Effect of Ferric Citrate Hydrate on Fibroblast Growth Factor 23, α-Klotho and Platelets

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Abstract

Background

Ferric citrate hydrate (FC) was approved as a phosphate binder for patients with non-dialysis-dependent (NDD) chronic kidney disease (CKD) and dialysis-dependent CKD in 2014 and as an oral iron preparation for patients with iron deficiency anemia (IDA) in 2021 in Japan.

Approximately 70% of patients with CKD have gastrointestinal disease, but when gastric acid secretion inhibitors (GASI) are used concomitantly, phosphate-binding effect of some phosphate-binders and iron replacement with oral iron preparations are attenuated. However, no data examined whether concomitant use of GASI interferes with the phosphate binding and iron replacement effects of FC.

Serum phosphate levels in the body are maintained within a normal range by coordinated regulation of bone-derived hormone fibroblast growth factor 23 (FGF23) and membrane protein alpha-klotho (α-klotho). As CKD progresses, levels of serum phosphate and FGF23 increase, whereas α-klotho level decreases. In other words, there is an inverse relationship between FGF23 and α-klotho levels. Separately from CKD progression, iron metabolism is associated with levels of FGF23. FC may have an impact on both FGF23 and α-klotho levels in patients with CKD from the aspect of iron replacement, however, no data examined.

FGF23 has been reported to be increased not only in CKD patients but also in IDA patients, and higher level of FGF23 is reported to be associated with cardiovascular risk. Platelet count is also increased in some patients with IDA, and higher level of platelet count is reported to be associated with cardiovascular risk. However, it is unclear whether

iron replacement with FC could decrease the higher levels of FGF23 and platelet count in IDA patients regardless of CKD status.

Methods

Firstly, two clinical studies of FC in CKD patients (12 weeks in NDD-CKD and 52 weeks in underlying hemodialysis) were retrospectively analyzed. Patients were divided into with or without concomitant administration of GASI, and levels of phosphate- and iron-related parameters were analyzed.

Secondly, a 24-week, randomized, open-label, multicenter trial in patients with undergoing hemodialysis was retrospectively analyzed. Patients taking non-iron-based phosphate binder(s) were randomized at a 1:1 ratio to continue non-iron-based phosphate binder(s) (control group) or switch to FC (FC group). During the study, levels of serum phosphate and hemoglobin (Hb) were controlled within the target ranges. Levels of intact FGF23 (full length of FGF23), C-terminal FGF23 (full-length FGF23 and C-terminal cleavage fragment), and α-klotho were analyzed. In addition, an association analysis of intact FGF23 or C-terminal FGF23 and α-klotho levels was conducted.

Finally, a randomized, open-label, multicenter, 24-week study in IDA patients with NDD-CKD and non-CKD was analyzed retrospectively. Patients were randomized at 1:1 to FC-low (500 mg/day) or FC-high (1000 mg/day). The impact of FC on intact FGF23 and C-terminal FGF23 levels, and high platelet count ($>35.2\times10^4/\mu L$ or $>45.0\times10^4/\mu L$) were evaluated.

Results

For the first investigation, among NDD-CKD patients (FC, 60 patients; placebo, 30 patients), 14 FC patients and 14 placebo patients used GASI. The adjusted mean differences (95% confidence interval) of changes from baseline to the end of treatment, or discontinuation (EOT) in serum phosphate were [-0.85 mg/dL (-1.70, -0.01) with GASI vs -1.61 mg/dL (-2.23, -0.98) without GASI, interaction p = 0.16], serum ferritin was [104.84 ng/mL (35.97, 173.71) with GASI vs 145.30 ng/mL (96.34, 194.25) without GASI, interaction p = 0.34], and transferrin saturation (TSAT) were 12.56% (-0.83, 25.95) with GASI vs 18.56% (8.15, 28.98) without GASI, interaction p = 0.49]. In patients with underlying hemodialysis, 95 out of 180 patients used GASI. There were no differences in the mean changes from baseline to the EOT in serum phosphate, serum ferritin, and TSAT levels between with and without GASI. In both studies, there were no differences in the mean doses of FC with and without GASI.

For the second investigation, patients with underlying hemodialysis were randomized to FC (n = 48) and control (n = 45) groups. The mean changes from baseline to the EOT in serum ferritin and TSAT were significantly increased in FC group compared with control group. The mean changes from baseline to the EOT in C-terminal FGF23 were significantly different between FC and control groups (mean ± standard deviation; −0.2 ± 0.8 $\log_e$ pg/mL vs. 0.2 ± 0.8 $\log_e$ pg/mL, respectively; p = 0.04). The mean changes from baseline to the EOT in intact FGF23 and α-klotho were not different between the groups. In addition, levels of intact FGF23 or C-terminal FGF23, and α-klotho were not significantly associated with each other in both groups.

For the last investigation, 73 IDA patients were randomized to FC-low (NDD-CKD n=21, non-CKD n=15) and FC-high (NDD-CKD n=21, non-CKD n=16) groups.

Regardless of CKD status, FC increased serum ferritin and TSAT levels and did not change intact FGF23 or serum phosphate levels, but decreased C-terminal FGF23 levels. By week 8, FC normalized platelet count in all patients with high platelet count at baseline.

Conclusion

The phosphate-binding and iron replacement effects of FC were not attenuated by concomitant administration of GASI in patients with NDD-CKD and underlying hemodialysis. In addition, under the condition in which levels of serum phosphate and Hb were maintained, iron replacement of FC decreased C-terminal FGF23 levels in patients with underlying hemodialysis, whereas intact FGF23 and α-klotho levels did not change. Furthermore, iron replacement of FC decreased C-terminal FGF23 and platelet count levels in IDA patients with NDD-CKD and non-CKD who have elevated levels of C-terminal FGF23 and platelet count at baseline.

Since the effects of phosphate binders and oral iron preparations are reportedly attenuated by concomitant administration of GASI, and the higher levels of C-terminal FGF23 and platelet count are associated with cardiovascular risk, the findings of FC present that FC can respond to medical needs to date and may contribute to reduce the cardiovascular risk in IDA patients regardless of CKD status.

Contents

Abbreviations

APTT	activated partial thromboplastin time
ATP	adenosine triphosphate
Ca*P	calcium–phosphate calculated variable
cCa	corrected serum calcium
CI	confidence interval
CKD	chronic kidney disease
CRP	C-reactive protein
eGFR	estimated glomerular filtration rate
EOT	end of treatment, or at discontinuation
FC	ferric citrate hydrate
FGF23	fibroblast growth factor 23
GASI	gastric acid secretion inhibitors
Hb	hemoglobin
IDA	iron deficiency anemia
iPTH	intact parathyroid hormone
JSDT	Japanese Society for Dialysis Therapy
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MHLW	Ministry of Health, Labor and Welfare
mKL	full-length transmembrane α-klotho
NDD	non-dialysis-dependent
OECD	Organization for Economic Co-operation and Development
PT-INR	prothrombin time-international normalized ratio
RDW	red blood cell distribution width
SD	standard deviation
sKL	soluble secreted α-klotho
sTfR	soluble transferrin receptor
TIBC	total iron-binding capacity
TSAT	transferrin saturation
WHO	World Health Organization
α-klotho	alpha-klotho

General Introduction

Chronic Kidney Disease

According to the Global Burden of Diseases, Injuries, and Risk Factors Study which uses published data from 354 diseases and 84 risk incidents, 697.5 million chronic kidney disease (CKD) patients were estimated in 2017 and the estimated prevalence rate increased by 29.3 % from 1990 to 2017[1]. It is also estimated that 1.2 million patients died globally caused by CKD, and the estimated mortality rate caused by CKD increased by 41.5% from 1990 to 2017[1].

In Japan, 13.3 million CKD patients were estimated, and the estimated prevalence rate was 13% in 2005[2], and the estimated number increased to 14.8 million in 2015 mainly due to an increase in the elderly population[3]. The Ministry of Health and Welfare (MHLW) in Japan reported that renal failure accounted for the eighth leading cause of death among the nation's population in 2017, and the Japanese Society for Dialysis Therapy (JSDT) reported that approximately 350,000 patients were receiving dialysis therapy in Japan and the mean age of new hemodialysis patients was 71.09 years old in 2021[4].

The Organization for Economic Co-operation and Development (OECD) Reviews of Public Health in 2019[5] reported that hemodialysis cost in Japan was approximately 400,000 Japanese yen per patient per month and the total medical cost for hemodialysis amounted to 1.57 trillion Japanese yen per year. It is an important problem from the increasing burden of the Japanese universal health insurance coverage system[6].

Globally, diabetes was the largest underlying disease leading to CKD and it accounted for 30.7% of CKD disability-adjusted life-years in 2017[1]. In Japan, JSDT also estimated that 40.2% of new hemodialysis patients were caused by diabetic nephropathy, and diabetes nephropathy was the largest causal attribution in 2021[4]. To prevent diabetes patients from leading to CKD and reduce the number of new hemodialysis patients, MHLW has been conducting various educational activities.

The kidneys filter all the blood continuously and remove wastes, toxins, and excess fluid in the body. In addition, kidneys control blood pressure by releasing the enzyme renin into the bloodstream, stimulating a peptide hormone erythropoietin to the production of

red blood cells in the bone marrow needed to carry oxygen, and keep bones healthy by maintaining the right balance of phosphate, calcium, parathyroid hormone, and vitamin D.

CKD is a condition in which kidneys are damaged and decreases their ability to keep the body healthy. The five stages of CKD (stages 1-5) are stratified by levels of estimated glomerular filtration rate (eGFR) (Table 1)[7]. Stage 1 of CKD has an eGFR of 90 mL/min/1.73m^2 or greater and mild damage to kidneys (albuminuria, proteinuria, hematuria), stage 2 of CKD has an eGFR between 60 and 89 mL/min/1.73m^2 and has mild damage to kidneys (albuminuria, proteinuria, hematuria), stage 3 of CKD has an eGFR between 30 and 59 mL/min/1.73m^2, and has moderate damage to kidneys (chronic renal insufficiency, early renal insufficiency), stage 4 of CKD has an eGFR between 15 and 29 mL/min/1.73m^2 and has moderate to severe damage to kidneys (chronic renal insufficiency, late renal insufficiency, pre-end stage renal disease) and stage 5 of CKD has an eGFR less than 15 mL/min/1.73m^2 and has severe damage to kidneys (renal failure, uremia, end-stage renal disease).

Table 1. CKD classification

CKD stage	Condition of kidneys	eGFR (mL/min/1.73m^2)
1	Mild damage (albuminuria, proteinuria, hematuria) with normal eGFR	90 and over
2	Mild damage (albuminuria, proteinuria, hematuria) with a mild decrease in eGFR	60 to 89
3	Moderate damage (chronic renal insufficiency, early renal insufficiency)	30 and 59
4	Moderate to severe damage (chronic renal insufficiency, late renal insufficiency, pre-end stage renal disease)	15 to 29
5	Severe damage	less than 15

Progression of CKD is associated with several complications, such as mineral and bone disorders, renal anemia, and cardiovascular disease. In the kidneys, phosphate excretion and 1-α-hydroxylation-vitamin D production occur, and CKD patients are associated with hyperphosphatemia due to decreased renal phosphate excretion and inadequate 1-α-hydroxylation-vitamin D production[8]. In the kidneys, a peptide hormone erythropoietin is secreted to produce red blood cells in the bone marrow and CKD patients are associated with renal anemia due to decreased erythropoietin secretion. Hyperphosphatemia is one of the risk factors associated with cardiovascular disease in CKD patients[9] and the treatment goals of CKD-associated mineral and bone disorders is to prevent the development of hyperphosphatemia[10], and CKD-associated renal anemia also increases morbidity and mortality from cardiovascular complications[8].

Treatment of Hyperphosphatemia

As a treatment for CKD-associated hyperphosphatemia, in addition to dietary modification, the use of phosphate-binding therapy is recommended[10]. Kidney Disease Outcomes Quality Initiative guidelines state that both calcium-based phosphate binders and other non-calcium-based, non-aluminum-based, non-magnesium-based phosphate binders are effective[11]. In dialysis patients who remain hyperphosphatemia (serum phosphate >5.5 mg/dL) despite the use of either of the phosphate binders, a combination of both calcium-based and non-calcium-based phosphate binders is recommended. However, the total dose of elemental calcium provided by the calcium-based phosphate binders is recommended not to exceed 1500 mg/day, and the total intake of elemental calcium (including dietary calcium) is recommended not to exceed 2000 mg/day to prevent hypercalcemia (corrected serum calcium >10.2 mg/dL)[11].

In Japan, calcium-based phosphate binder (calcium carbonate) and non-calcium-based phosphate binders, such as lanthanum carbonate, phosphate-binding polymers (bixalomer hydrochloride and sevelamer), and iron-based phosphate binders (ferric citrate hydrate and sucroferric oxyhydroxide) are available (Table 2). However, each drug has its own issues. Although calcium carbonate has an advantage of being cheaper than other non-calcium-based phosphate binders, there are concerns because the high dose use of calcium carbonate has been reported to be a risk of vascular calcification[12]. In lanthanum

carbonate, serious adverse reactions of intestinal perforation, ileus, gastrointestinal bleeding, and gastrointestinal ulcers have been reported in the package inserts. In phosphate-binding polymers, constipation has been reported primarily in the package insert. Additionally, in iron-based phosphate binders, diarrhea has been reported primarily in their package inserts.

Table 2. Phosphate binders in Japan

Classification		Generic Name
Calcium-based		calcium carbonate
Non-calcium-based	Lanthanum-based	lanthanum carbonate hydrate
	Iron-based	ferric citrate hydrate
		sucroferric oxyhydroxide
	Polymer	bixalomer
		sevelamer hydrochloride

Treatment of Renal Anemia

For CKD-associated renal anemia, the use of iron preparations and erythropoiesis-stimulating agents are recommended. In patients with CKD who are not on dialysis, erythropoiesis-stimulating agents is typically considered when the hemoglobin (Hb) level drops below 10 g/dL, and in patients on dialysis, erythropoiesis-stimulating agents is usually avoided unless the Hb level is between 9 and 10 g/dL[13]. The use of iron preparations is recommended in CKD patients to treat iron deficiency, prevent its development to use of erythropoiesis-stimulating agents, and reduce the dose of erythropoiesis-stimulating agents in patients receiving erythropoiesis-stimulating agents treatment[13]. The use of erythropoiesis-stimulating agents is associated with cardiovascular events[14], accordingly, practice patterns have shifted globally toward reduced the use of erythropoiesis-stimulating agents and increased iron preparations.

Iron Deficiency Anemia

World Health Organization Global Anemia estimates in 2021 reported that global anemia prevalence was 29.9% in women of reproductive age (15-49 years) and the prevalence was 29.6% in non-pregnant women of reproductive age, and 36.5% in pregnant women[15]. The most common cause of anemia is iron deficiency anemia (IDA)[16]. IDA is also frequently reported in chronic diseases, such as CKD, inflammatory bowel diseases, cancer, rheumatoid arthritis, and obesity[16]. In addition, World Health Organization Global Prevalence of Anemia in 2011 reported that the prevalence of anemia in Japanese women of reproductive age (15-49 years) was 22%, which was higher compared to other developed countries, and the prevalence of anemia among pregnant women was 31% in Japan[17].

Patients with IDA have symptoms, such as headache, paleness, fatigue[16], and can negatively impact patients' health-related quality of life and increase morbidity and mortality[18]. Oral iron preparations are recommended as the first-line therapy for IDA, however many patients do not tolerate oral iron preparations because of gastrointestinal side effects[19]. Intravenous iron preparations are useful for patients who are not tolerable to oral iron preparations, who have severe iron deficiency, and who need quick recovery, however, there are side effects of nausea, vomiting, pruritus, headache, and flushing[20].

Ferric Citrate Hydrate (Riona®)

Ferric citrate hydrate (FC, 250 mg tablet containing approximately 60 mg of elemental iron, Torii Pharmaceutical Co. Ltd., Tokyo Japan) has been approved in Japan as an oral iron-based phosphate binder in patients with CKD, including dialysis- and non-dialysis-dependent (NDD) patients in 2014[21-24], and it was approved as an iron preparation to treat IDA in 2021[25, 26].

FC has an unique formulation of interrelated complexes between ferric iron and citric acid. FC has a larger surface area than commercial-grade ferric citrate which is likely to make contributions to its rapid disintegration and dissolution[27, 28]. Accordingly, FC is soluble in a wide range of pH in the stomach, in the duodenum (iron absorption site) and in the small intestine (phosphate absorption site).

Research Purpose and Objectives

FC has two indications: the treatment of hyperphosphatemia in patients with CKD and the treatment of iron deficiency anemia. The purpose of this study is to determine whether FC is effective in these patients with or without gastric acid secretion inhibitors (GASI) and whether there is a beneficial therapeutic effect other than improvement in serum phosphate levels and iron metabolism, focusing on fibroblast growth factor 23 (FGF23), alpha-klotho (α-klotho), and platelet levels. Therefore, I conducted three studies, as described in Chapters I to III below.

GASI, such as proton pump inhibitors or histamine-2 receptor antagonists, are one of the most prescribed medications in the world, and approximately 70% of CKD patients reportedly suffered gastrointestinal complications[29]. Phosphate-binding effects of some phosphate binders were attenuated in the presence of GASI in patients with CKD[30, 31]. Furthermore, patients taking GASI showed decreased iron absorption and increased risk of iron deficiency, which were dose- and treatment-duration-dependent[32]. Phosphate binders and oral iron preparations which are difficult to be affected by GASI are desired, however, no report has been addressed, and FC may resolve the difficulty. I have investigated this issue in Chapter I.

FGF23 is a peptide hormone that regulates serum phosphate levels by promoting renal phosphate excretion and inhibiting intestinal phosphate absorption. α-klotho is expressed in the kidneys and acts as a co-receptor for FGF23. The α-klotho/FGF receptor complex binds to FGF23 with higher affinity than α-klotho or FGF receptor alone to stimulate FGF23 signaling[33]. In CKD patients, levels of FGF23 increase in response to impaired kidneys function to maintain serum phosphate level to be normal, on the other hand, levels of α-klotho decrease in response to impaired kidneys function[34]. Interestingly, iron deficiency is also associated with levels of FGF23[35]. There have been few clinical studies evaluating FGF23 and α-klotho simultaneously in CKD patients including undergoing hemodialysis. Although FGF23 level is assumed to be elevated and α-klotho level is assumed to be decreased in patients with underlying hemodialysis, iron replacement of FC may have an impact on levels of FGF23 and α-klotho, however, no report has been addressed. I have investigated this academically interesting issue in Chapter II.

Regardless of CKD status, patients with IDA have higher levels of FGF23[36]. The higher levels of FGF23 are associated with cardiovascular risk both in general population[37] and CKD patients with IDA[38]. In addition, iron deficiency also affects platelet count elevation, which has been reported to be associated with ischemic diseases such as thrombosis[39]. Iron replacement with FC in patients with IDA may decrease the elevated levels of FGF23 and platelet count. However, no report has been addressed. I have investigated this high-profile issue in Chapter III.

Chapter I:

Effect of Ferric Citrate Hydrate on the Concomitant Administration of Gastric Acid Secretion Inhibitors in Patients with Non-dialysis- dependent Chronic Kidney Disease and Hemodialysis

I-1. Introduction

Iron homeostasis is tightly regulated because iron is essential for several biological processes. An important process for the bioavailability of iron is the dissolution of dietary iron by gastric acid in the stomach at a low pH[40]. Patients taking gastric acid secretion inhibitors (GASI) attenuate the bioavailability of iron and exhibit elevated risk of iron deficiency. These risks are dose and treatment dependent[32]. Notably, iron deficiency is more common in patients who have undergone surgery of gastrectomy or gastric bypass[41, 42]. These findings endorse the indispensable role of lowering the pH in the stomach by gastric acid for maintaining homeostasis of iron.

The ferric iron (Fe^{3+}) from FC binds to the phosphate in the diet and forms an insoluble ferric phosphate, which leads to the fecal excretion of excessive serum phosphate[43]. Serum phosphate levels decrease by administration of FC in patients with non-dialysis-dependent (NDD)-CKD[21] or undergoing dialysis through this mechanism[22-24]. Moreover, ferric iron (Fe^{3+}) from FC undergoes enzymatic reduction to ferrous iron (Fe^{2+}) before being absorbed in the duodenum, the proximal portion of the small intestine[44, 45], and administration of FC contributes to the amelioration of anemia in patients with iron deficiency anemia (IDA) through this mechanism[25].

In a previous study in patients with undergoing hemodialysis, administration of FC with or without concomitant histamine-2 receptor antagonist showed comparable phosphate-binding efficacy[46]. These findings indicate that FC can dissolve in the presence of suppressed gastric acid secretion by histamine-2 receptor antagonists, and consistently exhibits its effect irrespective of the pH in the stomach.

An iron replacement with oral ferrous iron (ferrous sulfate) was attenuated when concomitant administration of omeprazole (proton pump inhibitor) in a rat model and patients with IDA[47, 48]. For the oral ferric iron preparations, no investigation has been conducted. Accordingly, it remains uncertain whether iron replacement with FC is influenced by the concomitant administration of GASI.

In this study, to clarify the impact of GASI on phosphate-binding and iron replacement with FC, a retrospective analysis was conducted using two clinical studies in patients with NDD-CKD and undergoing hemodialysis[21, 22].

I-2. Materials and Methods

This was a retrospective study using data from two clinical studies of FC in CKD patients with hyperphosphatemia. The GBA4-4 study was a 12-week, multicenter, randomized, double-blind, placebo-controlled, dose-titration study in NDD-CKD patients[21], and the GBA4-6 study was a 52-week, multicenter, open-label, dose-titration study in patients undergoing hemodialysis[22].

In the GBA4-4 study, patients with CKD who have hyperphosphatemia, who were ≥20 years old, with serum phosphate levels at ≥5.0 mg/dL and <8.0 mg/dL were included[21]. In the GBA4-6 study, patients with undergoing hemodialysis three times a week for a minimum of 3 months before initiating the study, serum phosphate levels at ≥3.5 mg/dL and <10.0 mg/dL with treatment for hyperphosphatemia or ≥6.1 mg/dL and <10.0 mg/dL without treatment for hyperphosphatemia were included[22].

Patients who exhibited gastrointestinal disease and had a history of surgery of either gastric resection or duodenectomy were excluded. The administration of FC was undertaken orally, thrice daily, just after meals. The initial dosage of FC was set at 1500 mg a day, which was subsequently modified to a maximum of 6000 mg a day. Target serum phosphate levels were set at ≥2.5 mg/dL to ≤4.5 mg/dL in the GBA4-4 study and ≥3.5 mg/dL to ≤6.0 mg/dL in the GBA4-6 study.

GASI utilization from 4 weeks prior to the initiation of the study until the end of treatment or discontinuation (EOT) was defined as concomitant administration of GASI. GASI was defined as proton pump inhibitors or histamine-2 receptor antagonists, specifically the following drugs (Omeprazole, Lansoprazole, Rabeprazole Sodium, Cimetidine, Ranitidine Hydrochloride, Famotidine, Nizatidine, Roxatidine Acetate Hydrochloride, and Lafutidine). The patients were divided into two cohorts: patients who were treated concomitantly with GASI and patients who were not treated concomitantly with GASI.

Parameters related to mineral and bone disorder [serum phosphate, corrected serum calcium (cCa), intact parathyroid hormone (iPTH), and calcium–phosphate calculated variable (Ca*P)] were analyzed. Patients with at least one assessment of mineral and bone disorder-related parameters were used for the efficacy analysis set. For each study, change from baseline to the EOT was calculated for all assessment parameters. Patients with at

least one assessment of iron-related parameters [transferrin saturation (TSAT), serum ferritin, serum iron, and total iron-binding capacity (TIBC)] and hemoglobin (Hb) were used for the safety analysis set.

In the GBA4-4 study, adjusted mean differences [(least square mean of FC) − (least square mean of placebo)] and 95% confidence intervals (CI) were used to determine the differences between the groups treated with FC and placebo. These values were compared by using an analysis of covariance. In the GBA4-6 study, changes from baseline to the EOT were used in a descriptive manner without performing further statistical analysis. SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA) were used for all statistical analyses.

The GBB4-4 and GBB4-6 studies were executed in alignment with the Declaration of Helsinki and related ethical policies and guidelines. The study protocols were approved by the institutional review boards. Written informed consent was obtained from all study participants before the initiation of the study.

I-3. Results

The GBA4-4 study collected data from 90 NDD-CKD patients. To ensure a balanced distribution, the patients were randomly assigned to two groups, with a ratio of 2:1 between the FC group (n = 60) and the placebo group (n = 30). In total, 3 patients from the FC group and 1 patient from the placebo group were excluded due to incorrect administration or missing records of effective evaluation. About a quarter in the FC group (13 out of 60 patients) and about half in the placebo group (13 out of 30 patients) received concomitant administration of GASI (Fig. I-1a).

The GBA4-6 study collected data from 180 patients undergoing hemodialysis. All these patients received treatment with FC and approximately half of these (95 out of 180 patients) received concomitant administration of GASI (Fig. I-1b).

In NDD-CKD patients in the GBA4-4 study, serum phosphate levels exhibited a lower level in the FC group compared to the placebo group, and there was no significant interaction with GASI utilization (interaction $p = 0.16$) (Table I-1). Similarly, in Ca*P and cCa levels, there was no significant interaction with GASI utilization (interaction $p = 0.14$ and interaction $p = 0.77$, respectively) (Table I-1). However, there was significant interaction with GASI utilization in iPTH levels (interaction $p = 0.02$) (Table I-1).

In both with and without GASI cohorts, serum ferritin levels gradually increased in the FC group compared to the placebo group (Fig. I-2a). Concurrently, in both with and without GASI cohorts, levels of TSAT exhibited an increasing trend over time in the FC group compared to the placebo group (Fig. I-2b). Between with and without GASI cohorts, significant interactions were not observed in levels of serum ferritin (interaction $p = 0.34$) and TSAT (interaction $p = 0.49$) (Table I-2). Similarly, significant interactions were not observed in levels of Hb, serum iron, and TIBC (Table I-2).

Between with and without GASI cohorts, the mean [± standard deviation (SD)] doses of FC were 3,240 (± 725) mg/day (n = 14) and 3,445 (± 831) mg/day (n = 46), respectively.

In patients undergoing hemodialysis in the GBA4-6 study, the levels of serum phosphate exhibited a parallel trend in both with and without GASI cohorts (Fig. I-5). Between with and without GASI cohorts, the mean changes (± SD) in serum phosphate from baseline to the EOT were −0.23 (± 1.41) mg/mL and 0.02 (± 1.69) mg/mL, respectively. Similarly, between with and without GASI cohorts, the changes in serum Ca*P, cCa, and iPTH from baseline to the EOT were comparable between with and without GASI cohorts (Table I-3).

Between with and without GASI cohorts, levels of serum ferritin (Fig. I-4a) and TSAT (Fig. I-4b) exhibited a gradual increase, and those levels exhibited almost the same. The mean changes (± SD) in serum ferritin from baseline to the EOT were 166.32 (±153.70) ng/mL and 155.16 (± 139.47) ng/mL, respectively, similarly those in TSAT were 16.60 (± 19.44) % and 16.02 (± 18.81) %, respectively. Comparable changes were observed in other parameters (Hb, serum iron, and TIBC) between with and without GASI cohorts (Table I-3).

The mean (± SD) doses of FC in the with and without GASI cohort were 2619 (± 1,113) mg/day (n = 95) and 2,854 (± 1,164) mg/day (n = 85), respectively.

I-4. Discussion

Since the acidic nature of gastric acid plays a crucial role in the bioavailability of iron[40], the increase in pH in the stomach resulting from concomitant administration of GASI could potentially impact on bioavailability of iron. Therefore, this retrospective study aimed to investigate whether the concomitant administration of GASI impacts the

phosphate-binding and iron replacement effects of FC. In the 12-week GBA4-4 study in patients with NDD-CKD, the FC group exhibited a decreasing trend in serum phosphate levels compared to the placebo group, independent of GASI utilization. In addition, compared to the placebo group, the FC group exhibited an increasing trend in levels of serum ferritin and TSAT, independent of GASI utilization. In the 52-week GBA4-6 study in patients with undergoing hemodialysis, FC consistently maintained lower serum phosphate levels regardless of GASI utilization. Also, FC effectively increased levels of serum ferritin and TSAT independent of GASI utilization. Additionally, because the amount of elemental iron depends on the dose of FC, and the dose of FC could significantly affect its effects, the mean doses of FC in both studies were similar in patients with and without the use of GASI. This study revealed that the concomitant administration of GASI is unlikely to impact the phosphate-binding and iron replacement effects of FC in patients with NDD-CKD and undergoing hemodialysis.

The phosphate-binding effect of other phosphate binders, calcium carbonate or lanthanum carbonate was attenuated when concomitant admiration of GASI in patients with undergoing hemodialysis[49-51]. In the present study, the concomitant admiration of GASI did not attenuate the phosphate-binding effect of FC in those patients. This finding aligns with a previous clinical study, which examined the impact on the phosphate-binding effect of FC when concomitant administration of histamine-2 receptor antagonists in patients with undergoing hemodialysis[46].

Iron replacement is strictly regulated in the body because low dose of iron or too much dose of iron levels can lead to health problems. The rate of absorption of ferric iron (Fe^{3+}) has been reported to be lower compared to that of ferrous iron (Fe^{2+}) due to the inferior solubility and bioavailability[52]. Although FC is a ferric iron (Fe^{3+}) product, FC is specifically formulated to possess a significant surface area, resulting in increasing solubility[28]. Consequently, such formulation likely accounts for the comparable dissolution behavior of iron from FC at a variety of pH[28]. Ferric Citrate (Auryxia®) has the same active ingredient as that of FC and exhibited a 3.08 times faster dissolution rate at pH 8 compared to the reagent grade of commercial ferric citrate[45]. It is considered that the characteristic properties of FC contribute to its effects as exhibited in the present study.

Since approximately 70% of patients with CKD have gastrointestinal complications[29], FC is considered beneficial from both a phosphate binder and iron preparation standpoint in patients with CKD who are concomitantly receiving GASI.

This was a retrospective study. The number of included patients was not sufficient to draw definitive conclusions. Since phosphate and iron play crucial roles in the body, they are individually regulated by bone derived hormone of fibroblast growth factor 23 (FGF23)[53] and liver derived hormone of hepcidin[54], respectively. Accordingly, it would also be interesting to investigate whether the concomitant administration of GASI impacts FGF23 and Hepcidin levels when administration of FC.

I-5. Conclusions

In patients with NDD-CKD and undergoing hemodialysis, the concomitant administration of GASI is unlikely to influence on phosphate-binding and iron replacement effects of FC.

I-6. Tables

Table I-1. Analysis of covariance interactions in mineral and bone disorder-related parameters in non-dialysis-dependent CKD patients (Efficacy analysis set)

	FC, n = 57						Placebo, n = 29						FC − Placebo[a]		
	With GASI, n = 13			Without GASI, n = 44			With GASI, n = 13			Without GASI, n = 16			With GASI	Without GASI	Interaction
Mean (SD)	BL	EOT	Change	BL	EOT	Change	BL	EOT	Change	BL	EOT	Change	FC−placebo, adjusted mean difference[b] (95% CI)		*P*-value[c]
Serum phosphate [mg/dL]	5.53 (0.59)	4.43 (1.57)	−1.10 (1.20)	5.70 (0.80)	4.35 (1.18)	−1.35 (1.31)	5.28 (0.64)	5.15 (0.85)	−0.13 (0.65)	5.81 (0.52)	6.01 (0.73)	0.21 (0.67)	−0.85 (−1.70, −0.01)	−1.61 (−2.23, −0.98)	0.16
Serum cCa [mg/dL]	8.74 (0.64)	8.96 (0.41)	0.22 (0.54)	8.58 (0.48)	8.78 (0.61)	0.20 (0.51)	8.61 (0.45)	8.67 (0.34)	0.06 (0.31)	8.54 (0.44)	8.48 (0.48)	−0.06 (0.40)	0.21 (−0.13, 0.55)	0.27 (0.02, 0.53)	0.77
iPTH [pg/mL]	317.2 (478.8)	273.1 (359.5)	−44.1 (135.1)	321.0 (260.1)	251.1 (179.6)	−70.0 (134.4)	223.2 (129.2)	203.5 (139.0)	−19.7 (71.2)	312.3 (202.6)	361.0 (280.7)	48.7 (125.1)	0.4 (−79.5, 80.3)	−116.3 (−175.5, −57.2)	0.02
Ca*P [(mg/dL)^2]	48.35 (6.43)	39.66 (13.82)	−8.69 (10.53)	48.86 (7.11)	37.86 (9.17)	−11.0 (10.56)	45.39 (5.66)	44.51 (6.66)	−0.88 (5.28)	49.54 (4.26)	50.87 (5.67)	1.34 (4.87)	−6.37 (−13.17, 0.43)	−12.66 (−17.68, −7.64)	0.14

[a]Analysis of covariance (covariate, baseline).

[b]Adjusted mean difference = [least square mean of FC] − [least square mean of placebo].

[c]*P*-value: Test for [adjusted mean difference of with GASI] vs [adjusted mean difference of without GASI].

BL, baseline; Ca*P, calcium–phosphate product; cCa, corrected calcium; CI, confidence interval; EOT, end of treatment or at discontinuation; FC, ferric citrate hydrate; GASI, gastric acid secretion inhibitor; iPTH, intact parathyroid hormone; SD, standard deviation.

Table I-2. Analysis of covariance interactions in iron-related parameters in non-dialysis-dependent CKD patients (Safety analysis set)

Mean (SD)	FC, n = 60						Placebo, n = 30						FC − Placebo[a]		
	With GASI, n = 14			Without GASI, n = 46			With GASI, n = 14			Without GASI, n = 16			With GASI	Without GASI	Interaction
	BL	EOT	Change	BL	EOT	Change	BL	EOT	Change	BL	EOT	Change	FC−placebo, adjusted mean difference[b] (95% CI)		*P*-value[c]
Serum iron [μg/dL]	69.9 (30.2)	97.0 (52.0)	27.1 (66.0)	72.0 (26.6)	107.7 (45.2)	35.7 (42.6)	72.4 (41.1)	79.9 (32.1)	7.5 (35.4)	56.9 (21.0)	61.3 (31.4)	4.3 (24.8)	18.2 (−12.4, 48.8)	39.8 (15.8, 63.8)	0.27
Serum ferritin [ng/mL]	39.41 (25.60)	149.24 (91.11)	109.83 (104.46)	78.00 (53.43)	220.68 (106.15)	142.68 (94.28)	134.75 (109.35)	112.26 (96.13)	−22.49 (74.85)	80.81 (76.49)	77.39 (67.89)	−3.43 (43.58)	104.84 (35.97, 173.71)	145.30 (96.34, 194.25)	0.34
TIBC [μg/dL]	273.4 (37.2)	238.6 (34.8)	−34.7 (29.6)	267.9 (44.7)	243.0 (35.2)	−24.8 (27.8)	254.0 (40.1)	257.0 (34.2)	3.0 (19.2)	271.6 (54.0)	270.1 (40.3)	−1.5 (19.0)	−31.1 (−46.7, −15.5)	−24.6 (−36.5, −12.7)	0.51
TSAT [%]	25.94 (11.52)	42.59 (25.58)	16.64 (30.13)	27.61 (11.34)	44.67 (19.53)	17.07 (17.56)	28.37 (15.28)	31.23 (11.44)	2.86 (14.68)	22.04 (9.59)	23.35 (12.75)	1.31 (9.75)	12.56 (−0.83, 25.95)	18.56 (8.15, 28.98)	0.49
Hb [g/dL]	10.08 (1.06)	10.80 (2.27)	0.72 (1.90)	10.30 (1.57)	10.68 (1.77)	0.38 (1.65)	10.99 (1.55)	11.02 (1.14)	0.03 (0.93)	10.11 (0.79)	10.19 (1.27)	0.08 (0.93)	0.39 (−0.71, 1.49)	0.37 (−0.46, 1.20)	0.98

[a]Analysis of covariance (covariate, baseline).

[b]Adjusted mean difference = [least square mean of FC] − [least square mean of placebo].

[c]*P*-value: Test for [adjusted mean difference of with GASI] vs [adjusted mean difference of without GASI].

BL, baseline; CI, confidence interval; EOT, end of treatment or at discontinuation; FC, ferric citrate hydrate; GASI, gastric acid secretion inhibitor; Hb, hemoglobin; SD, standard deviation; TIBC, total iron-binding capacity; TSAT, transferrin saturation.

Table I-3. Changes in mineral and bone disorder- and iron- related parameters in patients with undergoing hemodialysis

	With GASI, n = 95			Without GASI, n = 85		
Mean (SD)	BL	EOT	Change	BL	EOT	Change
Serum phosphate	5.46	5.23	−0.23	5.61	5.64	0.02
[mg/dL]	(1.13)	(1.07)	(1.41)	(1.36)	(1.53)	(1.69)
Serum cCa	9.21	9.07	−0.14	9.17	8.86	−0.30
[mg/dL]	(0.59)	(0.63)	(0.56)	(0.55)	(0.58)	(0.57)
iPTH	151.9	208.3	56.3	165.0	227.1	62.1
[pg/mL]	(107.0)	(130.8)	(119.0)	(144.6)	(144.5)	(129.0)
Ca*P	50.26	47.51	−2.75	51.45	49.84	−1.60
[$(mg/dL)^2$]	(10.66)	(10.43)	(13.07)	(12.72)	(13.42)	(14.84)
Serum iron	61.5	85.5	24.0	58.5	84.2	25.6
[μg/dL]	(21.0)	(35.9)	(39.6)	(20.5)	(38.3)	(40.2)
Serum ferritin	79.51	245.83	166.32	92.52	247.69	155.16
[ng/mL]	(73.10)	(165.33)	(153.70)	(88.95)	(174.60)	(139.47)
TIBC	250.8	211.3	−39.5	255.2	215.1	−40.1
[μg/dL]	(44.6)	(34.6)	(35.0)	(43.6)	(32.2)	(31.3)
TSAT [%]	24.94	41.54	16.60	23.30	39.32	16.02
	(8.50)	(18.57)	(19.44)	(8.38)	(17.34)	(18.81)
Hb [g/dL]	10.89	11.13	0.24	11.06	11.28	0.23
	(1.00)	(1.26)	(1.43)	(1.07)	(1.26)	(1.18)

BL, baseline; Ca*P, calcium–phosphate product; cCa, corrected calcium; EOT, end of treatment or at discontinuation; GASI, gastric acid secretion inhibitor; Hb, hemoglobin; iPTH, intact parathyroid hormone; SD, standard deviation; TIBC, total iron-binding capacity; TSAT, transferrin saturation.

I-1. Figures

Figure I-1a

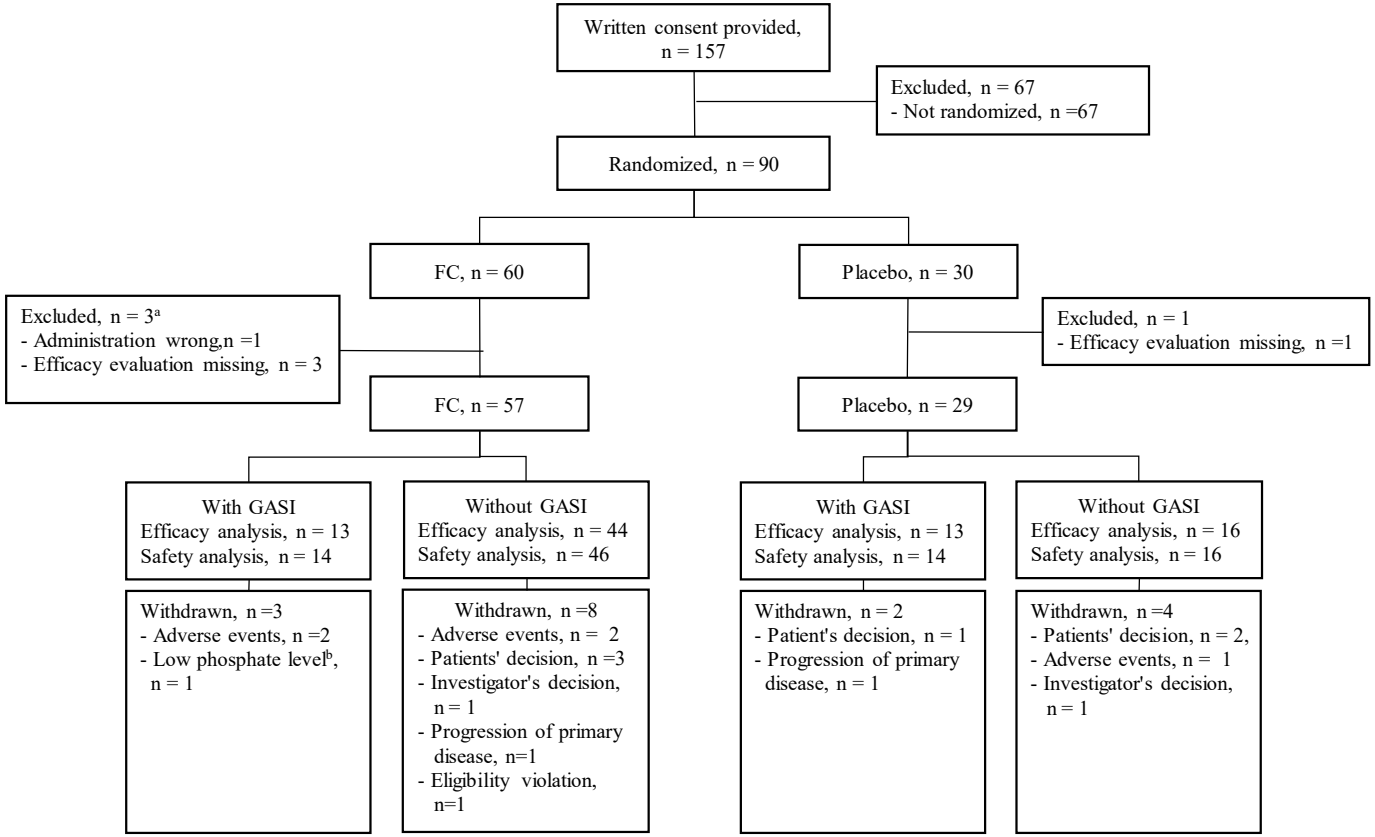


Figure I-1a. Flow diagram in GBB4-4 study.

a. Multiple reasons included, b. serum phosphate <2.5 mg/dL.

Figure I-1b

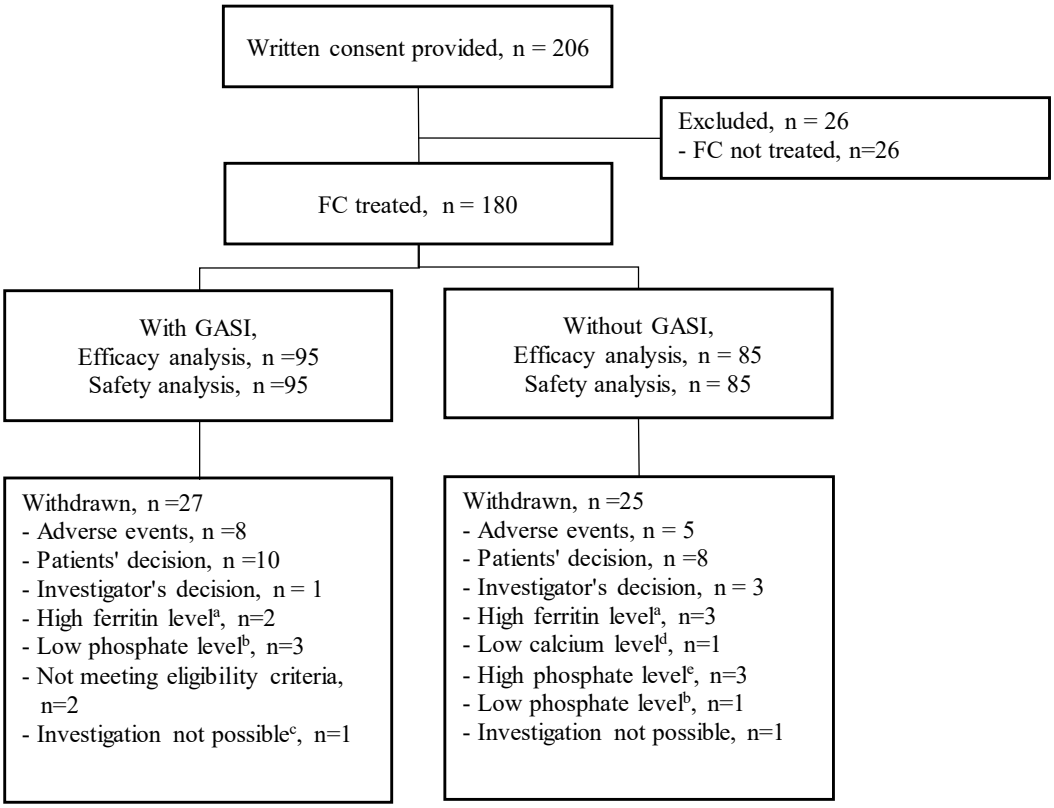


Figure I-1b. Flow diagram in GBB4-6 study.

a. Serum ferritin ≥800 ng/mL, b. Serum phosphate <3.0 mg/dL, c. The investigation was not possible for the patient's reason, d. serum corrected calcium <7.5 mg/dL, e. Serum phosphate ≥10.0 mg/dL.

Figure I-2

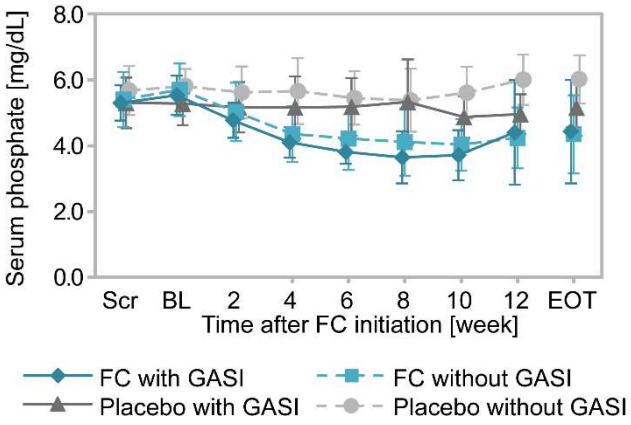


Figure I-2. Time-course fluctuations in serum phosphate from GBA4-4 study.

Blue-green lines, FC group; light black lines, placebo group; full lines, with GASI; dashed lines, without GASI, Data are presented as mean ± SD.

Figure I-3

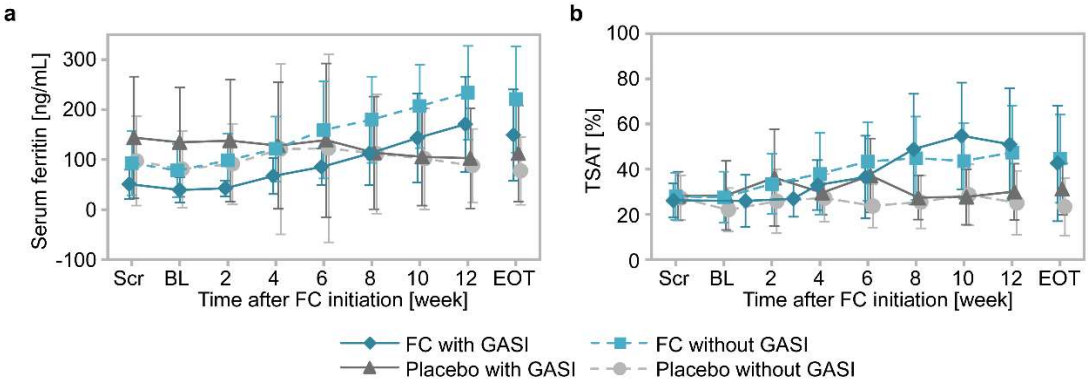


Figure I-3. Time-course fluctuations in serum ferritin and TSAT from GBA4-4 study.

a. Time-course fluctuation in serum ferritin, b. Time-course fluctuation in TSAT, FC group (blue-green lines), placebo group (light black lines), with GASI (full lines), without GASI (dashed lines), Data are presented as mean ± SD.

Figure I-4

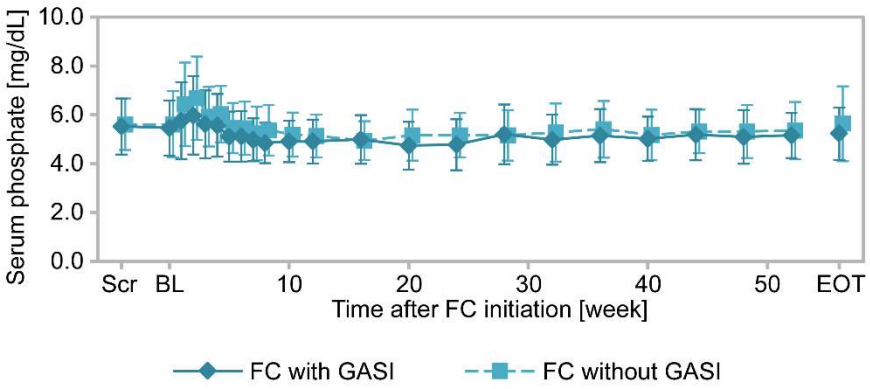


Figure I-4. Time-course fluctuations in serum phosphate from GBA4-6 study.

With GASI (Full line), without GASI (dashed lines), Data are presented as mean ± SD.

Figure I-5

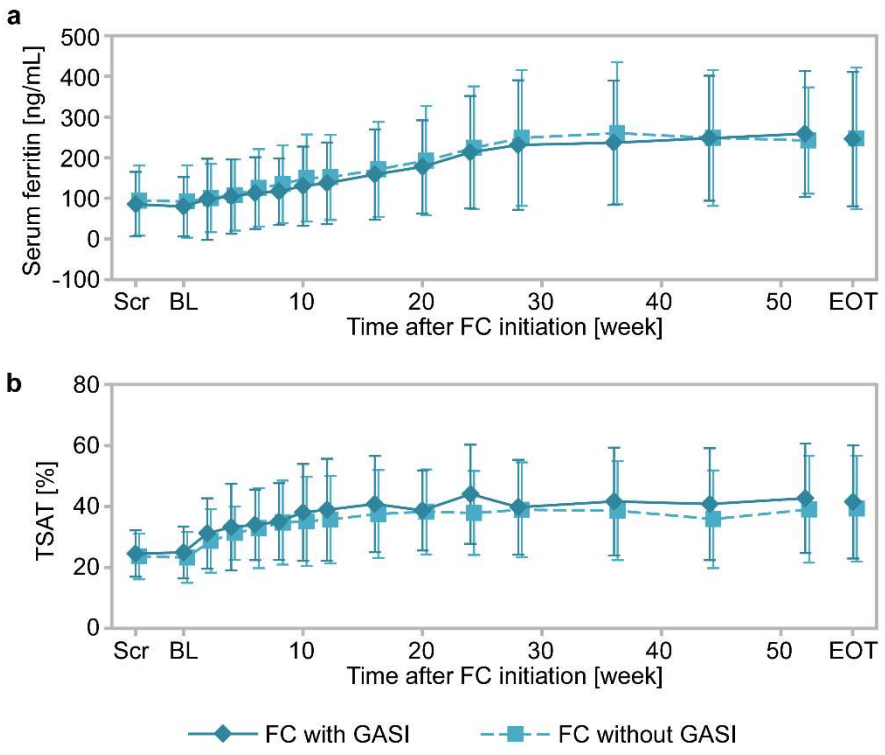


Figure I-5. Time-course fluctuations in serum ferritin and TSAT from GBA4-6 study.

a. Time-course fluctuation in serum ferritin, b. Time-course fluctuation in TSAT, with GASI (full line), without GASI (dashed line), Data are presented as mean ± SD.

Chapter II:

Effect of Ferric Citrate Hydrate on Fibroblast Growth Factor 23 and α-Klotho in Hemodialysis Patients

II-1. Introduction

Hyperphosphatemia and anemia are frequent clinical complications in patients with CKD. Hyperphosphatemia is associated with vascular calcification[55] and anemia is associated with cardiovascular events in patients with CKD[56]. A phosphotropic hormone FGF23 and a transmembrane protein alpha-klotho (α-klotho) have been identified as important contributors to maintain phosphate homeostasis in the body[57]

FGF23 represents an endocrine hormone that is expressed in bone and plays an essential biological role in preserving the delicate equilibrium levels of serum phosphate by promoting phosphate excretion from the kidneys and inhibiting phosphate absorption in the intestine[35, 58-60]. Notably, in patients with CKD, the levels of FGF23 exhibit a significant increase in response to a decrease in kidneys function, and in patients with severe kidneys damage, despite the elevated levels of FGF23, FGF23 cannot control their serum phosphate levels, and therefore hyperphosphatemia are observed[35]. Apart from preserving homeostasis of serum phosphate levels, FGF23 levels are associated with IDA[35], and IDA is reported to be associated with cardiovascular risk[61, 62].

α-klotho is a pleiotropic protein expressed in the kidneys, existing in the full-length membrane form (mKL) and the soluble circulating form (sKL)[63]. The mKL serves as a co-receptor for FGF23, creating a FGF receptor-klotho complex in the kidneys to promote the receptor's binding affinity for FGF23[33, 64]. This complex plays a crucial role in enhancing urinary phosphate excretion in the kidneys and inhibiting intestinal phosphate absorption in the intestine to control serum phosphate homeostasis. On the other hand, sKL is produced by cleavage of mKL[65, 66], and can be found in blood as well as in the cerebrospinal fluid. It is presumed that sKL possesses cytoprotective effects, including prevention of apoptosis, oxidative stress, and senescence[67]. The kidneys are mainly responsible for α-klotho expression and the major source of sKL. Levels of sKL decrease in response to impaired kidneys function contrary to levels of FGF23. Since the α-klotho/FGF receptor complex for stimulating FGF23 signaling decreases in response to impaired kidneys function[68], full-length FGF23 signaling is inhibited and it induces hypophosphatemia. The possibility of α-klotho serving as a potential biomarker of kidneys function has been reported based on the decreased α-klotho levels in patients with

CKD[69]. Decreased levels of sKL have been also associated with the prevalence of anemia in patients with CKD[70].

An inverse relationship between FGF23 and α-klotho levels was observed in patients with CKD[71]. However, few studies have examined this relationship in patients with underlying hemodialysis.

The present study retrospectively investigated the impact of iron replacement with FC on the relationship between FGF23 and α-klotho levels using data from the ASTRIO study [A Study examining The contribution to Renal anemia treatment with ferric citrate hydrate, Iron-based Oral phosphate binder] in patients with undergoing hemodialysis[72].

II-2. Material and Methods

The ASTRIO study is a randomized, open-label, active-controlled, multicenter, 24-week study (UMIN000019176) to investigate the effect of FC for treatment of renal anemia in patients undergoing hemodialysis, and the detailed study design was previously provided[72]. The study was executed in alignment with the Declaration of Helsinki and related ethical policies and guidelines. The study protocol was approved by the institutional review board of the Jikei University School of Medicine (Tokyo, Japan). Written informed consent was obtained from all study participants prior to the initiation of any study procedures.

The study participants were ≥ 20 years old, had been undergoing hemodialysis at least 12 weeks prior to the registration, and were prescribed one or more non-iron-based phosphate binders (either as a monotherapy or in combination therapy) to manage hyperphosphatemia. Additionally, at least 4 weeks prior to the registration, patients were receiving a constant dose of erythropoiesis-stimulating agents to treat renal anemia.

The study participants were randomly allocated into the FC or the control group at a 1:1 ratio (Fig. II-1). The initial dose of FC was 1500 mg a day [consisting of two tablets of FC (250 mg), thrice daily just after meals]. The dose of FC was controlled every week, with a maximum dose of 6000 mg a day to maintain the serum phosphate levels within the target range of 3.5 to 6.0 mg/dL[73]. Erythropoiesis-stimulating agents was administered to maintain Hb levels within the target range of 10.0 to 12.0 g/dL[74]. The use of all oral iron preparations, except for FC, was strictly prohibited. However, if the relevant

physicians have determined that it is necessary, utilization of intravenous iron preparation (saccharated ferric oxide) was allowed, particularly in cases when the patients exhibited TSAT level below 20% and serum ferritin level below 100 ng/mL.

Efficacy and safety biomarkers were evaluated every 4 weeks until the EOT (at week 24 or discontinuation; Fig. II-1). Levels of intact FGF23, C-terminal FGF23, and α-klotho have been measured at baseline, week 12, week 24, and the EOT. Before hemodialysis, measurement samples were collected.

Intact FGF23, C-terminal FGF23, and α-klotho were centrally measured at LSI Medience Corporation (Tokyo, Japan). Plasma levels of intact FGF23 were measured by the FGF23 ELISA kit (Kainos, Tokyo, Japan), plasma levels of C-terminal FGF23 were measured by the FGF23 Multi-Matrix ELISA kit (Biomedica Immunoassays, Vienna, Austria), and plasma levels of α-klotho were measured by the Human Soluble α-Klotho Assay kit (IBL International GmbH, Hamburg, Germany).

Descriptive statistics were used to summarize the baseline characteristics and the comparison differences between the FC group and the control group. The comparison differences were conducted using Student's *t*-test (continuous variables) or Fisher's exact test (categorical variables). Analysis of covariance was used to determine mean differences between the groups. Correlation analyses between levels at baseline or change from the baseline to the EOT of intact FGF23 or C-terminal FGF23 and α-klotho levels in each treatment group were conducted by Pearson's correlation coefficient. All statistical analyses were conducted using SAS version 9.3 or 9.4 (SAS Institute Inc., Cary, NC, USA).

II-3. Results

A total of 93 patients were enrolled. 48 patients were randomized to the FC group and 45 patients were randomized to the control group. In the FC group, two patients did not receive the study treatment, 8 patients withdrew due to adverse events and 4 patients withdrew by patients' intention, resulting in 34 out of 48 patients completing the 24-week study. In the control group, the 24-week study was completed by 41 of 45 patients. One patient from each group was excluded from the analyses due to a lack of data. For the evaluation of EOT, data from 82 out of 93 patients were available, with 40 patients from

the FC group and 42 patients from the control group (Fig II-2). There were no significant differences in patient characteristics between the FC group and the control group at baseline (Table II-1).

The changes in biomarkers from baseline to the EOT are summarized in Table II-2. Serum phosphate and Hb levels were maintained. For iron-related parameters, the adjusted mean differences from baseline to the EOT in TSAT and serum ferritin in the FC group compared to the control group were 79.5 ng/mL ($p < 0.001$) and 9.0% for TSAT ($p < 0.001$), respectively.

The time-course fluctuations of intact FGF23, C-terminal FGF23, or α-klotho were exhibited in Fig II-3 and Fig II-4. The levels of intact FGF23 and C-terminal FGF23 in the FC group showed a decreasing trend from baseline to the EOT, while those in the control group did not change throughout the study (Fig II-3A, Fig II-4A, Fig II-3B, and Fig II-4B). From baseline to the EOT, no changes in α-klotho were observed in either group (Fig II-3C, Fig II-4C). The exponential form of the log-adjusted mean difference in levels of intact FGF23 did not show a significant difference between the groups (0.8 $\log_e$ pg/mL; $p = 0.33$). In contrast, those in C-terminal FGF23 demonstrated statistically significant (0.7 $\log_e$ pg/mL; $p = 0.04$) (Table II-2).

The levels of intact FGF23, C-terminal FGF23, and α-klotho were exhibited in the scatter plot to examine the correlation between intact FGF23 or C-terminal FGF23 and α-klotho in terms of their levels at baseline (Fig II-5) and their changes from baseline to the EOT (Fig II-6). At baseline, no significant correlation was observed: between levels of intact FGF23 and α-klotho in the FC group (correlation coefficient $r = 0.11$, Fig II-5A); between levels of intact FGF23 and α-klotho in the control group (correlation coefficient $r = 0.03$, Fig II-5B); between levels of C-terminal FGF23 and α-klotho in the FC group (correlation coefficient $r = 0.12$, Fig II-5C); and between the levels of C-terminal FGF23 and α-klotho levels in the control group (correlation coefficient $r = 0.02$, Fig II-5D). No significant correlation was found in changes from baseline to the EOT: between levels of intact FGF23 and α-klotho in the FC group (correlation coefficient $r = 0.16$, Fig II-6A); between levels of intact FGF23 and α-klotho in the control group (correlation coefficient $r = 0.03$, Fig II-6B); between levels of C-terminal FGF23 and α-klotho levels in the FC

group (correlation coefficient r = 0.14, Fig II-6C); and between levels of C-terminal FGF23 and α-klotho in the control group (correlation coefficient r = −0.13, Fig II-6D).

No serious treatment-related adverse events were observed in either group. The rate of discontinuation attributable to adverse events was higher in the FC group (n=8) compared to the control group (n=1) (Fig II-2).

II-4. Discussion

An inverse correlation between FGF23 and α-klotho levels was reported in both healthy participants[75] and patients with NDD-CKD[76, 77]. Moreover, FGF23 and α-klotho levels are associated with renal anemia, in addition to the progression of CKD[35, 70]. Since few clinical studies simultaneously evaluated FGF23 and α-klotho levels in patients with undergoing hemodialysis, the present study investigated the relationship between the levels of FGF23 and α-klotho during the administration of FC compared with non-iron-based phosphate binders (control) in patients with undergoing hemodialysis retrospectively. Levels of serum ferritin increased significantly and levels of C-terminal FGF23 decreased significantly in the FC group compared with the control group, while the serum phosphate and Hb levels were controlled by phosphate binders and erythropoiesis-stimulating agents within the target ranges in both groups. No significant correlation was found between intact FGF23 or C-terminal FGF23 and α-klotho in either group.

The levels of FGF23 increase, while the levels of α-klotho decreases, in response to the disease progression in patients with CKD[53, 71, 77]. In a previous randomized trial of sevelamer carbonate in patients with NDD-CKD, no treatment effect on the levels of serum phosphate, intact FGF23, C-terminal FGF23, and α-klotho were observed[78]. When serum phosphate levels significantly decreased by administration of FC, intact FGF23 levels decreased in patients with NDD-CKD[21] and undergoing hemodialysis[79]. In the present study, levels of C-terminal FGF23 showed a significant decrease in the FC group compared to the control group in patients with undergoing hemodialysis when the condition of serum phosphate and Hb levels were controlled within the target ranges. Since iron deficiency is known to promote production and cleavage of FGF23 and leads

to increase in C-terminal FGF23 levels [35], these findings raise the possibility that iron replacement with FC resulted in decreased production and cleavage of FGF23.

In the present study, the lack of increase in α-klotho levels in the FC group may be due to the elevated intact FGF23 levels in patients with undergoing hemodialysis[53, 77]. The previous study reported that the initiation of hemodialysis led to a reduction in intact FGF23 levels, however, it did not influence α-klotho levels[80]. This finding strongly endorses the difficulty of increasing levels of α-klotho in patients with undergoing hemodialysis. α-klotho is known to enhance receptor signaling of FGF23 in the kidneys through promoting FGF23 receptor binding affinity[60]. Furthermore, it also plays a significant role in regulating FGF23 production in bones[81]. Consequently, as a negative feedback, expression of α-klotho might be suppressed by the elevated levels of FGF23 in patients with undergoing hemodialysis. The statistically significant reduction of levels of C-terminal FGF23 observed after administration of FC in the present study may not have been sufficient to promote the expression of α-klotho. In a rat model, production of α-klotho was recovered after ischemic acute renal failure[82]. Whether the production of α-klotho can be recovered in kidneys in patients with CKD remains unknown and further research is needed to have a better understanding. It is also the possibility of speculation that the administration of FC has not lead an increase in α-klotho levels, because of the stable Hb levels throughout the study. In patients with undergoing hemodialysis, it has been reported that the levels of α-klotho showed a positive correlation with Hb levels ($p < 0.05$)[83].

The present study had several limitations. Firstly, the sample size was small (n=93), and 8 patients in the FC group and 1 patient in the control group discontinued the treatment as a result of adverse events. Randomized patients to the FC group switched from their prior non-iron-based phosphate binders to FC at baseline. On the contrary, randomized patients to the control group maintained their prior non-iron-based phosphate binders during the study. These difference may have influenced the difference of the number of discontinuation due to adverse events. Secondly, the present study was conducted retrospectively, and the estimation of sample size was made based on the change in dose of erythropoiesis-stimulating agents per week. Consequently, the sample size might have been insufficient to investigate changes in intact FGF23, C-terminal FGF23, and α-klotho

levels, or their correlation relationships. Thirdly, levels of intact FGF23, C-terminal FGF23, and α-klotho were measured at limited points in time (baseline, weeks 12, week 24, and the EOT), which may have been insufficient for the 24 weeks duration of the study. Fourthly, to mitigate the risk of false discovery, multiple testing corrections would have been needed, however, such correction was not performed in the present study. Finally, α-klotho has two forms, mKL and sKL[63], however, this study solely evaluated sKL which can be detected in the blood circulation.

II-5. Conclusions

In conclusion, when maintaining serum phosphate and Hb levels, administration of FC in patients with undergoing hemodialysis significantly increased serum ferritin and TSAT levels, and decreased C-terminal FGF23 levels compared to patients who received non-iron-based phosphate binders. However, administration of FC did not change the levels of α-klotho, and any correlation relationships between levels of intact FGF23 or C-terminal FGF23 and α-klotho were not found.

II-6. Tables

Table II-1. Baseline characteristics

Characteristics	Control group (n = 45)	FC group (n = 46)	*P* value[a]
Age [years], mean ± SD	62.7 ± 12.7	63.3 ± 10.0	0.78[a]
Body weight before dialysis [kg], mean ± SD	62.91 ± 13.61	60.02 ± 10.67	0.26[a]
Male sex, n (%)	36 (80.0)	30 (65.2)	0.16[b]
Use of IV iron preparations, n (%)	6 (13.3)	4 (8.7)	0.52[b]
Serum phosphate [mg/dL], mean ± SD	5.15 ± 1.25	5.36 ± 1.15	0.42
Hb [g/dL], mean ± SD	10.47 ± 0.94	10.52 ± 0.70	0.78
TSAT [%], mean ± SD	21.2 ± 9.3	23.0 ± 9.8	0.36
Serum ferritin [ng/mL], mean ± SD	85.6 ± 85.8	105.7 ± 85.5	0.27
ESA dose[c] [IU/week], mean ± SD	5848.1 ± 4082.8	5735.4 ± 4933.3	0.91
intact FGF23 [pg/mL], mean ± SD	7883.1 ± 10243.8	11774.5 ± 14561.0	0.14
C-terminal FGF23 [pg/mL], mean ± SD	1185.8 ± 1608.6	1610.6 ± 2370.9	0.32
α-Klotho [pg/mL], mean ± SD	442.8 ± 239.1	400.2 ± 107.1	0.27

[a]Student's t-test; [b]Fisher's exact test; [c]Epoetin 200 IU, darbepoetin 1 μg, and epoetin beta pegol 1 μg are equivalent. ;
ESA, erythropoiesis-stimulating agent; FC, ferric citrate hydrate; Hb, hemoglobin;
IV, intravenous; SD, standard deviation; TSAT, transferrin saturation; FGF23, Fibroblast growth factor 23.

Table II-2. Changes in biomarker levels from baseline to end of treatment

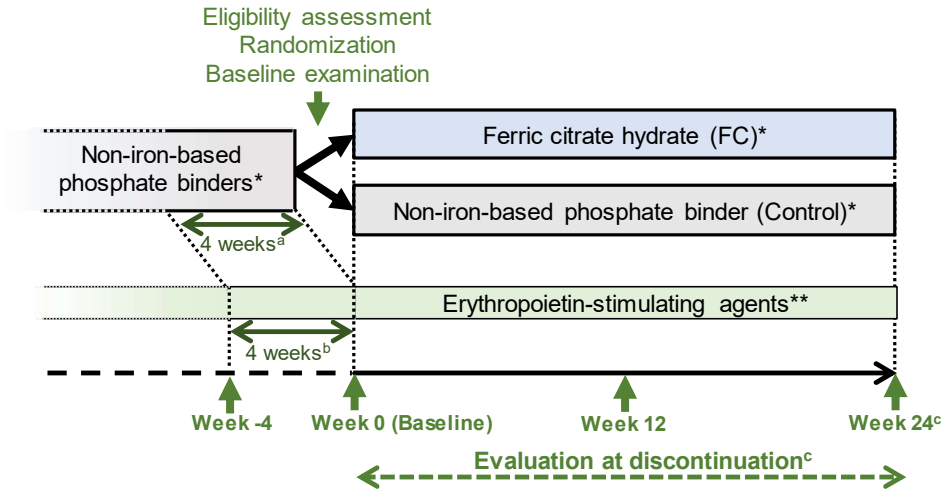
Variables	Control group (n = 42)			FC group (n = 40)			AMD	95% CI	*P*-value[a]
	BL	EOT	Change	BL	EOT	Change			
Serum phosphate [mg/dL]	5.15 (1.25)	5.04 (1.32)	−0.17 (1.53)	5.36 (1.15)	5.65 (1.39)	0.24 (1.59)	0.55	−0.03, 1.13	0.06
Hb [g/dL]	10.47 (0.94)	10.74 (1.14)	0.34 (1.73)	10.52 (0.70)	10.90 (1.23)	0.45 (1.33)	0.17	−0.34, 0.69	0.51
TSAT [%]	21.2 (9.3)	21.8 (10.8)	0.5 (11.8)	23.0 (9.8)	31.8 (13.6)	8.6 (12.1)	9.0	4.0, 13.9	< 0.001
Serum ferritin [ng/mL]	85.6 (85.8)	89.0 (97.4)	2.9 (79.3)	105.7 (85.5)	181.2 (108.2)	79.0 (81.5)	79.5	44.7, 114.4	< 0.001
intact FGF23 [$\log_e$ pg/mL][b]	8.1 (1.5)	8.3 (1.5)	0.1 (0.9)	8.5 (1.5)	8.4 (1.5)	−0.1 (0.8)	0.8[c]	0.6, 12	0.33
C-terminal FGF23 [$\log_e$ pg/mL][b]	6.3 (1.3)	6.5 (1.3)	0.2 (0.8)	6.6 (1.3)	6.3 (1.5)	−0.2 (0.8)	0.7[c]	0.5, 1.0	0.04
α-Klotho [pg/mL]	442.8 (239.1)	440.3 (153.5)	−8.9 (145.3)	400.2 (107.1)	399.7 (129.3)	2.0 (91.5)	−11.1	−51.2, 29.0	0.58

Data are shown as mean ± standard deviation except for AMD, 95% CI and *P*-value.

[a]Analysis of covariance (covariate: baseline); [b]Logarithmic transformation; [c]Exponential form of logarithmic adjusted mean difference; FC, ferric citrate hydrate; AMD, adjusted mean difference (FC – Control); CI, confidence interval; FGF23, BL, baseline; EOT, end of treatment; Hb, hemoglobin; TSAT, transferrin saturation; FGF23, Fibroblast growth factor 23.

II-7. Figures

Figure II-1



*Serum phosphate target: 3.5–6.0 mg/dL
**Hemoglobin target: 10.0–12.0 g/dL
[a]Use of non-iron-based phosphate binder for ≥4 weeks before randomization
[b]Dose and brand of ESA not changed for ≥4 weeks before randomization
[c]Evaluation at discontinuation was performed within 7 days after discontinuation if possible

Figure II-1. Study design.

Figure II-2

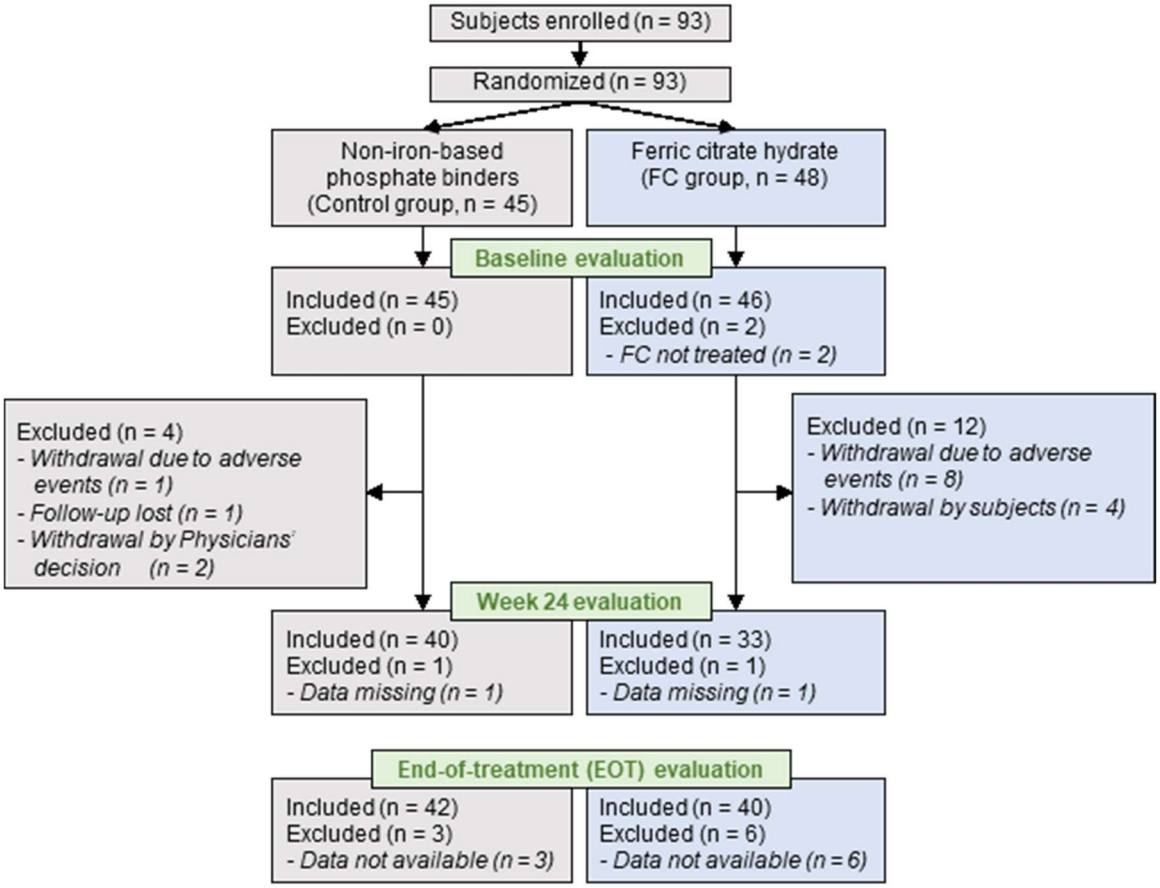


Figure II-2. Flow diagram.

Figure II-3

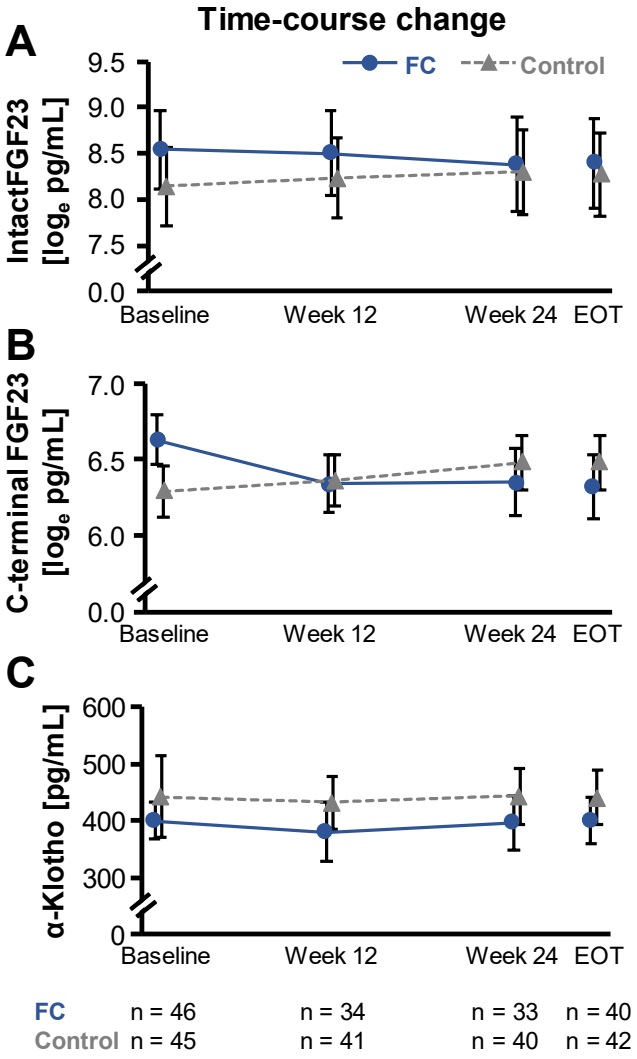


Figure II-3. Time-course fluctuations in intact FGF23, C-terminal FGF23, and α-Klotho from baseline to week 24 and end of treatment.

A. Intact FGF23, B. C-terminal FGF23, C. α-Klotho, Data are presented as mean and upper and lower limit of 95% CI.

Figure II-4

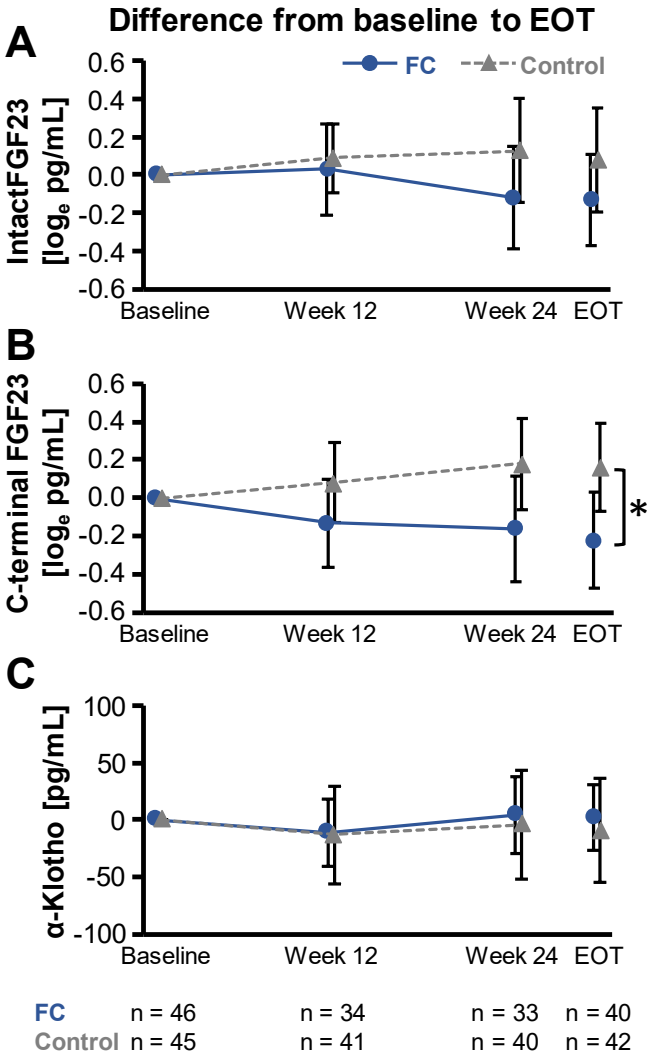


Figure II-4. Time-course fluctuations in difference in levels of intact FGF23, C-terminal FGF23, and α-Klothof from baseline to week 24 and end of treatment.

A. Intact FGF23, B. C-terminal FGF23, C. α-Klotho, * The difference between the FC and control groups (p = 0.04), Data are presented as mean and upper and lower limits of 95% CI.

Figure II-5

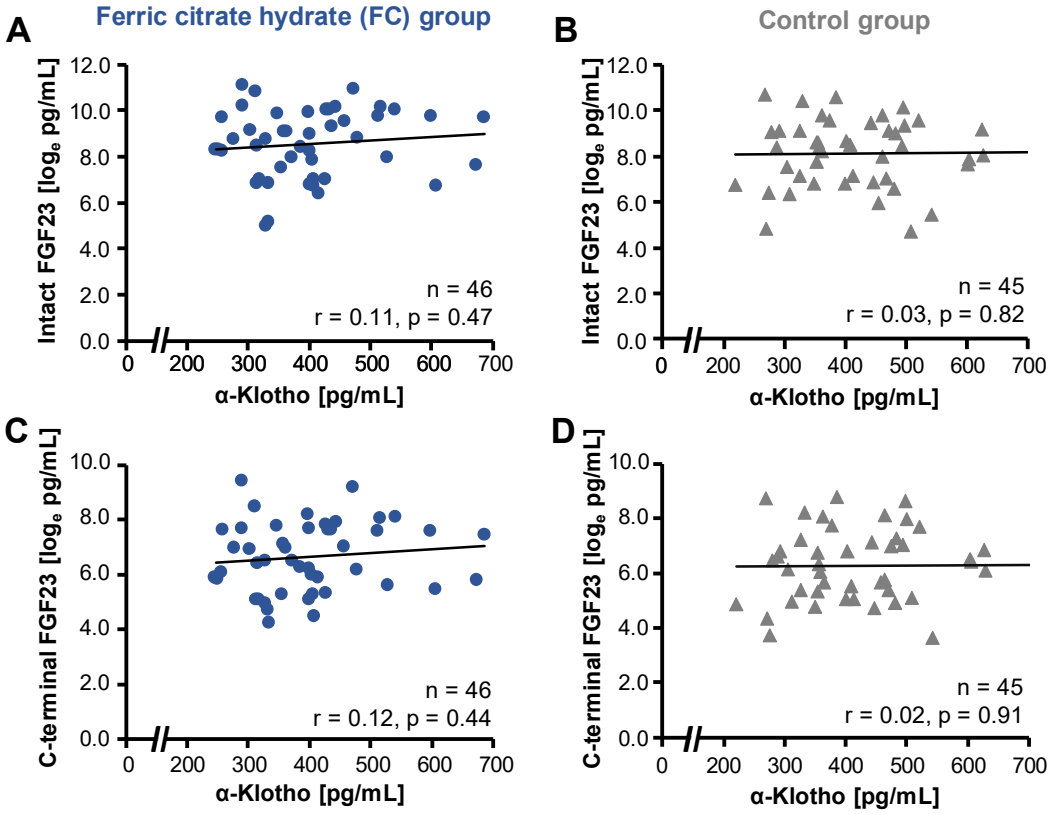


Figure II-5. Correlation relationship between baseline α-Klotho levels and intact FGF23 or C-terminal FGF23 levels.

A. α-Klotho versus intact FGF23 in the FC group, B. α-Klotho versus intact FGF23 in the control group, C. α-Klotho versus C-terminal FGF23 in the FC group, D. α-Klotho versus C-terminal FGF23 in the control group, r, Person's correlation coefficient.

Figure II-6

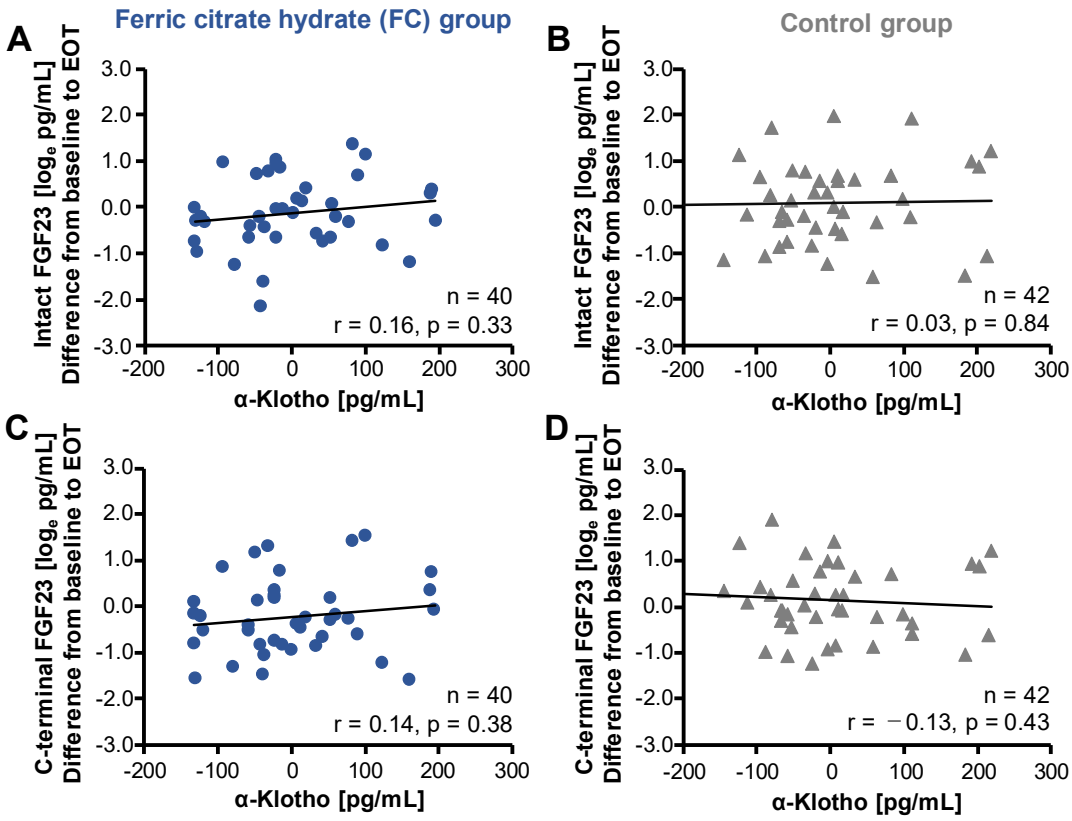


Figure II-6. Correlation relationship of changes between α-Klotho levels and intact FGF23 or C-terminal FGF23 levels from baseline to the EOT.

A. α-Klotho versus intact FGF23 in the FC group, B. α-Klotho versus intact FGF23 in the control group, C. α-Klotho versus C-terminal FGF23 in the FC group, D. α-Klotho versus C-terminal FGF23 in the control group, r, Person's correlation coefficient.

Chapter III:

Effect of Ferric Citrate Hydrate on Fibroblast Growth Factor 23 and Platelets in Non-dialysis-dependent Chronic Kidney Disease and Non-Chronic Kidney Disease Patients with Iron Deficiency Anemia

III-1. Introduction

According to the 2019 World Health Organization report, IDA is a global health problem[84]. IDA often complicates in patients with CKD, chronic heart failure, and other chronic conditions[16, 85, 86]. In addition to treating of its underlying cause, oral iron preparations are used as a first-line treatment for treating IDA.

Iron deficiency is a known risk-related factor for mortality and cardiovascular events[87, 88], and elevated levels of FGF23 are linked to increasing risks of each in general population[36, 37, 89]. FGF23 acts as a peptide hormone that regulates serum phosphate levels by promoting phosphate elimination in the kidneys and attenuating phosphate absorption in the gut. Iron deficiency promotes the production and cleavage of FGF23, which results in an increased levels of C-terminal cleavage fragments. Consequently, iron deficiency increases the FGF23 levels as measured by C-terminal FGF23 assays, which include measurements of both full-length FGF23 and its C-terminal cleavage fragments (C-terminal FGF23), whereas the levels of the full-length FGF23 identified exclusively by intact FGF23 assays (intact FGF23) does not change[90, 91].

Previous investigations have exhibited that the administration of intravenous iron preparations, ferric carboxymaltose and iron dextran, increased levels of Hb, serum ferritin, and TSAT[92]. A reduction in C-terminal FGF23 levels was observed in non-CKD patients with IDA. Although patients treated with iron dextran did not have significant changes in intact FGF23 levels, an increase in intact FGF23 levels was observed in patients administrated with ferric carboxymaltose. This increase in intact FGF23 levels consequentially triggered a subsequent decrease in serum phosphate levels[92]. No study has reported the impact of oral iron preparations on intact FGF23 and C-terminal FGF23levels in non-CKD patients with IDA.

IDA has the potential to exhibit an increase in platelet count, and high platelet count has been implicated in the pathogenesis of thrombotic events[38, 39]. In a previous study of patients with IDA from 1979 to 2019, 32.6% had a state of thrombocytosis (platelet count $> 45\times10^4/\mu L$), while 15.8% had thrombosis[38]. Anemia and thrombocytosis were induced in rats after administration of iron-deficient diet[93] and an increase in the size of thrombi has been observed in rat models of arterial and venous thrombosis[94]. Notably, oral iron succinate reduced platelet count to below $45\times10^4/\mu L$ in patients with IDA whose platelet

count was higher than $45\times10^4/\mu L$ at baseline[95]. Therefore, it can be postulated that iron replacement with oral iron preparations could be expected to reduce elevated platelet count in non-CKD patients with IDA and plausibly improve the potential risk of thrombotic events. However, no previous studies have reported the effect of oral iron preparations on platelet count in NDD-CKD patients with IDA.

Efficacy and safety of FC as an iron replacement therapy have been reported in NDD-CKD and non-CKD patients with IDA previously [26]. In the present study, the effects of FC on intact FGF23, C-terminal FGF23 and platelet count in NDD-CKD and non-CKD patients with IDA were retrospectively analyzed using data from the previous study[26].

III-2. Material and Method

A randomized, open-label, multicenter, uncontrolled, 24-week study registered at jRCT2080223943 was conducted across 31 centers in Japan between July 2018 and December 2019 to explore the efficacy and safety of FC in patients with IDA[26]. The study was executed in alignment with the Declaration of Helsinki and relevant ethical guidelines. The institutional review boards approved the study protocol. All participants in the study agreed to sign a written informed consent form prior to the any study procedures.

Major inclusion criteria encompassed Japanese patients aged ≥20 years old without and with CKD (set by eGFR <60 mL/min/1.73 m^2); whose Hb levels were found to be 8.0 ≤ Hb <11.0 g/dL and whose serum ferritin levels were <50 ng/mL in patients with CKD and <12.0 ng/mL in patients without CKD.

Major exclusion criteria included cases of anemia due to causes other than iron deficiency; whose levels of serum phosphate were <2.5 mg/dL or ≥4.5 mg/dL; who are scheduled to start maintenance dialysis (including surgical shunt or catheter placement); who are scheduled to conduct renal transplantation over the study's duration; have a history of a current malignant tumor or a previous malignant tumor within the previous 5 years; have received iron-free phosphate binders within the previous 2 weeks; have received oral iron, intravenous iron, or iron-containing medicines targeting hyperphosphatemia within the previous 4 weeks; and have received erythropoiesis-stimulating agents within the previous 12 weeks.

Dynamic equilibrium randomization in terms of Hb levels and without or with CKD at baseline, was conducted to perform a 1:1 allocation of patients into either the FC-low group (2 tablets of 250 mg tablet; 500 mg of FC/day) or the FC-high group (4 tablets of 250 mg tablet; 1000 mg of FC/day) (Figure 1). Administration of FC was once daily in the FC-low group, whereas in the FC-high group it was twice a day, just after meals.

Investigators discontinued administration of FC after week 8 if serum ferritin levels exceed predetermined levels of ≥50.0 ng/mL in patients with CKD and ≥25.0 ng/mL in patients without CKD as an example case in point.

The following drugs were prohibited to use throughout the study: (i) oral or intravenous iron preparations (ii) erythropoiesis-stimulating agents, (iii) protein anabolic hormones, testosterone enanthate, and mepitiostane, (iiii) drugs intended to improve absorption of oral iron preparations, (v) drugs for the management of hyperphosphatemia.

Erythrocyte-related parameters and iron-related parameters were evaluated at baseline, week 4, week 8, and the EOT (at week 24 or discontinuation), including Hb, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell count, red blood cell distribution width (RDW), serum iron, TSAT, TIBC, serum ferritin, hepcidin-25 and serum transferrin receptor (sTfR). Intact FGF23 and C-terminal FGF23 were evaluated at baseline, week 8, and the EOT. Serum phosphate, serum calcium, platelet count, prothrombin time-international normalized ratio (PT-INR), activated partial thromboplastin time (APTT), fibrinogen, and quantitative C-reactive protein (CRP), were evaluated at baseline, week 4, week 8, and the EOT.

To standardize the evaluation methods and procedures, all clinical laboratory tests were collectively evaluated at a central laboratory. Levels of serum intact FGF23 (full-length) were measured using the FGF23 ELISA Kit (Kainos, Tokyo, Japan), levels of plasma C-terminal FGF23 (full-length plus C-terminal cleavage fragment) were measured using the Human FGF23 (C-Term) ELISA Kit (Immutopics, Inc., CA, USA), levels of serum hepcidin-25 were measured using the Quantikine® ELISA Human Hepcidin Immunoassay (R&D Systems Inc., MN, USA), and levels of serum sTfR were measured by using the Access sTfR assay in Access immunoassay system (Beckman Coulter Inc., CA, USA).

The sample size was originally estimated to assess the iron replacement of FC and set to be 35 patients in each group (a cumulative total of 70)[26]. Patients who received FC and were evaluated at least one assessment of efficacy considered as the modified intention-to-treat population, and patients who had at least one assessment of safety considered as the safety analysis population. Changes from baseline to week 8 and the EOT within these populations were calculated with 95% CIs. For the platelet count analyses, the upper limit of normal for platelet count was set at $35.2\times10^4/\mu L$ (the 97.5th percentile of upper reference range for healthy adults in Japan)[96] and $45.0\times10^4/\mu L$ (the criteria for thrombocytosis by WHO)[97]. All statistical analyses were executed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), and all adverse events were categorized according to MedDRA version 21.0 using standardized terminology.

III-3. Results

Thirty-six patients were randomly allocated to the FC-low group: CKD n=21, non-CKD n=15, and 37 patients were randomly allocated to the FC-high group: CKD n=21, non-CKD n=16 (Table III-1). Within each of the two groups, 72 out of 73 patients (98.6%) completed the 8 weeks of treatment and 26 patients (35.6%) completed the full 24 weeks of treatment. In overall 47 patients (64.4%) discontinued due to adequate iron replacement after week 8: 41 patients (56.2%) or for some other reasons (Fig III-1). Mean treatment duration (± SD) within the FC-low group and the FC-high group were 127.0 ± 47.1 days for patients with CKD and 108.1 ± 30.7 days for patients with non-CKD, and 119.6 ± 43.1 days for patients with CKD and 107.6 ± 38.8 days for patients with non-CKD, respectively. Adverse events that lead to discontinuation in the FC-low group (n=2) were constipation and diarrhoea, hepatic enzyme increased and malaise. Adverse event that lead to discontinuation in the FC-high group (n=1) was abdominal distension. The modified intention-to-treat population and the safety population included all patients (n=73) (Fig III-1).

In both the FC-low and the FC-high groups, regardless of CKD status, there was a significant increase in mean levels of iron-related parameters; serum iron, TSAT, serum ferritin, hepcidin-25, and red blood cell related parameters; Hb, hematocrit, red blood cell count, MCV, MCH and MCHC, concurrently, TIBC and sTfR levels exhibited a decrease

from baseline to week 8 and from baseline to the EOT. (Table III-2, Table III-3). Furthermore, mean RDW and reticulocyte count levels tended to increase from baseline to week 4 in both groups, regardless of CKD status (Fig III-2, Fig III-3).

At baseline, levels of median intact FGF23 tended to be higher in patients with CKD patients compared in patients with non-CKD, whereas median levels of C-terminal FGF23 tended to be lower in patients with CKD compared in patients with non-CKD (Table III-4).

After administration of FC, there were no significant changes in median intact FGF23 and mean serum phosphate levels in both groups regardless of CKD status (Table III-4, Table III-5, Fig III-4, and Fig III-5). Meanwhile, in both groups, a decrease in median C-terminal FGF23 levels was observed from baseline to week 8 regardless of CKD status (Table III-4, Fig III-6).

At baseline, 1 (5.0%) patient with CKD and 8 (53.3%) patients with non-CKD in the FC-low group showed more than the predefined upper limit of platelet count (>35.2 × 10^4/μL). In the FC-high group, 3 (15.8%) patients with CKD and 8 (50.0%) patients with non-CKD showed more than the predefined upper limit of platelet count (>35.2 × 10^4/μL). In all 20 of these patients, FC administration resulted in a reduction of the platelet count below 35.2 × 10^4/μL by week 8 (Figure III-7). Similarly, FC administration corrected thrombocytosis in all patients who had the WHO definition of thrombocytosis (>45.0 × 10^4/μL) at baseline, including 1 (6.7%) patient with non-CKD in the FC-low group, 1 (5.3%) patient with CKD and 3 (18.8%) patients with non-CKD in the FC-high group (Figure III-7). In all 5 of these patients, FC administration reduced the platelet count below 35.2 × 10^4/μL by week 8 (Figure III-7)

After FC administration, the mean platelet count decreased from baseline to week 8 and from baseline to the EOT either in patients with CKD or non-CKD (Table III-5). However, clinically meaningful changes were not observed in patients whose platelet count under the predefined upper limit at baseline (≤35.2 × 10^4/μL), either in patients with CKD or non-CKD (Figure III-8).

In both groups, administration of FC did not result in changes in mean levels of serum calcium, PT-INR, APTT, fibrinogen, or CRP (Tables III-5 and III-6).

III-4. Discussion

Iron deficiency promotes production and cleavage of FGF23 and therefore it leads to increase in levels of C-terminal FGF23. Separately, iron deficiency increases platelet count in some patients. Each of increased levels of C-terminal FGF23 and platelet count is associated with an increased risk of mortality and cardiovascular events[37, 38, 87, 88, 98, 99]. This study aimed to assess the impacts of FC on levels of intact FGF, C-terminal FGF23, and platelet count in IDA patients with NDD-CKD and non-CKD.

Iron replacement with FC resulted in enhanced erythropoiesis, independent of CKD status. Mean levels of RDW and reticulocyte count showed a tendency to increase up to 4 weeks after administration of FC. The early elevation in level of reticulocyte count after administration of oral iron preparations is an indirect indicator of the effective iron used to produce red blood cells[100]. The elevation in level of RDW corresponds to increased level of reticulocyte count, attributable to the larger size of reticulocyte in comparison to mature red blood cells. Iron replacement with FC has been successfully used for producing red blood cells regardless of CKD status, as an increase in the mean Hb levels has also been observed.

To maintain serum phosphate levels in the narrow normal range, intact FGF23 and C-terminal FGF23 levels increase progressively in patients with CKD from the early CKD stages [53, 101]. In the present study, at baseline, median levels of intact FGF23 were higher in patients with CKD compared in patients with non-CKD. On the contrary, median C-terminal FGF23 levels were lower in patients with CKD compared in patients with non-CKD. As indicated by their lower levels of Hb, TSAT, and serum ferritin at baseline, severity of IDA was greater in the patients with non-CKD compared to the patients with CKD. The greater severity of IDA would result in greater stimulation of FGF23 production and proteolytic cleavage in patients with non-CKD. Furthermore, this is in line with previous research findings that proteolytic cleavage of FGF23 is suppressed in CKD status compared to non-CKD status in mice and humans for maintaining levels of serum phosphate within normal range[102, 103]. In the present study, proteolytic cleavage of FGF23 in CKD patients may be more suppressed than in patients with non-CKD, and C-terminal cleavage fragments induced by IDA may exhibit diminished in patients with CKD compared to those in patients with non-CKD. Consequently, these phenomena are

anticipated to bring reduced levels of C-terminal FGF23 in CKD patients relative to those in patients with non-CKD patients at the baseline.

Administration of FC did not impact on intact FGF23 levels regardless of CKD status, it is likely attributable to the amelioration of elevated production and proteolytic cleavage of FGF23 due to iron deficiency without altering the intact FGF23 levels.

Iron replacement with intravenous ferric carboxymaltose decreases levels of C-terminal FGF23 while concurrently increasing intact FGF23 levels induces hypophosphatemia[104]. Conversely, in the present study, an increase in levels of intact FGF23 and a consequent decrease in levels of serum phosphate were not observed. A previous study which compared the effect of FGF23 between oral iron of sodium iron citrate and intravenous saccharated iron oxide in undergoing hemodialysis patients with iron-deficiency, levels of serum phosphate were maintained during the study, but levels of intact FGF23 elevated in the intravenous iron group while remaining unchanged in the oral iron group, and levels of C-terminal FGF23 decreased in both oral and intravenous groups[105]. From the results of the previous study and the findings of the present study, oral iron preparations may be less likely to cause hypophosphatemia due to elevated levels of intact FGF23.

Another previous study using FC or oral sodium ferrous citrate for 12 weeks in NDD-CKD patients with iron deficiency[106], levels of intact and C-terminal FGF23 did not change. Unlike the previous study, the decrease in C-terminal FGF23 in the present study may reflect more severe patients with iron deficiency at baseline. In addition, it has been reported that levels of C-terminal FGF23 may be underestimated in data collection for serum compared to plasma[107]. Therefore, the present study which used data collection for plasma may have measured C-terminal FGF23 levels more sensitively and precisely than in the previous study, which used the data collection for serum. In a similar previous study, wherein ferric citrate (Auryxia®, Akebia Therapeutics Inc., MA, USA) or ferrous sulfate was administered for 12 weeks in NDD-CKD patients with iron deficiency[108], both levels of intact FGF23 and C-terminal FGF23 tended to decrease. Unlike this previous study, the lack of a decrease in levels of intact FGF23 in the present study might be linked to the higher mean levels of eGFR, and the lower mean levels of TSAT and serum ferritin at baseline. In another previous study in patients undergoing hemodialysis with iron deficiency of oral sodium ferrous citrate, a decrease was observed in levels of intact

FGF23, C-terminal FGF23, dose of erythropoiesis-stimulating agents and CRP without changes in levels of serum phosphate[109]. According to the other previous study, inflammation and erythropoietin promote the production and proteolytic cleavage of FGF23[110]. In the present study, use of erythropoiesis-stimulating agents was prohibited and the levels of CRP did not change during the study. Therefore, the decreased production of FGF23 by the levels of CRP and dose of erythropoiesis-stimulating agents did not occur.

A positive association between elevated C-terminal FGF23 levels and an increased risk of mortality was found in an observational study of the general population[37]. In another observational study, a significant increase in both the prevalence and incidence of anemia was observed in patients with mild to moderate NDD-CKD[111]. Furthermore, increased risk of mortality and heart failure due to iron deficiency has been reported through an intermediate role for C-terminal FGF23 in patients with mild to moderate NDD-CKD[112]. FGF23 may be attributed to a direct and toxic effect on cardiac function, because FGF23 has been reported to be involved in cardiac hypertrophy via FGF receptor 4. It is likely that FGF receptor 4 plays an important role for FGF23 in cardiac hypertrophy, and increased expression of FGF23 also increases FGF receptor 4 expression in rats and mice models[113, 114]. In mice models of CKD, administration of ferric citrate (Auryxia®) not only ameliorated cardiac function but also prolonged life span[115]. Taken together with the results of the present study, it is plausible that iron replacement with FC could potentially decrease risk of mortality or cardiovascular events by lowering elevated levels of C-terminal FGF23 in patients with IDA.

In the present study, the proportion of patients with higher platelet count (>35.2 × 10^4/μL) was lower in patients with CKD compared to patients with non-CKD. Platelets, as well as red blood cells, are produced by hematopoietic stem cells in the bone marrow, and erythropoietin exerts its influence upon hematopoietic stem cells. Therefore, decreased erythropoietin secretion in patients with CKD may be a possible reason for the difference in the proportion of patients with higher platelet count. On the other hand, higher relative risk for venous thromboembolism was observed in patients with CKD than in non-CKD in a community-based study[116].

Although the exact mechanism of why platelet count is elevated in some patients with IDA is not fully understood, it is known that iron deficiency has been shown to affect the differentiation of bone marrow-derived megakaryocytes to erythrocytes and to increase megakaryocyte lineage commitment in humans and mice[117].

Unlike the previous study of oral ferrous succinate[95], oral iron replacement with FC normalized platelet count to below $35.2 \times 10^4/\mu L$ or $45.0 \times 10^4/\mu L$ in IDA patients with CKD as well as in non-CKD. Although currently speculative, our results may suggest that administration of FC ameliorates iron deficiency induced unbalanced differentiation of hematopoietic stem cells regardless of CKD status.

Given the possibility that elevated platelet count in patients with IDA links to thrombosis risk[39], it could be postulated that administration of FC in IDA patients with elevated platelet count may lead to a risk reduction of thrombosis, by amelioration of elevated platelet count into the normal range.

Limitations of the present study are the sample size, which was set to assess the effect of iron replacement by administration of FC. Further studies are needed to determine the effect of FC on FGF23 and platelet count. Furthermore, it is noteworthy that the assessment points of FGF23 levels were limited. Considering the changes in FGF23 levels by an intravenous iron ferric carboxymaltose were observed within 24 hours after administration[104], data from earlier points in time after FC administration would need to be collected.

III-5. Conclusion

In conclusion, iron replacement with FC increased levels of TSAT and serum ferritin while decreasing levels of C-terminal FGF23 without affecting levels of intact FGF23 or serum phosphate in both NDD-CKD and non-CKD patients with IDA. Moreover, it normalized platelet count in patients with elevated platelet count at baseline. Future studies will be investigated whether these observed findings of FC could potentially lead to a reduction of mortality and cardiovascular events in patients with IDA regardless of CKD status.

III-6. Tables

Table III-1 Patient demographics (modified intention-to-treat population)

Characteristics	FC-low (n=36)		FC-high (n=37)	
	CKD (n=21)	non-CKD (n=15)	CKD (n=21)	non-CKD (n=16)
Age, years, mean ± SD	73.2 ± 14.0	45.2 ± 7.1	67.7 ± 13.2	46.7 ± 8.6
Sex, n (%)				
Male	7 (33.3)	0 (0.0)	4 (19.0)	0 (0.0)
Female	14 (66.7)	15 (100.0)	17 (81.0)	16 (100.0)
Menopausal status (females), n (%)				
Pre-menopause	2 (14.3)	15 (100.0)	5 (29.4)	15 (93.8)
Post-menopause	12 (85.7)	0 (0.0)	12 (70.6)	1 (6.3)
Primary cause of IDA [a], n (%)				
Uterine myoma	0 (0.0)	3 (20.0)	0 (0.0)	5 (31.3)
Adenomyosis uteri	0 (0.0)	4 (26.7)	0 (0.0)	1 (6.3)
Endometriosis	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.3)
Other	1 (4.8)	4 (26.7)	1 (4.8)	2 (12.5)
Unknown	20 (95.2)	4 (26.7)	20 (95.2)	9 (56.3)
eGFR, mean ± SD (mL/min/1.73 m^2)	41.2 ± 12.4	84.5 ± 14.3	41.8 ± 14.7	90.0 ± 14.7
The underlying cause of CKD [a] (n, %)				
Diabetic nephropathy	9 (42.9)	-	6 (28.6)	-
Chronic glomerulonephritis	0 (0.0)	-	3 (14.3)	-
Nephrosclerosis	8 (38.1)	-	9 (42.9)	-
Unknown	5 (23.8)	-	3 (14.3)	-
Other	0 (0.0)	-	5 (23.8)	-

[a] Multiple choices are allowed.

FC-low group, ferric citrate hydrate at 500 mg/day; FC-high group, ferric citrate hydrate at 1000 mg/day; SD, standard deviation; IDA, iron deficiency anaemia; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate (creatinine-based)

Table III-2: Time course of iron-related parameters and hemoglobin (modified intention-to-treat population)

Parameter		BL	Week 8	EOT	Change: BL to week 8	Change: BL to EOT
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD (95% CI)	Mean ± SD (95% CI)
Serum iron (µg/dL)						
CKD	FC-low (n=21) [a]	46.0 ± 24.5	70.0 ± 28.0	72.5 ± 25.2	23.1 ± 31.1 (8.5, 37.7)	26.5 ± 24.7 (15.3, 37.8)
	FC-high (n=21)	45.2 ± 23.8	67.6 ± 24.6	74.8 ± 24.1	22.3 ± 25.9 (10.6, 34.1)	29.6 ± 27.3 (17.1, 42.0)
Non-CKD	FC-low (n=15)	19.3 ± 14.9	63.9 ± 27.4	82.0 ± 22.6	44.6 ± 34.3 (25.6, 63.6)	62.7 ± 28.2 (47.1, 78.4)
	FC-high (n=16)	15.7 ± 4.0	83.4 ± 69.7	93.1 ± 84.3	67.8 ± 71.0 (29.9, 105.6)	77.4 ± 85.8 (31.7, 123.1)
Serum ferritin (ng/mL)						
CKD	FC-low (n=21) [a]	16.9 ± 9.1	35.8 ± 16.9	49.7 ± 20.6	18.8 ± 11.7 (13.3, 24.2)	32.9 ± 16.1 (25.5, 40.2)
	FC-high (n=21)	15.9 ± 8.1	44.0 ± 33.5	69.2 ± 57.5	28.1 ± 32.2 (13.4, 42.7)	53.3 ± 54.7 (28.4, 78.1)
Non-CKD	FC-low (n=15)	5.2 ± 2.6	22.7 ± 7.1	30.3 ± 8.5	17.5 ± 6.8 (13.8, 21.3)	25.2 ± 8.2 (20.6, 29.7)
	FC-high (n=16)	4.9 ± 1.9	20.8 ± 7.1	26.7 ± 14.0	15.9 ± 7.2 (12.1, 19.8)	21.8 ± 14.3 (14.2, 29.5)
TIBC (µg/dL)						
CKD	FC-low (n=21) [a]	382.3 ± 67.1	334.1 ± 53.5	318.2 ± 53.0	−46.0 ± 39.4 (−64.4, −27.5)	−64.1 ± 38.4 (−81.6, −46.6)
	FC-high (n=21)	378.5 ± 58.5	328.1 ± 66.0	310.2 ± 66.1	−50.3 ± 37.8 (−67.5, −33.1)	−68.2 ± 38.6 (−85.8, −50.7)
Non-CKD	FC-low (n=15)	448.2 ± 48.8	354.5 ± 31.7	344.5 ± 39.0	−93.7 ± 38.2 (−114.8, −72.5)	−103.7 ± 35.7 (−123.5, −83.9)
	FC-high (n=16)	436.9 ± 51.9	356.6 ± 35.8	350.2 ± 43.6	−80.3 ± 47.5 (−105.6, −55.0)	−86.7 ± 50.4 (−113.5, −59.8)
TSAT (%)						
CKD	FC-low (n=21) [a]	12.4 ± 6.7	20.8 ± 7.2	22.8 ± 7.3	8.1 ± 8.0 (4.4, 11.8)	10.3 ± 6.2 (7.5, 13.2)
	FC-high (n=21)	12.5 ± 7.8	21.3 ± 8.1	25.0 ± 8.7	8.8 ± 7.4 (5.4, 12.1)	12.5 ± 9.0 (8.4, 16.6)

Non-CKD	FC-low (n=15)	4.3 ± 3.5	18.1 ± 7.4	24.3 ± 7.8	13.8 ± 9.2 (8.7, 18.9)	19.9 ± 8.8 (15.1, 24.8)
	FC-high (n=16)	3.6 ± 1.3	23.5 ± 18.8	26.6 ± 21.0	19.9 ± 19.0 (9.8, 30.1)	23.1 ± 21.3 (11.7, 34.4)
Hepcidin-25 (ng/mL)						
CKD	FC-low (n=21) [a]	2.6 ± 3.6	10.9 ± 8.5	17.0 ± 9.8	8.3 ± 6.9 (5.1, 11.6)	14.5 ± 7.7 (11.0, 18.0)
	FC-high (n=21)	2.9 ± 3.5	15.1 ± 10.4	23.5 ± 16.5	12.2 ± 10.4 (7.5, 17.0)	20.6 ± 14.6 (13.9, 27.2)
Non-CKD	FC-low (n=15)	0.3 ± 0.6	7.9 ± 7.9	7.5 ± 6.0	7.6 ± 7.8 (3.3, 11.9)	7.2 ± 5.8 (3.9, 10.4)
	FC-high (n=16)	0.2 ± 0.1	5.5 ± 4.9	9.2 ± 8.1	5.3 ± 4.9 (2.7, 7.9)	9.0 ± 8.0 (4.7, 13.3)
sTfR (nmol/L)						
CKD	FC-low (n=21) [a]	24.3 ± 9.6	19.2 ± 4.4	17.2 ± 5.2	−5.1 ± 6.5 (−8.1, −2.0)	−7.1 ± 6.2 (−9.9, −4.3)
	FC-high (n=21)	28.7 ± 18.3	18.8 ± 6.0	17.2 ± 4.8	−10.0 ± 13.5 (−16.1, −3.8)	−11.5 ± 15.5 (−18.6, −4.5)
Non-CKD	FC-low (n=15)	44.4 ± 14.0	18.9 ± 5.0	16.7 ± 4.8	−25.5 ± 10.8 (−31.5, −19.5)	−27.7 ± 12.1 (−34.3, −21.0)
	FC-high (n=16)	44.5 ± 14.9	22.0 ± 6.4	21.5 ± 8.7	−22.5 ± 14.8 (−30.4, −14.6)	−23.0 ± 17.6 (−32.4, −13.6)
Hemoglobin (g/dL)						
CKD	FC-low (n=21) [a]	10.2 ± 0.8	11.4 ± 0.7	11.8 ± 1.0	1.3 ± 1.1 (0.8, 1.8)	1.6 ± 1.3 (1.0, 2.2)
	FC-high (n=21)	10.2 ± 0.6	11.9 ± 1.3	12.0 ± 1.4	1.7 ± 1.6 (0.9, 2.4)	1.8 ± 1.8 (1.0, 2.6)
Non-CKD	FC-low (n=15)	9.4 ± 0.6	12.4 ± 0.8	12.9 ± 0.9	3.0 ± 0.9 (2.5, 3.5)	3.5 ± 1.0 (2.9, 4.0)
	FC-high (n=16)	9.4 ± 0.6	12.4 ± 1.2	12.8 ± 1.7	3.0 ± 1.3 (2.3, 3.7)	3.4 ± 1.8 (2.4, 4.3)

[a] Week 8, n=20.

BL, baseline; EOT, end of treatment or at discontinuation; CI, confidence interval; FC-low group, ferric citrate hydrate at 500 mg/day; FC-high group, ferric citrate hydrate at 1000 mg/day; TIBC, total iron-binding capacity; TSAT, transferrin iron saturation, sTfR, soluble transferrin receptor, SD, standard deviation

Table III-3: Time course of erythrocyte-related parameters (modified intention-to-treat population)

Parameters		BL Mean ± SD	Week 8 Mean ± SD	EOT Mean ± SD	Change from BL to week 8 Mean ± SD	95% CI (BL to week 8)	Change from BL to EOT Mean ± SD	95% CI (BL to EOT)
Red Blood Cell Count (10^4/μL)								
CKD	FC-low (n=21) [a]	357.4 ± 30.6	381.5 ± 35.1	383.0 ± 38.4	25.3 ± 26.9	12.6, 37.9	25.6 ± 27.9	12.9, 38.3
	FC-high (n=21)	382.6 ± 48.5	416.3 ± 67.6	405.7 ± 60.9	33.7 ± 31.6	19.3, 48.1	23.1 ± 26.7	11.0, 35.3
Non-CKD	FC-low (n=15)	416.3 ± 41.4	458.8 ± 37.0	443.8 ± 45.5	42.5 ± 28.6	26.6, 58.3	27.5 ± 56.3	−3.7, 58.7
	FC-high (n=16)	411.6 ± 39.3	455.2 ± 38.5	448.6 ± 41.9	43.6 ± 25.1	30.2, 57.0	37.1 ± 28.7	21.8, 52.4
Hematocrit (%)								
CKD	FC-low (n=21) [a]	30.9 ± 2.3	34.4 ± 2.3	35.1 ± 3.2	3.5 ± 3.3	2.0, 5.1	4.2 ± 3.7	2.5, 5.9
	FC-high (n=21)	31.2 ± 1.7	35.9 ± 4.1	35.9 ± 4.3	4.7 ± 4.5	2.7, 6.7	4.7 ± 4.7	2.6, 6.8
Non-CKD	FC-low (n=15)	29.8 ± 1.6	37.9 ± 2.3	38.6 ± 3.2	8.0 ± 2.4	6.7, 9.4	8.7 ± 3.5	6.8, 10.7
	FC-high (n=16)	29.8 ± 1.8	37.8 ± 3.6	38.7 ± 4.7	8.0 ± 3.5	6.1, 9.9	8.8 ± 4.7	6.3, 11.3
MCV (fL)								
CKD	FC-low (n=21) [a]	86.8 ± 5.5	90.4 ± 4.3	91.9 ± 4.2	1.6 ± 1.2	1.0, 2.1	2.3 ± 1.9	1.4, 3.2
	FC-high (n=21)	82.6 ± 8.6	86.9 ± 4.7	88.9 ± 4.2	1.7 ± 2.0	0.8, 2.6	2.6 ± 2.7	1.4, 3.9
Non-CKD	FC-low (n=15)	72.3 ± 6.7	82.8 ± 5.6	87.3 ± 6.0	4.4 ± 1.9	3.4, 5.4	6.4 ± 3.0	4.8, 8.0
	FC-high (n=16)	73.0 ± 5.8	82.9 ± 3.8	86.0 ± 5.6	4.2 ± 2.7	2.8, 5.6	5.4 ± 3.3	3.7, 7.2
MCH (pg)								
CKD	FC-low (n=21) [a]	28.5 ± 2.2	30.1 ± 1.7	30.8 ± 1.7	3.5 ± 3.3	2.0, 5.0	5.0 ± 4.8	2.9, 7.2
	FC-high (n=21)	27.1 ± 3.4	28.8 ± 1.8	29.7 ± 1.5	4.3 ± 5.1	2.0, 6.6	6.3 ± 6.4	3.4, 9.3

Non-CKD	FC-low (n=15)	22.8 ± 2.6	27.2 ± 1.9	29.2 ± 2.3	10.5 ± 4.5	8.1, 13.0	15.0 ± 6.7	11.3, 18.7
	FC-high (n=16)	23.0 ± 2.3	27.2 ± 1.4	28.4 ± 2.1	9.9 ± 6.8	6.3, 13.6	13.0 ± 8.1	8.7, 17.3
MCHC (%)								
CKD	FC-low (n=21) [a]	32.8 ± 0.6	33.3 ± 0.6	33.5 ± 0.7	0.4 ± 0.4	0.2, 0.6	0.7 ± 0.7	0.4, 1.0
	FC-high (n=21)	32.7 ± 1.1	33.1 ± 0.5	33.4 ± 0.5	0.4 ± 0.9	−0.02, 0.8	0.7 ± 1.1	0.2, 1.2
Non-CKD	FC-low (n=15)	31.5 ± 0.8	32.8 ± 0.5	33.4 ± 0.9	1.3 ± 0.8	0.9, 1.8	1.9 ± 1.3	1.2, 2.6
	FC-high (n=16)	31.5 ± 0.7	32.7 ± 0.6	33.0 ± 0.6	1.2 ± 0.8	0.7, 1.6	1.5 ± 1.0	1.0, 2.0
RDW (%)								
CKD	FC-low (n=21) [a]	15.2 ± 1.8	16.6 ± 2.8	14.8 ± 2.2	1.4 ± 1.6	0.7, 2.1	−0.4 ± 1.5	−1.1, 0.3
	FC-high (n=21)	15.6 ± 2.1	17.7 ± 4.6	15.6 ± 3.0	2.2 ± 2.8	0.9, 3.4	0.02 ± 2.1	−0.9, 1.0
Non-CKD	FC-low (n=15)	18.3 ± 2.9	23.5 ± 2.1	16.0 ± 3.2	5.2 ± 3.2	3.5, 7.0	−2.3 ± 4.3	−4.7, 0.1
	FC-high (n=16)	18.0 ± 2.1	24.2 ± 4.3	18.4 ± 5.7	6.3 ± 3.1	4.6, 7.9	0.5 ± 5.5	−2.4, 3.4
Reticulocyte Count (10^4/μL)								
CKD	FC-low (n=21) [a]	4.2 ± 1.3	5.1 ± 1.6	4.7 ± 1.9	0.9 ± 1.7	0.2, 1.8	0.6 ± 1.6	−0.2, 1.3
	FC-high (n=21)	5.3± 1.7	6.0 ± 2.0	6.0 ± 2.3	0.6 ± 1.8	−0.2, 1.5	0.7 ± 2.2	−0.3, 1.7
Non-CKD	FC-low (n=15)	6.1 ± 2.8	5.8 ± 3.2	6.5 ± 3.1	−0.3 ± 2.7	−1.8, 1.2	0.4 ± 2.0	−0.7, 1.5
	FC-high (n=16)	5.3 ± 2.3	6.3 ± 3.1	6.2 ± 2.7	0.9 ± 2.6	−0.5, 2.3	0.9 ± 2.3	−0.4, 2.2

[a] Week 8, n=20

BL, baseline; EOT, end of treatment or at discontinuation; CI, confidence interval; SD, standard deviation; FC-low group, ferric citrate hydrate at 500 mg (approximately 120 mg elemental iron)/day; FC-high group, ferric citrate hydrate at 1000 mg (approximately 240 mg elemental iron)/day; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width

Table III-4: Time course of fibroblast growth factor 23 (modified intention-to-treat population)

Parameters		BL Median (Q1, Q3)	Week 8 Median (Q1, Q3)	EOT Median (Q1, Q3)	Change from BL to week 8 Median (Q1, Q3)	95% CI (BL to week 8)	Change from BL to EOT Median (Q1, Q3)	95% CI (BL to EOT)
Intact FGF23 (pg/mL)								
CKD	FC-low	64.00	63.00	62.10	1.00	−9.30, 13.40	2.60	−10.10, 14.40
	(n=21) [a]	(48.10, 82.20)	(53.70, 77.65)	(52.00, 74.60)	(−9.35, 13.90)		(−10.10, 14.40)	
	FC-high	58.20	54.50	56.30	0.90	−11.10, 7.30	1.30	−7.10, 5.20
	(n=21)	(45.10, 72.50)	(49.60, 68.90)	(48.10, 72.10)	(−11.10, 7.30)		(−7.10, 5.20)	
Non-CKD	FC-low	40.80	41.70	37.40	−2.60	−6.50, 5.40	−3.90	−11.70, 6.00
	(n=15)	(37.50, 47.20)	(35.10, 49.10)	(30.90, 51.30)	(−6.50, 5.40)		(−11.70, 6.00)	
	FC-high	35.90	37.85	39.05	−1.90	−5.80, 3.20	−3.40	−5.10, 5.30
	(n=16)	(30.10, 48.75)	(28.00, 43.35)	(29.90, 45.35)	(−7.10, 2.65)		(−5.20, 5.15)	
C-terminal FGF23 (RU/mL)								
CKD	FC-low	159.00	108.50	104.00	−58.00	−181.00, −12.50	−71.90	−181.00, −11.00
	(n=21) [a]	(135.00, 390.00)	(85.25, 132.00)	(90.40, 118.00)	(−227.50, −12.25)		(−181.00, −11.00)	
	FC-high	188.0	110.00	114.00	−66.00	−265.70, −27.00	−74.00	−265.7, −18.9
	(n=21)	(136.00, 361.00)	(91.50, 135.00)	(89.30, 153.00)	(−265.70, −27.00)		(−265.70, −18.90)	
Non-CKD	FC-low	1010.00	102.00	96.10	−725.00	−1124.00,	−745.00	−1124.00,
	(n=15)	(260.00, 1240.00)	(83.60,122.00)	(73.00, 155.00)	(−1124.00, −168.50)	−168.50	(−1124.00, −183.00)	−183.00
	FC-high	775.00	104.50	104.50	−649.50	−988.00,	-666.50	−1117.00,
	(n=16)	(394.00, 1285.00)	(65.50, 138.00)	(70.75, 144.00)	(−1127.00, −326.65)	−299.40	(−1191.50, −319.40)	−298.40

[a] Week 8, n=20

BL. Baseline; CI, confidence interval; FC-low group, ferric citrate hydrate at 500 mg/day; FC-high group, ferric citrate hydrate at 1000 mg /day; EOT, end of treatment or at discontinuation

Table III-5: Time courses of serum phosphate, platelet count, serum calcium, and CRP (safety analysis population)

Parameters		BL Mean ± SD	Week 8 Mean ± SD	EOT Mean ± SD	Change from BL to week 8 (Mean ± SD)	95% CI (BL to week 8)	Change from BL to EOT (Mean ± SD)	95% CI (BL to EOT)
Serum phosphate (mg/dL)								
CKD	FC-low (n=21) [a]	3.56 ± 0.72	3.63 ± 0.49	3.57 ± 0.43	0.11 ± 0.60	−0.18, 0.39	0.01 ± 0.58	−0.25, 0.27
	FC-high (n=21)	3.32 ± 0.48	3.36 ± 0.42	3.40 ± 0.51	0.03 ± 0.44	−0.17, 0.23	0.08 ± 0.48	−0.14, 0.30
Non-CKD	FC-low (n=15)	3.69 ±0.30	3.75 ± 0.42	3.67 ± 0.56	0.06 ± 0.50	−0.21, 0.33	−0.02 ± 0.63	−0.37, 0.33
	FC-high (n=16)	3.34 ± 0.69	3.46 ± 0.63	3.47 ± 0.52	0.12 ± 0.57	−0.19, 0.42	0.13 ± 0.60	−0.19, 0.45
Platelet count (10^4/μL)								
CKD	FC-low (n=21) [a]	24.27 ± 7.17	21.89 ± 5.25	21.58 ± 6.22	−1.78 ± 4.05	−3.67, 0.11	−2.69 ± 4.10	−4.56, −0.82
	FC-high (n=21) [b]	26.56 ± 8.58	25.09 ± 6.97	23.69 ± 6.62	−1.56 ± 7.28	−5.07, 1.95	−2.87 ± 4.58	−5.01, −0.72
Non-CKD	FC-low (n=15)	33.25 ± 9.02	24.35 ± 5.47	24.91 ± 6.95	−8.91 ± 5.36	−11.88, −5.94	−8.34 ± 5.32	−11.28, −5.40
	FC-high (n=16)	36.99 ± 8.38	29.58 ± 4.41	28.63 ± 4.64	−7.41 ± 6.25	−10.74, −4.08	−8.36 ± 7.34	−12.27, −4.45
Serum calcium (mg/dL)								
CKD	FC-low (n=21) [a]	9.22 ± 0.70	9.34 ± 0.48	9.25 ± 0.56	0.16 ± 0.43	−0.05, 0.36	0.03 ± 0.41	−0.15, 0.22
	FC-high (n=21)	9.00 ± 0.31	9.23 ± 0.45	9.10 ± 0.34	0.23 ± 0.32	0.09, 0.38	0.11 ± 0.25	−0.01, 0.23
Non-CKD	FC-low (n=15)	9.04 ± 0.14	9.18 ± 0.34	9.10 ± 0.43	0.14 ± 0.31	−0.03, 0.31	0.06 ± 0.43	−0.18, 0.30
	FC-high (n=16)	9.08 ± 0.28	9.27 ± 0.25	9.22 ± 0.24	0.19 ± 0.23	0.07, 0.31	0.14 ± 0.32	−0.04, 0.31
CRP (mg/dL)								
CKD	FC-low (n=21) [a]	0.14 ± 0.23	0.14 ± 0.14	0.15 ± 0.30	0.01 ± 0.20	−0.09, 0.10	0.01 ± 0.33	−0.14, 0.16
	FC-high (n=21)	0.23 ± 0.63	0.34 ± 0.94	0.39 ± 0.80	0.11 ± 1.15	−0.42, 0.63	0.16 ± 1.03	−0.31, 0.63
Non-CKD	FC-low (n=15)	0.14 ± 0.44	0.15 ± 0.40	0.14 ± 0.41	0.01 ± 0.08	−0.03, 0.05	0.003 ± 0.03	−0.02, 0.02
	FC-high (n=16)	0.08 ± 0.09	0.06 ± 0.08	0.07 ± 0.07	−0.02 ± 0.08	−0.06, 0.03	−0.01 ± 0.07	−0.05, 0.03

[a] Week 8, n=20; [b] Week 8, n=19;

BL, baseline; EOT, end of treatment; CI, confidence interval; FC-low group, ferric citrate hydrate at 500 m/day; FC-high group, ferric citrate hydrate at 1000 mg/day; SD, standard deviation

Table III-6: **The time course of coagulation-related parameters (safety analysis population)**

Parameters		BL Mean ± SD	Week 8 Mean ± SD	EOT Mean ± SD	Change from BL to week 8 Mean ± SD	95% CI (BL to week 8)	Change from BL to EOT Mean ± SD	95% CI (BL to EOT)
PT-INR								
CKD	FC-low (n=21) [a]	1.07 ± 0.25	1.03 ±0.19	1.03 ±0.16	−0.04 ± 0.11	−0.09, 0.02	−0.04 ±0.13	−0.11, 0.02
	FC-high (n=21)	1.08 ±0.35	1.14 ±0.60	1.09 ± 0.43	0.07 ± 0.27	−0.06, 0.19	0.01 ± 0.14	−0.05, 0.08
Non-CKD	FC-low (n=15)	0.99 ± 0.04	0.96 ± 0.05	0.96 ± 0.05	−0.03 ± 0.07	−0.07, 0.01	−0.03 ± 0.06	−0.06, 0.003
	FC-high (n=16)	0.97 ± 0.04	0.97 ± 0.06	0.97 ± 0.04	0.001 ± 0.06	−0.03, 0.03	0.003 ± 0.04	−0.02, 0.02
APTT (sec)								
CKD	FC-low (n=21) [a]	33.33 ± 4.10	33.39 ± 3.42	33.19 ± 3.73	0.27 ± 2.76	−1.02, 1.55	−0.15 ± 2.91	−1.47, 1.18
	FC-high (n=21)	33.02 ± 2.69	33.65 ± 3.54	33.23 ± 3.94	0.63 ± 2.60	−0.55, 1.81	0.21 ± 2.49	−0.93, 1.35
Non-CKD	FC-low (n=15)	31.01 ± 2.39	32.27 ± 3.22	32.17 ± 2.80	1.26 ± 2.44	−0.09, 2.61	1.16 ± 2.32	−0.12, 2.44
	FC-high (n=16)	30.72 ± 3.28	32.73 ± 3.39	32.01 ± 3.08	2.01 ± 2.72	0.56, 3.46	1.29 ± 1.88	0.29, 2.29
Fibrinogen (mg/dL)								
CKD	FC-low (n=21) [a]	324.5 ± 75.1	327.5 ± 61.9	307.7 ± 68.3	13.8 ± 48.9	−9.1, 36.6	−16.8 ± 61.9	−45.0, 11.4
	FC-high (n=21)	329.4 ± 94.2	331.8 ± 60.7	328.0 ± 81.9	2.4 ± 69.6	−29.3, 34.0	−1.4 ± 91.7	−43.2, 40.3
Non-CKD	FC-low (n=15)	285.2 ± 60.4	291.7 ± 60.3	267.7 ± 41.5	6.5 ± 35.4	−13.1, 26.1	−17.5 ± 53.0	−46.9, 11.8
	FC-high (n=16)	279.0 ± 47.6	291.5 ± 56.5	289.2 ± 53.3	12.5 ± 39.7	−8.7, 33.7	10.2 ± 36.2	−9.1, 29.5

[a] Week 8, n=20;

BL, baseline; EOT, end of treatment or at discontinuation; CI, confidence interval; SD, standard deviation; FC-low group, ferric citrate hydrate at 500 mg (approximately 120 mg elemental iron)/day; FC-high group, ferric citrate hydrate at 1000 mg (approximately 240 mg elemental iron)/day; PT-INR, prothrombin time-international normalized ratio; APTT, activated partial thromboplastin time

III-7. Figures

Figure III-1

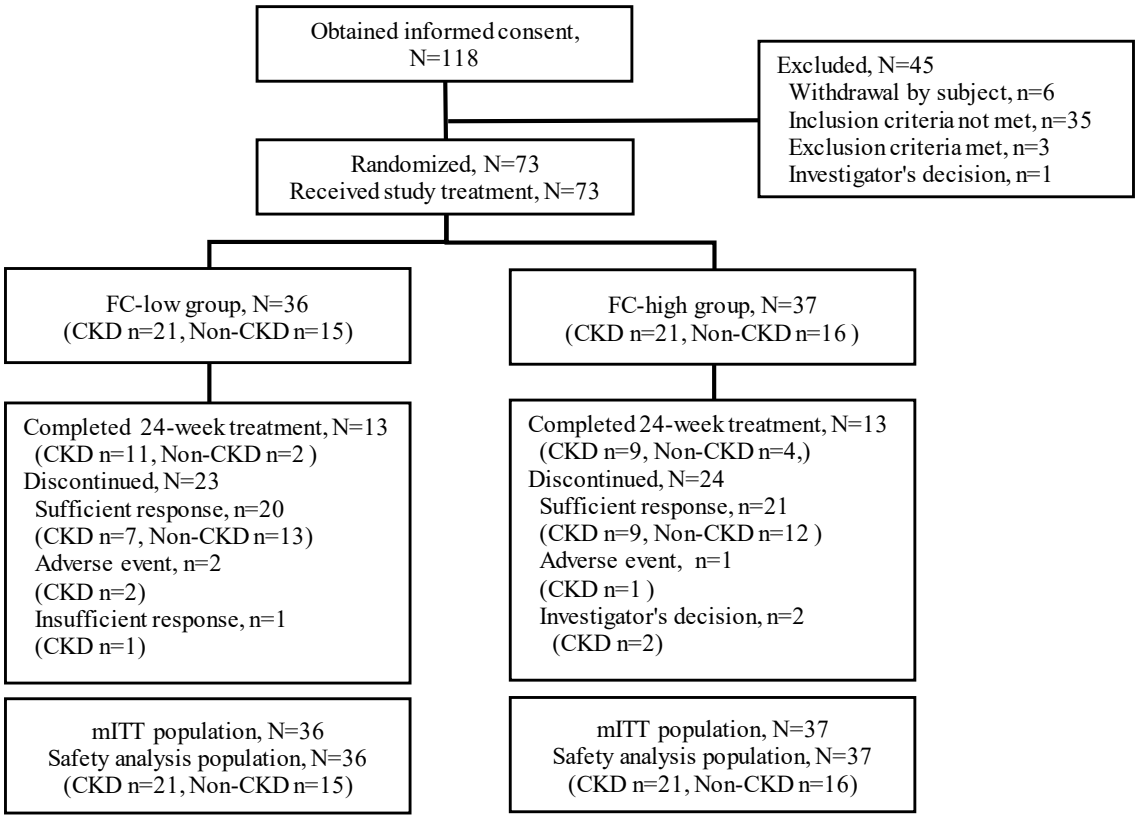
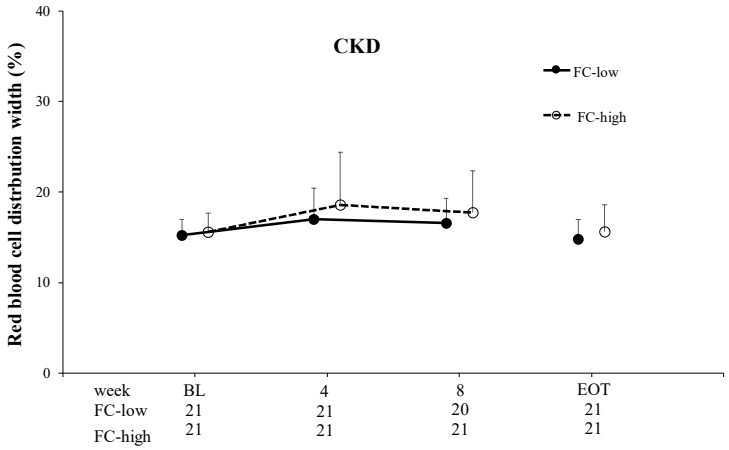


Figure III-1 Patient Flow.

Figure III-2

A



B

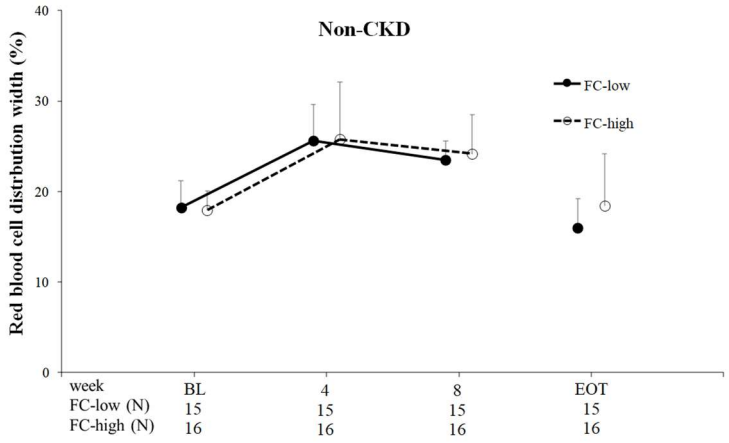
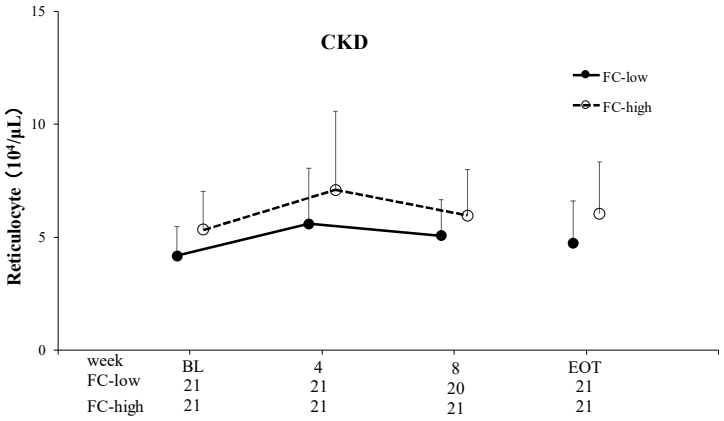


Figure III-2-Time-course fluctuations of red blood cell distribution width from baseline to week 8 and end of treatment.

A. CKD patients, B. non-CKD patients, FC-low (black circles), FC-high (white circles), Data are presented as mean + SD.

Figure III-3

A



B

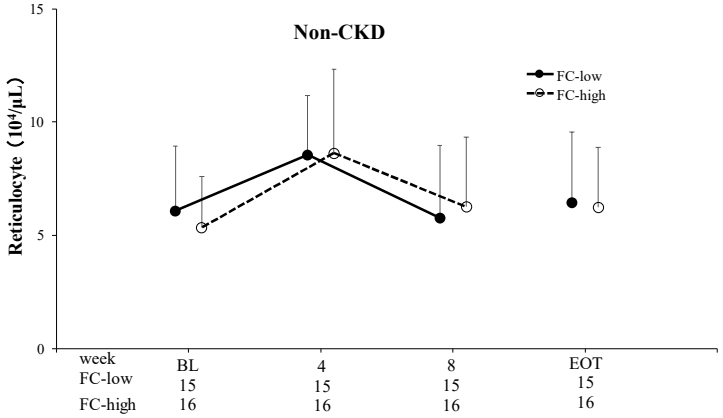
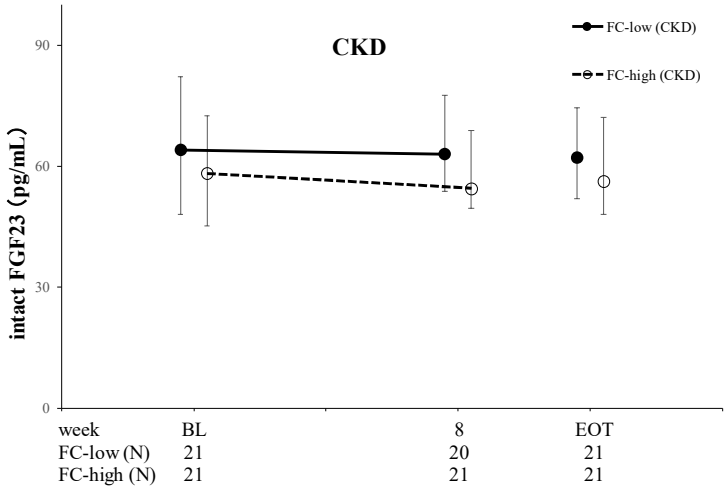


Figure III-3-Time-course fluctuations of reticulocyte count from baseline to week 8 and end of treatment.

A. CKD patients, B. non-CKD patients, FC-low (black circles), FC-high (white circles), Data are presented as mean + SD.

Figure III-4

A



B

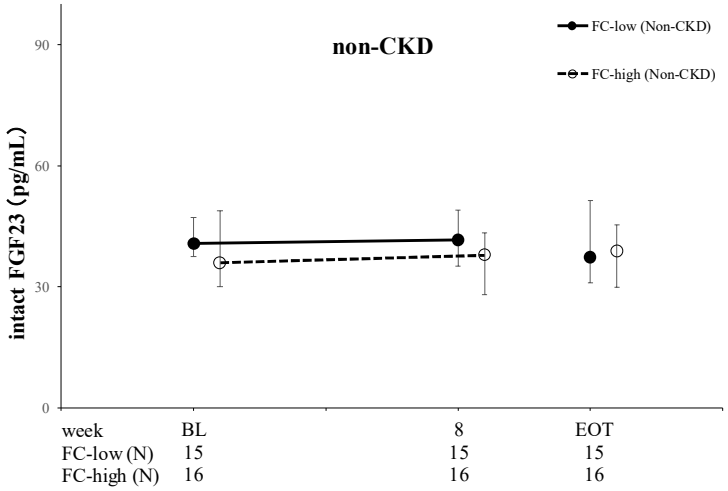
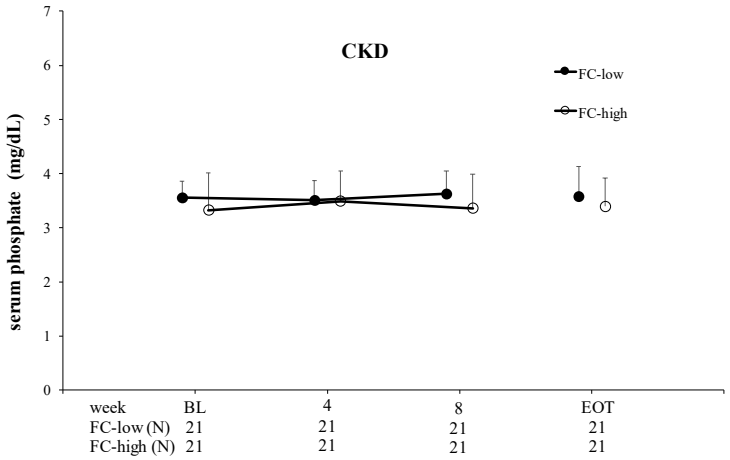


Figure III-4 Time-course fluctuations of intact FGF23 from baseline to week 8 and end of treatment.

A. CKD patients, B. non-CKD patients, FC-low (black circles), FC-high (white circles), Data are presented as median (Q1, Q3).

Figure III-5

A



B

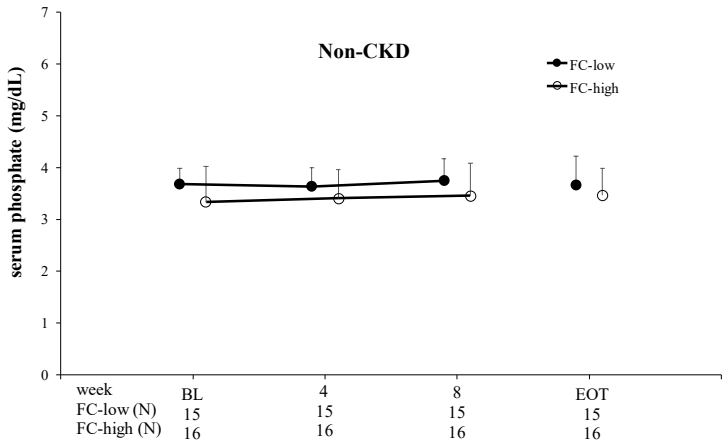
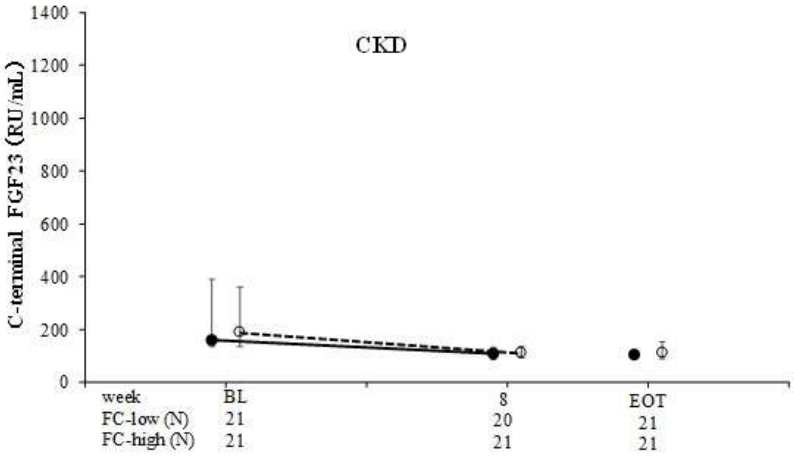


Figure III-5 Time-course fluctuations of serum phosphate from baseline to week 8 and end of treatment.

A. CKD patients, B. non-CKD patients, FC-low (black circles), FC-high (white circles), Data are presented as mean + SD.

Figure III-6

A



B

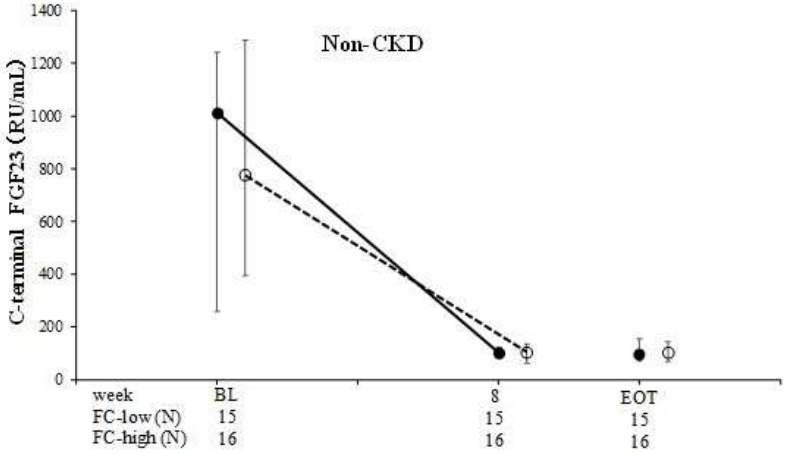


Figure III-6 Time-course fluctuations of C-terminal FGF23 from baseline to week 8 and end of treatment.

A. CKD patients, B. non-CKD patients, FC-low (black circles), FC-high (white circles), Data are presented as median (Q1, Q3).

Figure III-7

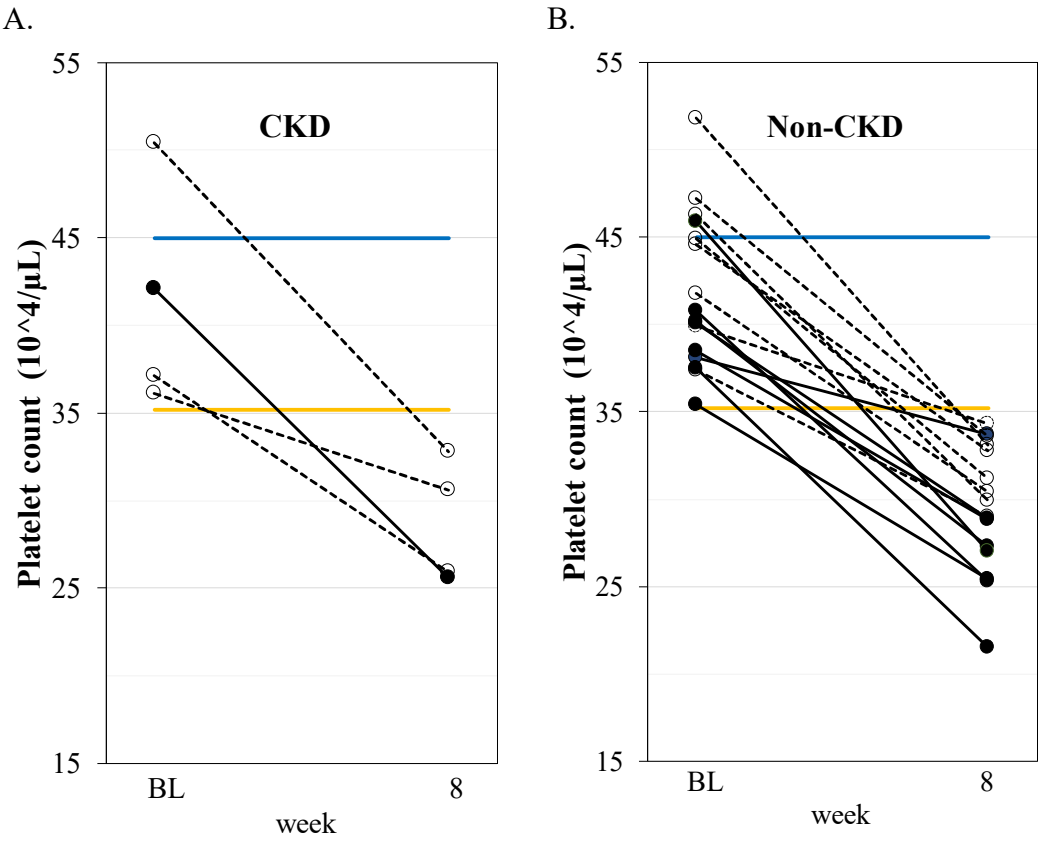


Figure III-7: **Changes in platelet count in patients with high platelet count from baseline to week 8.**

A. CKD patients, B. non-CKD patients, FC-low (black circles), FC-high (white circles), the orange line is 35.2 × 10^4/μL which is 97.5% upper reference limit in healthy adult in Japan, and the blue line is 45.0 × 10^4/μL which is a criteria in WHO definition of thrombocytosis.

Figure III-8

A. B.

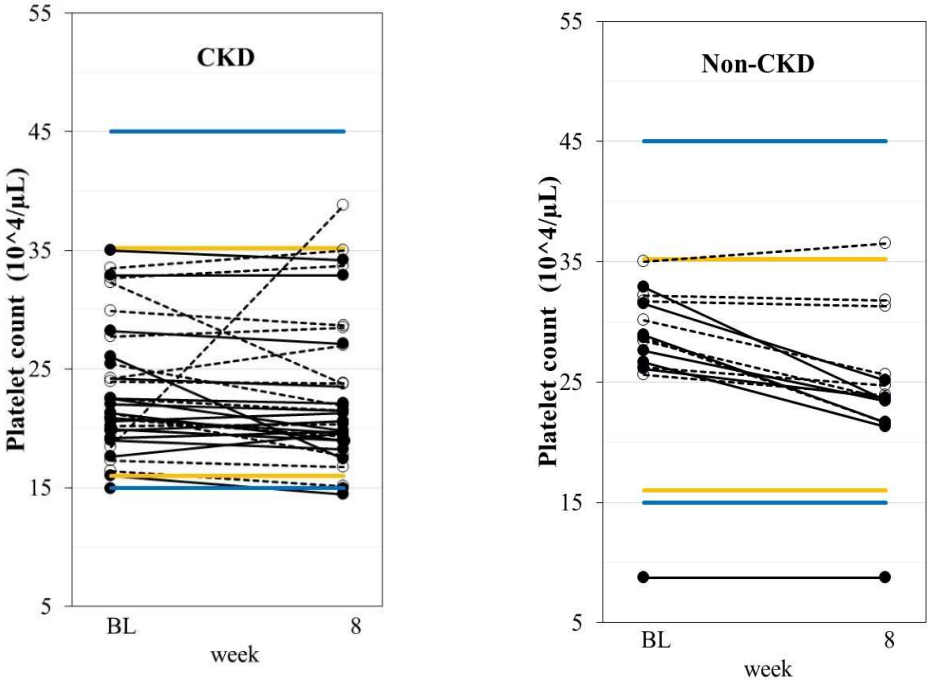


Figure III-8: **Changes in platelet count in patients without high platelet count from baseline to week 8.**

A. CKD patients, B. non-CKD patients, FC-low (black circles), FC-high (white circles), the orange lines are 35.2 × 10^4/μL and 16.0× 10^4/μL which are 97.5% upper reference limit and 2.5% lower reference limit in healthy adult in Japan, respectively, the blue lines are 45.0 × 10^4/μL and 15.0 × 10^4/μL which are criteria in WHO definition of thrombocytosis and thrombocytopenia, respectively.

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General Discussion

Two indications for FC are the treatment of hyperphosphatemia in patients with CKD, and the treatment of IDA. Phosphorus is one of the essential minerals for good health and present as a component of bones and teeth. In addition, phosphorus binds to proteins and lipids, and they are components of cell membranes and nucleic acids (deoxyribonucleic acid and ribonucleic acid) that carry genetic information. Furthermore, phosphorus plays an important role in maintaining life and activity as a component of adenosine triphosphate (ATP), which is responsible for energy production. Iron is also one of the essential minerals that are indispensable for maintaining good health. Iron is a component of Hb in red blood cells and plays an important role in transporting oxygen to tissues throughout the body. In addition, iron is a constituent of enzymes and plays an important role in energy metabolism for ATP synthesis.

FC is a drug related to serum phosphate level and iron metabolism. I conducted three studies, summarized in Chapter I, II and III of this dissertation, and found that FC can be effective with or without GASI and improve FGF23 and platelet count levels.

To conduct my research on FC, the following hypotheses were made. Firstly, FC has unique characteristics that may be soluble in a wide range of pH in the stomach, in the duodenum (oral iron absorption site) and in the small intestine (phosphate-binding site). In addition, FC has a large surface area that enables its rapid disintegration and dissolution. In terms of clinical application, the pH independent solubility of FC, is likely to play an important role in both the phosphate-binding and iron replacement effects of FC, and may address an unmet medical need for patients concomitantly taking GASI. Secondly, FGF23, a hormone known to regulate serum phosphate level, and α-klotho, a protein known to be a co-receptor for FGF23, are among the biomarkers of high interest. However, it is unclear whether FGF23 has any activity other than regulating serum phosphate level, whether α-klotho has any activity other than being a co-receptor for FGF23, and how α-klotho expression is regulated in the kidneys. FC may affect FGF23 and α-klotho in terms of phosphate-binding and iron replacement, and then may provide new insights. Finally, it has been suggested that elevated FGF23 level may be associated with the risk of cardiovascular events, and both non-clinical and clinical studies are underway to investigate this. It has also been suggested that elevated platelet count may be associated

with the risk of cardiovascular events such as thrombosis, but the mechanisms are under investigation from both non-clinical and clinical perspectives. Since iron deficiency has been reported to increase FGF23 and platelet count, the iron replacement of FC may affect elevated FGF23 and platelet count. This may provide new insights.

In Chapter I, I hypothesized that the concomitant administration of GASI would not interfere with the dissolution of FC and attenuate its effects on phosphate-binding and iron replacement, and therefore investigated it in patients with NDD-CKD and undergoing hemodialysis. The hypothesis was verified in those patients. It is considered that the characteristic properties of FC, to be soluble in a wide range of pH, promotes its solubility at higher pH levels under concomitant administration of GASI, and therefore, the concomitant administration of GASI did not interfere with the effects of FC. When GASI were used concomitantly, phosphate-binding effect of some phosphate binders and iron replacement with oral iron preparations were attenuated. Accordingly, this finding will be useful for CKD patients who are concomitantly administered GASI.

In Chapter II, I hypothesized that iron replacement with FC may have an impact on both levels of FGF23 and α-klotho in CKD patients, specifically, I expected that levels of C-terminal FGF23 decreased, and levels of α-klotho increased by administration of FC. This hypothesis was partially verified in patients with undergoing hemodialysis. When maintaining levels of serum phosphate and Hb within the target ranges, administration of FC decreased C-terminal FGF23 levels compared with the control group, while α-klotho levels did not change. Furthermore, any significant correlation relationships were not found between levels of C-terminal FGF23 and α-klotho. It is considered that iron replacement with FC led to decreased FGF23 production and subsequent cleavage, and therefore C-terminal FGF23 levels decreased. One of the reasons of the lack of increase in α-klotho levels might be attributed to the study population. Kidneys in patients undergoing hemodialysis are severely damaged and it would be difficult to increase the expression of α-klotho.

In Chapter III, I hypothesized that iron replacement with FC has an impact on elevated levels of FGF23 and platelet count in patients with IDA, and investigated it in IDA patients with NDD-CKD and non-CKD. This hypothesis was verified that iron

replacement with FC decreased elevated levels of C-terminal FGF23 and platelet count in those patients. This was assumed to be an effect of iron replacement by FC.

Considering the results of these investigations collectively, administration of FC would be useful in CKD patients, and IDA patients regardless of CKD status. In a situation that phosphate binders and oral iron preparations are less sensitive to GASI are desired, phosphate-binding and iron replacement effects of FC were not affected by the concomitant administration of GASI. In addition, elevated levels of C-terminal FGF23 and platelet count are independently associated with cardiovascular risk, but administration of FC decreased elevated levels of them in patients with IDA regardless of CKD status.

Conclusion

In conclusion of this dissertation, important findings in my investigations are i) concomitant administration of GASI did not interfere with the phosphate-binding and iron replacement effects of FC in patients with NDD-CKD and undergoing hemodialysis, ii) iron replacement of FC decreased C-terminal FGF23 levels in patients with undergoing hemodialysis, under the conditions of levels of serum phosphate and Hb were controlled, whereas intact FGF23 and α-klotho levels did not change, iii) administration of FC decreased elevated C-terminal FGF23 and platelet count in patients with IDA regardless of CKD status.

Since GASI is used in many patients with CKD, the result of my investigation of i) concomitant administration of GASI did not interfere with the phosphate-binding and iron replacement effects of FC, is favorable for those patients. Since the higher levels of C-terminal FGF23 are reported to be associated with cardiovascular risk in patients with CKD including patients with undergoing hemodialysis, the result of my investigation of ii) iron replacement with FC decreased C-terminal FGF23 in patients with undergoing hemodialysis, may contribute to decrease the risk in those patients. Furthermore, since the higher levels of C-terminal FGF23 and platelet count are associated with cardiovascular risk in IDA patients regardless CKD status, the result of my investigation of iii) administration of FC decreased elevated levels of C-terminal FGF23 and platelet count in patients with IDA regardless of CKD status, may contribute to decrease the risk in those patients as well.

Considering the results of these investigations collectively, administration of FC would be useful in CKD patients, and IDA patients regardless of CKD status. I expect that future studies should investigate whether these findings of FC actually lead to a reduction of cardiovascular events in CKD patients and IDA patients.

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