Original Article

Cross-linked iron dextran is an efficient oral phosphate binder in the rat

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Abstract *Background.* There is a need for alternative oral phosphate binders. *In-vitro* studies showed that iron(III)oxide–hydroxide-modified cross-linked dextran is a promising, insoluble phosphate-binding agent. The present study was designed to assess its *in-vivo* efficacy and safety in the rat.

Study, design and methods. Iron(III)oxide–hydroxide modified dextran beads were mixed with normal rat feed in a proportion of 8% by weight. With this formula rats were fed for 4 weeks. A control group received the same diet without added phosphate binder. Samples of blood, urine, and faeces were taken from each animal before the phosphate binder was administered, 2 weeks later, and at the end of the examination period (day 29). Phosphate, calcium, iron were analysed in the blood samples. Calcium and phosphate concentrations were determined in the urine, phosphate, calcium, and iron concentrations in the excrements. Stability of the material in the duodenum was also simulated.

Results and conclusions. The results demonstrate an excellent phosphate-binding capacity of the material and a good tolerance during the intestinal passage. No significant chemical or enzymatic degradation, histological alterations, or other treatment-related macroscopic findings were recorded. The present efficacy and toxicity study has shown effective phosphate binding with no toxicity and no iron release after ingestion of this novel phosphate binding agent. We propose clinical evaluation studies to assess whether similar efficacy and safety can be shown in humans.

Key words: hyperphosphataemia; iron(III)oxide–hydroxide modified dextran beads; phosphate binder

Introduction

Secondary hyperparathyroidism and renal osteodystrophy are the main complications generally observed in advanced chronic renal failure [1–4]. Hyperphosphataemia plays an important role in their genesis. It is mainly the result of accumulation of phosphate as consequence of diminished renal excretion.

The cornerstone of treatment of hyperphosphataemia is the oral administration of phosphate binders [5]. Today there is consensus that aluminium-containing phosphate binders should be avoided [6,7]. Nevertheless, use of alternative phosphate binders, mainly calcium carbonate and calcium acetate, is limited in many patients by side-effects, particularly hypercalcaemia [8,9]. Consequently there is consensus that alternative phosphate binders would be highly desirable.

Recently we developed a novel type of phosphate binder, viz. iron(III)oxide–hydroxide modified dextran [10]. We described its properties with respect to phosphate binding from aqueous solutions and whole blood [11,12]. Here we present results of *in-vitro* and *in-vivo* studies addressing the issues of efficacy and toxicity of this compound as an oral phosphate binder in rats.

Subjects and methods

Materials

The cross-linked polysaccharide dextran Dormagel® N 25 C, originally used for the purification and deionization of solutions, was obtained from Pfeifer and Langen, Dormagen, Germany. The exclusion limit is 6000 daltons and the bead size 150–300 μm. The dextran gel was converted to an iron(III)oxide–hydroxide derivative as described [10–12] by reaction with a concentrated iron(III)chloride solution followed by the addition of concentrated sodium hydroxide solution to a final pH of 13. The material was washed to neutrality with water and dried at 60°C.

Determination of electrolytes and histological investigations

Phosphate, calcium, magnesium, and iron concentrations in serum, plasma, faeces, and urine were photometrically determined according to established methods. The iron content

of the phosphate binder was determined by atomic absorption spectroscopy (AAS), phosphate concentrations by the inductively coupled plasma (ICP) technique. The histological investigations were done in the laboratory of Research and Consulting Company AG, Itingen, Switzerland.

Phosphate adsorption studies

Aqueous phosphate solutions (1–25 mmol/l) were used to determine phosphate binding capacities and dependence on pH (1–7.4), temperature (5–45°C, pH 7) and phosphate concentration (pH 7). The phosphate binder was suspended in the corresponding phosphate solution and the mixture was shaken for 7 days to ensure equilibrium binding. The material was removed by filtration and phosphate concentration was determined in the supernatant.

Simulation of phosphate binder passage through the intestines

The phosphate binder (7 g) was added to 1800 ml of a phosphate solution (6.4 mmol/l, pH 1.8, sodium 140 mmol/l, potassium 2.1 mmol/l, chloride 115 mmol/l) and agitated by a paddle mixer at 100 r.p.m. The pH value was raised in steps of 0.25 units by the addition of solid tris(hydroxymethyl) aminomethane, and samples (1 ml) were drawn from the supernatant at 15-min intervals over a period of 180 min. Then 1200 ml of a phosphate solution (2.25 mmol/l) were added, the mixture adjusted to pH 7.5, and further samples were taken every 15 min from the supernatant. Iron and phosphate concentrations in the supernatant samples were determined photometrically.

Iron and phosphate resorption in the duodenum

Pig duodenum sections of equal size (18–20 cm), immersed in aerated Ringer solution, were inverted to place the mucosa outside, filled with 30 ml of Ringer solution and tied on both ends. The sacs were immersed in 1.5 l of Ringer solution supplemented with 6.4 mmol/l phosphate and 0.5 mM NADH. Phosphate binder (7 g) was added to the experimental tissue incubation. The samples were incubated for 4 h at 37°C under slow agitation. The sacs were opened, and the inner fluid (volume, 50 ml) was removed and analysed for iron and phosphate concentration. Ferrous and ferric ions were differentiated by the inclusion, or omission, of ascorbate prior to photometric determination.

Studies in the rat, necropsy and histopathology

Nineteen male rats (210 g) were divided into two groups. Group 1 (control group) included seven animals and group 2 (phosphate binder, PB group) 12 animals. The phosphate binder was admixed to commonly used rat food in a proportion of 8% by weight. The iron content of the phosphate binder was about 18%. The diet was offered *ad libitum* to group 2 for 4 weeks. Six animals of this group were killed after 28 days, and the other six animals were kept for a 36-day treatment-free recovery period. The control group received the same diet without added phosphate binder. At the end of scheduled treatment periods, the animals were anaesthetized by intraperitoneal injection of sodium pentobarbitone and killed by exsanguination. Necropsy was performed on all animals. At post-mortem examination all organs were examined. Samples of the following tissues and

organs were collected from the animals and fixed in phosphate-buffered neutral 4% formaldehyde solution (10% formalin): adrenal glands, epididymides, femur, heart, kidneys, large intestine, liver, small intestines, spleen, stomach, testes, and thyroids, including parathyroid glands. The tissues of all animals were trimmed, processed, embedded in paraffin wax, sectioned to an approximate thickness of 4 µm and stained with haematoxylin and eosin. The sections were examined by light-microscopy.

Samples of blood, urine, and faeces were taken from each animal before the phosphate binder was administered, 2 weeks later, and at the end of the examination period (day 29). Phosphate, calcium, iron and clinical–chemical parameters such as total protein, haemoglobin, blood gas and acid–base-status were analysed in the blood samples. Calcium and phosphate concentrations were determined in the urine, phosphate, calcium, and iron concentrations in the excrements. The behaviour of the rats was observed during the entire period.

Results

In vitro binding characteristics

Binding characteristics of the phosphate adsorbent were investigated with respect to temperature, pH value, and phosphate concentration (Figure 1). Maximum binding capacity (0.73 mmol phosphate/g) is obtained at pH 2–3. It decreases to about 70% when the pH is raised to 7.5 (Figure 1a). The dependence of binding capacity on the temperature exhibits an almost linear relationship in the range from 5 to 45°C (Figure 1b). Phosphate adsorption reaches saturation at phosphate concentrations above 20 mmol/l (Figure 1c).

Simulation of intestinal passage

Passage of the phosphate binder through the small and large intestine was simulated *in vitro* to investigate the stability and capacity of the binder during variation of the pH value from 1.8 to 7.5 over a period of 6 h. The results shown in Figure 2 demonstrate that the adsorption capacity of the phosphate binder is at its maximum at pH 3–4 (0.81 mmol phosphate/g) and decreases to about 0.65 mmol/g phosphate binder at a neutral pH 7.5 (Figure 2a). The ratio between adsorbed phosphate and binder material can be considered excellent. Iron release from phosphate binder at different pH values was also analysed during the simulation (Figure 2b). Only 0.05 mmol (2.7 mg) iron per gram dry weight would be released during the time required for passage through the intestines.

Enzymatic mobilization of iron

The physiological mechanism of iron uptake in the duodenum includes reduction of trivalent to divalent iron catalysed by a NADH-requiring ferric reductase [13]. We tested for the possible action of this enzyme on the iron(III)oxide–hydroxide dextran beads in experiments with pig duodenum sections, outlined under Subjects and methods. During 4 h contact of 7 g
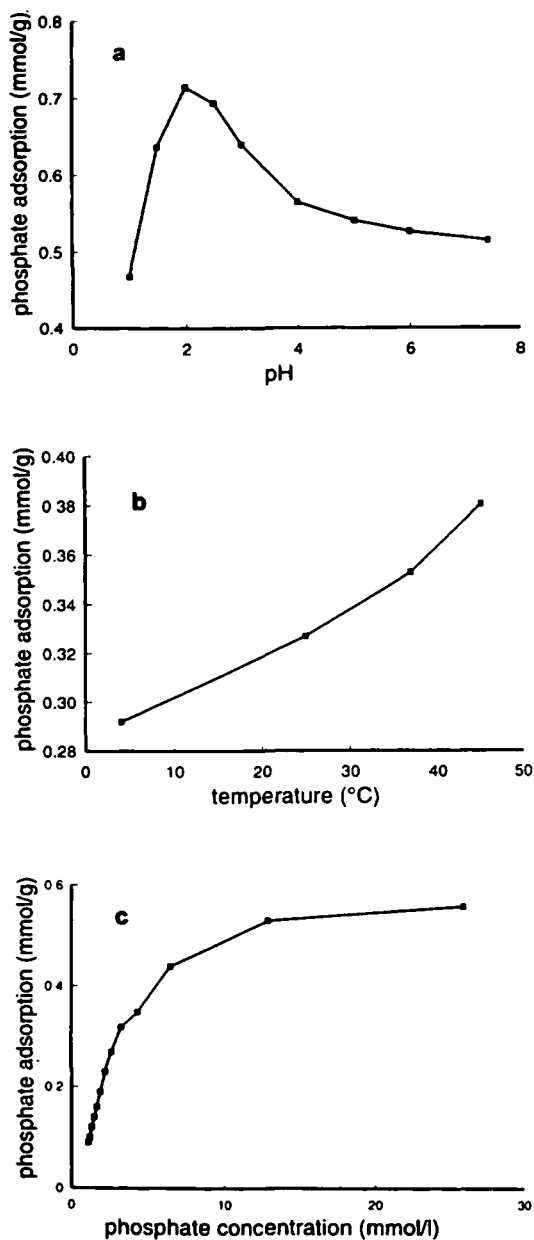
Fig. 1. Phosphate-binding characteristics of iron(III)oxide–hydroxide modified dextran beads. a pH dependence (phosphate 3.2 mmol/l; 22°C). b Temperature dependence (phosphate 3.2 mmol/l; pH 7.4). c Saturation behaviour (pH 7.4, 22°C).

Fig. 2. Simulation of intestinal passage of phosphate binder. a Adsorption of phosphate. Initial phosphate concentrations: period 0–180 min, 6.4 mmol/l; period 180–360 min, 4.75 mmol/l; open circles represent the pH profile. b Release of iron; open circles represent the pH profile.

material with the mucosal surface at 37°C, in the presence of 0.5 mmol/l NADH, only 0.046 mmol iron (0.03 mmol Fe^{2+} and 0.016 mmol Fe^{3+}) were transported through the duodenum. This value is only slightly higher than the small amount of endogenous iron (0.034 mmol) found in a control experiment without phosphate binder. Thus, enzymatic iron liberation from the iron(III)oxide–hydroxide dextran complex is very limited. This is in accordance with the chemical stability of this type of iron complex above pH 4 as shown in Figure 2b.

Phosphate uptake from a 2.7-mmol/l solution by the mucosa was also measured in these experiments. As
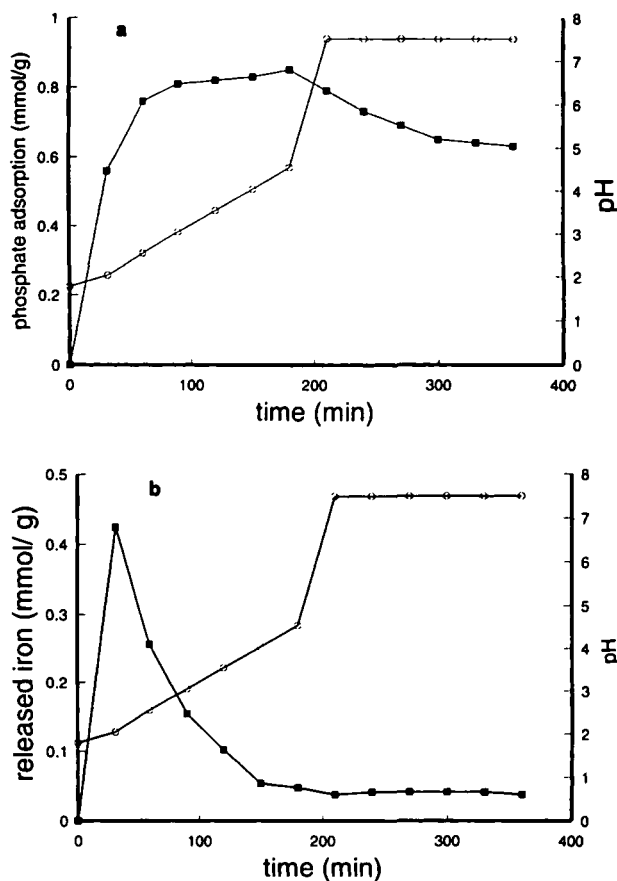
expected, in the presence of the phosphate binder the value was four times lower than in a control tissue sample (0.011 mmol vs 0.046 mmol in identical duodenum sections).

In-vivo studies with rats

Phosphate and calcium plasma concentrations of the rats fed with phosphate-binder-containing food (PB group) differed little from those in the control group (Figure 3a). However, the average amount of renally excreted phosphate (0.05 mmol/l) was 15 times lower in the PB group (Figure 3b). There was no difference between both groups with respect to the average iron concentrations. Furthermore neither group showed any change of the calcium plasma level. However, an increase of the average renal calcium excretion was observed in the PB group (4.6 mmol/l) versus the control group (1.1 mmol/l). Analysis of the excrements revealed an elevated amount of phosphate (PB group: 0.118 mmol/g; control group: 0.040 mmol/g) indicating that dietary phosphate had been efficiently eliminated by the phosphate binder.

Starting from an average body weight of 210 g the average weights of both groups were nearly identical
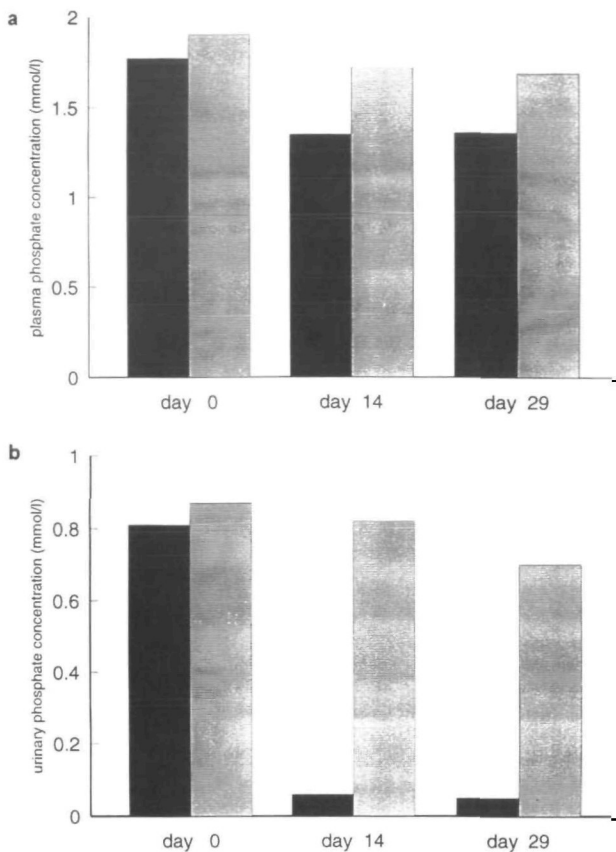
Fig. 3. Effect of phosphate binder-containing diet on plasma and urinary phosphate in rats. The amount of iron(III)oxide–hydroxide modified dextran beads admixed to the standard diet was 8% by weight. Black bars, experimental animals (12); open bars, control group (7) on standard diet. a Mean values of plasma phosphate level (standard deviation in control group s, 0.10 mmol/l; in experimental group, s, 0.17 mmol/l), b Mean values of urinary phosphate excretion (control group s, 0.05 mmol/l; experimental animals, s, 0.11 mmol/l).

(i.e. 305 g in the control group and 303 g in the PB group) at the end of the trial. The amount of food intake of the PB group was 20% higher than that of the control group (22.7 g/day in the PB group, 18.9 g/day in the control group). With regard to food digestion, the PB group digested about 15% less food (6.15 g per gram weight increase) than the control group (5.34 g food per gram weight increase). No animal died during the trial. The behaviour and activity of the treated rats did not show any remarkable changes.

No treatment-related macroscopic findings were recorded in the animals, either at necropsy or on histopathological examination. Likewise, the phosphate binder produced no microscopic signs of pathology apart from three findings within the normal range of morphological alterations commonly observed in rats of this strain and of this age.

Discussion

A strongly reduced phosphate uptake (for example, 25–32 mmol or 800–1000 mg P per day), is difficult to ensure for prolonged periods of time because of the unpredictabilities associated with diets, patient behaviour, and influence of drugs. The aim in the prevention and the treatment of hyperphosphataemia caused by chronic renal failure is a reduction of serum phosphate levels below 1.8 mmol/l (5.4 mg/dl) [14]. Medication with commonly used phosphate binders containing aluminium or calcium is associated with a high risk of toxicity, including bone disease and aluminium dementia from aluminium-containing antacids [15,16], hypercalcaemia, as well as soft-tissue calcification and gastrointestinal problems such as diarrhoea or constipation from antacids containing calcium [17,18].

As demonstrated in the *in-vitro* studies, the maximum phosphate binding capacity (0.73 mmol mmol phosphate/g phosphate binder) of the novel type of phosphate binder, i.e. iron(III)oxide–hydroxide modified dextran beads, is obtained at pH values of about pH 2–3 in the presence of high phosphate concentrations. At pH values above 5 the phosphate binding capacity is still in the range of 0.5 mmol phosphate/g phosphate binder. These conditions are found in the gastrointestinal tract.

The *in-vivo* study shows that intake of food admixed with the phosphate binder (8% by weight) causes substantial reduction of urinary phosphate excretion without affecting dietary intake of phosphate. Binding of phosphate in the intestine could be directly substantiated by measuring faecal phosphate excretion which showed a marked increase.

From these studies we calculate that 1 g of phosphate binder will adsorb under *in-vivo* conditions approximately 0.65 mmol of phosphate. Extrapolating this value for a 70-kg human being, the ingestion of approximately 15 g of this novel type of phosphate binder would be necessary per day in order to treat hyperphosphataemia.

Dietary intake of the phosphate binder investigated was associated with small but significant reduction of faecal calcium excretion and increased renal calcium excretion. Whether this is a transient non-steady-state phenomenon, secondary to changes in intestinal transit time, or an indirect effect mediated via higher rates of production of $1,25(OH)_2$ vitamin D_3 and increased active intestinal absorption of calcium, requires further study. Previous studies have shown that reduction of phosphate in a diet decreases the PTH level and increases the $1,25(OH)_2$ vitamin D_3 level [18–21]. Moreover, these studies revealed an increased calcium excretion in the urine, an increased intestinal calcium absorption and occasionally an increase in the total ionized serum calcium.

Release of iron from the phosphate binder is a legitimate concern. The *in-vitro* studies, however, showed, that the maximum amount of iron released, is 0.05 mmol per gram of phosphate binder. We emphasize that in the *in-vivo* studies no change in plasma iron concentration was observed. It is well known that iron deficiency is common in uraemic patients [22,23] and iron requirements may even increase substantially in uraemic patients treated with

recombinant human erythropoietin. Release of such modest amounts of iron may therefore not be unwelcome.

No toxic effects were noted by necropsy and histopathology in this short-term (4–8 weeks) study. Although longer studies are clearly required, these preliminary results are encouraging. No unwanted side-effects were recorded. We emphasize that body weight and food consumption, very sensitive indicators of toxicity, were not altered significantly.

In conclusion, the above studies hold promise that iron(III)oxide–hydroxide modified dextran beads may be of use as an effective and safe oral phosphate binder in uraemic patients. Currently existing phosphate binders are unsatisfactory (i) because of the risk of aluminium toxicity when aluminium-containing phosphate binders are used, and (ii) because of the risk of hypercalcaemia when calcium carbonate or calcium acetate are used. The above efficacy and toxicity study in animals is sufficiently encouraging to warrant analogous short-term studies in uraemic patients.

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Editor's note

Please see also the Editorial Comment by Ritz and Hergesell (pp. 766–768 in this issue).