Original Research

Effect of cross-linked chitosan iron (III) on vascular calcification in uremic rats

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Impact statement

Vascular calcification (VC) is a common complication due to CKD-related bone and mineral disorder (BMD) and is characterized by deposition of calcium in vessels. Effective therapies are not yet available but new phosphorus chelators can prevent complications from CV. We tested the effect of chitosan, a new phosphorus chelator, on the VC of uremic animals. It has recently been proposed that chitosan treatment may be effective in the treatment of hyperphosphataemia. However, its action on vascular calcification has not been investigated yet. In this study, we demonstrated that chitosan reduced the calcium content in the aorta, suggesting that this may be a therapeutic approach in the treatment of hyperphosphatemia by preventing CV.

Abstract

Cross-linked chitosan iron (III) is a chitin-derived polymer with a chelating effect on phosphorus, but it is untested in vascular calcification. We evaluated this compound's ability to reduce hyperphosphatemia and its effect on vascular calcification in uremic rats using an adenine-based, phosphorus-rich diet for seven weeks. We used a control group to characterize the uremia. Uremic rats were divided according the treatment into chronic kidney disease, CKD-Ch-Fe(III)CL (CKD-Ch), CKD-calcium carbonate, or CKD-sevelamer groups. We measured creatinine, phosphorus, calcium, alkaline phosphatase, phosphorus excretion fraction, parathyroid hormone, and fibroblast growth factor 23. Vascular calcification was assessed using the aortic calcium content, and a semi-quantitative analysis was performed using Von Kossa and hematoxylin–eosin staining. At week seven, rats in the chronic kidney disease group had higher creatinine, phosphorus, phosphorus excretion fraction, calcium, alkaline phosphatase, fibroblast growth factor 23, and aortic calcium content than those in the Control group. Treatments with cross-linked chitosan iron (III) and calcium carbonate prevented phosphorus increase (20%–30% reduction). The aortic calcium content was lowered by 88% and 85% in the CKD-Ch and CKD-sevelamer groups, respectively. The prevalence of vascular changes was higher in the chronic kidney disease and CKD-calcium carbonate (62.5%) groups than in the CKD-Ch group (37.5%). In conclusion, cross-linked chitosan iron (III) had a phosphorus chelating effect similar to calcium carbonate already available for clinical use, and prevented calcium accumulation in the aorta.

Keywords: Vascular calcification, bone mineral disorder, hyperphosphatemia, chitosan, phosphate binder, chronic kidney disease

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Introduction

Vascular calcification (VC) is a major complication of metabolic disorders that are caused by chronic kidney disease (CKD), and is highly associated with increased cardiovascular risk and mortality.[1] Previously considered to be related to aging and ensuing atherosclerosis, hypertension and diabetes, VC is now well recognized as being a result of phenotypic changes of the smooth muscle cells

due to a CKD-related bone and mineral disorder (BMD) active process, which primarily involves hyperphosphatemia.[2–4]

Some *in vitro* studies observed increased calcium deposition in the vascular smooth muscle cells in culture media containing high phosphorus concentrations.[5,6] The expression of proteins such as alkaline phosphatase (AP), osteocalcin, and osteopontin is also usually found in mineralized tissues, and is enhanced in calcified vascular smooth muscle cells.[7] Moreover, uremic animals that were administered a high phosphorus diet had important calcification of the arterial media, concomitant with high levels of the phosphaturic hormone fibroblast growth factor 23 (FGF-23) and osteopontin.[4,8]

Phosphorus chelators are chemical compounds that are targeted to restore phosphorus homeostasis in CKD patients, blocking the absorption of phosphorus and eliminating it in the stool.[9] Clinical trials have shown that the use of calcium-based chelators, such as calcium carbonate and acetate, is associated with VC progression and higher mortality, thus highlighting the need of developing new effective chelating drugs to reduce serious adverse effects.[10–12] New formulations have included calcium-free, iron-based compounds with potential phosphorus chelating activity.[13–15] Among these calcium-free formulations, sevelamer hydrochloride is the most widely used. It has considerable clinical chelating activity, but its use is limited by its high cost.[16] Chitosan is a chitin-derived polymer with a pair of free electrons that are linked to a nitrogen atom, which serves as a supporting element for metallic phosphorus-absorbent compounds.[8,17] Iron is a transition metal that can establish a strong bond to phosphate. After the inclusion of the iron molecule in the chemical structure of the Chitosan, creating the Ch-Fe(III)CL, the new complex is insoluble at acidic pH in the stomach, prevents release and absorption of iron, and then provides an available phosphorus binding surface.[17] Previous *in vitro* and *in vivo* studies with diet phosphorus overload models have shown the efficacy of iron-III chitosan (Ch-Fe(III)CL) as a chelator.[17–19] However, the phosphorus-chelating effect of Ch-Fe(III)CL in VC has not yet been tested in a model of CKD-induced hyperphosphatemia.

The objective of the present study was to evaluate the phosphorus chelating effect of Ch-Fe(III)CL in an experimental model of uremia, to evaluate its effect on VC and other markers of CKD-related BMD, and to compare its therapeutic potential against other phosphorus chelators.

Materials and methods

Statement of ethics

All procedures were performed in accordance with the Brazilian Federal Law (11,794, 8 October 2008) and the guidelines of the National Council for the Control of Animal Testing. The study was approved by the Ethics Committee of Animal Use of Federal University of Juiz de Fora, Protocol number 031/2013.

Animals and experimental protocol

A total of 56 male Wistar rats, aged between 8 and 12 weeks and weighing about 250 g, were used. The animals were obtained from the rat colony of the Reproductive Biology Center of the Federal University of Juiz de Fora, where all experiments were performed. They were divided into five groups (Figure 1). In Protocol 1 (model study), we fed rats in the Control group with a standard diet and compared these rats to rats in a CKD group that were fed with a 0.75% adenine-enriched diet for four weeks and a 0.1% adenine-enriched diet for the following three weeks.[20] The adenine-enriched diet contained 1% phosphorus (Pragsoluções, Jaú, Brazil). The Control and CKD groups received vehicle (1 mL/100 g of weight of distilled water); with gavage for four weeks, starting from week 3. We evaluated eight animals from each group at weeks 4 (day 28) and 7 (day 49).

In Protocol 2 (treatment study), we compared the treatment groups to the CKD group. Rats with uremia that was induced according to Protocol 1 were randomly divided into four groups (eight in each group) and treated with gavage for four weeks, starting from week 3, as follows:

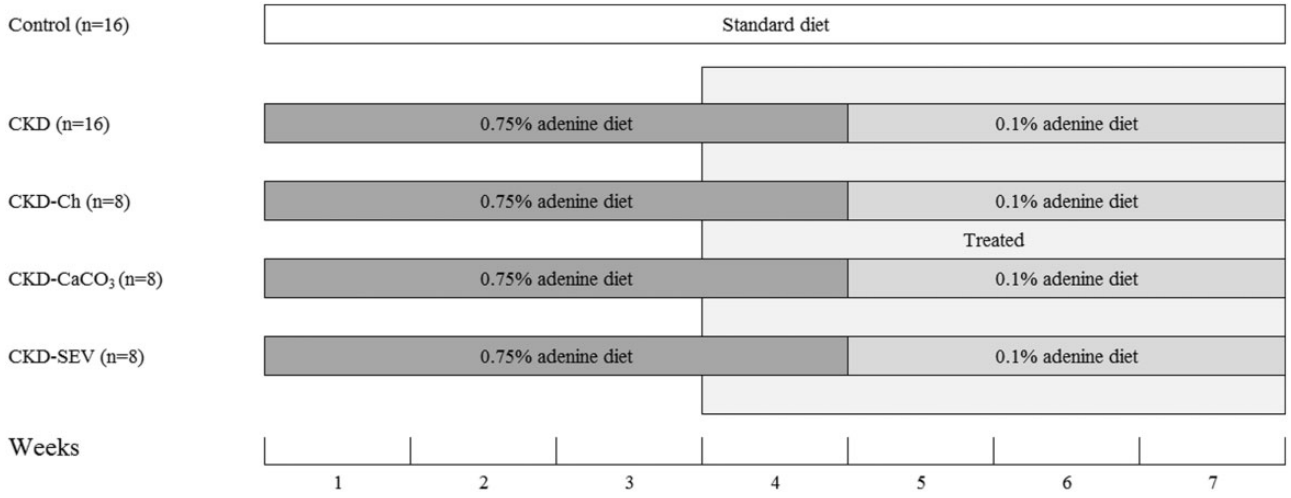

Figure 1. Study design. The animals were divided into five groups: Control ($n = 16$), chronic kidney disease (CKD) ($n = 16$), CKD-cross-linked chitosan iron (III) (CKD-Ch) ($n = 8$), CKD-calcium carbonate (CKD-$CaCO_3$) ($n = 8$) and CKD-sevelamer (CKD-SEV) ($n = 8$).

CKD group –treated with distilled water (1 mL/100 g of weight); CKD-Ch group – treated with 30 mg/kg/day of Ch-Fe(III)CL,[18] CKD-$CaCO_3$ group – treated with 500 mg/kg/day of calcium carbonate,[11,13] and CKD-SEV group – treated with 500 mg/kg/day of sevelamer[21] (Figure 1).

Chitosan (240 kD, Purifarma, São Paulo, Brazil) was dissolved in a 0.1 M $Fe(NO_3)_3$ aqueous solution (Vetec, Rio de Janeiro, Brazil) over 4 h, then precipitated with acetone, resulting in an orange precipitate, which was filtered and washed with acetone to remove excess $Fe(NO_3)_3$, and vacuum-dried. This product was then added to a 15% glutaraldehyde solution in acetone (Merck, Cotia, Brazil) for 24 h, filtered and washed with water to remove excess glutaraldehyde.[17–19] All drugs were dissolved in distilled water.

On the day prior to euthanasia, the animals were weighed and kept in metabolic cages for 24 h for urine sampling and accurate evaluation of food consumption. Blood samples were collected via a cardiac puncture with the rats under anesthesia of 10 mg/kg of xylazine and 90 mg/kg of ketamine i.p. (König, Avellaneda, Argentina), followed by diaphragm rupture for euthanasia.

Biochemical parameters

We assessed serum and urinary creatinine, serum and urinary phosphorus (to calculate the phosphorus excretion fraction (PeF)), serum calcium, and AP (Labmax, Labtest Diagnóstica, Lagoa Santa, Brazil). We also determined the serum parathyroid hormone (PTH) (Rat Intact PTH (ELISA) Kit, Immutopics, San Clemente, USA) and serum FGF-23 (FGF-23 ELISA Kit, Cloud-Clone Corp. Houston, USA) using ELISA (R&D Systems, Minneapolis, USA).

VC

Quantification of aortic calcium content. After we euthanized the rats, we harvested approximately 1.5 cm of the abdominal aorta at −80°C. Each fragment was dehydrated in an incubator (Sterilifer, Diadema, Brazil) at 60°C for 24 h and then crushed, weighed, and solubilized in a hydrochloric acid solution (HCl) (Vetec, Belo Horizonte, Brazil) at 0.6 N (40 mL HCl/g of tissue) for 48 h. The solution was centrifuged at 3000 r/min for 10 min. The calcium concentration (Calcium Dry–Reagent Set, Pointe Scientific, Canton, USA) in the supernatant was measured in a spectrophotometer (YKSI, Shanghai, China) at a wavelength of 570 nm. The calcium concentration in the abdominal aorta was expressed as milligrams of calcium per gram of dry weight, and, in this particular experiment, we evaluated five animals from each group.[22,23]

Semi-quantitative morphologic evaluation of the vessels. The morphologic changes of the aortic arch, as well as the presence of calcium deposits, were assessed histologically. The thoracic aorta was divided into two fragments, fixed in 10% formalin, and submitted to standard histological processing. The aortic fragments of each animal were divided into 5 μm sections, stained with hematoxylin–eosin and Von Kossa staining, and put transversally in the same block. We evaluated 20 fields for each animal under an optical microscope using the 400× objective, 8 animals per group, attributing the following score to each animal:[24]

1. Normal: no lesions (Figure 2(a));
2. Mild lesion: total or partial discontinuity of the intima or elastic fibers (Figure 2(b));
3. Moderate lesion: total or partial discontinuity of the intima or elastic fibers, and loss of the normal architecture within the media (Figure 2(c));
4. Severe lesion: total or partial discontinuity of the intima or elastic fibers, and loss of the normal architecture within the media plus calcification (Figure 2(d)).

Animals with a score of 0 or 1 were classified as without lesions or with an incipient vascular lesion (no injury or mild injury), and those with scores of 2 or 3 as presenting with an established vascular lesion (severe injury).

Statistical analysis

Values are expressed as means and standard deviations. Normality was assessed with the Shapiro–Wilk test. The Control and CKD groups were compared using Student's *t*-test. The CKD and treated CKD groups were compared using the analysis of variance test, followed by Tukey's test. We considered *P*-values <0.05 as statistically significant. All statistical analyses were performed using SPSS 13.1® (IBM Corporation, Chicago, USA).

Results

Mortality and metabolic and biochemical parameters of Control and CKD groups (Protocol 1)

Death did not occur in the rats in Protocol 1. Comparisons of the metabolic parameters of rats in the Control and CKD groups at weeks 4 and 7, and those of the biochemical parameters obtained from the sera and urine of rats in both groups, are shown in Table 1. The rats in the CKD group had lower food intake and body weight at both time points than the rats in the Control group. The rats in the CKD group had higher levels of serum creatinine, phosphorus, AP, and PeF at week 4 than rats in the Control group. We observed partial recovery of kidney function at week 7, after reduction of adenine concentration in the diet. However, differences between the Control and CKD animals were still present, with a progressive increase of PeF and AP. FGF-23 progressively raised in the CKD group throughout the experiment, with a significant increase at week 7. The serum PTH was higher in the CKD group than in the Control group only at week 4.

VC in control and CKD groups (Protocol 1)

We found no differences between Control and CKD groups at week 4. Animals from the CKD group had higher calcium content in the aorta at week 7 when we compared to Control group (3.43 ± 2.82 vs. 0.29 ± 0.11 mg/g, $P = 0.038$) (Figure 2(e)).

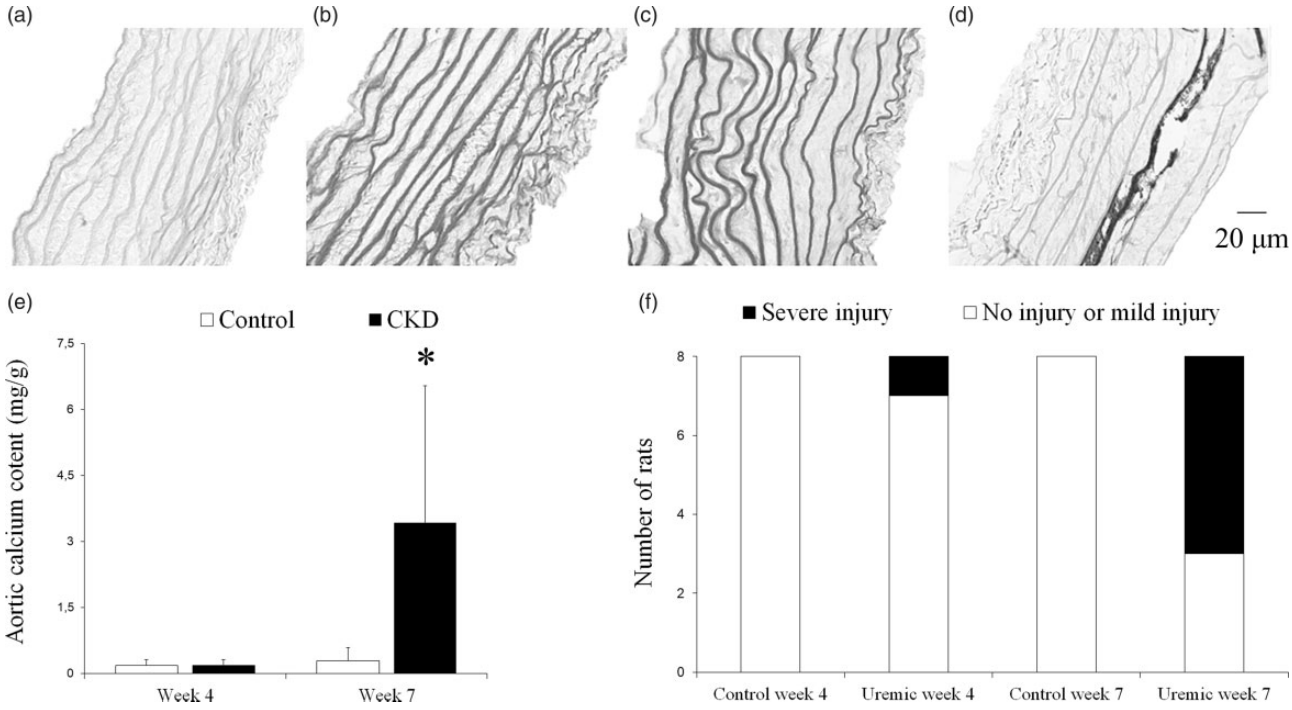

Figure 2. Vascular calcification evaluation of the Control and CKD groups at weeks 4 and 7. (a–d) Scores to evaluate the prevalence of vascular disorders. (a) Normal. (b) Mild lesion. (c) Moderate lesion. (d) Severe lesion. (e) Calcium content of the abdominal aorta in mg calcium/g tissue. Mean ± standard deviation ($n = 5$). Student's *t*-test was used to compare the groups. *$P < 0.05$. (f) Prevalence of vascular disorders in animals classified as no initial or vascular injury, or injury to vascular injury was established ($n = 8$).

Rats in the Control group did not have any vascular morphological changes at any time point. Among the rats in the CKD group, only one (12.5%) had a well-established vascular lesion (score 2 or 3) at week 4; however at week 7, a higher prevalence of such lesions (62.5%), including calcified areas, was seen in this group than at week 4 (Figure 2(f)).

Mortality and metabolic and biochemical parameters in CKD and treated CKD groups (Protocol 2)

Death did not occur in the rats in Protocol 2. Among rats in the CKD-Ch, CKD-$CaCO_3$, and CKD-SEV groups, we did not observe modifications in weight, food consumption, and serum creatinine, serum phosphorus, serum calcium, AP, PTH, and FGF-23 levels compared to rats in the CKD group (Table 2). We observed a trend towards lower serum phosphorus values in the CKD-Ch group (8.62 ± 1.73 mg/dL) when compared to the CKD group (10.95 ± 2.29 mg/dL, $P = 0.057$) and lower PeFs in CKD-Ch than in CKD groups (40.54 ± 16.13 vs. $71.57 \pm 20.22\%$, $P = 0.006$), respectively, suggesting that Ch-Fe(III)CL has a chelating action on phosphorus, as well as the phosphate binder $CaCO_3$ also reduced PeF. We observed a tendency to reduce PeF values in the CKD-SEV group ($43.27 \pm 36.20\%$) when compared to the CKD group ($71.57 \pm 20.22\%$, $P = 0.08$). There were no differences in the other biochemical parameters when we compared CKD-Ch to CKD-SEV (Table 2).

VC in CKD and treated CKD groups (Protocol 2)

After seven weeks, CKD rats exhibited increased levels of calcium content in the abdominal aorta (3.43 ± 2.82 mg/g) and the treatment with Ch-Fe(III)CL reduced 88% of calcium content in the aorta (0.40 ± 0.06 mg/g, $P = 0.043$), as well as 85% with SEV (0.51 ± 0.33 mg/g, $P = 0.05$) (Figure 3(a)). The semi-quantitative evaluation of vascular lesions revealed that vascular changes were present in 62.5%, 62.5%, and 75% of animals in the CKD, CKD-$CaCO_3$, and CKD-SEV groups, respectively, while well-established vascular lesions were seen in only 37.5% of animals in the CKD-Ch group. Ch-Fe(III)CL was the only chelator that reduced the prevalence of moderate-to-severe lesions (Figure 3(b)).

Discussion

In this study, we reproduced the model of chronic tubulointerstitial nephritis by adding adenine to the diet, which led to an increase in serum creatinine, phosphorus, calcium, and AP, as well as elevation in PeF, FGF-23, and PTH. We opted for this adenine-induced nephropathy model because it is currently the most frequently used to reproduce the clinical aspects of BMD-CKD.[20,25] By putting the rats on a four-week 0.75% adenine diet followed by a 0.1% adenine diet for three weeks, we attempted to maintain a longer lasting renal lesion, without causing excessive weight loss or increasing mortality.[26] Phosphorus has an important role in CKD progression and promotes VC; hence, we included a slightly higher concentration of phosphorus (1%) in the diet that was offered to the animals.[4,8,13] Both time length of the diet with adenine and the increase of phosphorus in the diet were targeted to increase the possibility of development of BMD-CKD and VC.[20,27] With the modification of the adenine concentration, we found a

reduction in both weight and food intake during the diet with 0.75% adenine, but a lower rate of weight loss and an increase in food intake towards the end of the experiment. These results are similar to those previously described by other authors.[13,20,26]

Ch-Fe(III)CL polymer derived from chitin can effectively bind phosphorus, thus lowering serum phosphorus concentrations in rats with hyperphosphatemia induced by the addition of phosphorus to the water and in diabetic rats.[18,19] Thus, we decided to test it in an experimental model of uremia. We opted to initiate treatment three weeks after induction of uremia to mimic the conservative treatment of CKD.[28–30] In this model, we showed that none of the tested chelators modified progression of CKD, as measured by creatinine, as other authors reported.[13,20,31,32] In previous clinical studies, the range of the chelating effect, measured by the percentage of phosphorus reduction, varied between 20% and 50%.[23–27,31] Although the rats in the CKD group had hyperphosphatemia, none of the treatments reduced the levels of phosphorus, that were similar to those of normal animals, which suggests that the renal

Table 1. Metabolic and biochemical parameters of the Control and CKD groups in weeks 4 and 7.[a]

Parameters	Week 4	Week 7
Body weight (g)		
Control	265.4 ± 14.9	283.1 ± 14.5
CKD	138.6 ± 10.5*	190.7 ± 25.8*
Food intake (g)		
Control	18.5 ± 2.6	18.9 ± 2.7
CKD	13.3 ± 3.4*	16.1 ± 2.4*
Serum creatinine (mg/dL)		
Control	0.41 ± 0.21	0.47 ± 0.25
CKD	1.64 ± 0.26*	0.95 ± 0.21*
Serum phosphorus (mg/dL)		
Control	7.11 ± 0.71	6.82 ± 0.49
CKD	14.70 ± 4.87*	10.95 ± 2.29*
PeF (%)		
Control	14.79 ± 9.70	20.07 ± 16.82
CKD	57.44 ± 45.26*	71.57 ± 20.22*
Serum calcium (mg/dL)		
Control	9.45 ± 1.10	8.42 ± 1.10
CKD	10.74 ± 1.73	10.43 ± 1.85*
Alkaline phosphatase (U/L)		
Control	212.62 ± 59.64	205.7 ± 62.39
CKD	452.71 ± 153.43*	561.0 ± 90.90*
PTH (pg/dL)		
Control	214.84 ± 53.31	272.30 ± 192.22
CKD	3769.40 ± 1693.62*	5940.20 ± 5740.35
FGF-23 (pg/dL)		
Control	6.85 ± 0.60	7.42 ± 1.96
CKD	75.83 ± 59.91	81.36 ± 37.17*

[a]Data are shown mean ± standard deviation ($n = 8$). Student's t-test was used to compare the groups.
*$P < 0.05$ in the CKD group vs. Control. The dosages of PTH and FGF-23 were used in five animals from each group.
CKD: chronic kidney disease; PeF: phosphorus excretion fraction; PTH: parathyroid hormone; FGF-23: fibroblast growth factor 23.

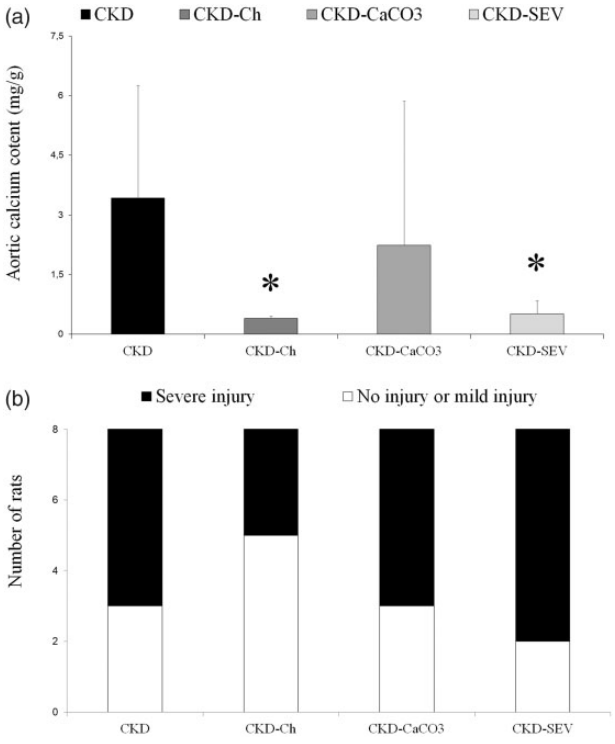

Figure 3. Vascular calcification evaluation of the CKD, CKD-Ch, CKD-$CaCO_3$ and CKD-SEV groups after seven weeks. (a) Calcium content in the abdominal aorta in mg calcium/g tissue, mean ± standard deviation ($n = 5$). Student's t-test was used to compare the groups. *$P < 0.05$. (b) Prevalence of vascular changes in animals that were classified as without initial vascular injury or injury, or a vascular injury. Mean ± standard deviation ($n = 8$).

Table 2. Metabolic and biochemical parameters of the CKD groups: CKD, CKD-Ch, CKD-$CaCO_3$, and CKD-SEV after seven weeks.[a]

Parameters	CKD	CKD-Ch	CKD-$CaCO_3$	CKD-SEV
Body weight (g)	190.73 ± 25.78	199.46 ± 9.06	198.41 ± 27.08	189.69 ± 17.72
Food intake (g)	16.06 ± 2.38	15.94 ± 3.90	10.63 ± 6.05	14.19 ± 3.28
Serum creatinine (mg/dL)	0.95 ± 0.21	0.83 ± 0.32	0.87 ± 0.52	0.88 ± 0.36
Serum phosphorus (mg/dL)	10.95 ± 2.29	8.62 ± 1.73	8.71 ± 1.77	8.95 ± 1.37
PeF (%)	71.57 ± 20.22	40,54 ± 16.13*	34.63 ± 15.32*	43.27 ± 36.20
Serum calcium (mg/dL)	10.43 ± 1.85	10.34 ± 1.21	9.38 ± 1.57	9.37 ± 2.24
Alkaline phosphatase (U/L)	561.00 ± 90.90	442.13 ± 113.70	452.88 ± 99.13	554.00 ± 114.00
PTH (pg/dL)	5940.20 ± 5740.35	1514.00 ± 309.97	2495.00 ± 1312.40	7362.60 ± 7552.72
FGF-23 (pg/dL)	81.36 ± 37.17	70.88 ± 11.89	68.11 ± 35.65	99.64 ± 54.50

[a]Data shown as a mean ± standard deviation ($n = 8$). Student's t-test was used to compare the treated groups vs. CKD group.
*$P < 0.05$. PTH and FGF-23 were administered in five animals from each group.
CKD: chronic kidney disease; CKD-Ch: CKD-Ch-Fe(III)CL; CKD-$CaCo_3$: CKD-calcium carbonate; CKD-SEV: CKD-sevelamer; PeF: phosphorus excretion fraction; PTH: parathyroid hormone; FGF-23: fibroblast growth factor 23.

lesion was not severe enough to allow us to demonstrate more significant results, or that a longer period of treatment would be needed. However, the Ch-Fe(III)CL and $CaCO_3$ treatment reduced PeFs at the end of the experiment, assuring a chelator effect by reducing phosphorus overload. Similar to a model of CKD induced by adenine that was previously described,[26] our model found that the calcium serum levels were raised in the CKD group, but remained unchanged after chelation treatment.

In CKD patients, the phosphorus levels are initially controlled by FGF-23 and PTH.[33] One of the consequences of the increase of FGF-23 is an increase of PeF, which lowers serum phosphorus.[31] In fact, previous studies using adenine models have shown that high FGF-23 and high PeF were seen within four weeks.[13,29] We found this particular pattern in the CKD group at both observed time points, but treatment with chelators did not reduce the FGF-23 levels. However, it is known that reduction in FGF-23 levels due to the use of phosphorus chelators is not always observed.[29,31]

The biochemical markers AP and PTH, despite traditionally linked to the process of bone remodeling, can be often associated with the occurrence of VC.[4,8] The levels of PTH and AP were elevated in the rats in the CKD group, whereas treatment with Ch-Fe(III)CL and $CaCO_3$ tended to reduce these levels. A variation in intra-group values of PTH in CKD experimental models is frequently observed in the literature,[14,22,29,30] which can partly explain why we did not find significant differences after treatment.

Among a number of models, the adenine models are the ones in which a higher prevalence of VC (probably due to a higher degree of BMD) can be seen. However, the occurrence and severity of vascular lesions are directly related to the time and intensity of renal dysfunction and overload of the phosphorus diet.[29,34] Animals with CKD experienced partial recovery of renal function, but at the end of seven weeks, we could observe some evidence of VC through Von Kossa staining, as well as a high degree of calcium content in the aorta. A few reports have studied a 0.75% adenine model for a period of eight weeks and demonstrated VC through Von Kossa staining. However, in those reports, the animals had creatinine levels two to four times higher than those in our study.[28,30,32]

In CKD group, we found an increase in the FeP, i.e. an increase in the bioavailability of phosphorus and also an increase in the calcium content in the aorta. Calcium levels in the aortic tissue decreased in the groups treated with Ch-Fe(III)CL, as well as the PeF. This effect was similar to the observed in CKD group treated with SEV. The CKD group treated with Ch-Fe(III)CL developed less marked vascular wall changes than the other treatment groups, highlighted by a lower frequency of established lesion scores, meaning less severe forms of vascular lesions. Other studies have shown benefits of using compounds based on Fe;[13–15] however, it is still unknown the possible toxic effects of iron on CKD-related BMD. In addition to the phosphorus chelating effect, Ch-Fe(III)CL is a totally non-absorbable and low cost compound. Another point to be highlighted is the low dose of the complex used to obtain the phosphorus chelating effect. The dose used in this study was equivalent to approximately 2 g/day for a 70 kg person and represents half of the doses commonly used by other chelators.

This study has some limitations that should be noted. We believe that when we tried to reduce mortality, the changes induced in this study were probably not severe enough to fully reproduce the vascular lesions that were needed to highlight the benefit of treatment. The best indicator of this pattern is that the partial recovery of creatinine observed with the exchange of 0.75% adenine diet for 0.10% adenine; however, the renal dysfunction was still present. This limitation could be corrected with a longer experiment interval. In models of a less marked pattern of lesions, collection of all of the aortic tissue for general evaluation of VC could have enhanced the percentage of lesions, as done by other authors.[8,35] As an attempt to minimize this possible limitation, we standardized the region that was used for aortic fragment sampling for the Von Kossa staining.

We conclude that Ch-Fe (III) CL had a chelating effect on phosphorus, characterized by the reduction of PeF, similar to $CaCO_3$, already in clinical use. In addition, treatment with Ch-Fe (III) CL was able to prevent calcium deposition in the abdominal aorta and attenuated moderate to severe vascular lesions.

Authors' contributions: Helady Sanders-Pinheiro designed the study and prepared the first draft of the paper. She is guarantor. Barbara Bruna Abreu de Castro designed the study, prepared the first draft of the paper and did the experimental work and statistical analysis of the data. Paulo Giovani de Albuquerque Suassuna, Moises Carminatti, Julia Bianchi Brito, Wagner Vasques Dominguez, Ivone Braga de Oliveira, Vanda Jorgetti, Melani Ribeiro Custodio contributed to the experimental work. Wander Barros do Carmo designed the study, did the experimental work and statistical analysis of the data. All authors revised the paper critically for intellectual content and approved the final version. All authors agree to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

DECLARATION OF CONFLICTING INTERESTS

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