



# Conventional and novel impacts of ferric citrate on iron deficiency anemia and phosphorus metabolism in rats

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**ABSTRACT.** Ferric citrate is an oral iron-based phosphate binder, being known to affect iron status and improve iron deficiency anemia (IDA) in chronic kidney disease (CKD) patients. We examined whether oral administration of ferric citrate could change iron status and improve anemia without affecting phosphorus metabolism in iron deficiency anemia rats. In Normal rat study, normal rats were fed a diet containing 0.3 or 3% ferric citrate for 11 days for setting the dose and administration period of ferric citrate. The effects of ferric citrate on iron status- and phosphorus metabolism-related parameters were evaluated using blood and urine samples. Next, an iron deficiency anemia was induced by feeding iron-depleted diet in rats. After 7 days of starting the iron-depleted diet, 0.3% ferric citrate was administered for 7 days by dietary admixture. Iron status- and phosphorus metabolism-related parameters were evaluated with blood and urine samples. In Normal rat study, 3% ferric citrate treatment increased serum iron level and transferrin saturation (TSAT), and decreased serum phosphorus level, intact fibroblast growth factor 23 (iFGF23) level, and urinary phosphorus excretion, but 0.3% ferric citrate treatment showed no effects. On the other hand, in Iron deficiency anemia rat study, 0.3% ferric citrate treatment increased iron status-related parameters and improved anemia, but did not show any apparent changes in phosphorus metabolism-related parameters. In conclusion, ferric citrate could have hematopoietic effects without affecting phosphorus metabolism, and could be a potential option for the treatment of IDA in patients without CKD.

**KEY WORDS:** ferric citrate, iron deficiency anemia, phosphorus metabolism

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Anemia represents about a third of the world's population, and has been a serious and global public health problem that affects maternal and child mortality, physical performance. Half the cases are due to iron deficiency [10]. Thus, iron deficiency anemia (IDA) is the most common and most treatable of all anemias. There is an evolving understanding that iron deficiency can lead to symptoms independent of anemia and can be associated with a variety of diseases [5]. For example, iron deficiency and IDA are important signs of gastrointestinal hemorrhage. Two main mechanisms are known to exist in induction of iron deficiency [10]. One is an absolute deficiency, and the other is functional one. Absolute iron deficiency means that total body iron stores are low or exhausted, and functional iron deficiency is a status of functional failure in iron supply. Functional iron deficiency can be induced in several chronic inflammatory diseases, including chronic kidney disease (CKD) [10].

On the other hand, patients with CKD frequently develop many complications, including hyperphosphatemia, ectopic calcification, secondary hyperparathyroidism, and anemia. Among them, CKD mineral bone disorders (CKD-MBD), including hyperphosphatemia, are associated with an increased risk of fracture, cardiovascular disease and death [4, 14]. Therefore, CKD patients with hyperphosphatemia needs to use phosphate binders in order to restore phosphate balance in addition to treatment for anemia.

In such a treatment environment for CKD, iron-containing phosphate binders are used for hyperphosphatemia. Among them, ferric citrate is an iron-based compound with distinctive chemical characteristics and a mechanism of action that render it dually effective as a therapy in patients with CKD; it has been approved as a phosphate binder for the control of serum phosphate levels

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in adult CKD patients treated with dialysis and as an iron replacement product for the treatment of IDA in adult CKD patients not treated with dialysis [7]. In fact, it has been reported that ferric citrate improved hyperphosphatemia, and would induce increases in hemoglobin, transferrin saturation (TSAT) and ferritin [6, 16]. It has been also reported that ferric citrate hydrate led to few serious adverse events in organ systems usually affected by iron overload, and improved iron parameters in patients with CKD and iron deficiency anemia without negatively perturbing serum phosphate [7]. Ferric citrate could be a safe and efficacious treatment for IDA due to the clinical results in patients with nondialysis-dependent CKD [6, 7]. From these clinical results mentioned above, there is a possibility that ferric citrate could exert not only phosphate reducing effect but also hematopoietic effect in advanced CKD patients. It is expected that, in theory, successful treatment of hyperphosphatemia and anemia in patients with CKD could help avoid adverse clinical outcomes; [2] however, with the exception of renal replacement, no interventions have yet been proven to improve outcomes.

As we mentioned above, IDA is common not only in CKD patients but also in patients with other diseases. In such IDA patients with normal renal function, it is unclear whether ferric citrate could have an improving effects on IDA. In addition, if ferric citrate had hematopoietic effects on IDA, it is also unknown whether ferric citrate might reduce serum phosphorus level by inhibiting phosphorus absorption, whose serum phosphorus level within the reference level. Actually, clinical usage of ferric citrate for IDA patients has not been reported yet.

In this study, at first, we conducted Normal rat study for setting the dose and administration period of ferric citrate. Based on the results, 0.3% was set as the ferric citrate dose, and one week was set as the administration period. Next, according to the above experimental conditions, we examined whether ferric citrate could exert hematopoietic effects in IDA rats, and evaluated whether ferric citrate could affect phosphorus metabolism at that time.

## MATERIALS AND METHODS

### *Experimental design*

Ferric Citrate was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). The experimental protocols about Normal rat study and Iron deficiency anemia rat study were approved by the Experimental Animal Ethical Committee of Japan Tobacco Inc. First, we conducted Normal rat study for setting the dose and administration period of ferric citrate. We examined which dose of ferric citrate would have iron replenishment effects in Normal rat study. At the same time, we evaluated the effects on phosphorus metabolism. Next, we conducted Iron deficiency anemia rat study for examining the effects of ferric citrate on iron status in anemic condition by reference to the results in Normal rat study. We evaluated the effects on phosphorus metabolism also in Iron deficiency anemia rat study.

### *Normal rat study*

Male Sprague-Dawley rats, 6 weeks of age, were purchased from Charles River Japan (Yokohama, Japan) and fed a standard powder chow CRF-1, (Oriental Yeast Co., Ltd., Tokyo, Japan) containing 13.8 mg iron, 1,220 mg phosphorus and 810 mg calcium/100 g diet. The rats were housed in bracket cages or metabolic cages and food and water were supplied *ad libitum*.

At Day 1 (the 1st day of food admixture of ferric citrate), the rats were divided into 3 groups that were matched with respect to body weight ( $n=6$ ). A mixed diet containing 0.3 or 3% ferric citrate was fed to the rats for 11 days, containing 65.1 mg iron or 526.8 mg iron per 100 g diet, respectively. From Days 7 to 8, the urine were collected at metabolic cages. At Days 1, 8 and 12, blood samples were collected via the tail vein, and blood levels of hemoglobin, calcium, phosphorus and intact fibroblast growth factor 23 (iFGF23) were measured. Hemoglobin and red blood cell (RBC) levels were measured using an automated hematology analyzer Sysmex KX-21 (SYSMEX Corp., Kobe, Japan), and iFGF23 levels were determined using a sandwich ELISA kit (Kainos Laboratories, Tokyo, Japan).

### *Iron deficiency anemia rat study*

Male Sprague-Dawley rats, 6 weeks of age, were purchased from Charles River Japan and fed a AIN-93G diet (Oriental Yeast Co., Ltd.) containing 3.5 mg iron, 160 mg phosphorus and 500 mg calcium /100 g diet and water *ad libitum*.

On Day 1, rats were fed an iron-depleted diet (iron-depleted AIN-93G, Oriental Yeast Co., Ltd, Tokyo, Japan). After 7 days of iron depleted diet feeding (at Day 8), rats were divided into 2 groups: Control group and 0.3% Ferric citrate group (each group had 6 animals). Zero point three % ferric citrate diet contains 51.3 mg iron/100 g diet. Dietary administration of ferric citrate was carried out for 7 days (from Day 8 to 15). Animals in Normal group were fed a normal diet (AIN-93G) throughout the experimental period. On Days 1, 8 and 15 (at the end of treatment), the body weights were measured and blood samples were collected via the tail vein. From Days 14 to 15, the urine were collected at metabolic cages. Blood levels of hemoglobin, RBC, calcium, phosphorus and iFGF23 were measured as described above and below.

### *Blood and urinary biochemistry*

Phosphorus concentrations were measured by using a phosphorus/inorganic phosphorus measurement kit (Kyowa Medex Co., Ltd., Tokyo, Japan). Calcium concentrations were measured by using a calcium assay kit (Sekisui Medical Co., Tokyo, Japan). Creatinine concentrations were measured by using a creatinine assay kit (Kainos Laboratories). Levels of serum iron or unsaturated iron binding capacity (UIBC) were measured by using an iron or UIBC measurement kit (Shino-Test Corp., Tokyo, Japan). Transferrin saturation (TSAT) was calculated by serum iron level and UIBC.

### Statistics

Bartlett's test for homoscedasticity was performed to compare the ferric citrate groups with the Control group (Normal rat study). If homoscedasticity was found, Dunnett's multiple comparison test was performed, and if heteroscedasticity was found, Steel's multiple comparison test was performed. A test for homoscedasticity was performed by the F test to compare the Normal group with Control group, or the Control group with 0.3% Ferric citrate group (Iron deficient rat study). If homoscedasticity was found, Student's *t* test was performed, and if heteroscedasticity was found, the Welch test was performed.

## RESULTS

### *The effects of ferric citrate on anemia-related parameters and phosphorus metabolism-related parameters (Normal rat study)*

In Normal rat study, the food consumption in ferric citrate groups was almost the same with that of Control group during the administration period (Control group, 24.4 g/day; 0.3% Ferric citrate, 25.4 g/day; 3% Ferric citrate group, 25.4 g/day). We examined the effects of ferric citrate on both anemia-related parameters and phosphorus metabolism-related parameters. Regarding anemia-related parameters, serum iron level in 3% Ferric citrate group was significantly higher than that in Control group, although hemoglobin and RBC levels in the ferric citrate groups were not significantly different from Control group (Fig. 1A–C). In addition, serum UIBC in 3% Ferric citrate group was significantly lower than that in Control group (Fig. 1D), and TSAT in that group was also significantly higher than that in Control group (Fig. 1E). Total iron binding capacity (TIBC) was not changed by ferric citrate treatment (data not shown). On the other hand, 0.3% ferric citrate treatment did not show any apparent changes in anemia-related parameters in Normal rat study. Then, the significant decreases in serum phosphorus level and excretion amount of phosphorus, and increase in serum calcium level were observed in 3% Ferric citrate group (Fig. 2A–C). As an additional investigation about phosphorus metabolism, we measured iFGF23 concentration in blood. As a result, it was shown that 3% ferric citrate treatment decreased iFGF23 level significantly (Fig. 2D). In addition, as for mineral metabolism-related parameters, urinary excretion amount of calcium were significantly elevated in 3% Ferric citrate group (Table 1), and serum level and urinary excretion amount of creatinine were not significantly changed in ferric citrate treatment groups (serum creatinine level at Day 12;  $0.54 \pm 0.17$ ,  $0.94 \pm 0.32$  and  $0.70 \pm 0.35$  mg/dl; urinary excretion amount of creatinine;  $9.5 \pm 0.9$ ,  $9.1 \pm 1.0$ ,  $8.9 \pm 0.7$  mg/day; Control group, 0.3% Ferric citrate group, 3% Ferric citrate group, respectively). Zero point three % ferric citrate treatment did not show any changes also in phosphorus metabolism-related parameters in normal condition.

### *The effects of ferric citrate on parameters associated with anemia and phosphorus metabolism in an iron-deficient anemic condition (Iron deficiency anemia rat study)*

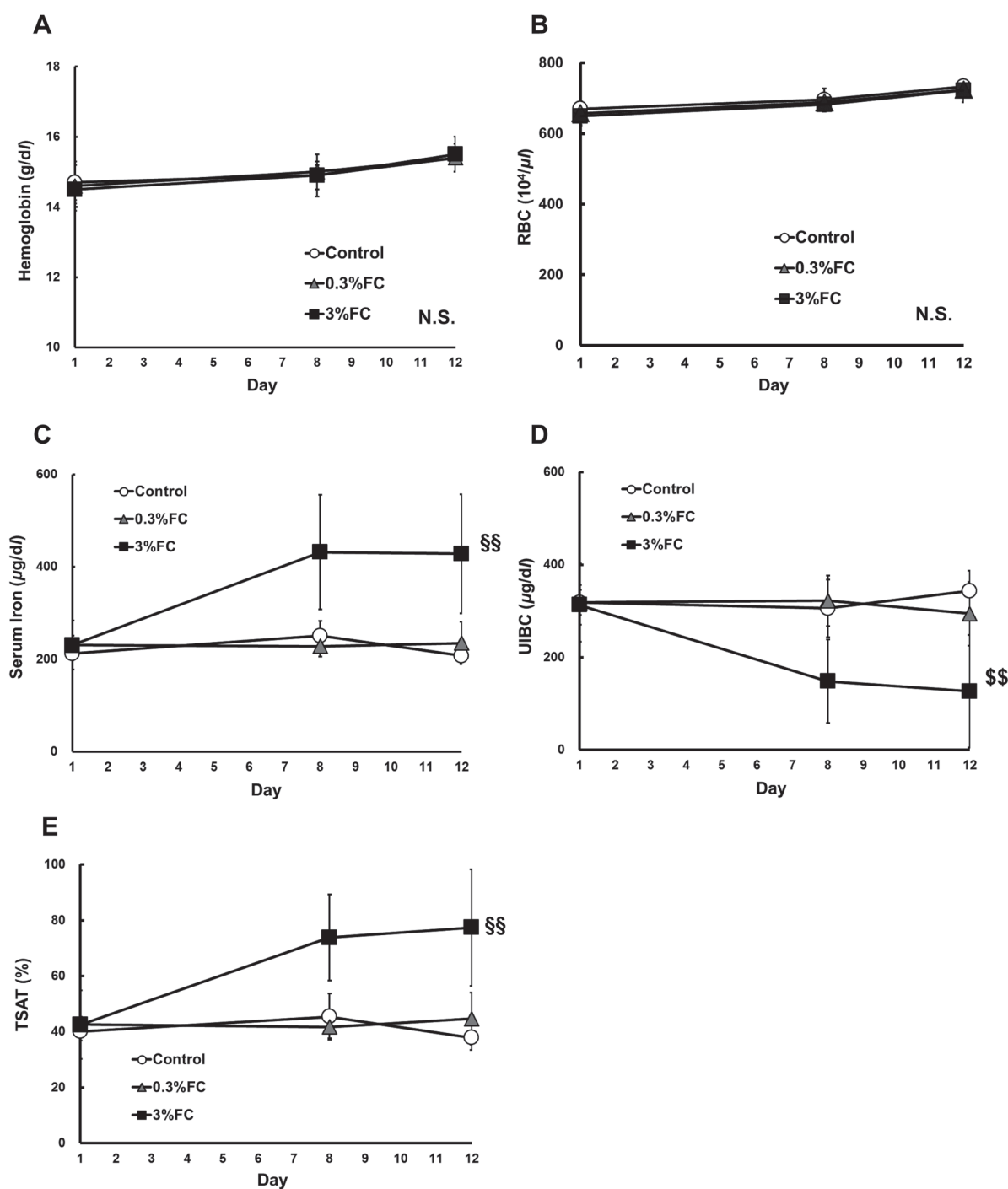
Next, we examined the effects of ferric citrate in rats fed an iron-depleted diet, and investigated whether an oral iron agent could exert hematopoietic effects in anemic condition. As shown in Normal rat study, 3% ferric citrate treatment showed iron replenishment effects, and also affected phosphorus metabolism-related parameters. But, 0.3% ferric citrate treatment did not affect both of them in normal condition. On the other hand, ability of iron absorption may increase in IDA condition than in normal condition [1]. In other words, 0.3% ferric citrate treatment might have increasing effects on iron related parameters in iron-depleted condition. Thus, we set 0.3% as a dose of ferric citrate for examining the effects on iron status in Iron deficiency anemia rat study.

After feeding of an iron-depleted diet, hemoglobin in the Control group was significantly decreased compared to that in Normal group at Day 8 (Fig. 3A). Furthermore, RBC in Control group tended to be decreased at Day 8 (Fig. 3B). At Day 15, hemoglobin values in 0.3% Ferric citrate group were significantly increased compared to that in Control group, and were almost the same with those in Normal group. Similar increases and related decreases were also seen in RBC, serum iron, UIBC and TSAT in 0.3% Ferric citrate group (Fig. 3B–E).

On the other hand, as for phosphorus metabolism-related parameters, serum inorganic phosphorus concentrations in 0.3% Ferric citrate group were not changed by iron administration compared to those in Control group (Table 2). In addition, 0.3% ferric citrate treatment did not significantly change serum calcium, iFGF23, and creatinine levels (Table 2). Treatment with 0.3% ferric citrate did not significantly change urinary phosphorus excretion, calcium excretion and creatinine excretion (Table 3).

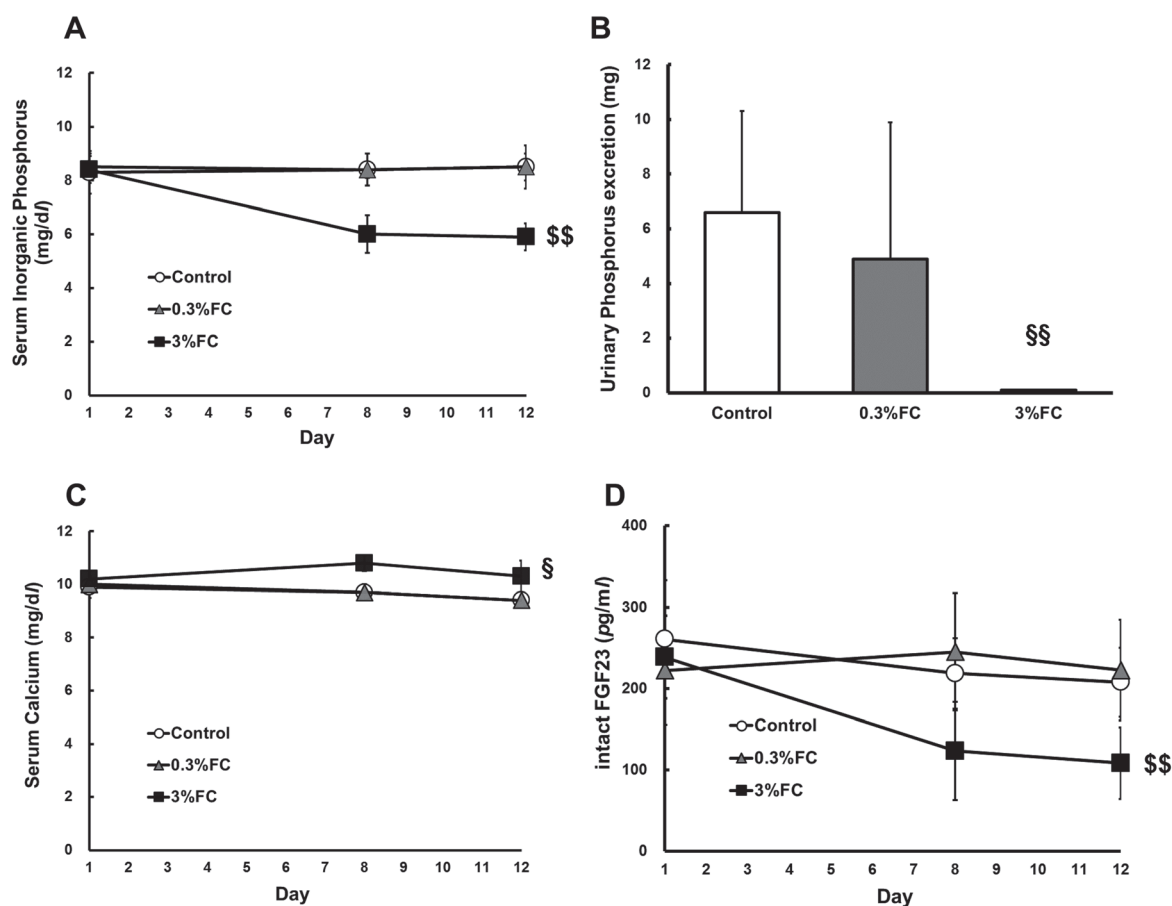
## DISCUSSION

In Normal rat study, as for iron status parameters, serum iron concentration and TSAT were increased by 3% ferric citrate treatment, although no changes were seen in hemoglobin and RBC levels. Therefore, it was considered that one part of administered iron was absorbed into body, and seems to be that 3% ferric citrate treatment could affect iron status. Regarding phosphorus metabolism parameters, serum phosphorus concentration and urinary phosphorus excretion in 3% Ferric citrate group were significantly decreased. In addition, it was shown that serum calcium concentration and urinary calcium excretion were increased by 3% ferric citrate treatment, being similar with non-clinical study results about ferric citrate hydrate [8]. Phosphorus and calcium homeostasis is strictly regulated mainly by kidney, bone, intestine and parathyroid glands [9]. Increases in serum phosphorus can lead to a decrease in serum ionized calcium. Decreases in serum ionized calcium stimulate release of parathyroid hormone (PTH). PTH increases phosphorus and calcium resorption/release from bone, increases calcium absorption and phosphorus excretion via the kidneys. Thus, it is possible that inhibitory effect of ferric citrate on phosphorus absorption



**Fig. 1.** Effects of Ferric citrate on anemia related parameters (Normal rat study). (A) Hemoglobin, (B) red blood cell (RBC), (C) serum iron, (D) unsaturated iron binding capacity (UIBC) and (E) transferrin saturation (TSAT). Data are the mean  $\pm$  standard deviation. N.S. means not significant.  $^{SS}P < 0.01$  (Dunnnett's multiple comparison test);  $^{SS}P < 0.01$  (Steel's multiple comparison test) vs. Control group.

could induce serum calcium increase via serum phosphorus decrease. Furthermore, iFGF23 level was decreased in 3% Ferric citrate group. FGF23 is a bone-derived factor that plays an important role in the metabolism of phosphorus and vitamin D [11]. Particularly, FGF23 is secreted for maintaining serum phosphorus concentration via regulation of an urinary phosphorus excretion and production of 1,25-dihydroxyvitamin D3 (1,25 (OH) 2 D3) in the kidneys [12, 13]. Thus, it was suggested that 3% ferric citrate treatment induced decrease in iFGF23 level via inhibitory effects on phosphorus absorption. Taken together, 3% ferric citrate treatment exerted phosphorus reducing effects and supplemented iron into body, and these results of 3% ferric citrate treatment are consistent with the previous reports in CKD patients [3, 6, 17]. Thus, it was suggested that ferric citrate would have not only inhibitory effects on phosphorus absorption but also hematopoietic effects.



**Fig. 2.** Effects of Ferric citrate on phosphorus metabolism related parameters (Normal rat study). (A) Serum inorganic phosphorus, (B) urinary phosphorus excretion, (C) serum calcium and (D) serum intact fibroblast growth factor 23 (iFGF23). Data are the mean  $\pm$  standard deviation.  $^{SS}P < 0.01$  (Dunnett's multiple comparison test);  $^{\$}P < 0.05$ ;  $^{SS}P < 0.01$  (Steel's multiple comparison test) vs. Control group.

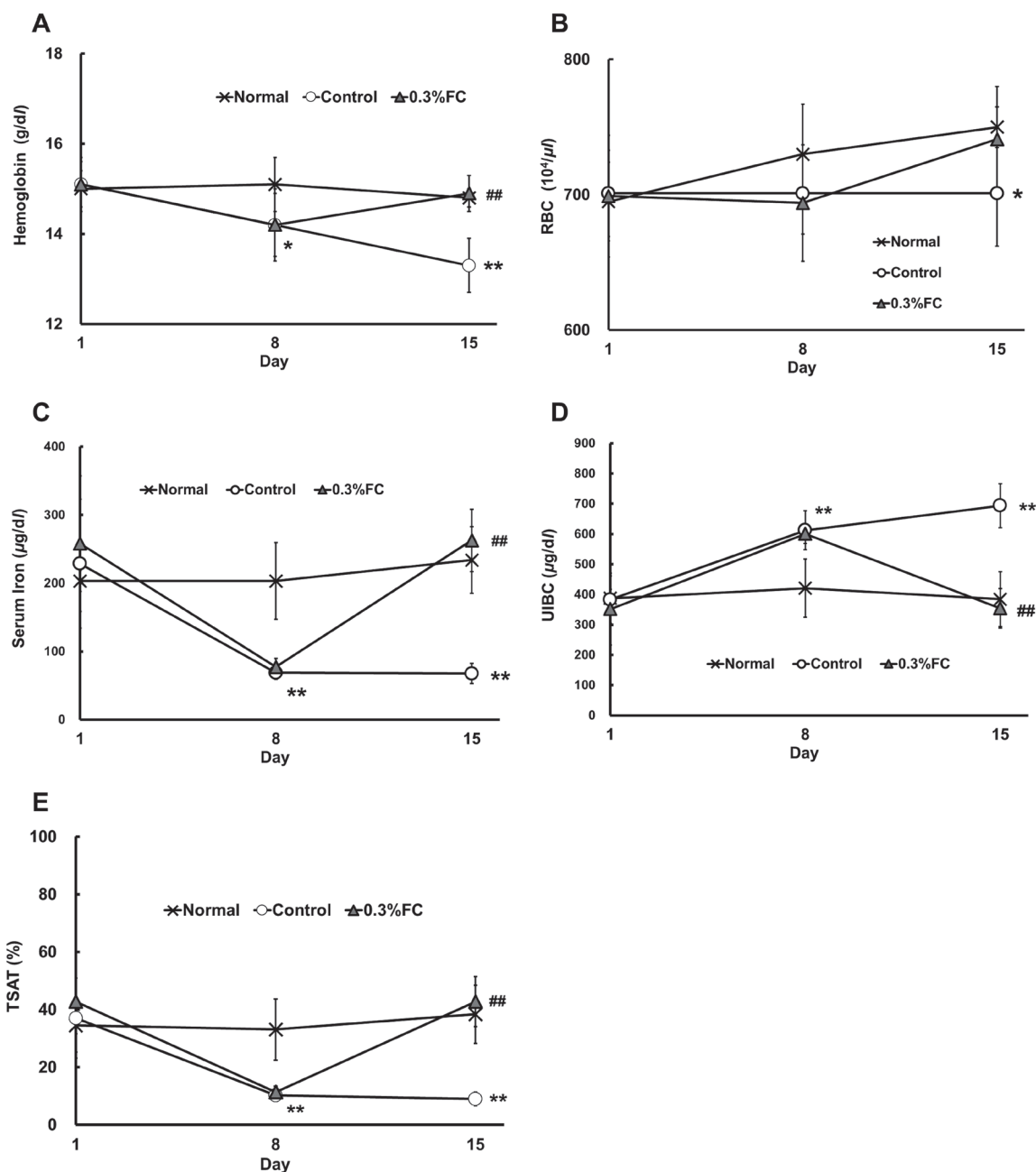
**Table 1.** Body weight and urinary calcium excretion (Normal rat study)

|         | Body weight (g)  | Urinary excretion (Day7–8)  |   |
|---------|------------------|-----------------------------|---|
|         |                  | Calcium (mg)                |   |
| Day 1   |                  |                             |   |
| Control | 319.8 $\pm$ 12.7 | -                           | - |
| 0.3%FC  | 321.2 $\pm$ 8.6  | -                           | - |
| 3%FC    | 320.8 $\pm$ 8.0  | -                           | - |
| Day 8   |                  |                             |   |
| Control | 358.5 $\pm$ 13.0 | 1.0 $\pm$ 0.5               | - |
| 0.3%FC  | 360.7 $\pm$ 16.2 | 0.9 $\pm$ 0.5               | - |
| 3%FC    | 354.5 $\pm$ 14.5 | 2.7 $\pm$ 0.4 <sup>a)</sup> | - |
| Day 12  |                  |                             |   |
| Control | 374.1 $\pm$ 10.7 | -                           | - |
| 0.3%FC  | 380.4 $\pm$ 17.7 | -                           | - |
| 3%FC    | 369.7 $\pm$ 16.0 | -                           | - |

FC, Ferric citrate. -, not examined. Data are the mean  $\pm$  standard deviation. a)  $P < 0.01$  vs. Control group.

On the other hand, 0.3% ferric citrate treatment did not induce any significant changes in serum iron concentration, TSAT, hemoglobin and RBC level, indicating that few amount of iron would be absorbed due to the homeostasis of iron in this normal condition (Normal rat study). Furthermore, 0.3% ferric citrate treatment could not show significant decrease in serum phosphorus level and urinary phosphorus excretion, being similar with the results in the previous study about ferric citrate hydrate [8]. Unchanging urinary phosphorus excretion would mean that 0.3% ferric citrate treatment did not induce phosphorus-retaining effect by suppressing urinary excretion. In addition, 0.3% ferric citrate treatment did not affect iFGF23 levels. Although we have measured neither serum intact PTH concentration nor serum 1,25 (OH)<sub>2</sub> D<sub>3</sub> concentration in this study, supplemental data in the previous report showed that 0.3% ferric citrate treatment did not change both of them [8]. As for treatment period of ferric citrate, we also measured serum phosphorus, serum calcium and serum iFGF23 concentrations at Day 12. Each value at Day 12 was almost the same with that at Day 8, respectively. Thus, it was suggested that the effects of ferric citrate treatment on phosphorus metabolism were almost stable at Day 8. Taken together, it would be possible that 0.3% ferric citrate treatment could not affect phosphorus metabolism in normal rats with normal renal function.

Next, we examined the effects of 0.3% ferric citrate treatment on iron status and phosphorus metabolism in an iron-insufficient



**Fig. 3.** Effects of Ferric citrate on parameters associated with anemia in iron deficiency anemia condition (Iron deficiency anemia rat study). (A) Hemoglobin, (B) red blood cell (RBC), (C) serum iron, (D) unsaturated iron binding capacity (UIBC) and (E) transferrin saturation (TSAT). Data are the mean  $\pm$  standard deviation. Statistics analysis was performed by comparing Normal group and Control group, or Control group and 0.3%FC group. \* $P < 0.05$ ; \*\* $P < 0.01$ , Control group vs. Normal group. ## $P < 0.01$ , 0.3%FC group vs. Control group.

condition. We used AIN-93G diet and iron-depleted AIN-93G diet in Iron deficiency anemia rat study for inducing iron deficiency instead of CRF-1 diet used in Normal rat study. There is no obvious difference of phosphorus and iron related parameters in normal breeding among these two kinds of diet, although the composition is different among them. Thus, there could be no problem with conducting Normal rat study as a pilot study for Iron deficiency anemia rat study.

In Iron deficiency anemia rat study, at first, iron-depleted diet induced the reduction in serum iron concentration, TSAT and hemoglobin level, leading to anemic condition. In 0.3% ferric citrate treatment group, serum iron level, TSAT and hemoglobin were increased, indicating that this dosage of ferric citrate would have potential to accelerate hematopoiesis. It might be possible that ferric citrate could show hematopoietic effects independently of phosphorus metabolism in iron deficiency anemia, because ability of iron absorption may increase in IDA condition than in normal condition [1]. It should be also considered that the effects of 0.3% ferric citrate treatment on iron status and hematopoiesis may depend on iron status of recipient.

As for phosphorus metabolism, phosphorus metabolism related parameters in Control group at Day 1 in Normal rat study are

**Table 2.** Serum inorganic phosphorus, serum calcium, serum intact fibroblast growth factor 23 (iFGF23) and serum creatinine (Iron deficiency anemia rat study)

|         | Serum parameters             |                          |                |                    |
|---------|------------------------------|--------------------------|----------------|--------------------|
|         | Inorganic Phosphorus (mg/dl) | Calcium (mg/dl)          | iFGF23 (pg/ml) | Creatinine (mg/dl) |
| Day 1   |                              |                          |                |                    |
| Normal  | 9.7 ± 0.4                    | 9.9 ± 0.1                | 248.1 ± 70.3   | 0.42 ± 0.13        |
| Control | 10.1 ± 0.5                   | 9.9 ± 0.2                | 250.2 ± 88.0   | 0.43 ± 0.11        |
| 0.3%FC  | 10.0 ± 0.7                   | 10.3 ± 0.1 <sup>a)</sup> | 248.8 ± 57.2   | 0.44 ± 0.14        |
| Day 8   |                              |                          |                |                    |
| Normal  | 8.5 ± 0.7                    | 9.9 ± 0.2                | 262.9 ± 48.7   | 0.78 ± 0.56        |
| Control | 8.1 ± 0.9                    | 9.8 ± 0.6                | 220.5 ± 29.0   | 0.55 ± 0.20        |
| 0.3%FC  | 7.8 ± 0.6                    | 9.8 ± 0.2                | 254.0 ± 83.1   | 0.35 ± 0.08        |
| Day 15  |                              |                          |                |                    |
| Normal  | 7.9 ± 0.3                    | 9.3 ± 1.0                | 266.0 ± 53.7   | 0.43 ± 0.12        |
| Control | 7.9 ± 0.6                    | 10.0 ± 0.3               | 303.1 ± 110.6  | 0.59 ± 0.35        |
| 0.3%FC  | 7.6 ± 0.6                    | 9.8 ± 0.2                | 336.5 ± 78.3   | 0.41 ± 0.06        |

FC, Ferric citrate. Data are the mean ± standard deviation. a)  $P < 0.01$  vs. Control group.

**Table 3.** Body weight, urinary excretion amount of inorganic phosphorus, calcium and creatinine (Iron deficiency anemia rat study)

|         | Body weight (g) | Urinary excretion (Day 14–15) |                  |                     |
|---------|-----------------|-------------------------------|------------------|---------------------|
|         |                 | Inorganic Phosphorus (mg/day) | Calcium (mg/day) | Creatinine (mg/day) |
| Day 1   |                 |                               |                  |                     |
| Normal  | 339.5 ± 14.7    | -                             | -                | -                   |
| Control | 355.6 ± 16.8    | -                             | -                | -                   |
| 0.3%FC  | 349.7 ± 25.3    | -                             | -                | -                   |
| Day 8   |                 |                               |                  |                     |
| Normal  | 367.1 ± 21.6    | -                             | -                | -                   |
| Control | 390.4 ± 23.6    | -                             | -                | -                   |
| 0.3%FC  | 384.6 ± 36.7    | -                             | -                | -                   |
| Day 15  |                 |                               |                  |                     |
| Normal  | 398.0 ± 25.7    | 5.7 ± 2.2                     | 0.88 ± 0.38      | 9.7 ± 2.4           |
| Control | 417.3 ± 35.7    | 6.6 ± 4.8                     | 1.0 ± 0.28       | 10.9 ± 2.3          |
| 0.3%FC  | 418.0 ± 39.0    | 4.0 ± 2.0                     | 1.0 ± 0.38       | 11.2 ± 2.0          |

FC, Ferric citrate. -, not examined. Data are the mean ± standard deviation.

comparable to those in Control group at Day 8 in Iron deficiency anemia rat study (Fig. 2, Tables 2 and 3). For example, the serum phosphorus level in Normal rat study and Iron deficiency anemia rat study was  $8.3 \pm 0.8$  mg/dl and  $8.1 \pm 0.9$  mg/dl, respectively. In addition, referring to the report [15] that contains serum data in IDA patients, the serum phosphate levels are within the range of 2.5 to 4.5 mg/dl which is the standard value for healthy individuals. Therefore, it was suggested that there was no difference in phosphorus metabolism between normal condition and anemic condition. Taken together, in the case of normal renal function, it could be possible to extrapolate the effects on phosphorus metabolism in IDA condition from those in normal condition. Then, we set 0.3% as the dose of ferric citrate and one week as the administration period. Considering the above-mentioned phosphorus metabolism together, it is also considered that 0.3% ferric citrate could not affect phosphorus metabolism also in that anemic condition. Actually, 0.3% ferric citrate treatment did not change serum concentrations or urinary excretions of phosphorus or calcium compared with those in Control group, like Normal rat study. In addition, ferric citrate treatment did not affect serum iFGF23 or serum phosphorus, suggesting that this iron supplementation with 0.3% ferric citrate treatment may not affect phosphorus metabolism.

Ferric citrate is an iron-based compound, and has been approved in the USA as a phosphate binder for the control of serum phosphorus levels in adult patients with CKD on dialysis and also as an iron replacement product indicated for the treatment of iron deficiency anemia in adult patients with CKD not on dialysis [7]. It is approved in Japan for the improvement of hyperphosphatemia in patients with CKD. Despite these indicated uses, it has been ambiguous whether oral administration of ferric citrate could change iron status and improve anemia without affecting phosphorus metabolism in iron-deficiency anemia patients without CKD. In this study, it was shown that ferric citrate could exert hematopoietic effects without inhibiting phosphorus absorption in rats with normal renal function.

In conclusion, ferric citrate could have hematopoietic effects without affecting phosphorus metabolism, and could be a potential option for the treatment of IDA in patients without CKD.

**CONFLICT OF INTEREST.** Akio Iida and Mutsuyoshi Matsushita are employees of Japan Tobacco Inc. Takeshi Ohta and Takahisa Yamada have no conflict of interest.

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