3.0 mEq/L	38	48	77	95	
Intact PTH (pg/ml)	508 ± 187	765 ± 468	554 ± 292	726 ± 270	<0.001
Serum calcium (mg/dl)	9.7 ± 0.5	10.0 ± 0.5	9.3 ± 0.8	9.9 ± 0.3	<0.001
Serum phosphorus (mg/dl)	6.0 ± 1.1	6.0 ± 1.6	6.3 ± 1.2	6.0 ± 1.2	0.55
Parathyroid gland volume (mm^3)					
total volume	377 ± 240	1479 ± 1029	343 ± 268	1076 ± 349	<0.001
volume of the largest gland	231 ± 130	1065 ± 1034	216 ± 154	667 ± 181	<0.001

Plus-minus values are mean ± SD.

Achievement of Intact PTH Targets

Mean intact PTH levels declined progressively both in group S and in group L during the first 8 weeks and reached a plateau by the end of the study (Figure 1A). During the efficacy-assessment phase, 46% of patients in group S and 32% of those in group L reached the primary end point of a mean intact PTH level <180 pg/ml; this difference was not statistically significant ($P = 0.17$; Figure 2A). The percent change in intact PTH levels was comparable between the two groups throughout the study (Figure 1B). During the efficacy-assessment phase, mean intact PTH decreased by 55% from baseline in group S and by 58% in group L (Figure 2B). The proportions of patients whose intact PTH levels decreased by >30% did not differ between the two groups (88 *versus* 76%; $P = 0.16$; Figure 2A). Logistic-regression analysis showed that the presence of one or more enlarged glands at baseline did not predict either the likelihood of achieving the primary end point (odds ratio, 0.54; 95% confidence interval, 0.20 to 1.46) or the secondary end point of a reduction in mean intact PTH level by >30% (odds ratio, 0.45; 95% confidence interval, 0.13 to 1.52).

Other Biochemical Parameters

After cinacalcet initiation, mean calcium levels in both groups transiently declined and remained stable throughout the study, with slightly higher levels in group L than in group S (Figure 1C). Serum phosphorus levels decreased slightly during the first 8 weeks and gradually increased thereafter in both groups (Figure 1D). The percent changes in serum calcium and

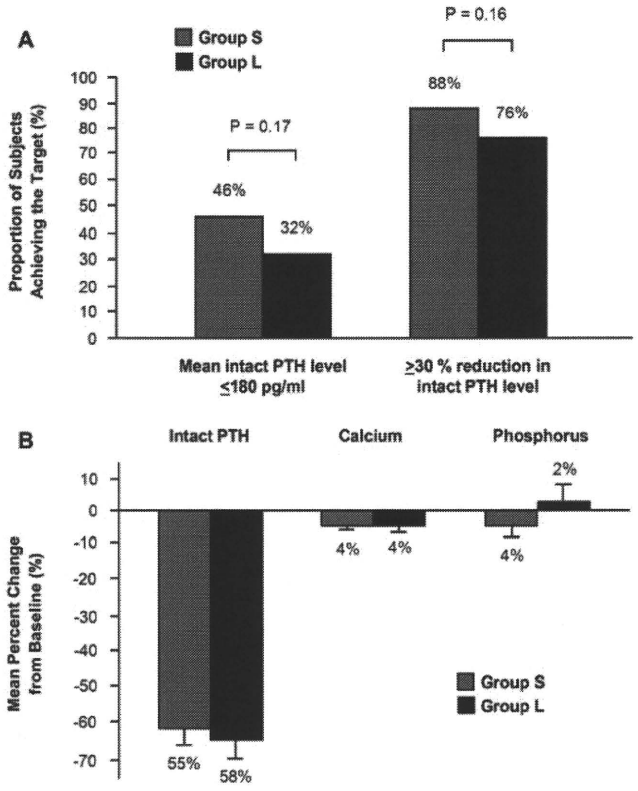

Figure 2. (A) Proportion of patients with serum intact PTH <180 pg/ml or >30% reduction in the PTH level from baseline. (B) Mean (±SEM) percent change from baseline to assessment values for intact PTH, calcium, and phosphorus.

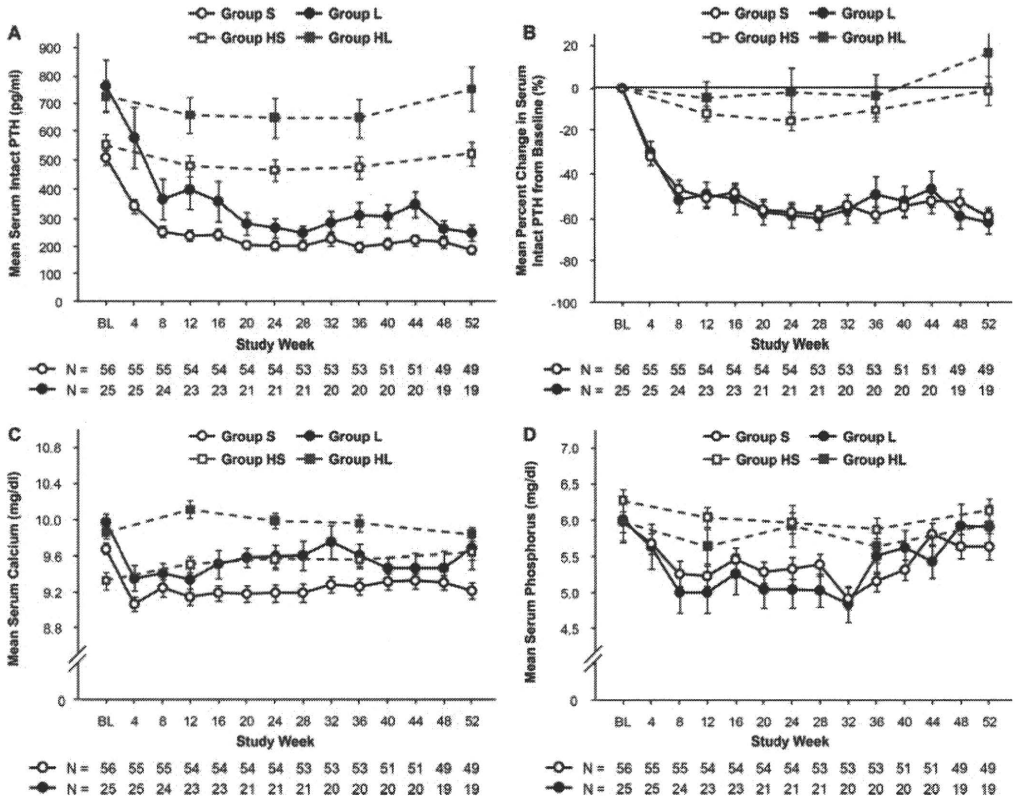

Figure 1. Mean (±SEM) serum intact PTH (A), mean (±SEM) percent change in the PTH level from baseline at each time point (B), mean (±SEM) serum calcium (C), and mean (±SEM) serum phosphorus (D). BL, baseline.

phosphorus levels from baseline during the efficacy-assessment phase were comparable between group S and group L (Figure 2B). The proportion of patients achieving the target calcium and phosphorus levels remained unchanged from baseline in both groups: 84% of patients in group S achieved the calcium target, compared with 56% in patients in group L (group S *versus* group L; $P = 0.009$), and 59% of patients in group S achieved the phosphorus target, compared with 60% in patients in group L (group S *versus* group L, $P = 0.56$). The proportion of subjects who achieved simultaneous control of PTH, calcium, and phosphorus was significantly higher in group S than in group L (23 *versus* 4%; $P = 0.029$).

Effect of Cinacalcet on Parathyroid Gland Volume

A total of 213 parathyroid glands were detected by ultrasonography at baseline. Among these, 179 glands in 65 patients were followed at 26-week intervals until the end of the study. Nine glands disappeared during the study. Overall, treatment with cinacalcet was associated with significant reductions in the total volume of each patient's glands: mean ± SD at baseline, week 26, and week 52 were 649 ± 490 mm^3, 552 ± 468 mm^3, and 527 ± 462 mm^3, respectively ($P = 0.003$). The percent change from baseline at week 52 in total gland volume was significantly correlated with reduced intact PTH levels ($r = 0.30$, $P = 0.02$). There was also a significant correlation between the absolute change in total gland volume and the absolute change in intact PTH levels ($r = 0.37$, $P = 0.002$).

The volume of each parathyroid gland also decreased significantly during the study: mean ± SD at baseline, week 26, and week 52 were 236 ± 272 mm^3, 200 ± 266 mm^3, and 191 ± 252 mm^3, respectively ($P < 0.001$). With stratification by baseline gland volume, significant volume reductions were observed both in glands with baseline volume >500 mm^3 (780 ± 357 mm^3, 627 ± 468 mm^3, and 571 ± 453 mm^3 at baseline, week 26, and week 52, respectively; $P = 0.01$) and in glands with baseline volume <500 mm^3 (152 ± 116 mm^3, 134 ± 128 mm^3, and 132 ± 129 mm^3 at baseline, week 26, and week 52, respectively; $P = 0.02$; Figure 3). At baseline, there was a significant correlation between total gland volume and intact PTH levels ($r = 0.23$, $P = 0.04$), but this correlation disappeared at the end of the study. Exclusion of the disappeared glands during the study did not change these results.

Medication Use

At the end of the study, 96% of subjects in group S and 89% of those in group L received daily doses of either 25 or 50 mg of cinacalcet. The mean ± SD dose of cinacalcet in group L was slightly, but not significantly, higher than that in group S (36.7 ± 14.5 mg/d *versus* 42.8 ± 20.9 mg/d; $P = 0.19$; Table 2). At baseline, the mean ± SD dose of intravenous vitamin D sterols, expressed as calcitriol equivalents, was significantly higher in group L compared with that in group S (2.18 ± 1.07 μg/d *versus* 3.06 ± 1.65 μg/d; $P < 0.01$), and the use and dose of vitamin D sterols remained relatively unchanged in both groups throughout the study. The use of calcium carbonate in group S increased gradually throughout the study, whereas such changes were transient among patients in group L. The dose of calcium carbonate remained constant in both groups. The proportion of patients receiving sevelamer hydrochloride remained constant in both groups during the study, with a slight decrease in the mean daily dose.

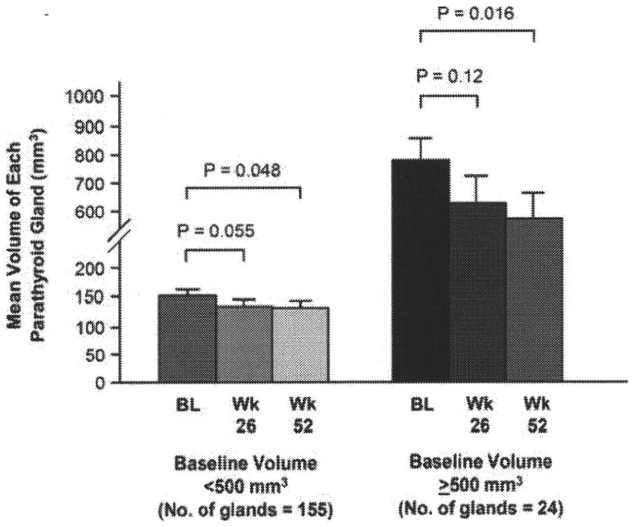

Figure 3. Mean (±SEM) parathyroid gland volume, estimated by ultrasonography, before and after 26 and 52 weeks of treatment with cinacalcet, stratified according to the baseline gland volume. BL, baseline.

Safety

A total of 39% of patients in group S and 52% of those in group L experienced at least one adverse event (Table 3). The most common adverse events were stomach discomfort, nausea, and vomiting, all of which tended to occur more frequently in group L than in group S. Gastrointestinal events were typically of mild to moderate severity, but 5% of patients discontinued cinacalcet use because of these adverse effects. Hypocalcemia (serum calcium <7.5 mg/dl) occurred exclusively in patients belonging to group S (7%), and one patient was withdrawn from the study. There were three deaths during the study, but none were attributed to cinacalcet treatment.

Comparison with Historical Controls

Mean intact PTH, calcium, and phosphorus levels in historical controls remained relatively unchanged during the retrospective period, both in group HS and in group HL (Figure 1). At the end of follow-up, only 8% of patients in group HS achieved an intact PTH <180 pg/ml whereas no patients achieved this in group HL.

The mean ± SD volume of each parathyroid gland in historical controls increased significantly in glands with baseline volume <500 mm^3 (133 ± 135 mm^3 and 183 ± 229 mm^3 at baseline and week 52, respectively; $P < 0.001$) and not significantly in glands with baseline volume >500 mm^3 (655 ± 172 mm^3 and 695 ± 275 mm^3 at baseline and week 52, respectively; $P = 0.31$) during the 52-week observation period. These changes were in sharp contrast to those of cinacalcet trial participants in whom parathyroid gland volume progressively decreased regardless of pretreatment parathyroid size (group S

Table 2. Use of cinacalcet hydrochloride and concomitant medications for secondary hyperparathyroidism

	Group S				Group L			
	Baseline (n = 56)	Week 12 (n = 55)	Week 26 (n = 53)	Week 52 (n = 49)	Baseline (n = 25)	Week 12 (n = 24)	Week 26 (n = 21)	Week 52 (n = 19)
Cinacalcet hydrochloride (mg/d)		29.3 ± 9.9	32.7 ± 13.2	36.7 ± 14.5		28.7 ± 10.0	35.7 ± 19.9	42.8 ± 20.9
Intravenous vitamin D sterols								
subjects (%)	71	65	62	65	72	54	52	68
calcitriol dose equivalents[a] (μg/wk)	2.18 ± 1.07	2.28 ± 1.16	2.32 ± 1.25	2.39 ± 1.33	3.06 ± 1.65	3.11 ± 1.37	2.86 ± 1.30	3.32 ± 2.00
Oral vitamin D sterol use (%)	21	25	25	29	12	21	29	32
Calcium carbonate								
subjects (%)	59	73	79	82	40	75	62	53
calcium carbonate dose (mg/d)	2506 ± 1067	2737 ± 1144	2779 ± 1370	2755 ± 1408	2030 ± 868	2044 ± 805	2023 ± 831	2150 ± 1156
Sevelamer hydrochloride								
subjects (%)	68	64	64	65	72	67	67	63
sevelamer hydrochloride dose (mg/d)	3026 ± 1048	3100 ± 980	2977 ± 1019	2930 ± 1069	3167 ± 1222	2878 ± 1224	2942 ± 1351	2625 ± 1517

Plus-minus values are mean ± SD.
[a]1.5 μg of calcitriol = 10 μg of maxacalcitol.

Table 3. Adverse events in >3% of patients

Events	All patients (n = 81), %	Group S (n = 56), %	Group L (n = 25), %
At least one adverse event	43	39	52
Stomach discomfort	16	13	24
Nausea	9	4	20[a]
Vomiting	5	2	12
Diarrhea	5	4	8
Muscle cramp	4	4	4
Hypocalcemia	4	7	0

[a]$P < 0.05$ *versus* group S.

versus group HS, $P < 0.001$; group L *versus* group HL, $P = 0.003$; Figure 4). The likelihood of achieving a >30% reduction in total gland volume was greater among cinacalcet-treated patients than historical controls (odds ratio, 5.05; 95% confidence interval, 2.07 to 12.31) and was consistent across subgroups stratified by age, sex, duration of dialysis, baseline biochemical variables, the presence of enlarged glands, or the use of intravenous vitamin D (Figure 5).

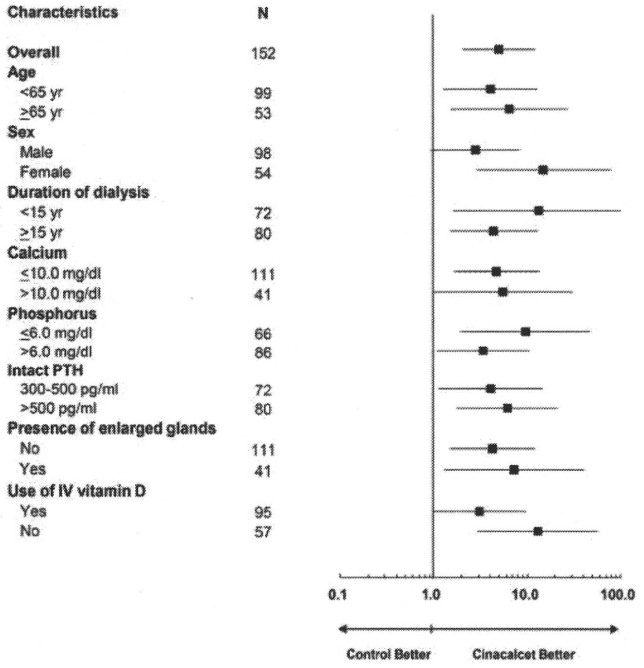

Figure 5. Stratified analysis of the likelihood of achieving a >30% reduction in total gland volume in cinacalcet-treated patients as compared with historical controls.

Discussion

In this multicenter, open-label study, treatment with cinacalcet effectively reduced serum PTH levels in hemodialysis patients with moderate to severe SHPT, even in those who had markedly enlarged parathyroid glands. This treatment resulted in a rapid decline in serum PTH levels by >50% in both groups with or without enlarged glands, and this response was sustained over 52 weeks. The proportion of patients who achieved intact PTH <180 pg/ml was slightly greater in group S compared with group L, but this was not statistically significant. We also observed a significant reduction in parathyroid gland volume during treatment with cinacalcet regardless of pretreatment size. Analysis of additional historical controls showed that in patients with SHPT the volume of parathyroid gland progressively increased with traditional therapy alone, which further highlights the effect of cinacalcet to induce a reduction in parathyroid gland volume.

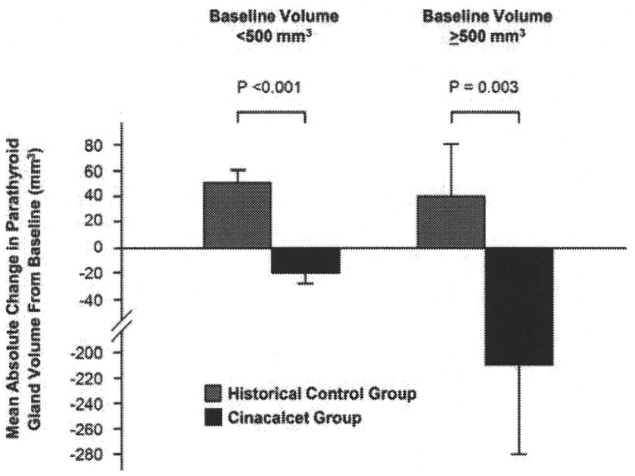

Figure 4. Mean (±SEM) absolute change in parathyroid gland volume from baseline to week 52 in historical controls and cinacalcet trial participants.

It is well known that, in patients with SHPT, the presence of parathyroid enlargement is a major determinant of response to treatment with vitamin D sterols (11–13). This finding is attributed to the observation that most of the enlarged glands removed from severe SHPT patients exhibit a nodular pattern of hyperplasia and decreased VDR expression (6,9,10), which could render the parathyroid glands less responsive to the biologic actions of vitamin D. In contrast, the present study showed that treatment with cinacalcet, an allosteric activator of the CaSR, is effective in suppressing PTH secretion even in patients with enlarged glands, in which the expression of CaSR is likely to be markedly decreased (7–9). This is not, however, surprising because experimental studies have shown that CaSR signaling to suppress PTH secretion is largely preserved *in vitro*, even in the setting of reduced CaSR expression (32). Cinacalcet therapy has also been shown to upregulate the expressions of VDR (33) and CaSR (34), which may facilitate the inhibitory effects of vitamin D sterols and cinacalcet on PTH secretion in these patients.

In this study, we also observed a significant reduction in parathyroid gland volume concomitantly with PTH suppression during treatment with cinacalcet. Such a reduction in parathyroid gland volume has also been reported by Meola *et al.* (26). However, contrary to their results, the current study demonstrated that a reduction in parathyroid gland volume by cinacalcet could occur even in enlarged glands with a baseline volume larger than 500 mm^3, further supporting the effective-

ness of cinacalcet in advanced parathyroid hyperplasia. There are two possible mechanisms for the reduction in parathyroid gland volume during therapy with cinacalcet. One possibility is a reduction in cell volume in response to decreased demand for PTH synthesis. Support for this hypothesis was provided by an experimental study by Chin *et al.*, which showed that, in uremic rats treated with calcimimetics, the reduction in parathyroid volume was attributable solely to a decrease in volume of the parathyroid cells (27). Although cell hypertrophy is considered to play only a minor role compared with cell proliferation in the pathogenesis of parathyroid enlargement (1), the observed degree of reduction in parathyroid size in the current study could be sufficiently explained by a reduction in cell hypertrophy. The other possibility is a reduction in cell number as a result of increased apoptosis. The effects of cinacalcet on parathyroid cell apoptosis, however, appear to be inconsistent depending on the experimental model. Mizobuchi *et al.* showed that high concentrations of calcimimetics induce apoptosis in parathyroid cells from uremic rats *in vitro* (34), whereas several investigators were not able to detect apoptotic parathyroid cells *in vivo* in calcimimetic-treated uremic rats (35,36). In clinical settings, the use of cinacalcet for treating SHPT has been linked with increased oxyphil/chief cell ratios (37) and cystic degeneration (26). The question of whether these morphologic changes were caused by the apoptosis of parathyroid cells is intriguing and worthy of further investigation.

The results of the current trial highlight the effectiveness of cinacalcet in patients with advanced parathyroid hyperplasia. This supports the notion that cinacalcet therapy can be an alternative to parathyroidectomy for these patients. However, it should be noted that, in our study, the proportion of subjects who achieved simultaneous control of PTH, calcium, and phosphorus were significantly lower in patients with enlarged glands compared with those without. Adverse gastrointestinal events also occurred more frequently in patients with enlarged glands. Given that some patients develop resistance to cinacalcet therapy and need to be referred for surgery (37,38), the observed difficulty in managing mineral metabolism and the limited use of cinacalcet because of safety concerns might imply future resistance to cinacalcet in these patients. Studies of much longer duration will be required to examine this possibility.

The strength of this study lies in its prospective design, long follow-up period, uniform data acquisition, and standardized ultrasonography protocol. Furthermore, it should be acknowledged that our study protocol permitted alteration of vitamin D dosage only on the grounds of patient safety, resulting in a relatively constant dosage of vitamin D sterols during the study period. This enabled us to isolate the effects of cinacalcet on parathyroid hyperplasia from those of vitamin D sterols. Our results, therefore, support the hypothesis that cinacalcet directly induces a reduction in parathyroid gland volume, although this effect might be partly mediated by cinacalcet-induced upregulation of parathyroid VDR (33).

There are, however, several limitations that should be considered. First, historical controls were used to evaluate the effect of cinacalcet on parathyroid gland volume. This could result in biases in patient selection and data collection, and thus it can be argued that certain baseline differences may have favored the cinacalcet-treated group. Second, the sample size was small, and the distribution of subjects between groups was unbalanced. These limitations may have resulted in low statistical power to detect differences between groups. Third, the study protocol was designed according to the JSDT guideline for the management of SHPT (30); thus, the cinacalcet dosage was increased to achieve intact PTH levels <180 pg/ml, a relatively lower value compared with that of previous studies (18–20) and the target range of the KDIGO guideline (39). Such aggressive suppression of PTH secretion might have facilitated the effect of cinacalcet on parathyroid gland volume in our study, but further studies are needed to examine whether treatment to achieve this level of PTH results in improved outcome. Finally, the evaluation of parathyroid gland volume was performed solely by neck ultrasonography. Interoperator coefficients of variation were not studied and are therefore unavailable. According to the reported sensitivity of ultrasonography in SHPT (40), a certain number of parathyroid glands, particularly small ones, might be missed by ultrasound study. It is also possible that a few patients with an ectopic, enlarged parathyroid gland were misclassified into group S or group HS.

Conclusions

Cinacalcet is effective in lowering serum PTH levels and concomitantly inducing a reduction in parathyroid gland volume in moderate to severe SHPT, even in patients with enlarged parathyroid glands. Whether the reduction in parathyroid gland volume induced by cinacalcet results in the long-term controllability of SHPT and an improvement in clinical outcomes warrants further investigation.

Acknowledgments

The authors thank the following investigators who participated in the study: Dr. Kazuko Itoh, Dr. Kazutaka Matsushita, and Dr. Makoto Sakai. The authors also thank Mr. Kensuke Moriwaki for assistance in the statistical analysis and Mr. Yasushi Shimizu and Ms. Chiemi Suzuki for their valuable technical assistance. This study was supported in part by grants from the ROD-21 Clinical Research Group (to S.N.) and The Kidney Foundation, Japan (JKFB08-29, to A.F.). Preliminary results of this study were presented in part at the World Congress of Nephrology in Milan on May 24, 2009.

Disclosures

M.F. has received lecture fees and a grant from Kyowa Hakko Kirin Co., Ltd., Japan, and Chugai Pharmaceutical Co., Ltd., Japan, and was a member of the advisory committee on clinical trials of cinacalcet in Japan. The other authors have no conflicts of interests.

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Therapeutic Apheresis and Dialysis

Therapeutic Apheresis and Dialysis 14(4):417–423
doi: 10.1111/j.1744-9987.2010.00813.x

Association Between Indoxyl Sulfate and Skeletal Resistance in Hemodialysis Patients

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Abstract: Skeletal resistance to parathyroid hormone (PTH) in uremia is known, although the mechanism of resistance is not fully elucidated. To clarify the roles of indoxyl sulfate, which is a uremic toxin, in skeletal resistance, we examined the relationship between indoxyl sulfate and biochemical markers of bone turnover in hemodialysis patients. We obtained blood samples from 47 hemodialysis patients and measured serum indoxyl sulfate, intact PTH, oxidative stress marker 8-hydroxy-2′-deoxyguanosine (8-OHdG), and various biochemical markers. The serum concentrations of alkaline phosphatase (ALP) and bone-specific alkaline phosphatase (BAP) were used as bone formation markers, and the concentration of tartrate-resistant acid phosphatase 5b (TRACP-5b) was used as a bone resorption marker. Serum indoxyl sulfate levels were much higher in hemodialysis patients than healthy subjects. Multiple regression analysis shows that indoxyl sulfate correlated negatively with ALP ($\beta = -1.897$, $P = 0.042$) and BAP ($\beta = -0.310$, $P = 0.029$), independent of intact PTH; however, indoxyl sulfate did not correlate with TRACP-5b or 8-OHdG. These findings suggest that indoxyl sulfate may relate skeletal resistance to PTH in uremia. **Key Words:** Bone turnover, Hemodialysis, Indoxyl sulfate, Oxidative stress, Parathyroid hormone resistance.

The effect of parathyroid hormone (PTH) on bone is down-regulated in uremia; therefore, PTH levels two to three times higher than the upper limit of normal range are required to keep bone turnover within a normal range in dialysis patients (1). This impaired PTH function in uremia is termed "skeletal resistance to PTH".

The mechanism of PTH resistance in uremia involves several factors: the reduction of PTH receptor in bones (2–5), 7–84 PTH (6,7), uremic toxin (8–10), osteoprotegerin (OPG) (11,12), and a decrease in bone morphogenetic protein-7 (BMP-7) (13–15). Indoxyl sulfate, a uremic toxin, is regarded as a factor that induces skeletal resistance to PTH. It is known to increase in patients with chronic kidney disease (16) and is considered to accelerate the progression of chronic kidney disease (17), atherosclerosis (18), and cardiac damage (19) through oxidative stress.

As a mechanism of skeletal resistance to PTH, we have previously demonstrated that uptake of indoxyl sulfate by osteoblasts occurs via the organic anion transporter (OAT) 3 and augments oxidative stress, thereby impairing osteoblast function and down-regulating PTH receptor expression (9). Down-regulation of PTH receptor mRNA in osteoblasts along with the development of renal dysfunction has been shown in a rat model (3,5). The administration of an oral charcoal adsorbent, which inhibits the accumulation of indoxyl sulfate, has been shown to reverse the down-regulation of *PTH* receptor gene in osteoblasts and improve low bone turnover disease (8). In humans, osteoblast PTH receptor mRNA in end-stage renal failure has been down-regulated in comparison to healthy individuals (4); however, no study has yet investigated the relationship between indoxyl sulfate and skeletal resistance to PTH in patients with renal dysfunction. In this study we

Received June 2009; revised December 2009.
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examine the relationship between indoxyl sulfate and biochemical markers of bone turnover in hemodialysis patients to determine whether indoxyl sulfate is involved in skeletal resistance to PTH in human.

PATIENTS AND METHODS

Patient selection

We enrolled patients from a single dialysis unit (Hara Genitourinary Hospital, Kobe, Japan). Medically stable patients undergoing hemodialysis for at least one year were selected as candidates for this study. Diabetic patients and patients taking vitamins C or E were excluded from this study. Written consent was obtained from all participants prior to their enrolment in the study, and the study was approved by the Local Ethics Committees. Forty-seven patients fulfilling the eligibility criteria participated in the study.

Laboratory methods

Blood samples were obtained from the participants after an overnight fast before dialysis, two days after the last hemodialysis treatment. Serum was separated by centrifugation immediately after the blood samples were collected and aliquots stored at –80°C. Hemoglobin, albumin, creatinine, urea nitrogen, calcium, phosphate, alkaline phosphatase (ALP), and C-reactive protein (CRP) were measured by standard laboratory techniques using automatic analyzers. We measured the serum levels of intact PTH by an electro-chemiluminescence immunoassay (Roche-Diagnostics, Basel, Switzerland), serum levels of bone-specific alkaline phosphatase (BAP) by an enzyme immunoassay (Quidel Corporation, San Diego, CA, USA), serum levels of tartrate-resistant acid phosphatase 5b (TRACP 5b) by a novel fragment absorbed immunocapture enzyme assay (FAICEA) method using two monoclonal antibodies (Nitto Boseki, Fukushima, Japan), serum levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) using a commercially available competitive ELISA kit (Japan Institute for Control of Aging, Shizuoka, Japan) by diluting the samples, and serum levels of indoxyl sulfate by a high-performance liquid chromatography technique (Fushimi Pharmaceutical, Kagawa, Japan). The ELISA kit can measure 8-OHdG values ranging from 0.125 to 10 ng/mL using a monoclonal specific antibody, N45.1 (20).

Statistical analysis

All data are represented as mean ± SD or frequency (%). We conducted univariate and multivariate linear regression analyses to evaluate factors influencing biochemical markers of bone turnover (ALP, BAP, and TRACP 5b) and 8-OHdG. In multiple regression analysis, indoxyl sulfate, gender, age, duration of hemodialysis, and variables that achieved statistical significance ($P < 0.05$) in univariate analysis were included. Comparison of 8-OHdG between patients using a cellulose membrane and those using polymethylmethacrylate (PMMA) or polysulfone (PS) membranes was performed with the Student's t-test. The value $P < 0.05$ was considered to be statistically significant. All analyses were performed with SPSS II for Windows, version 11.01J (SPSS, Chicago, IL, USA).

RESULTS

Patient characteristics

The clinical characteristics of the study population are shown in Table 1. Sixty-eight percent of the participants were male and the average age was 61.5 ± 11.7 years. All women were postmenopausal. The mean duration of hemodialysis was 18.1 ± 5.4 years. Average serum levels of indoxyl sulfate in the participants were 37.6 ± 16.5 μg/mL, which were much higher than those in healthy subjects previously reported (21). Eighteen patients used a cellulose membrane and twenty-nine patients used synthetic (PMMA or PS) membranes.

TABLE 1. *Clinical characteristics of the study population*

	All patients ($N = 47$)
Age (years)	61.5 ± 11.7 (range 30–83)
Male, N (%)	32 (68%)
Duration of HD (years)	18.1 ± 5.4 (range 11.0–31.4)
HD time (hours per week)	12.2 ± 0.5
Hemoglobin (g/dL)	10.5 ± 1.1
Albumin (g/dL)	3.8 ± 0.4
Creatinine (mg/dL)	11.9 ± 3.0
Urea nitrogen (mg/dL)	71.1 ± 14.0
Ca (mg/dL)	9.3 ± 0.6
P (mg/dL)	5.8 ± 1.3
Intact PTH (pg/mL)	253 ± 270
ALP (IU/L)	291 ± 115
BAP (U/L)	36.8 ± 15.9
TRACP 5b (mU/dL)	193 ± 147
CRP (mg/dL)	0.17 ± 0.16
8-OHdG (ng/mL)	0.82 ± 0.25
Indoxyl sulfate (μg/mL)	37.6 ± 16.5
Type of dialysis membrane	
Cellulose, N (%)	18 (38%)
PMMA, N (%)	12 (26%)
Polysulfone, N (%)	17 (36%)

ALP, alkaline phosphatase; BAP, bone-specific alkaline phosphatase; CRP, C-reactive protein; HD, hemodialysis; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; P, phosphate; PMMA, polymethylmethacrylate; PTH, parathyroid hormone; TRACP 5b, tartrate-resistant acid phosphatase 5b.

TABLE 2. *Correlation between bone turnover markers and clinical variables*

	ALP		BAP		TRACP 5b	
Variables	r	*P*-value	r	*P*-value	r	*P*-value
Age (years)	0.183	0.218	0.124	0.477	−0.063	0.717
Duration of HD	0.392	0.006	0.431	0.010	0.284	0.098
Ca	−0.051	0.732	−0.095	0.587	0.235	0.174
P	−0.116	0.436	0.078	0.656	0.350	0.039
Intact PTH	0.352	0.015	0.656	<0.001	0.771	<0.001
Albumin	−0.172	0.248	−0.050	0.776	0.091	0.603
BMI	0.019	0.901	−0.045	0.799	−0.239	0.167
Indoxyl sulfate	−0.287	0.050	−0.337	0.048	−0.120	0.494

ALP, alkaline phosphatase; BAP, bone-specific alkaline phosphatase; BMI, body mass index; HD, hemodialysis; P, phosphate; PTH, parathyroid hormone; TRACP 5b, tartrate-resistant acid phosphatase 5b.

Effect of indoxyl sulfate and other clinical variables on biochemical markers of bone turnover

The correlation between biochemical markers of bone turnover (ALP, BAP, and TRACP 5b) and clinical variables in univariate analysis is shown in Table 2. A significant negative correlation was observed between indoxyl sulfate and BAP. Indoxyl sulfate also correlated negatively with ALP, although the correlation was not statistically significant. There was no correlation between indoxyl sulfate and TRACP 5b. ALP and BAP significantly correlated with the duration of hemodialysis and intact PTH. TRACP 5b significantly correlated with phosphate and intact PTH.

In multiple regression analysis, indoxyl sulfate correlated negatively with ALP (Table 3) and BAP (Table 4), independent of PTH. There was no correlation between indoxyl sulfate and TRACP 5b (Table 5). ALP correlated with the duration of hemodialysis and intact PTH. BAP also correlated with intact PTH. Furthermore, TRACP 5b correlated with intact PTH.

Effect of indoxyl sulfate and other clinical variables on 8-OHdG

The correlation between oxidative stress marker 8-OHdG and clinical variables in univariate analysis is shown in Table 6. There was no correlation between indoxyl sulfate and 8-OHdG; however, 8-OHdG correlated significantly with CRP.

All the study participants were divided into two groups based on membrane types (cellulose, $N = 18$; synthetic, $N = 29$). There was no significant difference in 8-OHdG between the two groups (cellulose vs. synthetic; 0.81 ± 0.28 vs. 0.82 ± 0.22 ng/mL, $P = 0.91$).

In multiple regression analysis, indoxyl sulfate did not correlate with 8-OHdG (Table 7). Only gender correlated significantly with 8-OHdG.

TABLE 3. *Independent effect of clinical variables on alkaline phosphatase by multiple regression analysis (adjusted $R^2 = 0.255$)*

Significant variables	β	SE	*P*-value
Male gender	15.949	34.003	0.642
Age (years)	2.298	1.334	0.093
Duration of HD (years)	6.004	2.908	0.045
Intact PTH (pg/mL)	0.128	0.057	0.031
Indoxyl sulfate (μg/mL)	−1.897	0.904	0.042

This model included gender, age, duration of hemodialysis, intact parathyroid hormone, and indoxyl sulfate. HD, hemodialysis; PTH, parathyroid hormone.

TABLE 4. *Independent effect of clinical variables on bone-specific alkaline phosphatase by multiple regression analysis (adjusted $R^2 = 0.502$)*

Significant variables	β	SE	*P*-value
Male gender	−6.766	4.402	0.135
Age (years)	0.102	0.158	0.524
Duration of HD (years)	0.476	0.449	0.298
Intact PTH (pg/mL)	0.030	0.008	<0.001
Indoxyl sulfate (μg/mL)	−0.310	0.135	0.029

This model included gender, age, duration of hemodialysis, intact parathyroid hormone, and indoxyl sulfate. HD, hemodialysis; PTH, parathyroid hormone.

TABLE 5. *Independent effect of clinical variables on tartrate-resistant acid phosphatase 5b by multiple regression analysis (adjusted $R^2 = 0.560$)*

Significant variables	β	SE	*P*-value
Male gender	−19.566	38.448	0.615
Age (years)	0.441	1.626	0.788
Duration of HD (years)	−3.609	3.915	0.365
Phosphate (mg/dL)	25.385	17.861	0.166
Intact PTH (pg/mL)	0.391	0.068	<0.001
Indoxyl sulfate (μg/mL)	−0.615	1.176	0.605

This model included gender, age, duration of hemodialysis, phosphate, intact parathyroid hormone, and indoxyl sulfate. HD, hemodialysis; PTH, parathyroid hormone.

TABLE 6. *Correlation between 8-hydroxy-2′-deoxyguanosine and clinical variables*

Variables	r	*P*-value
Age	0.317	0.064
Duration of HD	0.128	0.463
Ca	–0.228	0.187
P	–0.224	0.197
Intact PTH	0.151	0.387
Hemoglobin	–0.128	0.464
Albumin	–0.263	0.126
CRP	0.415	0.015
Indoxyl sulfate	–0.090	0.534

CRP, C-reactive protein; HD, hemodialysis; P, phosphate; PTH, parathyroid hormone.

DISCUSSION

Several mechanisms have been suggested for skeletal resistance in uremia; however, the pathophysiology of skeletal resistance to PTH has not been fully elucidated. This study demonstrated a negative correlation between indoxyl sulfate and bone formation markers in hemodialysis patients, independent of PTH. Thus, this result indicates that patients who accumulate indoxyl sulfate show suppressed bone formation. This result is in agreement with our previous findings that have demonstrated improvement in rat bone formation by preventing the accumulation of indoxyl sulfate in blood (8). The *PTH* receptor gene in osteoblasts is down-regulated depending on the concentration of indoxyl sulfate in vitro (9), and inhibiting the accumulation of indoxyl sulfate reverses the down-regulation of the *PTH* receptor gene in osteoblasts (8). Human osteoblast PTH receptor is known to be down-regulated in end-stage renal failure (4); therefore, we hypothesize that the accumulation of indoxyl sulfate in blood is a cause of the down-regulation of *PTH* receptor gene in human osteoblasts.

The effect of PTH on both osteoclasts and osteoblasts similarly decreased in uremia (22). The mechanism of osteoclast differentiation and activation has been elucidated at molecular levels recently (23). Receptor activator of nuclear factor κB ligand (RANKL), which is produced by osteoblasts, stromal cells, T cells, and other sources, binds and activates receptor activator of nuclear factor κB (RANK) on the surface of osteoclasts. RANK–RANKL interactions lead to osteoclast differentiation and activation. These interactions are prevented by OPG that binds to and thereby inactivates RANKL. Since serum OPG levels are increased in chronic kidney disease patients (11,12), high serum OPG levels are considered to result in osteoclast inactivation. Mozar et al. demonstrated that indoxyl sulfate inhibits the differentiation of osteoclast-like cells (24). We could not show the relationship between indoxyl sulfate and the bone resorption marker in this study. We previously reported that bone resorption is suppressed less than bone formation in a uremic rat model (3). Reduced suppression of bone resorption in uremia could explain why we could not observe any correlation between indoxyl sulfate and the bone resorption marker in this study. Further studies in vitro and in vivo will be necessary to clarify whether indoxyl sulfate influences osteoclast function.

Uptake of indoxyl sulfate via OAT1 and OAT3 induces oxidative stress in proximal tubular cells (17). Oxidative stress induced by indoxyl sulfate activates plasminogen activator inhibitor-1 (PAI-1) and nuclear factor-κB (NF-κB), which is related to the progression of renal disease. Furthermore, indoxyl sulfate has shown a significant positive correlation with the oxidative stress marker pentosidine in hemodialysis patients (18). We demonstrated that indoxyl sulfate increases oxidative stress in osteoblasts via OAT3 (9); however, we could not show a positive correlation between indoxyl sulfate and oxidative stress in this study. Oxidative stress correlates positively with many factors, including age, diabetes, anemia, erythropoietin (25), intravenous iron administration, ferritin (26), CRP (27), cellulose membranes (28), and malnutrition (29). Although we analyzed some factors that are related to 8-OHdG, we did not identify a relationship between 8-OHdG and all the aforementioned factors; therefore, the factors that we did not analyze may influence the relationship between indoxyl sulfate and 8-OHdG.

Gender and menopausal status correlate with bone metabolism (30–34). Serum ALP, BAP, and TRACP5b levels in women were higher than those in men, but were not statistically significant in our study (data not shown). Since the number of female participants was only 15, the statistical power was possibly not sufficient to detect the correlation between gender and ALP, BAP, or TRACP5b. Indoxyl sulfate

TABLE 7. *Independent effect of clinical variables on 8-hydroxy-2′-deoxyguanosine by multiple regression analysis (adjusted $R^2 = 0.432$)*

Significant variables	β	SE	*P*-value
Male gender	–0.306	0.074	<0.001
Age (year)	0.0005	0.003	0.852
Duration of HD (year)	0.003	0.007	0.621
CRP (mg/dL)	0.448	0.238	0.070
Indoxyl sulfate (μg/mL)	–0.002	0.002	0.354

This model included gender, age, duration of hemodialysis, C-reactive protein, and indoxyl sulfate. CRP, C-reactive protein; HD, hemodialysis.

negatively correlated with BAP and ALP in multiple regression analysis including, gender as independent variables; therefore, we believe that indoxyl sulfate negatively correlated to BAP and ALP, independent of gender.

Nutritional status possibly influences bone metabolism in hemodialysis patients (35,36). In our study, the body mass index and serum albumin levels did not correlate with biochemical markers of bone turnover. In addition, indoxyl sulfate negatively correlated with BAP and ALP when we included the nutritional status and performed multiple regression analysis (data not shown); therefore, we believe that indoxyl sulfate negatively correlates with BAP and ALP, independent of the nutritional status.

When toxins or biomolecules in hemodialysis patients are evaluated, removal by hemodialysis sometimes influences the results. Indoxyl sulfate (37), ALP, BAP (38), and TRACP5b (39) are poorly removed by dialysis. Since PTH is adsorbed by some membranes, changes in PTH during hemodialysis are influenced by dialysis membranes (40); however, since PTH has a very short half life, serum PTH levels before hemodialysis are possibly less influenced by dialysis membrane. Serum levels of 8-OHdG after hemodialysis are different according to each dialysis modality (41–44); however, there was no statistically significant correlation between the serum levels of 8-OHdG and the type of membrane employed in this study. Therefore, we believe that the type of membrane used had little effect on the serum levels of these toxins and biomolecules in this study.

Our study has several limitations. First, the sample size is relatively small; therefore, we could not analyze many factors that influence the relationship between indoxyl sulfate and skeletal resistance to PTH. However, we could demonstrate that indoxyl sulfate is negatively correlated with bone formation independent of major factors such as PTH. Second, as this was a cross-sectional study, we could not determine whether indoxyl sulfate suppresses bone formation, although we showed that there is a negative correlation between indoxyl sulfate and bone formation. To confirm that accumulation of indoxyl sulfate in blood suppresses bone formation, it is necessary to investigate whether decreasing serum levels of indoxyl sulfate improves low bone turnover in humans. Third, we did not assess additional oxidative markers and antioxidants. Fourth, we did not assess other uremic toxins—phenylacetic acid may be one of candidate toxins that can interfere with the bone metabolism (10). Further studies will be necessary to investigate the relation between other uremic toxins and skeletal resistance to PTH in uremia. Finally, we did not assess bone histomorphometric parameters obtained by bone biopsy, which is the gold standard for the assessment of bone turnover. Although BAP and TRACP 5b have been useful markers and correlated well with bone histomorphometric parameters in patients with end-stage renal failure (38,39,45–48), biochemical markers of bone turnover still have some limitations for the assessment of bone turnover (49–51). Further studies assessed by bone histomorphology would be necessary to support our results.

CONCLUSION

We demonstrated that indoxyl sulfate correlated negatively with bone formation markers independent of PTH. In contrast, indoxyl sulfate did not correlate with bone resorption and oxidative stress markers. These findings suggest that the accumulation of indoxyl sulfate in blood may induce, at least in part, low bone turnover in hemodialysis patients.

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AJKD
Original Investigation

Effect of Sevelamer and Calcium-Based Phosphate Binders on Coronary Artery Calcification and Accumulation of Circulating Advanced Glycation End Products in Hemodialysis Patients

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Background: Some trials have indicated that coronary artery calcification progresses more slowly in sevelamer-treated dialysis patients than in those using calcium-based binders. Effects of phosphate binders on circulating advanced glycation end products (AGEs) are unknown.

Study Design: Randomized trial with parallel-group design.

Setting & Participants: 183 adult (aged >20 years) patients on maintenance hemodialysis therapy at 12 dialysis facilities with a mean vintage of 118 ± 89 (median, 108) months. Dialysate calcium concentration was 2.5 mEq/L, and dietary calcium was not controlled.

Intervention: Patients were randomly assigned to 12 months of treatment with sevelamer (n = 91) or calcium carbonate (n = 92).

Outcomes & Measurements: Primary outcome measures were change from baseline in coronary artery calcification score (CACS) determined at study entry and completion using multislice computed tomography and the proportion of patients with a ≥15% increase in CACS. Blood parameters were determined at study entry and 2-week intervals, and levels of plasma pentosidine, a representative AGE, were determined at study entry, 6 months, and study completion.

Results: 79 (86.8%) and 84 (91.3%) patients in the sevelamer and calcium-carbonate arms completed the treatment, respectively. Both binders were associated with an increase in mean CACS: 81.8 (95% CI, 42.9-120.6) and 194.0 (139.7-248.4), respectively ($P < 0.001$ for both). After adjustment for baseline values, the increase in the sevelamer group was 112.3 (45.8-178) less ($P < 0.001$). Percentages of patients with a ≥15% increase in CACS were 35% of the sevelamer group and 59% of the calcium-carbonate group ($P = 0.002$). Plasma pentosidine levels increased with sevelamer treatment ($P < 0.001$). Sevelamer use was associated with decreased risk of a ≥15% increase in CACS regardless of baseline blood parameters, pentosidine level, and CACS.

Limitations: Treatment duration was relatively short, some sevelamer-treated patients (7 of 79) received calcium carbonate, and washout could not be performed.

Conclusions: The data suggest that sevelamer treatment slowed the increase in CACS and suppressed AGE accumulation.

Am J Kidney Dis. 57(3):422-431.

INDEX WORDS: Advanced glycation end products; coronary artery calcification; hemodialysis; low-density lipoprotein (LDL) cholesterol; pentosidine; sevelamer hydrochloride.

Coronary artery calcification occurs often in dialysis patients[1,2] and is predictive of cardiovascular morbidity and mortality in patients with end-stage renal disease.[3-7] Disturbed mineral metabolism assumes particular importance in vascular calcification in patients with end-stage renal disease.[8-11] Increased levels of serum phosphorus and calcium and calcium-phosphorus product in dialysis patients are associated independently with increased risk of arterial calcification[12,13] and cardiovascular mortality.[13-17] In vitro, exposure to high concentrations of phosphate, calcium, or both causes calcification of human smooth

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Received October 13, 2009. Accepted in revised form October 26, 2010. Originally published online January 18, 2011.

Trial registration: www.umin.ac.jp/ctr/; study number: UMIN000002150.

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0272-6386/$36.00
doi:10.1053/j.ajkd.2010.10.055

muscle cells[18,19] and rat aortic rings.[20] However, dietary restriction and dialysis are ineffective in controlling hyperphosphatemia, and most dialysis patients require phosphate-binder therapy.

Calcium-based phosphate binders have been the first-choice binder for dialysis patients. However, use of calcium-based phosphate binders has been questioned because calcium intake is higher in dialysis patients with coronary artery calcification than in those without,[2] and calcium-based phosphate-binder dose correlates with the severity of arterial calcification.[7,21] An option is to decrease dialysate calcium concentrations; however, adequate dialysate calcium concentrations presently are a subject of debate.[22,23] Sevelamer, a nonabsorbed mineral-free phosphate binder, decreases serum phosphorus and parathyroid hormone (PTH) levels in dialysis patients[24,25] and has decelerated the progression of coronary artery calcification[26-31] and improved mortality.[32] These effects of sevelamer have been attributed to its ability to improve key parameters, including low-density lipoprotein (LDL) cholesterol and PTH.[26-32]

Advanced glycation end products (AGEs) are a group of heterogeneous compounds formed through nonenzymatic oxidative and nonoxidative reactions between proteins and reactive carbonyl compounds derived from carbohydrates and lipids.[33] AGE levels and oxidative stress are both increased in uremic patients and have been related to cardiovascular disease.[34-36] Recently, evidence was provided that an increase in levels of plasma pentosidine, an AGE, is associated with the progression of coronary artery calcification in dialysis patients.[37,38]

The present randomized trial examined the effects of sevelamer and calcium carbonate on the progression of coronary artery calcification and plasma pentosidine concentrations in hemodialysis (HD) patients.

METHODS

Patients

Adult (aged >20 years) patients undergoing maintenance HD therapy at 12 participating dialysis facilities in Japan were enrolled. Exclusion criteria were serious gastrointestinal disease (dysphagia, active untreated gastroparesis, severe motility disorder, intestinal surgery, and markedly irregular bowel function), alcohol or drug dependence or abuse, active malignancy vasculitis, or poorly controlled diabetes or hypertension deemed by the investigator to interfere with appropriate and safe study execution.

Written informed consent was obtained from all patients before study-related procedures were performed. The study was approved by the institutional review board at each participating organization and conducted in compliance with the Declaration of Helsinki.

Study Design and Procedures

We based target sample-size calculation on data from Chertow et al[26]; namely, mean ± standard deviation coronary artery calcification score (CACS) of 151 ± 471 and −46 ± 692 after 52 weeks of treatment with calcium carbonate and sevelamer, respectively. A 2-group t test with 2-sided α error rate of 5%, 80% power, and common standard deviation of 471 estimated that 91 patients per group would allow detection of a significant difference in absolute change from baseline CACS between the sevelamer and calcium-carbonate groups.

Patients were randomly assigned at the coordinating center between April 1, 2004, and December 30, 2005, in a 1:1 fashion to open-label treatment with sevelamer hydrochloride or calcium carbonate. Investigators were informed of patient allocation using concealed envelopes. The study was completed on December 30, 2006. Sevelamer hydrochloride (Renagel, 250-mg tablets; Chugai Pharmaceutical Co Ltd [www.chugai-pharm.co.jp] or Phosblock, 250-mg tablets, Kyowa Hakko Kirin Co Ltd. [www.kyowa-kirin.co.jp]) was prescribed in the sevelamer arm. When serum phosphorus level could not be controlled to <6.5 mg/dL in the sevelamer arm, 9 g/d of sevelamer with up to 1.5 g/d of precipitated calcium carbonate was allowed. In the calcium-carbonate arm, only calcium carbonate was used. Study duration was 12 months. Multislice computed tomography (CT) was performed at study entry and completion.

Investigators were instructed to control serum calcium, phosphorus, PTH, and dyslipidemia every 2 weeks. Although this study predated publication of the National Kidney Foundation's KDOQI (Kidney Disease Outcomes Quality Initiative) clinical practice guideline for bone disease and metabolism in chronic kidney disease,[39] clinical practice included target values <10.2 mg/dL for serum calcium, <6.5 mg/dL for serum phosphorus, <65 mg^2/dL^2 for calcium-phosphorus product, and 150-300 pg/mL for PTH. Investigators were free to modify the dose of phosphate binders. In general, when serum calcium level was >10.5 mg/mL, either the calcium carbonate dose was decreased or vitamin D_3 dose was decreased or discontinued; when serum phosphorus level was >6.5 mg/dL, phosphate-binder doses were increased. During the trial, dialysate calcium concentration was 2.5 mEq/L, dietary calcium intake was not controlled, no estimate of patient adherence (pill count) was performed, and no patient received calcimimetics. Investigators and clinicians were blinded to results of multislice CT. Baseline medical conditions were based on clinical diagnoses and assessed using chart review.

Laboratory Measurements

Biochemical parameters were determined at baseline and 2-week intervals. Serum glucose, creatinine, urea, phosphorus, calcium, LDL and high-density lipoprotein cholesterol, and triglycerides were measured using an Hitachi Model 7700 (Hitachi Electronics Co Ltd, www.hitachi.co.jp). PTH was determined using an Elecsys PTH analyzer (Roche Diagnosis, www.roche.com).

Pentosidine Measurement by High-Performance Liquid Chromatography

Plasma pentosidine concentration was determined at study entry, 6 months into treatment, and at study completion. Fresh heparinized plasma samples were obtained before dialysis. Pentosidine concentrations were determined using reverse-phase high-performance liquid chromatography as described previously[40,41] with synthetic pentosidine as a standard.

Imaging Procedure

All CT was performed at the Tokai University Hospital on 1 CT scanner by 2 radiologists who were blinded to patient information. CT was performed using a 64-slice multislice CT scanner (Somatom Cardiac Sensation 64; Siemens Medical Solutions, www.siemens.com). The CT protocol consisted of electrocardiogram-

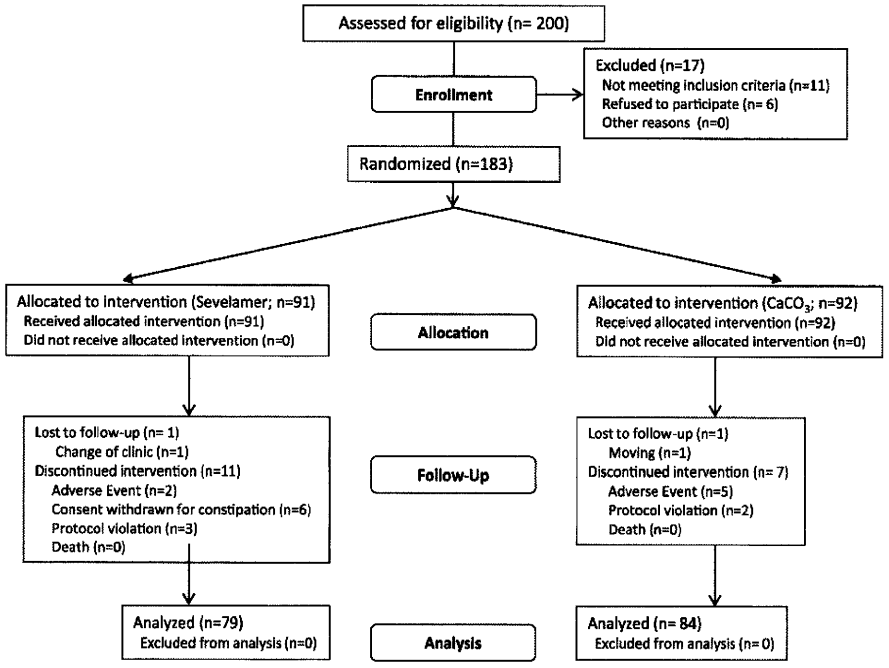

Figure 1. Patient disposition. A total of 200 hemodialysis patients initially were screened; 17 were excluded on the basis of the exclusion criteria, and 183 patients were randomly assigned to 12-month treatment with either sevelamer hydrochloride (n = 91) or calcium carbonate ($CaCO_3$; n = 92). Of those, 163 patients (79 patients in the sevelamer arm and 84 patients in the calcium-carbonate arm) completed the study.

gated acquisition of the entire heart, with the patient scanned in the supine position in the cranio-caudal direction and after deep inspiration. Acquisition parameters were collimation of 64×0.6 mm with z-flying focal spot for the simultaneous acquisition of 64 overlapping 0.6-mm slices, rotation time of 0.33 seconds, pitch of 0.3, tube voltage of 120 kV, tube-current time product of 200 mA, and slice thickness of 3.0 mm. Calcium scoring was performed by the 2 radiologists according to Agatston scoring[42] on the reconstructed image sets using commercially available software (syngo Ca Scoring; Siemens Medical Solutions) using the standard lower threshold of 130 HU.

Statistical Analysis

Continuous variables are expressed as mean ± standard deviation and compared using Student t test or Pearson χ^2 test. A last-value-carried-forward approach was used for biochemical parameters. The difference between groups in proportions of patients with a ≥15% increase in CACS was analyzed using χ^2 test. Differences in the mean change in each variable were evaluated using analysis of covariance. Logistic regression was used to examine the relationships between risk of a ≥15% increase in CACS and calcium carbonate treatment compared with sevelamer treatment. Odds ratios also were adjusted for each variable. All statistical analyses followed the intent-to-treat principle.

Changes in calcification were evaluated as absolute change in CACS (final value minus baseline value) and relative change in CACS (proportion of patients with a ≥15% increase in CACS). These 2 outcome measures were used for analyses because the former more heavily weighs patients with extensive baseline calcification and the latter carries the risk of more heavily weighing those with less extensive baseline calcification.

All probability values are 2 tailed. $P < 0.05$ is considered significant. All statistical analyses were performed using PASW Statistics 18 (SPSS Inc, www.spss.com) and R, version 2.10.1 (www.r-project.com).

RESULTS

Patients

Two hundred patients were screened; 17 were excluded on the basis of the established criteria, and 183 patients were randomly assigned to sevelamer (n = 91) or calcium-carbonate therapy (n = 92; Fig 1). Seventy-nine (86.8%) and 84 (91.3%) participants in the sevelamer and calcium-carbonate arms completed the 12 months of treatment, respectively. Of sevelamer-

Table 1. Baseline Characteristics of Study Participants

	Sevelamer	Calcium Carbonate	P
No. of participants	91	92	
Men/women	52/39	47/45	0.5
Age (y)	59 ± 12	57 ± 12	0.3
HD vintage (mo)	105 ± 84	119 ± 92	0.3
Primary cause of CKD (%)			
Diabetes	23	19	0.5
Chronic glomerular nephritis	59	57	0.9
Others	18	24	0.3
Smoking (%)	17	22	0.5
Hypertension (%)	55	59	0.7
Coronary artery disease (%)	9	5	0.2
Vitamin D_3 medication (%)	60	68	0.3
Statin medication (%)	8	11	0.6
Phosphate-binder use before study entry (%)			
Sevelamer	39	26	0.1
Calcium carbonate	25	40	0.1
Sevelamer + calcium carbonate	32	29	0.6
Other combinations	3	4	0.7

Note: Unless otherwise indicated, continuous variables expressed as mean ± standard deviation. Differences between groups were analyzed using t test for continuous variables and χ^2 test for categorical variables.

Abbreviations: CKD, chronic kidney disease; HD, hemodialysis.