Effects of cinacalcet treatment on serum soluble Klotho levels in haemodialysis patients with secondary hyperparathyroidism

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Abstract
Background. Klotho is a transmembrane protein that acts as a co-factor for fibroblast growth factor factor 23 (FGF23). Klotho also exists as a soluble circulating protein, but its role in secondary hyperparathyroidism (SHPT) is largely unknown.

Methods. We measured serum soluble Klotho levels in 51 haemodialysis patients, who participated and completed a 52-week, multicentre, open-label single-arm trial that examined the effectiveness of cinacalcet for treating SHPT.

Results. After 12 weeks of cinacalcet treatment, serum soluble Klotho decreased significantly (P=0.03) but only marginally from 398 pg/mL [interquartile range (IQR), 268–588 pg/mL] to 378 pg/mL (IQR, 266–568 pg/mL) and returned to baseline levels. There were no significant associations between the changes in soluble Klotho levels and changes in any other parameters of mineral metabolism, including serum calcium, phosphorus, intact parathyroid hormone and FGF23.

Conclusion. Despite significant alterations in mineral and bone metabolism during treatment with cinacalcet, this resulted in only small and transient reductions in serum levels of soluble Klotho.

Keywords: cinacalcet hydrochloride; FGF23; haemodialysis; secondary hyperparathyroidism; soluble Klotho

Introduction
Klotho, originally identified as an anti-ageing protein [1], is a single-pass transmembrane protein that acts as a co-receptor for fibroblast growth factor 23 (FGF23) [2, 3]. Klotho is also cleaved and released into the circulation and functions as a humoral factor. Recent experimental studies suggest that this soluble form of Klotho plays a role in the regulation of mineral homeostasis. Secreted Klotho enhances calcium re-absorption in the distal nephron by activating the transient receptor potential v-5 [4, 5] and also functions as a phosphaturic substance via its enzymatic action on renal sodium-dependent phosphate transporter 2a [6]. However, the role and regulation of soluble Klotho in patients with end-stage kidney disease (ESKD) is largely unknown. In this study, we examined the effects of cinacalcet hydrochloride, an allosteric modulator of the calcium-sensing receptor (CaSR) [7–9], on serum soluble Klotho levels in haemodialysis patients with secondary hyperparathyroidism (SHPT).

Materials and methods

Study design
This was a post hoc analysis of a 52-week, multicentre, open-label single-arm trial that examined the effectiveness of cinacalcet in maintenance haemodialysis patients with SHPT. The study procedures have been described elsewhere [9]. The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the institutional review board of Kobe University School of Medicine, which waived the need for additional written informed consent for the post hoc analysis.

Measurements

Serum samples were collected prospectively at baseline and after 12, 24 and 52 weeks. Serum soluble Klotho levels were determined using a sandwich ELISA kit according to the manufacturer’s protocol (Immuno-Biological Laboratories, Co., Ltd, Gunma, Japan). This assay detects circulating soluble Klotho by using two monoclonal antibodies that specifically recognize the extracellular domain of Klotho. The intra- and inter-assay coefficients of variation were <10% [10]. Serum full-length FGF23 levels were determined using a sandwich ELISA kit (Kainos Laboratories, Tokyo, Japan). Serum intact parathyroid hormone (PTH) levels were determined using an electrochemiluminescence immunoassay (Elecsys PTH; Roche Diagnostics, Mannheim, Germany). Serum calcium levels were corrected for albumin concentration using Payne’s formula [11].

Statistical analysis

Data are reported as mean ± SD or median [interquartile range (IQR)] as appropriate. Associations between parameters were examined by Spearman’s rank test. Changes from baseline in biochemical parameters were analysed using repeated measures analysis of variance (ANOVA) followed by Bonferroni post hoc test. As serum intact PTH, FGF23 and soluble Klotho levels were not normally distributed, the values were log transformed for the ANOVA. P < 0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics 20 (IBM SPSS, Tokyo, Japan).
Results

Study population and baseline results

Of the 81 patients enrolled in the original trial, 51 completed the 52-week trial and had serum samples available for analysis (Table 1). At baseline, median serum soluble Klotho was 398 pg/mL (IQR, 268–588 pg/mL). The serum levels of soluble Klotho were associated significantly with serum calcium levels ($r = 0.29$, $P = 0.04$), but not with age ($r = -0.21$, $P = 0.1$), duration of dialysis ($r = 0.08$, $P = 0.6$), phosphorus ($r = -0.23$, $P = 0.1$), intact PTH levels ($r = 0.02$, $P = 0.9$) or FGF23 levels ($r = -0.05$, $P = 0.7$).

Effect of cinacalcet on serum soluble Klotho

Treatment with cinacalcet resulted in significant and sustained reductions in serum calcium, phosphorus, intact PTH and FGF23 levels (Table 2). In contrast, soluble Klotho levels showed a small but significant decrease after 12 weeks of treatment [378 pg/mL (IQR, 266–568 pg/mL), $P = 0.03$] and then returned to baseline values (Figure 1). There were no significant associations between the changes in soluble Klotho levels from baseline to Week 12 and changes in any other parameters of mineral metabolism, including serum calcium ($r = 0.11$, $P = 0.9$), phosphorus ($r = 0.16$, $P = 0.3$), intact PTH ($r = -0.87$, $P = 0.5$) and FGF23 ($r = 0.22$, $P = 0.1$).

Discussion

Treatment with cinacalcet has marked effects on biochemical parameters of SHPT [7–9] and also lowers serum FGF23 levels [12, 13]. However, in this study, cinacalcet treatment of SHPT resulted in only small and transient reductions in serum levels of soluble Klotho.

In patients with ESKD, Klotho expression is decreased significantly in both the kidney [14] and hyperplastic parathyroid glands [15, 16]. A recent experimental study also showed very low renal, plasma and urinary levels of Klotho in mice with chronic kidney disease (CKD) and proposed that CKD is a state of Klotho deficiency [17]. In line with these observations, we found that serum levels of soluble Klotho were modestly reduced in patients on haemodialysis compared to values reported in healthy adults [10]. Further studies are, however, needed to determine the source and metabolism of circulating soluble Klotho in patients with ESKD.

The mechanisms responsible for the small transient reduction in soluble Klotho levels during cinacalcet treatment are unknown; however, the absence of associations between changes in soluble Klotho and changes in other parameters of SHPT suggests that cinacalcet has a direct effect on circulating soluble Klotho. Whether CaSR regulates secretion of soluble Klotho from the kidney or parathyroid should be examined in future studies.

Recently, Klotho has been shown to directly inhibit phosphate uptake by vascular smooth muscles in vitro [17]. This study, however, found that cinacalcet did not increase serum soluble Klotho, despite its beneficial effect of attenuating vascular calcification [18]. Further

| Table 1. Baseline demographic, clinical and biochemical data of the 51 patientsa |
|-----------------------------|-------------------|-------------------|-------------------|-------------------|
| Age (years)                | 63 ± 12           |                  |                  |                  |
| Sex (%)                     | 61 Male           | 39 Female        |                  |                  |
| Duration of dialysis (months) | 177 ± 101         |                  |                  |                  |
| Primary cause of renal failure (%) | 65 Glomerulonephritis | 18 Diabetes | 2 Hypertension | 12 Others |
| Use of vitamin D sterols (%) | 90 Maxacalcit      | 39 Sevelamer hydrochloride | 22 Calcitriol | 29 Oral vitamin D |
| Use of phosphate binders (%) | 94 Calcium carbonate | 43 Sevelamer hydrochloride | 80 Sevelamer hydrochloride | 80 Sevelamer hydrochloride |
| Serum calcium (mg/dL)       | 9.7 ± 0.5         |                  |                  |                  |
| Serum phosphorus (mg/dL)    | 6.0 ± 1.1         |                  |                  |                  |
| Intact PTH (pg/mL)          | 488 (358–699)     |                  |                  |                  |
| Serum FGF23 (pg/mL)         | 14 750 (2876–22 907) |                  |                  |                  |
| Serum soluble Klotho (pg/mL) | 398 (268–588)     |                  |                  |                  |

aData are means ± SD for normally distributed variables and median (IQR) for variables with skewed distributions.

| Table 2. Laboratory and medication data during treatment with cinacalcetb |
|-----------------------------|-------------------|-------------------|-------------------|
| Biochemical parameters      |
| Serum calcium (mg/dL)       | 9.7 ± 0.5         | 9.1 ± 0.6         | 9.2 ± 0.7         | 9.2 ± 0.7         | <0.001 |
| Serum phosphorus (mg/dL)    | 6.0 ± 1.1         | 5.2 ± 1.1         | 5.3 ± 1.1         | 5.7 ± 1.3         | <0.001 |
| Intact PTH (pg/mL)          | 488 (358–699)     | 217 (125–316)     | 179 (113–264)     | 183 (131–268)     | <0.001 |
| Serum FGF23 (pg/mL)         | 14 750 (2876–22 907) | 5112 (1851–17 206) | 7071 (1966–17 206) | 5498 (1073–17 055) | <0.001 |
| Medications                 |
| Cinacalcet hydrochloride (mg/day) | 0                  | 29.9 ± 10.9       | 34.4 ± 14.5       | 36.5 ± 15.6       | <0.001 |
| Intravenous vitamin D sterols Subjects (%) | 61                  | 63                 | 63                 | 59                 | 0.5 |
| Calcitriol dose equivalentsc (µg/week) | 2.22 ± 1.12       | 2.32 ± 1.19       | 2.19 ± 1.05       | 2.29 ± 1.23       | 0.8 |

aData are means ± SD for normally distributed variables and median (IQR) for variables with skewed distributions.

bP-value for repeated measures ANOVA or Cochran’s Q-test, as appropriate.

c1.5 µg calcitriol = 10 µg maxacalcit.
investigations are warranted to determine whether replacement of soluble Klotho or treatment to increase soluble Klotho levels results in improved outcome in patients receiving haemodialysis.

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Conflict of interest statement. H.K. has received honoraria from Kyowa Hakko Kirin and Chugai Pharmaceutical. T.K. has received honoraria from Kyowa Hakko Kirin, Chugai Pharmaceutical and Bayer Japan. M.F. has acted as a consultant for Kyowa Hakko Kirin, Bayer Japan and Novartis; has received honoraria from Kyowa Hakko Kirin, Chugai Pharmaceutical, Bayer Japan, Novartis, Genzyme and Abbott Japan and has received grants/research support from Kyowa Hakko Kirin, Chugai Pharmaceutical and Bayer Japan. The other authors declare that they have no conflict of interests.


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