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Cross-linking chitosan-Fe(III), an oral phosphate binder: studies in vitro and in vivo

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Abstract

The objective was to evaluate the in vitro and in vivo phosphate binding properties of cross-linked chitosan iron (III) (CH-Fe(III)-CL), a potential oral phosphate binder for treating hyperphosphatemia. At equilibrium, the in vitro phosphate binding of CH-Fe(III)-CL was 23.6 mg g^{-1} for simulated gastrointestinal conditions. In hyperphosphatemic rats, CH-Fe(III)-CL was similar to iron sulfate in reducing serum phosphate by about 35%. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cross-linked chitosan iron (III); Hyperphosphatemia; Phosphate

1. Introduction

Phosphorus is an essential element involved in multiple metabolic processes and the transfer and storage of high-energy compounds (Helikson et al., 1997). Hyperphosphatemia may be a problem resulting in chronic hemodialysis patients, which are in positive phosphate balance (Hergesell and Ritz, 1999). Treatment of hyperphosphatemia is necessary in order to prevent secondary hyperparathyroidism and renal osteodystrophy in patients with chronic renal failure undergoing dialysis (Jing and Yamaguchi, 1992). Phosphate-binding drugs, in order to sequester phosphate in the intestinal tract and to reduce entry of phosphate into the extracellular fluid space, have been successfully used to control hyperphosphatemia. However, these drugs may be problematic due to the occurrence of side effects such as hypercalcemia (calcium carbonate, calcium acetate) and toxicity (aluminum-based compounds) (Hergesell and Ritz, 1999; Kuroda et al., 1995). Several studies suggest that ferric compounds bind dietary phosphate and lower serum phosphate when given orally and even intravenously (Spengler et al., 1994; Hergesell and Ritz, 1999; Hsu et al., 1999). Kuroda et al. (1995) and Hsu et al. (1999) have reported that intestinal absorption of phosphorus was inhibited by a high concentration of iron in iron(II) citrate and iron(III) citrate, respectively, presumably resulting from the formation of a complex with phosphorus in the intestine.

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Chitosan, a polyaminosaccharide, is the deacetylated derivative of chitin, a naturally occurring polymer found in the shells of crustacea, the cuticles of insects and cell walls of some fungi (Lim and Wan, 1998). Due to such properties as biocompatibility, biodegradability and bioactivity, this biopolymer has been considered for use in a variety of functions including flocculation and coagulation in food processing, in heavy-metal ion recovery from waste waters na in the fabrication of structural matrices for food, cosmetic, biotechnological and biomedical applications (Delben et al., 1990). Not hydrolysable by the digestive enzymes in man, its chemical structure is similar to that of cellulose (Jing et al., 1997). Considering the extra electron pair of nitrogen, chitosan can form polymer–metal complexes with many metal cations, and is thus used as a metal-ion sorbent. Jing and Yamaguchi (1992) demonstrated that the phosphate-binding capacity of iron (II)-chitosan is greater than aluminum hydroxide for a dilute phosphate solution. In this study, we investigated a new type of phosphate binder iron-contained, a cross-linked chitosan iron (III) (CH-Fe(III)CL) prepared from chitosan.

2. Materials and methods

2.1. Materials

Chitosan [degree of deacetylation = 0.76; molecular weight (M_v) 65 000, determined by Mark–Houwink equation] was obtained through basic hydrolysis of chitin (Rathke and Hudson, 1994; Rodrigues et al., 2000). The CH-Fe(III)-CL (85mg g^{-1} of iron) was prepared and characterized by methods reported in the literature (Fagundes et al., 2001). The chitosan was dissolved in a $Fe(NO_3)_3$ 0.1 M aqueous solution for 4 h. An orange precipitated was obtained after addition of the acetone. The solid was filtered and washed with acetone, to remove excess of $Fe(NO_3)_3$, and finally dried in vacuum. Later, the solid was put in contact with glutaraldehyde 15% (in acetone) solution for 2 h (Fagundes et al., 2001). The polymer was characterized following the procedure described in the literature (Rodrigues et al., 1999). All other chemicals were reagent grade and used as received.

2.2. In vitro study

2.2.1. Phosphate adsorption

30 mg of the CH-Fe(III)-CL was shaken (150 rpm) for 2 h in 20 ml of KH_2PO_4 1000 mg l^{-1}, pH 1.5 (pH of gastric juice) in thermostatically-controlled water at 37.5 ± 0.5 °C. The CH-Fe(III)-CL was removed by filtration and unbound KH_2PO_4 was determined by molybdenum blue spectrophotometric methods (Colina et al., 1996). Then, the same material was shaken for 2 h at 20 ml of KH_2PO_4 100 mg dl^{-1}, pH 7.5 (pH of intestinal juice). The binder was removed by filtration and unbound KH_2PO_4 was determined. In another experiment, 30 mg of the CH-Fe(III)-CL was shaken for 4 h at 20 ml of KH_2PO_4 1000 mg l^{-1}, pH 7.5. The concentration of unbound KH_2PO_4 was determined. The quantity of KH_2PO_4 adsorbed by polymer was obtained by subtracting the concentration in the supernatant solution from the initial concentration.

2.2.2. Elution iron

30 mg of the CH-Fe(III)-CL was shaken (150 rpm) for 2 h at 20 ml of HCl, pH 1.5 and 20 ml KH_2PO_4 pH 7.5, in thermostatically-controlled water at 37.5 ± 0.5 °C. The CH-Fe(III)-CL was removed by filtration and the iron concentration of the solution was determined by using a 1.10-phenantroline spectrophotometric method (Jing et al., 1997).

2.3. In vivo study

Eight-week-old male Wistar rats were used (body weight of 140–160 g). Animals were housed in Biotério Setorial of Univali during the treatment and were separated in three groups with five rats in each group. All the groups received commercial chow ad libitum during the treatment and received water with sodium phosphate 1.2% (Schwarz et al., 1985) during 30 days for hyperphosphatemia induction. After 30 days, the animals only received the phosphate binder (31 mg kg^{-1}) CH-Fe(III)-CL (iron = 2.6 mg kg^{-1}), (9

mg kg^{-1}) iron sulfate (iron = 2.6 mg kg^{-1}), or 1 ml of the water for 15 days.

At the end day, 15 animals were fasting during 12 h and specimens of blood were obtained. Serum phosphorus, iron and calcium were determined. The phosphorus was measured by UV assay of molibdate (340 nm) (Colina et al., 1996). Iron was measured by colorimetric assay of Cromazurol B (Garcic, 1979). Calcium was measured by colorimetric assay of Cresolftaleina complexona (Lorenz, 1981). All samples were measured in triplicate. The analyses were carried out in a spectrofotometer UV/Vis Shimadzu UV 1601.

2.4. Statistical analysis

Data are expressed as mean ± S.D. The significance of the difference between the means of tests and control studies was established by analysis of variance (ANOVA) followed by the Dunnett test.

3. Results

3.1. In vitro study

The amount of lost iron for complex CH-Fe(III)-CL, after the contact with the solution of HCl pH 1.5, corresponded to 16% of the initial amount. On the other hand loss of iron was not observed after contact with phosphate solution buffer pH 7.5.

When oral binder is used, it goes through the stomach to the intestine. The pH changes from 1.5 to 7.5. The binder should maintain the high phosphate binding capacity, in spite of the changing pH. The adsorption phosphate capacities of the CH-Fe(III)-CL are shown in Table 1. The adsorption phosphate capacity after pH 1.5 for 2 h and pH 7.5 for 2 h was 23.6 mg g^{-1}.

Table 1
Effect of pH exchange on phosphate adsorption: initial KH_2PO_4 concentration 1000 mg l^{-1}, 30 mg of the CH-Fe(III)-CL, solution volume 50 ml, temperature 37 °C

Contact time (h)		Adsorption capacity (mg g^{-1})
pH 1.5	pH 7.5	
2	0	7.5
2	2	23.6
0	4	28.3

Table 2
Effect of CH-Fe(III)-CL and $Fe_2(SO_4)_3$ on serum phosphate, calcium and iron in hyperphosphatemic rats

Group	Ca (mg dl^{-1})	Fe (μg dl^{-1})	PO_4 (mg dl^{-1})
Control	8.114 ± 0.231	165.5 ± 24.91	8.180 ± 0.540
$Fe_2(SO_4)_3$	7.952 ± 0.502	186.5 ± 56.17	5.135 ± 0.826*
CH-Fe-CL	8.120 ± 0.369	190.1 ± 41.29	5.504 ± 0.502*

The values represent mean ± S.D. of five determinations.
* $P < 0.01$ vs. control group, Dunnett test.

3.2. In vivo study

Before the treatment with the phosphate binder, the animals received water with sodium phosphate (1.2%) ad libitum. The water containing sodium phosphate was effective in increase serum phosphorus of rats after 4 weeks.

The concentration of phosphate binder (CH-Fe(III)-CL and $Fe_2(SO_4)_3$) orally administered to the animals was 31 mg kg^{-1} during 15 days. The $Fe_2(SO_4)_3$ was administered for the animals to verify if the iron serum level was altered.

The effect of binders after the experiment on serum phosphorus, calcium and iron are shown in Table 2. Serum phosphorus in rats treated with CH-Fe(III)-CL or $Fe_2(SO_4)_3$ decreased significantly compared with control rats after 2 weeks of treatment. Mean serum phosphate decreased 32.7% in group treated with CH-Fe(III)-CL and 37.2% in group treated with $Fe_2(SO_4)_3$. CH-Fe(III)-CL and $Fe_2(SO_4)_3$ decreased phosphate equally well.

4. Discussion

The low iron elution in pH 1.5 is ascribed to polymer insolubility, due to glutaraldeyde and chitosan crosslinking, resulting in formation of Schiff bases (Monteiro and Airoldi, 1999).

Phosphate binding onto CH-Fe(III)-CL depended on pH. Maximum binding occured at pH 7.5. In this pH, HPO_4^{2-} is the predominant species, and the complex $[FeHPO_4]^+$ formations at the polymer surface (Fagundes et al., 2001). At a lower pH, the neutral species H_3PO_4 is dominant and results in a decrease of sorption efficiency. Additionally, the decrease in the sorption efficiency also can be attributed to the loss of iron during the permanence of the complex in contact with HCl.

Various iron(III) compounds are effective oral phosphate binders, like ferric ammonium citrate (Hsu et al., 1999), polynuclear iron hydroxide (Hergesell and Ritz, 1999), ferric polymaltose complex (Chang et al., 1999), and iron(III)-sucrose (Yamaguchi et al., 1999). Intestinal absorption of phosphate is inhibited by formation of an insoluble complex $[Fe(HPO_4)]^+$, at pH 7.5, at the binder surface.

Mean plasma phosphate in treated rat with either CH-Fe(III)-CL or $Fe_2(SO_4)_3$ was about 72%, compared the control. The phosphate adsorption capacity of CH-Fe(III)-CL is greater than those reported by Kuroda et al. (1995), for sodium ferrous citrate in clinical study. These results can be explained through the stability constants of the formed complex. According to the theory of acidity-basicity of Person, hard acids form complex more strongly with hard bases, while soft acids form complex more strongly with soft bases (Huheey, 1981). By the classification of Person, Fe^{3+} it is a hard acid while Fe^{2+} is an borderline acid (Huheey, 1981). On the other hand, HPO_4^{2-} is a hard base. The stability constant of $[FeHPO_4]$, is lower than the $[FeHPO_4]^+$, $\log K$ 3.60 and 8.36, respectively, ($K = [M][L]/[ML]$) (Smith and Martel, 1975).

There was no significant change in serum-calcium and serum-iron between treated groups and control group, consistent with the observation of Hergesell and Ritz (1999) and Chang et al. (1999), in clinical studies with iron (III) compounds. However, unlike Kuroda et al. (1995), in studies with for sodium ferrous citrate, in this case, no significant changes was observed in the levels in serum-calcium, since CH-Fe(III)-CL does not form complex with calcium.

5. Conclusion

CH-Fe(III)-CL is an effective oral phosphate binder. Cross-linked chitosan decreased iron elution. The serum phosphate after CH-Fe(III)-CL decreased, in comparison with control group. No significant change in serum calcium and serum iron was observed in treated and control rats. The result shows that CH-Fe(III)-CL is an alternative oral phosphate binder.

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