ORIGINAL ARTICLE

Cholecalciferol supplementation reduces soluble Klotho concentration in hemodialysis patients

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KEY WORDS

bone metabolism, cholecalciferol, fibroblast growth factor 23, hemodialysis, Klotho

ABSTRACT

INTRODUCTION Low levels of vitamin D are linked to numerous adverse clinical conditions in hemodialysis (HD) patients, including disturbances of mineral and bone metabolism and increased mortality. Klotho, a molecule involved in such processes as phosphate homeostasis and aging, exists in 2 forms: a transmembrane protein acting as a coreceptor for fibroblast growth factor 23 (FGF-23) and soluble form, which is formed by cleavage of the extracellular domain of this molecule.

OBJECTIVES The aim of the study was to evaluate the effect of cholecalciferol supplementation on soluble Klotho levels in HD patients.

PATIENTS AND METHODS This was a prospective, open-label trial examining the effects of cholecalciferol supplementation on selected laboratory markers in 22 patients on HD. Vitamin D deficiency was assessed by the measurement of 25-hydroxyvitamin D [25(OH)D] levels. Soluble Klotho, intact FGF-23, intact parathormone (iPTH), and markers of bone formation and resorption were measured at baseline and after 12 weeks of cholecalciferol supplementation.

RESULTS The levels of 25(OH)D increased, while those of iPTH and cross-linked C-telopeptide of type 1 collagen decreased significantly. Cholecalciferol treatment reduced the median concentration of soluble Klotho (from 438.73 pg/ml; interquartile range, 257.99–865.51 pg/ml; to 370.94 pg/ml; 181.72–710.91 pg/ml; $P < 0.05$). FGF-23 levels were not affected by the treatment.

CONCLUSIONS Supplementation with cholecalciferol in HD patients decreases soluble Klotho levels without affecting the FGF-23 concentration. Replenishment of vitamin D stores results in a decrease in iPTH levels and reduced bone resorption.

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INTRODUCTION Patients with chronic kidney disease are decimated by premature death. The causes of the unacceptably high mortality in this population remain unknown. Moreover, large clinical trials did not bring any spectacular outcomes resulting in life prolongation of this vulnerable group.[1] It is thought that disturbances in bone mineral metabolism may be one of the possible culprits responsible for excessive mortality of hemodialysis (HD) patients. Recently, a lot of attention has been paid to interactions between Klotho and fibroblast growth factor 23 (FGF-23) in renal patients.

Klotho is a transmembrane protein, which forms a complex with FGF receptor 1 (FGFR1). This process increases the receptor's affinity to FGF-23, also known as phosphatonin. Formation of FGF-23, FGFR1, and Klotho complex reduces renal phosphate reabsorption and decreases the synthesis of active vitamin D and parathormone (PTH).

The extracellular part of Klotho may be shed forming soluble Klotho, which is thought to serve as a humoral factor and regulate the function of calcium channels and sodium-phosphate transporter 2a as well as inhibit insulin and insulin growth factor-1 signaling.

Interestingly, both FGF-23 and Klotho are involved in clinical states not directly connected

to the disturbances of mineral metabolism. The FGF-23 concentration is elevated in renal patients[2,3] and has been associated with left ventricular hypertrophy,[2] coronary disease extent,[4] and all-cause mortality in renal patients.[5] A polymorphism in the Klotho gene has been reported to be associated with life span, coronary artery disease, and stroke.[6-8]

Recent reports showed that a few factors used routinely during the treatment process of HD patients alter the FGF-23 level. For example, cinacalcet reduces,[9,10] and active vitamin D[11] or intravenous iron[12] increases, the concentration of this hormone.

Data regarding the effect of pharmacological agents on the Klotho level are scarce. It was reported that treatment with cinacalcet transiently reduces the Klotho concentration in HD patients.[13] Furthermore, there is evidence that at least actions of soluble Klotho may be vitamin-D dependent. Recently, de Borst et al.[14] suggested that tumor necrosis factor-α-converting enzyme (TACE or ADAMS 17), which is upregulated in vitamin D deficiency, might be involved in the cleavage of the extracellular part of Klotho and, by that, might participate in its downregulation.

Vitamin D deficiency is common among HD patients.[15] It has been linked to glucose intolerance,[16] disturbances of bone metabolism,[17] bone fractures,[18] and increased mortality.[19]

To our knowledge, the effect of nutritional vitamin D supplementation on the Klotho level in HD patients has not been studied. Therefore, the aim of this study was to evaluate the effect of cholecalciferol supplementation on FGF-23, soluble Klotho, intact PTH (iPTH), and markers of bone metabolism in HD patients.

PATIENTS AND METHODS

Study design This was a 12-week, prospective, single-center, open-label trial of prevalent HD patients. Patients enrolled in the study were in stable medical condition, were older than 18 years, had iPTH levels higher than 100 ng/l, and 25-hydroxyvitamin D [25(OH)D] levels lower than 75 nmol/l (30 ng/ml). All patients underwent hemodialysis 3 times a week for 4 to 4.5 hours a day using low-flux dialyzers with bicarbonate-buffered dialysate. Blood flow ranged from 180 to 300 ml/min with a dialysate flow at 500 ml/min. The exclusion criteria were as follows: evidence of cancer, active infection, an unstable medical condition, or hypercalcemia within the previous month. It was shown that cinacalcet, an allosteric modulator of calcium-sensing receptor, which decreases PTH production through increased sensitivity of calcium sensing receptor to ambient calcium, lowers FGF-23 concentration.[9,10] Thus its use during the preceding month and during the study period was prohibited. Serum and EDTA-plasma were collected at baseline and at 12 weeks. Prior to enrollment, all patients provided written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki. The research protocol was reviewed and accepted by the local ethics committee.

Intervention Patients received oral cholecalciferol (Juvit D3, Hasco Lek S.A., Poland), which was prescribed once weekly for 12 weeks after mid-week HD session at dialysis unit ensuring 100% adherence. Dosing was based on baseline 25(OH)D levels as described previously by Matias et al.[20]: patients with 25(OH)D levels lower than 37 nmol/l (15 ng/ml) received 50 000 IU (2.5 ml) of cholecalciferol once a week, and those with 25(OH)D levels between 37 and 75 nmol/l (15–30 ng/ml) received 10 000 IU (0.5 ml) of cholecalciferol once weekly. Serum calcium and phosphorus levels were measured monthly. During the study, doses of phosphate binders and vitamin D analogue (alfacalcidol, Alfadiol, GKS Pharmaceuticals) remained unchanged.

Laboratory measurements Serum 25(OH)D, iPTH, cross-linked C-telopeptide of type 1 collagen (CTX), and procollagen I N-terminal propeptide (PINP) were determined with electrochemiluminescence immunoassays (Elecsys Vitamin D3, PTH, Beta CrossLaps, P1NP Roche Diagnostics, Mannheim, Germany). Intact FGF-23 (Immutopics Inc., San Clemente, California, United States) and serum soluble Klotho (IBL International Gmbh, Hamburg, Germany) were measured using sandwich enzyme-linked inmunosorbent assay according to the manufacturer's protocol.

Statistics Data are presented as mean ± standard deviation or median (interquartile range; IQR) as appropriate. Their distribution was evaluated with the Shapiro–Wilk test for normality. Skewed data (iPTH, FGF-23, Klotho, CTX) were transformed with a common logarithm to achieve the Gaussian distribution. Changes from baseline were analyzed with the *t* test for dependent variables or the Wilcoxon signed-rank test depending on meeting the assumptions. A two-tailed *P*-value less than 0.05 was considered statistically significant. All calculations were performed with Statistica 9.1 for MS Windows (StatSoft Inc.; Tulsa, Oklahoma, United States).

RESULTS

Study population and biochemical tests The baseline characteristics of the studied population are presented in the TABLE.

The supplementation of cholecalciferol for 12 weeks resulted in a significant increase in 25(OH)D levels compared with the baseline values (39.61 ±15.48 nmol/l vs. 87.11 ±31.13 nmol/l; $P < 0.0001$) and a decrease in iPTH levels (304.15 ng/l [IQR, 131.60–1113.00 ng/l] vs. 226.45 ng/l [64.68–853.80 ng/l]; $P < 0.01$). Serum calcium and phosphorous levels did not change during the study (2.03 ±0.27 mmol/l vs. 2.01 ±0.24 mmol/l, $P = 0.63$, and 1.71 ±0.43 mmol/l vs. 1.80 ±0.33 mmol/l, $P = 0.30$, respectively).

TABLE Baseline characteristics of the patients (n = 22)

Variable	Value
sex (male/female)	13/9
age, y	69 (20–84)
dialysis vintage, y	3 (1–23)
calcium carbonate, n (%)	22 (100)
alfacalcidol, n (%)	10 (45)
serum calcium, mmol/l	2.03 ±0.27
serum phosphate, mmol/l	1.71 ±0.43
iPTH, ng/l	304.15 (131.60–1113.00)
25(OH)D, nmol/l	39.61 ±15.48
soluble Klotho, pg/ml	438.73 (257.99–865.51)
FGF-23, pg/ml	145.66 (1.33–976.16)
PINP, ng/ml	284.45 (61.03–1200.00)
CTX, ng/ml	2.25 (0.65–6.00)

Data are presented as number (percentage), mean ± standard deviation, or median (interquartile range).

Abbreviations: CTX – cross-linked C-telopeptide of type 1 collagen, FGF-23 – fibroblast growth factor 23, iPTH – intact parathyroid hormone, IQR – interquartile range, PINP – procollagen I N-terminal propeptide, 25(OH)D – 25 hydroxyvitamin D

Klotho levels decreased significantly in the course of treatment compared with the baseline values (438.73 pg/ml [257.99–865.51] pg/ml vs. 370.94 pg/ml [181.72–710.91 pg/ml]; $P < 0.05$). The FGF-23 concentration did not change from baseline (145.66 pg/ml [1.33–976.16 pg/ml] vs. 119.99 pg/ml [10.01–1174.66 pg/ml]; $P = 0.85$).

The concentration of PINP was unaffected by cholecalciferol treatment (284.45 ng/ml [61.03–1200 ng/ml] vs. 257.10 ng/ml [130.90–1200.00 ng/ml]; $P = 0.65$). The CTX level decreased significantly from baseline (2.25 ng/ml [0.65–6.00 ng/ml] vs. 1.66 ng/ml [IQR 0.57–6.00 ng/ml]; $P < 0.05$).

Bivariate correlations The Klotho level did not correlate with age ($r = -0.09$, $P = 0.71$) or the levels of calcium ($r = -0.22$, $P = 0.32$), phosphorous ($r = 0.02$, $P = 0.92$), 25(OH)D ($r = -0.11$, $P = 0.62$), iPTH ($r = 0.02$, $P = 0.95$), FGF-23 ($r = 0.11$, $P = 0.63$), PINP ($r = 0.07$, $P = 0.77$), and CTX ($r = 0.18$, $P = 0.42$).

The magnitude of the change in the Klotho level was not associated with the change of 25(OH)D (ΔKlotho and Δ25(OH)D, $r = 0.16$, $P = 0.49$), CTX (ΔKlotho and ΔCTX, $r = 0.05$, $P = 0.83$), iPTH (ΔKlotho and ΔiPTH, $r = 0.30$, $P = 0.20$).

The degree of increase in 25(OH)D levels was associated with the change of iPTH levels at the limit of statistical significance (Δ25[OH]D and ΔiPTH, $r = -0.44$, $P = 0.055$) but not with ΔCTX (Δ25[OH]D and ΔCTX, $r = -0.35$, $P = 0.13$). The change in the iPTH value was significantly correlated with alterations in the CTX concentration (ΔiPTH and ΔCTX, $r = 0.66$, $P < 0.01$).

DISCUSSION To our knowledge, this is the first study to have investigated the potential effect of vitamin D replenishment on soluble Klotho levels in HD patients. Our results showed a reduction of soluble Klotho levels in these patients after treatment with cholecalciferol. The possible mechanism that could explain this mechanism is unknown, although it may be speculated that the replenishment of vitamin D stores normalizes increased activity of TACE/ADAMS 17, which is involved in shedding of Klotho extracellular domain as hypothesized by de Borst et al.[14] Interestingly, cinacalcet, another agent used in the treatment of secondary hyperparathyroidism, was also shown to transiently decrease serum Klotho levels.[13] Thus, it seems that the factors affecting mineral metabolism modulate soluble Klotho levels, although further studies are needed to elucidate this mechanism. It has to be underlined, however, that the effect of vitamin D replenishment with cholecalciferol on Klotho expression is unknown because we measured only its soluble form.

Klotho is mainly expressed in the kidneys, parathyroid glands, and choroid plexus[21] and is severely reduced in patients with renal impairment.[22,23] In agreement with the previous reports, soluble Klotho levels in our patients were lower than those reported in healthy subjects,[24] although increased concentrations were also described.[25] These discrepancies may result from the fact that the origin of soluble Klotho in this population is still unknown.

Another compelling finding of our study is that we observed no effect of cholecalciferol supplementation on the FGF-23 level despite a significant increase in the 25(OH)D concentration. Similar results were obtained by Stubbs et al.[26] This is in contrast to the effect of treatment with active vitamin D and its analogues, which increase the FGF-23 concentration.[11]

It may be hypothesized that supplementation with nutritional vitamin D results in tissue synthesis of calcitriol, which exerts mainly paracrine or autocrine actions and the obtained systemic calcitriol concentrations are not high enough to induce the production of FGF-23. It is tempting to speculate about potential benefits of vitamin D replenishment with cholecalciferol without the effect on the FGF-23 concentration, especially in light of the recent data which indicated that FGF-23 directly induces cardiac hypertrophy.[27]

A decrease in iPTH concentrations in the course of cholecalciferol treatment is in agreement with the previous reports,[28] although neutral effects have also been reported.[26] The magnitude of the observed decrease was small but, nonetheless, it was accompanied by a significant decrease in bone resorption, which was reflected by a drop in the CTX concentration. This decline was associated with a decrease in iPTH levels induced by an increase in 25(OH)D levels as reflected by significant correlations.

A single-arm, open-label design of this trial is a potential limitation. To minimize the bias, each patient's data before entering the study served as their own control. Moreover, a small study

sample size may hamper the detection of potential effects of normalizing 25(OH)D levels, although even small studies reported beneficial results.[26] The design of our study did not allow us to establish the clinical context for the observed changes, as we did not evaluate patient-related outcomes, which is yet another limitation.

In conclusion, our study showed that supplementation with cholecalciferol in HD patients decreases soluble Klotho levels without affecting the FGF-23 concentration. Replenishment of vitamin D stores results in a decrease in iPTH levels, which is accompanied by reduced bone resorption.

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Cholekalcyferol obniża stężenie rozpuszczalnej formy Klotho u chorych hemodializowanych

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SŁOWA KLUCZOWE

cholekalcyferol, czynnik wzrostu fibroblastów 23, hemodializa, Klotho, metabolizm kostny

STRESZCZENIE

WPROWADZENIE Niskie stężenia witaminy D są wiązane z wieloma niekorzystnymi stanami klinicznymi wśród chorych hemodializowanych, takimi jak zaburzenia gospodarki mineralnej i kostnej oraz zwiększona śmiertelność. Klotho, molekuła biorąca udział m.in. w takich procesach jak gospodarka fosforanowa oraz starzenie się, występuje w dwóch postaciach: białko przezbłonowe pełniące funkcję koreceptora dla czynnika wzrostu fibroblastów 23 (*fibroblast growth factor 23* – FGF-23) oraz forma rozpuszczalna, która powstaje poprzez oddzielenie części zewnątrzkomórkowej Klotho.

CELE Celem przeprowadzonego badania była ocena wpływu podawania cholekalcyferolu na stężenie rozpuszczalnej formy Klotho u pacjentów hemodializowanych.

PACJENCI I METODY W prospektywnym, otwartym badaniu oceniano wpływ suplementacji witaminy D przy pomocy cholekalcyferolu na wybrane parametry laboratoryjne w populacji 22 chorych hemodializowanych. Niedobór witaminy D oceniano na podstawie stężenia 25-hydroksywitaminy D [25(OH)D]. Rozpuszczalna forma Klotho, cała cząsteczka FGF-23, cała cząsteczka parathormonu (*intact parathormone* – iPTH) oraz markery kościotworzenia i resorpcji były oceniane przed i po 12 tygodniach podawania cholekalcyferolu.

WYNIKI Stężenie 25(OH)D wzrosło, natomiast iPTH i C-końcowego usieciowanego telopeptydu kolagenu typu 1 znacznie się obniżyło. Leczenie cholekalcyferolem powodowało spadek mediany stężenia rozpuszczalnej formy Klotho (z 438,73 pg/ml; rozstęp międzykwartylowy: 257,99–865,51 pg/ml; do 370,94 pg/ml; 181,72–710,91 pg/ml; $p < 0,05$). Leczenie nie wpłynęło na stężenie FGF-23.

WNIOSKI Leczenie cholekalcyferolem chorych hemodializowanych obniża stężenie rozpuszczalnej formy Klotho bez wpływu na stężenie FGF-23. Uzupełnienie niedoboru witaminy D powoduje spadek stężenia iPTH oraz zmniejszoną resorpcję kostną.

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