

BRIEF REPORT

Determination of the Elimination Half-Life of Fibroblast Growth Factor-23

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Context: Tumor-induced osteomalacia (TIO) is a rare paraneoplastic disease caused by mesenchymal tumors that secrete fibroblast growth factor-23 (FGF-23), a newly-described vitamin D and phosphate-regulating hormone. Surgical removal of the tumor, the ectopic source of circulating FGF-23, offers the opportunity to determine the elimination half-life of FGF-23.

Objective: The aim of the study was to determine the elimination half-life of FGF-23.

Patients/Methods: The tumors were removed from three patients with TIO, and serum samples were taken every 30 min for up to 72 h after the operation. FGF-23 was measured by both a C-terminal/

intact assay and an intact assay, and the elimination half-life was determined by one phase exponential decay methodology.

Setting: The Mark O. Hatfield Clinical Research Center of the National Institutes of Health, a tertiary referral clinical research center, was the setting for the study.

Results: The elimination life of FGF-23 as determined by C-terminal/intact and intact assays was 46 ± 12 and 58 ± 34 min, respectively.

Conclusions: The plasma half-life of serum FGF-23 is in the range of 46–58 min. (*J Clin Endocrinol Metab* 92: 2374–2377, 2007)

TUMOR-INDUCED OSTEOMALACIA (TIO), also known as oncogenic osteomalacia, is a rare paraneoplastic syndrome characterized by hypophosphatemia, renal phosphate wasting, osteomalacia, and inappropriately low 1,25(OH)₂-vitamin D₃ (1,25-D). It is often associated with progressive bone pain, muscle weakness, fatigue, nonhealing and recurrent fractures, as well as secondary hyperparathyroidism (1–3). TIO is caused by phosphaturic mesenchymal tumors (PMTs) of mixed connective tissue type that ectopically secrete molecules known as “phosphatonins” (4–6). To date, fibroblast growth factor-23 (FGF-23) appears to be the most clinically significant phosphatonin in TIO (7–9).

FGF-23 has emerged as an important regulator of both phosphate and vitamin D metabolism. The primary physiological source of FGF-23 appears to be bone cells (10–13).

FGF-23 inhibits both phosphate reabsorption and 1- α hydroxylation of 25-hydroxyvitamin D₃ at the proximal renal tubule (14–21). Much remains to be learned about FGF-23, including its physiological regulation and serum half-life. An earlier report determined the half-life of FGF-23 to be 21.5 min (22). We determined the biological half-life of FGF-23 in three patients with TIO by measuring serum FGF-23 for up to 3 d after surgical removal of PMTs and analyzed the data by one phase exponential decay methodology.

Patients and Methods

Patients

Patient 1 was a 57-yr-old man who developed bone pain and weakness 2 yr before diagnosis. Initially, when multiple areas of increased tracer uptake were seen on bone scan, a diagnosis of multiple myeloma was entertained. However, laboratory studies were consistent with TIO and showed low serum phosphorus, ratio of tubular reabsorption rate for phosphorus to the glomerular filtration rate (TmP/GFR), and 1,25-D. PTH was slightly elevated (Table 1). Functional studies [whole body sestamibi, octreotide, and positron emission tomography (PET) scans] showed increased uptake in the left groin (anterior medial thigh). Anatomical studies [computed tomography (CT) and magnetic resonance imaging (MRI) scans] identified a nodular soft tissue lesion in the left femoral triangle. The lesion was excised, and serum FGF-23 was measured every 30 min for the first 120 min and then less frequently for 2 d after the operation. The tumor was characterized as an angiolipoma with additional features consistent with a PMT. The patient's serum phosphorus and FGF-23 levels have remained in the normal range since the tumor was removed.

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Abbreviations: CT, Computed tomography; 1,25-D, 1,25(OH)₂-vitamin D₃; FGF, fibroblast growth factor; MRI, magnetic resonance imaging; PET, positron emission tomography; PHEX, phosphate regulating gene with homology to endopeptidases on the X-chromosome; PMT, phosphaturic mesenchymal tumor; RU, relative units; TIO, tumor-induced osteomalacia; TmP/GFR, ratio of tubular reabsorption rate for phosphorus to the glomerular filtration rate.

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TABLE 1. Biochemical parameters

| | Patient 1 | | Patient 2 | | Patient 3 | |
|--------------------------------------|------------|--------------------------|------------|--------------------------|------------|--------------------------|
| | Presurgery | Postsurgery ^a | Presurgery | Postsurgery ^b | Presurgery | Postsurgery ^b |
| C-terminal FGF-23 (18–108 RU/ml) | 294 | 61 | 636 | 125 | 854 | 110 |
| Intact serum FGF-23 (9–50 RU/ml) | 153 | 7 | 1097 | 5 | 680 | 10 |
| Serum phosphorus (2.5–4.8 mg/dl) | 1.5 | 4.5 | 1.9 | 2.6 | 2.3 | 4.0 |
| Serum 1,25 vitamin D (22–67 pg/ml) | 16 | 87 | 29 | 92 | 23 | 28 |
| Serum 25 vitamin D (10–68 ng/ml) | 24 | 29 | 22 | 18 | 20 | 32 |
| Serum calcium (2.05–2.50 mmol/liter) | 2.04 | 2.19 | 2.28 | 2.19 | 2.21 | 2.31 |
| PTH (6.0–40.0 pg/ml) | 55 | 24 | 34.9 | 28 | 8.3 | 9.4 |
| TmP/GFR (2.78–4.18 mg/dl) | 1.05 | 3.10 | 1.30 | 2.25 | 0.75 | 2.80 |

Normal ranges are shown in *parentheses*.

^a 48 h postsurgery.

^b 72 h postsurgery.

Patient 2 was a 38-yr-old man who had 5 yr of symptoms including bilateral foot pain and ankle swelling, bone pain, mostly in his back and ribs, fractures with minimal trauma, and proximal muscle weakness. Laboratory studies showed low serum phosphorus and TmP/GFR, with an inappropriately low-normal serum 1,25-D. Serum PTH was high-normal, and alkaline phosphatase was elevated (Table 1). Bone scan revealed multiple areas of increased activity. Whole body sestamibi scan was negative, PET/CT scan and octreoscan revealed intense increased uptake in the head of the left fibula, suspicious for tumor. MRI showed a 1.7-cm lesion in the head of the left fibula, abutting the superomedial cortex of the fibular head and occupying about one third of the marrow space. The tumor was removed surgically, and pathology of the excised tumor revealed a spindle cell neoplasm with features of a PMT. As in patient 1, serum FGF-23 was measured 30 min for the first 120 min, and then less frequently for 3 d after the operation. Serum phosphorus returned to normal by postoperative d 5. The patient's serum phosphorus and FGF-23 levels have remained in the normal range since the tumor was removed.

Patient 3 was a 47-yr-old man who presented 5 yr earlier with a progressive illness that included bone pain, weakness, and multiple fractures. On x-ray he was found to have multiple rib fractures, and bone scan revealed numerous fractures throughout the skeleton. Serum phosphorus, TmP/GFR, and 1,25-D were low (Table 1). Renal function and serum 25 (OH) vitamin D levels were normal, and PTH was mildly elevated. A suggestive, but atypical lesion was localized to the fat pad of the heel of the left foot with a combination of ¹¹¹In-pentetreotide octreotide scan, PET/CT scan, and MRI. The fact that this site was the likely source of FGF-23 was confirmed by selective venous catheterization of the left leg and the demonstration of a gradient in the FGF-23

concentrations between the proximal and distal lower extremity. The FGF-23 concentrations in a peripheral vein and multiple proximal veins in the lower extremity were 428–578 relative units (RU)/ml, whereas the concentration in the low superficial femoral vein was 1201 RU/ml.

The left heel lesion was removed, and serial serum FGF-23 concentrations (intact and C-terminal protein) were measured every 30 min for the first 120 min, and then less frequently after that, for 2 d after the operation. Histology of the tumor was consistent with a PMT and demonstrated spindle cells, clusters of osteoclast-like giant cells, foci of poorly formed cartilaginous metaplasia, and a multifocal appearance with a few hemangiopericytoma-like vessels. The patient's serum phosphorus and FGF-23 levels have remained in the normal range since the tumor was removed in January 2005.

All three patients had normal renal and hepatic function.

Methods

TmP/GFR was calculated by the method of Bijvoet *et al.* (23). FGF-23 was measured by two assays: one that detects both C-terminal and intact FGF-23 (C-terminal/intact, Immotopics, Inc., San Clemente, CA) and intact ELISA assay (Kainos Laboratories, Inc., Tokyo, Japan), according to the manufacturers' specifications. Plasma samples were taken just before tumor removal, every 30 min for the first 120 min, and periodically for up to 3 d postoperatively. The elimination half-life ($t_{1/2}$) of FGF-23 was calculated by a method fitting the data to a one phase exponential decay equation (Prism 4; GraphPad Software, San Diego, CA), using the following equation: $N = N_0 e^{-(0.693/t_{1/2})t} + plateau$, where N is the FGF-23 concentration at a given time point, N_0 is the initial

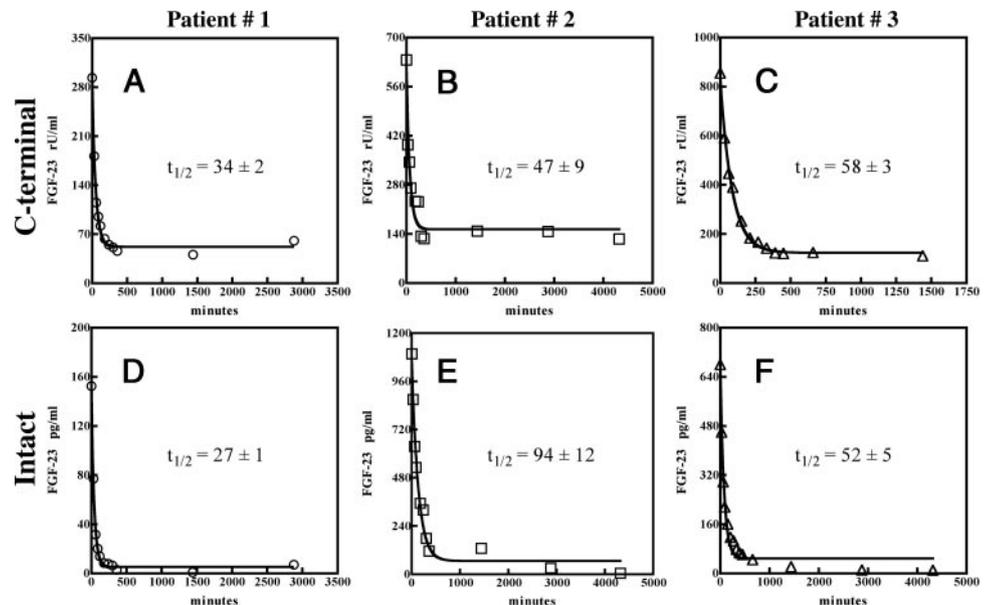


FIG. 1. Elimination half-life of FGF-23. FGF-23 was measured by the C-terminal/intact assay (A–C), and intact assay (D–F). The values for patient 1 (A and D), patient 2 (B and E), and patient 3 (C and F) are shown. FGF-23 was measured at the time points indicated after tumor removal. Calculated half-life ($t_{1/2}$) for each patient by each assay is inset in each panel. Combined values for the half-life for the three patients as determined by the C-terminal/intact assay was 46 ± 12 min, and by the intact assay 58 ± 34 min.

FGF-23 concentration, and the plateau is the final steady-state FGF-23 concentration.

Results

There was a rapid decline in the serum concentration of FGF-23 upon tumor removal (Fig. 1). The calculated elimination half-life of FGF-23 for the three patients, as calculated from the C-terminal/intact assay measurements, was: 34 ± 2 (1 SD), 47 ± 9 , and 58 ± 3 min (Fig. 1), for a mean half-life of 46 ± 12 min. The half-life, as calculated by the intact assay was: 27 ± 1 , 94 ± 12 , and 52 ± 5 min (Fig. 1), for a mean half-life of 58 ± 34 min. After the initial precipitous drop in FGF-23, there was a sustained plateau level of 52, 153, and 123 RU/ml (mean, 109 ± 52), in patients 1, 2, and 3, respectively, when FGF-23 was measured by the C-terminus assay. In the intact assay, the plateau level approached the detection limit of the assay.

Discussion

By performing serial measurement of plasma FGF-23 after tumor removal and using a one phase exponential decay method, we calculated the elimination half-life of FGF-23 to be between 46 and 58 min. Because the elimination half-life of the hormone after tumor removal has been found to be very similar to the biological half-life in other endocrine tumors, this result probably reflects the biological half-life of FGF-23 (24). However, it is possible that the elimination half-life of tumoral FGF-23 may not be the same as that of biologically normal FGF-23 produced by osteogenic cells, which have been shown to be the physiological source of FGF-23. FGF-23 is most likely glycosylated under normal physiological conditions (25), and tumors sometimes function using nonphysiological posttranslational modification machinery (26). Thus, given the fact that glycosylation may play a role in secretion, and/or metabolism and elimination (27), it is possible that the half-life reported here may only reflect what occurs in the case of tumors. Furthermore, many PMTs also produced PHEX (phosphate regulating gene with homology to endopeptidases on the X-chromosome) (28). PHEX, which is the protein mutated in X-linked hypophosphatemia, plays an as yet undefined role in phosphate metabolism, and it is possible that tumoral PHEX could play a role in the FGF-23 degradation in this disease.

The half-life we determined in these patients by this method was significantly greater than that found by Takeuchi *et al.* (21.5 *vs.* 46–57 min; Ref. 22). This is likely due the different methods used to calculate the half-life. Takeuchi *et al.* (22) used a semilog transformation on serum from three time points. When the data were fit to both semilog transformation and single phase exponential decay models, the data fit the latter better. It is likely that the calculation of the half-life by this model and using more time points represents the biological half-life of FGF-23. However, some of the variation in the half-life seen in these three patients may simply reflect individual variation in FGF-23 clearance. It is interesting to note, however, that in all three patients there was a sustained level of C-terminal FGF-23 beyond 72 h after tumor removal. This could reflect a very long half-life for the removal of the C-terminal fragment from the serum, as has

been suggested to be the case in postrenal transplantation hypophosphatemia (29), sustained secretion of C-terminal fragment by some other tissue(s), or a source of FGF-23 from another biological compartment (cerebrospinal fluid, for example). Differential secretion of C-terminal and intact molecule FGF-23 has been shown to occur in tissues (cells) lacking polypeptide GalNAc-transferase T₃, a glycosylase involved in FGF-23 metabolism (27). It would not have been useful to measure serum C-terminal FGF-23 values beyond 72 h in an effort to determine the elimination half-life of C-terminal FGF-23, because physiological production of FGF-23 resumes 2–5 d after tumor removal.

In summary, measurement of the disappearance of FGF-23 from the circulation after surgical excision of FGF-23-producing tumors indicates that the elimination half-life, and thus circulating half-life of FGF-23, is between 46 and 58 min.

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