Original Research

Iron-based phosphorus chelator: Risk of iron deposition and action on bone metabolism in uremic rats

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Impact statement

Hyperphosphatemia has a major impact in CKD owing to its association with increased cardiovascular mortality. Therefore, controlling hyperphosphatemia is essential in patients with CKD, by using medications that can reduce serum phosphate levels, and improve bone metabolism, thereby decreasing the risk of vascular calcification. Phosphate chelators, such as iron oxyhydroxide and iron citrate, satisfactorily reduce phosphatemia, but their effects on bone and mineral metabolism and the risk of iron overload still need to be assessed. The cross-linked chitosan-Fe (III) polymer used in this study bears similarities to iron oxyhydroxide, and preclinical studies have already demonstrated its phosphate chelating activity. Investigating its action on bone metabolism and iron overload in animal models of CKD may provide a new treatment option for BMD-CKD.

Abstract

Phosphate chelators are frequently used in patients with chronic kidney disease (CKD). New iron-based chelators remain understudied and offer a promising therapeutic option for the control of bone and mineral disorders of chronic kidney disease (BMD-CKD). We assessed the effect of the phosphorus chelator, chitosan-iron III (CH-FeCl), compared to calcium carbonate ($CaCO_3$) in BMD-CKD and the potential iron overload in uremic rats. Thirty-two animals were divided into four groups, namely the control, CKD, CKD/CH-FeCl, and CKD/$CaCO_3$ groups. CKD was induced by adding 0.75% (4 weeks) and 0.1% (3 weeks) adenine to the diet. The chelators were administered from week 3 through week 7. The renal function, BMD-CKD markers, and histomorphometry of the femur were assessed at week 7. The CKD group showed a significant increase in creatinine (83.9 ± 18.6 vs. 41.5 ± 22.1 μmol/L; $P = 0.001$), phosphate (3.5 ± 0.8 vs. 2.2 ± 0.2 mmol/L; $P = 0.001$), fractional excretion of phosphorus (FEP) (0.71 ± 0.2 vs. 0.2 ± 0.17; $P = 0.0001$), and FGF23 (81.36 ± 37.16 pg/mL vs. 7.42 ± 1.96; $P = 0.011$) compared to the control group. There was no accumulation of serum or bone iron after the use of CH-FeCl. The use of chelators reduced the FEP (control: 0.71 ± 0.20; CKD/CH-FeCl: 0.40 ± 0.16; CKD/$CaCO_3$ 0.34 ± 0.15; $P = 0.001$), without changes in the serum FGF23 and parathyroid hormone levels. Histomorphometry revealed the presence of bone disease with high remodeling in the uremic animals without changes with the use of chelators. The CH-FeCl chelator was efficient in reducing the FEP without iron accumulation, thereby paving the way for the use of this class of chelators in clinical settings in the future.

Keywords: Hyperphosphatemia, chitosan, renal insufficiency, chronic, fibroblast growth factor 23, chronic kidney disease-mineral and bone disorder, iron overload

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Introduction

The progressive loss of renal function leads to important metabolic changes, such as elevation in the serum levels of phosphorus (P), fibroblast growth factor 23 (FGF23), and parathyroid hormone (PTH), and the reduction in the serum levels of calcitriol, thereby resulting in the bone and mineral disorder of chronic kidney disease (BMD-CKD).[1–4] Hyperphosphatemia is one of the most frequent metabolic changes occurring as chronic kidney disease (CKD) progresses and contributes to the development of secondary hyperparathyroidism and vascular and bone complications.[5,6] The control of hyperphosphatemia in CKD commonly requires the combination of a P-restricted diet with the use of P chelators.[7,8] Calcium-free P chelators, such as sevelamer and lanthanum carbonate, are currently preferred due to the increased risk of vascular calcification by calcium chelators.[9] These chelating agents are effective in reducing phosphatemia, in addition to the serum FGF23 levels.[10,11] This reduction may contribute to the improvement of survival in patients with CKD as high serum FGF23 levels are associated with increased mortality, although long-term studies are not available to corroborate this claim.[12,13]

Fe-based chelators have currently become an option for the control of hyperphosphatemia, since they do not cause calcium overload[14–18] and effectively reduce the serum PTH and FGF23 levels.[15,19–22] However, to date, their action on bone tissue was assessed only in one experimental study that used sucroferric oxyhydroxide.[23] The risk of Fe overload, and its consequent bone deposition should be considered in patients with CKD, given their high frequency of use for the treatment of anemia secondary to CKD,[24] especially when using compounds that facilitate intestinal Fe absorption, such as ferric citrate.[25,26]

The phosphorus chelator CH-FeCl is a polymer of chitosan and iron in the ferric form (FeIII). This complex demonstrates a chelating effect without causing Fe deposition in the tissues when administered orally at low doses to rats with normal renal function and diabetes mellitus.[27–30] These data revealed its efficacy and safety, although its action on bone tissue has not yet been investigated.

Therefore, the objective of this study was to compare the effects of CH-FeCl and calcium carbonate ($CaCO_3$) chelators on bone tissue and other BMD-CKD markers, in addition to assessing the risk of Fe overload in the bone tissue of rats with adenine-induced uremia.

Materials and methods

Ethical procedures

All applicable guidelines for the care and use of animals were followed according to the Brazilian Federal Law 11.794 (2008) of the Brazilian National Council for Animal Experimentation Control and approved by the Ethics Committee for the Use of Animals in Research of the Federal University of Juiz de Fora (protocol 031/2013, CEUA-UFJF).

Experimental protocol

This study used 32, 8- to 12-week-old Wistar rats, weighing approximately 250 g, obtained from the colony of the Center for Reproductive Biology of the Federal University of Juiz de Fora. The animals were housed on acclimatized shelves at a temperature of 22 °C and subjected to 12/12-h light/dark cycles. All animals were fed a pellet diet containing 0.7% calcium, 1.0% phosphate, and 22.0% protein (PragSoluções Biociências, Jaú, Brazil). Water and food were available *ad libitum*. The animals were divided into four groups after one week of acclimatization with eight animals in each group (n = 32 animals), namely the control, CKD, CKD/CH-FeCl, and CKD/$CaCO_3$ groups. Uremia was induced in the CKD, CKD/CH-FeCl, and CKD/$CaCO_3$ groups by feeding the animals with a diet enriched with adenine (PragSoluções Bioscências, Jaú, SP) according to the following pattern: 0.75% adenine was added to the diet until week 4 (CKD-induction period) and a diet with 0.1% adenine was maintained until the end of the study (CKD maintenance period) for a total of seven weeks.[31] The animals in the control group received an adenine-free diet (PragSoluções Biociência, Jaú, SP) during the same period. CH-FeCl 30 mg/kg/day was administered daily by gavage from week 3 until the end of the study.[28] Similarly, $CaCO_3$ was administered at a dose of 500 mg/kg/day.[20] We opted to compare the chelating effect to $CaCO_3$ despite the knowledge of its potential calcium overload because this compound is still widely used in clinical practice.[32,33] The animals in the control and CKD groups were also administered 0.9% saline solution by gavage during the same period (Figure 1).

On the day before euthanasia, the animals were kept in metabolic cages and their urine was collected for 24 h to determine the urinary volume and perform biochemical analyses.

The animals were euthanized at the end of week 7 with an intraperitoneal injection of xylazine 10 mg/kg (König SA, Avellaneda, Argentina) and ketamine 90 mg/kg (König SA, Avellaneda, Argentina), and exsanguination, followed by diaphragm rupture.[30] Blood samples were collected via cardiac puncture. The collected serum and urine samples were stored at –80°C for subsequent biochemical analyses. The right femur of each animal was removed for bone histomorphometry and Fe deposition analysis. The fluorescent bone marker oxytetracycline (Terramicina®; Pfizer Animal Health, New York, NY, USA) was

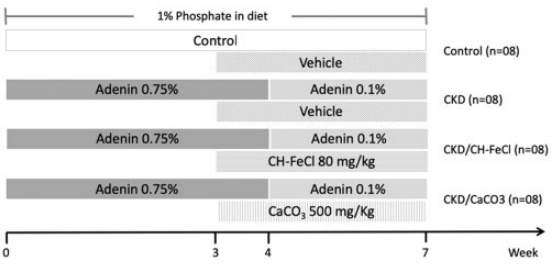

Figure 1. Study design evaluating the effect of the cross-linked chitosan-Fe (III) complex and calcium carbonate on the bone. CKD: chronic kidney disease; CH-FeCl: Chitosan-Fe (III) complex cross-linked; $CaCO_3$: calcium carbonate.

administered intraperitoneally at a dose of 30 mg/kg on days 37, 38, 44, and 45 of the protocol to assess the dynamic parameters of bone formation.[34]

Preparation of the CH-FeCl complex

The polymer used in this study was prepared according to a previous study.[28] The iron concentration in the complex was 80 mg/g, as measured by a Shimadzu UV1600 spectrophotometer (Shimadzu, Kyoto, Japan). The entire procedure was conducted at the Chemistry Laboratory of the University of Vale do Itajaí (UNIVALI, Itajaí, SC, Brazil).

Assessed parameters

Biochemical analysis. Serum creatinine, phosphate, total calcium, and Fe levels, and urinary creatinine and phosphate levels were measured using a Labmax Progress automatic analyzer (Labtest Diagnostica S.A., Lagoa Santa, MG). The urinary fractional excretion of phosphorus (FEP) was calculated from the serum and urinary creatinine levels and serum and urinary phosphate levels using the following equation:

$$[\text{urinary phosphate (mmol/L)} / \text{serum phosphate (mmol/L)}] \times [\text{serum creatinine (μmol/L)} / \text{urinary creatinine (μmol/L)}] \times 100.[35]$$

The serum PTH levels (Rat Intact PTH ELISA Kit, Immutopics, San Clemente, CA, USA) and serum FGF23 levels (FGF-23 ELISA Kit, Cloud-Clone Corp., Houston, USA) were determined by the ELISA method (R&D Systems, Minneapolis, USA).

Bone histomorphometry. The removed femur was processed as described previously.[34,36] Its distal portion was sliced into 5-μm- and 10-μm-thick sections using a Polycut S microtome equipped with a tungsten carbide blade (Leika, Heidelberg, Germany). The 5-μm sections were stained with 0.1% toluidine blue (pH = 6.4), and at least two non-consecutive sections were examined per sample. The 10-μm sections were not stained and were used to analyze the oxytetracycline staining using an ultraviolet light source.[32,34]

The structural, static, and dynamic parameters of bone formation and resorption were analyzed at the region of the distal metaphysis at 195 μm from the growth cartilage in a total of 30 fields (250× magnification) using a semi-automatic image analyzer and Osteomeasure (Osteometrics Inc., Atlanta, GA, USA). The structural parameters included the trabecular volume (BV/TV, %), number of trabeculae (Tb.N, μm), and trabecular thickness (Tb.Th, μm). The bone formation indices included the osteoid thickness (O.Th, μm), osteoid surface (OS/BS,%), osteoblastic surface (Ob.S/BS, %), mineralizing surface (MS/BS, %), and mineral apposition rate (MAR, μm/day). The bone formation rate (BFR/BS, $\mu m^3/\mu m^2$/day) was calculated using the MAR × MS/BS ratio derived from these measurements. The bone resorption indices included the resorption surface (ES/BS, %) and osteoclastic surface (Oc.S/BS, %). The histomorphometric indices were presented according to the nomenclature recommended by the American Society of Bone and Mineral Research-ASBMR.[36,37]

Iron overload. The bone Fe content was determined by Perls Prussian blue staining. We considered iron accumulation if >20% of the trabeculae were covered by Fe. The analyses were performed by an operator blinded to the animals group at the Laboratory of Medical Research-16 (LIM/16), Hospital das Clínicas, School of Medicine, University of São Paulo (HCFMUSP, São Paulo, SP, Brazil).

Statistical analysis

The results are presented as mean ± SD or median (minimum-maximum). The *t*-test was used for comparing two groups and the ANOVA (*post-hoc* Bonferroni) was used to compare more than two groups if the data showed normal distribution. The Mann-Whitney test was used to compare two groups and the Kruskal-Wallis test was used for more than two groups when the data showed non-normal distribution. *P*-values <0.05 were considered statistically significant. IBM SPSS Statistics 21 (SPSS Inc., Chicago, USA) was used for all analyses.

Results

A total of 32 animals were used in the experiments, and there were no deaths. The animals' weights and food intake were not affected by treatment with CH-FeCl and $CaCO_3$.

Biochemical parameters

The experimental model induced CKD. After the introduction of adenine diet, in CKD group, serum creatinine levels increased (141.5 ± 17.7 vs. 36.2 ± 17.7 μmol/L; $P = 0.0001$ in week 4, and 83.9 ± 18.6 vs. 41.5 ± 22.1 μmol/L; $P = 0.001$ in week 7) and GFR levels reduced (0.15 ± 0.03 vs. 1.8 ± 1.0 mL/min; $P = 0.006$ in week 4, and 0.31 ± 0.14 vs. 1.8 ± 0.8 mL/min; $P = 0.001$ in week 7), compared to control group.

The use of chelators did not significantly change the serum creatinine levels. The CKD group also showed higher levels of serum phosphate (3.5 ± 0.8 vs. 2.2 ± 0.2 mmol/L; $P = 0.001$), alkaline phosphatase (561 ± 90.9 vs. 205.75 ± 62.39 U/L; $P = 0.0001$), FGF23 (81.36 ± 37.16 vs. 7.42 ± 1.96 pg/mL; $P = 0.011$), and FEP (0.71 ± 0.2 vs. 0.2 ± 0.16; $P = 0.0001$), and lower hemoglobin levels (10.01 ± 0.71 vs. 14.66 ± 0.89 g/dL; $P = 0.0001$) compared to control group. On the other hand, the serum Fe levels did not differ between the groups (Table 1).

The use of CH-FeCl and $CaCO_3$ chelators in the CKD groups reduced the FEP compared to the CKD group without chelator use (CKD: 0.71 ± 0.2; CKD/CH-FeCl: 0.40 ± 0.16, $P = 0.008$; CKD/$CaCO_3$: 0.34 ± 0.15, $P = 0.001$). A decrease of approximately 20% was observed in the mean serum phosphate levels between the CKD and CKD-treated groups, although this difference was not statistically significant (CKD: 3.5 ± 0.8 mmol/L; CKD/CH-FeCl: 2.8 ± 0.6 mmol/L, $P = 0.203$; CKD/$CaCO_3$:

Table 1. Biochemical data: Laboratory parameters of all groups at the 4 and 7 weeks.

Parameter	Control ($n = 8$)	CKD ($n = 8$)	CKD/CH-FeCl ($n = 8$)	CKD/$CaCO_3$ ($n = 8$)
4 week				
Serum creatinine (μmol/L)	36.2 ± 17.7	141.5 ± 17.7*	150.3 ± 17.7	176.8 ± 61.9
Glomerular filtration rate (mL/min)	1.8 ± 1.0	0.15 ± 0.03*	0.28 ± 0.47	0.11 ± 0.04
7 week				
Serum creatinine (μmol/L)	41.5 ± 22.1	83.9 ± 18.6*	73.37 ± 23.8	76.9 ± 45.1
Glomerular filtration rate (mL/min)	1.8 ± 0.8	0.31 ± 0.14*	0.63 ± 0.37	0.98 ± 0.46
Phosphate (mmol/L)	2.2 ± 0.2	3.5 ± 0.8*	2.8 ± 0.6	2.8 ± 0.5
Total serum calcium (mmol/L)	2.1 ± 0.3	2.6 ± 0.5*	2.6 ± 0.3	2.3 ± 0.4
Fraction excretion of phosphorus	0.20 ± 0.16	0.71 ± 0.20*	0.40 ± 0.16#	0.34 ± 0.15#
Alkaline phosphate (U/L)	205.75 ± 62.39	561.0 ± 90.9*	432.71 ± 119.39	452.87 ± 99.13
PTH (pg/mL)	183.4 (149.9–611.2)	505.7 (119.7–1535)	154.9 (102.2–179.2)	208.5 (142.3–438.7)
FGF23 (pg/mL)	7.42 ± 1.96	81.36 ± 37.16*	70.88 ± 11.88	68.11 ± 35.64
Total serum iron (μmol/L)	37.1 ± 4.9	54.6 ± 29.5	47.7 ± 12.1	45.4 ± 10.3
Hemoglobin (g/dL)	14.66 ± 0.89	10.01 ± 0.71*	9.86 ± 1.68	9.31 ± 1.01

Values expressed as mean ± SD, median (min – max). The dosages of PTH and FGF23 were performed in five animals from each group.
CKD: chronic kidney disease; CH-FeCl: complex cross-linked chitosan iron(III); $CaCO_3$: calcium carbonate.
*$P < 0.05$ for CKD vs. Control.
#$P < 0.05$ compared to the group CKD.

Table 2. Histomorphometric analysis of the trabecular bone parameters in the femur.

	Control ($n = 8$)	CKD ($n = 8$)	CKD/CH-Fel ($n = 8$)	CKD/$CaCO_3$ ($n = 8$)
Structural parameter				
Trabecular volume (BV/TV, %)	22.64 ± 3.71	25.69 ± 15.83	15.25 ± 1.68	19.04 ± 11.09
Trabecular number (Tb.N, mm)	4.20 ± 0.35	4.46 ± 2.48	3.20 ± 0.64	3.32 ± 2.03
Trabecular thickness (Tb.Th, μm)	54.03 ± 8.57	57.78 ± 17.92	51.15 ± 15.22	59.63 ± 7.89
Trabecular separation (Tb.Sp, μm)	185.25 ± 18.63	265.23 ± 229.92	273.53 ± 63.53	332.35 ± 200.79
Formation parameter				
Osteoid thickness (O.Th, μm)	0.97 (0.5–1.5)	4.99* (2.63–25.7)	3.47 (1.6–4.1)	1.84# (1.4–2.4)
Osteoid surface (OS/BS, %)	1.87 ± 1.13	33.20 ± 21.33*	21.39 ± 9.27	13.81 ± 10.43
Osteoblast surface (Ob.S/BS, %)	1.71 ± 0.97	22.79 ± 11.67*	17.10 ± 8.21	11.76 ± 9,18
Mineralizing surface (MS/BS, %)	1.58 ± 0.77	2.59 ± 0.86	3.96 ± 1.45	6.59 ± 3.38
Mineral apposition rate (MAR, μm/day)	0.37 ± 0.20	1.32 ± 0.66*	0.57 ± 0.25	0.51 ± 0.24
Bone formation rate (BFR/BS, $\mu m^3/\mu m^2$/day)	0.01 (0.0–0.01)	0.03* (0.02–0.04)	0.03 (0.01–0.03)	0.03 (0.01–0.06)
Resorption parameter				
Eroded surface (ES/BS, %)	3.67 ± 0.91	14.20 ± 7.42*	12.75 ± 5.32	12.73 ± 4.66
Osteoclast surface (Oc.S/BS, %)	1.09 (0.32–1.13)	5.32* (0.34–7.19)	5.29 (1.05–6.07)	3.32 (1.53–5.58)

Values expressed as mean ± SD, median (min – max).
CKD: chronic kidney disease; CH-FeCl: complex cross-linked chitosan iron(III); $CaCO_3$: calcium carbonate.
*$P < 0.05$ CKD vs. Control.
#$P < 0.05$ compared to the group CKD.

2.8 ± 0.5 mmol/L, $P = 0.239$). A similar result was observed for the PTH levels (CKD: 505.7 pg/mL; CKD/CH-FeCl: 154.9 pg/mL, $P = 0.153$; CKD/$CaCO_3$: 208.5 pg/mL, $P = 0.189$). The serum levels of total calcium, alkaline phosphatase, and FGF23 were higher in all CKD groups, albeit without significant differences among them (Table 1).

Bone histomorphometry

The structural bone parameters did not differ among the groups. However, the bone formation and resorption parameters differed between the control and CKD groups. The animals with CKD showed bone disease with high remodeling, which was characterized by an increase in the OS/BS, O.Th, Ob.S/BS, ES/BS, Oc.S/BS, MAR, and BFR/BS. The use of chelators did not affect the values of the above-mentioned parameters (Table 2).

Perls staining did not detect Fe deposition in the bone tissues of any of the groups.

Discussion

Following CKD induction, the animals showed a significant elevation in serum levels of phosphate, alkaline phosphatase, PTH, FGF23, and FEP, in addition to changes in histomorphometric parameters, reflecting the expected pattern due to BMD and in accordance with the degree of CKD found in this experimental model.[37–39] The use of the chelators, CH-FeCl and $CaCO_3$, reduced the FEP without inducing a serum or bone Fe overload; however, a significant effect in serum FGF-23 and PTH levels was not seen.

The effects of Fe-based chelators in reducing phosphatemia are similar to those of other chelators, such as sevelamer and $CaCO_3$.[7,18] However, the results of clinical studies regarding the reduction in the FGF23 and PTH levels are still controversial.[21,22,40] Experimental studies found that sucroferric oxyhydroxide induced a reduction in the FGF23 and PTH levels only at higher doses, such as at a concentration of 5% (of the compound) in the animals'

diet, while it did not alter phosphatemia and the PTH and FGF23 levels at low doses.[20,23] The characteristics of the compound used in our experiments were similar to those of sucroferric oxyhydroxide. It consists of a complex between chitosan (a polysaccharide-based biopolymer) and Fe in its ferric form Fe (III) subjected to a cross-linking process, which renders it insoluble.[27,28,41]

This study found a significant reduction in the FEP and a tendency towards a decrease in phosphatemia and PTH levels in the CH-FeCl and CaCO3 groups, although the FGF-23 levels were not reduced. Despite the similarity between CH-FeCl and sucroferric oxyhydroxide, the Fe content of these compounds significantly differs, which could have contributed to the limited reduction in the analyzed parameters: sucroferric oxyhydroxide contains 21% Fe (III) (210 mg/g)[20,42] and CH-FeCl contains 8% Fe (III) (80 mg/g). The dose of Fe provided to the animals in our experiment was approximately 1 mg of Fe (III), which induced a mean decrease of 20% in phosphatemia, while Burger *et al.* also used 1 mg of Fe (III) but found a 32% reduction in phosphatemia in the studied rats.[28] Another study, by Yaguchi *et al.*, used sucroferric oxyhydroxide at a concentration of 5%, providing a daily dose of Fe (III) of approximately 190 mg, and obtained a mean reduction of 45% in phosphatemia.[23] Therefore, the low Fe concentration used in our study could have contributed to the non-significant reduction in the serum levels of phosphate, PTH, and FGF23, despite the reduction in the FEP.

The CKD adenine-induced model was chosen because it presents similarities to the development of CKD in humans and because it has been used in studies for cardiovascular assessment and bone metabolism disorder in CKD.[43] Contrary to our expectations, we did not find changes in the serum calcium levels in the chelator-treated and untreated CKD groups. An increase in the serum calcium levels was observed in other experimental models that used adenine,[20,23] possibly due the impairment of bone mineralization induced by the direct action of adenine on the osteoblasts.[42,44]

Our findings showed increased bone formation and resorption, as well as a deficit in bone mineralization, corresponding to a disease with high remodeling.[23,45] The use of chelators did not result in the expected changes in the histomorphometric parameters, unlike the results of the studies of Iida *et al.* and Yaguchi *et al.* that demonstrated a better balance between the osteoblastic and osteoclastic activities and a reduction in non-mineralized tissue.[23,42] However, these authors successfully improved the biochemical and histomorphometric parameters only at high concentrations of iron compounds. The effect of the studied polymer may be dose dependent considering that FGF23 inhibits bone mineralization and PTH acts on the bone formation and resorption parameters;[46–48] thus, higher doses may be required to obtain better control over FGF23, PTH, and, consequently, renal osteodystrophy (ROD). On the other hand, the influence of other factors on bone metabolism, such as metabolic acidosis, vitamin D deficiency, and uremic toxins, should be considered.[49,50]

A recent study by Custódio *et al.* analyzed 604 bone biopsies from dialysis patients and demonstrated bone Fe accumulation in 29.1% of patients, revealing that the bone overload of this compound is an event to be considered in ROD analyses.[24] It is important to eliminate the risk of bone Fe deposition in CKD patients, since these drugs are commonly used in this population and bone Fe accumulation is related to changes in bone metabolism.[51,52] Previous studies reported low intestinal Fe absorption and non-significant tissue accumulation in the animals tested with the CH-FeCl polymer.[27–29] However, to date, no study has investigated the presence of Fe in bone tissue after the use of this phosphate chelator. We also observed similar serum iron levels in animals and found variability in CKD groups, with or without phosphorus chelator treatment. As CKD progresses, intestinal iron absorption decreases due to elevated hepcidin levels. Although we did not assess hepcidin levels in our study, this modified iron metabolism has already been reported in studies that used the adenine-induced CKD model.[53,54] Our findings also showed no Fe overload in the bone tissue of the uremic animals, demonstrating its safety at the administered dose. In addition to its chelating activity, the CH-FeCl polymer possibly contributes to the reduction of the serum levels of uremic toxins, since chitosan has an antioxidant effect and an indoxyl-sulfate lowering capacity,[55–60] acting as an adjuvant therapy for the treatment of cardiovascular and bone complications of CKD.[60,61] This potential effect of the CH-FeCl polymer was not investigated, but it should be considered in future studies.

Our study has limitations, such as the lack of serum vitamin D and ferritin levels dosage, which would be the ideal marker of Fe overload, which was justified by the low volume of the blood samples obtained from the animals. Moreover, the low concentration of Fe used as chelator probably influenced the study results, making further studies comparing CH-FeCl with other Fe-based chelators interesting.

In conclusion, CH-FeCl possesses the ability to reduce the FEP similar to $CaCO_3$, without causing a Fe overload in bone tissues, and may be considered as a safe potential chelator in CKD. Future studies on this adenine model are required to determine a more effective dose of the compound, optimize the desired results, and further assess the potential benefits of chitosan in the management of CKD.

AUTHORS' CONTRIBUTIONS

All authors participated in the design of the study, and interpretation and analysis of the data and review of the manuscript. WBC, BBAC, VJ, MRC, and LCM conducted the experiments, CAR supplied critical reagents [phosphorus chelator chitosan-iron III (CH-FeCl)]; WBC, PAVC, and HS wrote the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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