Adsorption of Dietary Phosphate in Gut with Anion Exchange Resin

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Abstract
To evaluate the effectiveness of anion exchange resin in the binding of dietary phosphate in the digestive system, we carried out in vitro and in vivo studies of phosphate binding by comparing the anion exchange resin PAA-B, which has the same chemical structure as Sevelamer HCl (Renagel®), with CaCO₃. In in vitro phosphate-binding experiments, the anion exchange resin bound less phosphate at pH 7 than did CaCO₃. In vivo phosphate-binding experiments demonstrated that the levels of phosphorus excretion in the feces of rats treated with PAA-B increased to the same degree as those of rats treated with CaCO₃; however, PAA-B treatment resulted in gastrointestinal transport delay. These data suggest that anion exchange resin has a bright prospect as an effective drug for treating hyperphosphatemia if the adverse gastrointestinal effects can be overcome.

1 Introduction
Hyperphosphatemia is an inevitable problem in patients with chronic end-stage renal disease and who are on hemodialysis. The control of blood phosphorus levels may improve the probability of survival for these patients.[1] Because dietary restriction alone is usually inadequate to control blood phosphorus levels in renal failure patients, the administration of CaCO₃ has been used as a treatment to promote dietary phosphate excretion. The potency of CaCO₃ in binding phosphorus, however, is modest, and the removal of intestinal phosphate requires large doses. Another disadvantage is that high doses of CaCO₃ may cause hypercalcemia when it administered with vitamin D. The efficacy of CaCO₃ treatment has also been reported to become diminished when the frequently used histamine H₂-receptor antagonist is employed to treat end-stage renal disease.[2] Ion exchange resins play important roles in certain medical treatments, and therapy with such resins may constitute a promising alternative to the use of CaCO₃. Sevelamer HCl, a nonabsorbed phosphate-binding polymer, has been developed for the treatment of hyperphosphatemia in adult patients undergoing hemodialysis and has received a favorable evaluation in a clinical study.[3] A poly(allylamine hydrochloride) cross-linked with epichlorohydrin, the resin is known chemically as poly(allylamine-co-N,N’-diallyl-1,3-diamino-2-hydroxypropane) hydrochloride (PAA-B). The aim of the present research is to clarify this resin’s actual
capacity to bind phosphate in vitro and in vivo as well as to compare PAA-B and CaCO₃ with respect to any disadvantages in gastrointestinal transit.

2 Experimental

Male Sprague-Dawley rats weighing 200 to 250 g and ddY mice weighing 25 to 30 g were used in the present studies. All experimental procedures used were approved by the internal animal use committee of the Kurume University School of Medicine.

We used three phosphate binders in this study: PAA-B, which has the same chemical structure as Sevelamer HCl, Dowex 1X2, which is a quaternary ammonium salt-type anion-exchange resin prepared with styrene cross-linked with divinylbenzene, and CaCO₃.

2.1 phosphate binding in vitro

For in vitro phosphate-binding experiments, we followed the methods of Sheikh et al. with minor modifications.[4] NaH₂PO₄·H₂O was dissolved in deionized water to a concentration of 15 mmol dm⁻³ elemental phosphorus. The phosphate binders were added to the phosphate solution to give a final volume of 100 cm³. The phosphate solutions were then titrated to pH 3, 5, 7, 9, and 11 by the addition of concentrated HCl and NaOH. Because the pH of the phosphate solutions drifted over time, the solutions were retitrated to their initial pH immediately after the addition of the binder solution. The flasks containing the solutions were kept sealed in a bath thermostated to 37°C and were shaken at 60 strokes per minute. Samples for phosphorus assays were taken just before titration at the initial pH and were centrifuged at 3000 rpm for 10 min. The supernatant was filtered sequentially through filter paper (#4; Whatman Inc) and a 0.2-µm filter (Advantec Inc) before analysis. Phosphorus was measured by the method of Fiske and Subbarow.[5]

2.2 phosphate binding in vivo

Rat phosphorus excretion experiments were performed with a 2×2 crossover design. The rats were housed individually in metabolic cages in a room with controlled temperature (23°C ± 2°C) and humidity (55 ± 10%) and with the dark period maintained from 18:00 to 6:00 hours. The rats were fed with rodent chow powder (CA-1, Clea Japan Inc). The diets for tested groups were supplemented with 5.0% by weight of PAA-B, Dowex 1X2, or CaCO₃. The rats were divided into three groups (n = 7 per group) for monitoring the consumption of measured diets of food and water. All groups were fed ad libitum for four days to acclimate the animals to the diet without phosphate binder, and then they were fed diets with or without phosphate binder for four days. Excreted feces and urine were collected meticulously during the next 48 h. The rats were then fed the diet without phosphate binders for four days and fed again with diets containing phosphate binder with the same quantitative collection of feces and urine. Collected feces were weighed, lyophilized, ground into powder, ashed in an oven at 600°C, and added to 2 mol dm⁻³ HCl to dissolve the phosphate. Volumes and weights of collected urine samples were measured, mixed, and diluted directly with 2 mol dm⁻³ HCl. The phosphorus concentrations in feces and urine samples were determined by the above method.

2.3 Assessment of gastrointestinal transit

Phosphate binder suspended in an aqueous solution of mannitol,D-[1-¹⁴C] for a liquid transport or mixed with cellulose (Nicotiana tabacum), [¹⁴C(U)] and nonlabeled cellulose for a solid transport was administered orally to conscious mice. The mice were killed by cervical dislocation after 20 min and 40 min, respectively. The gastrointestinal tract was removed and divided into a stomach section and ten identical lengths of small intestine.
These sections extended from the cardiac part of the stomach to the ileocecum. Excised parts of the gastrointestinal tract were added to a liquid scintillator and were measured with a liquid scintillation counter (Beckman LS6500). Data were expressed as percentages of total radiocount of stomach and small intestine of each mouse.

3 Results and Discussion

In vitro phosphate binding by CaCO₃, PAA-B, and Dowex 1X2 were studied at pH 3, 5, 7, 9, and 11 (Fig. 1). At pH 7, CaCO₃ bound more phosphate than did PAA-B or Dowex 1X2, but the phosphate-binding capacity of CaCO₃ was markedly reduced below pH 5. In the PAA-B and Dowex 1X2 experiments, the pH range from 3 to 7 had little influence on phosphate binding. These results suggest that an anion exchange resin such as PAA-B is better than CaCO₃ for binding phosphate in the acidic intragastric environment.

Figure 2 indicates the phosphorus mass balance experiments in normal rats. Fed phosphorus is principally excreted outside the body; the phosphorus unabsorbed in gastrointestinal duct to the feces and superfluous phosphorus in vivo to the urine. Therefore, the changes in fecal and urinary phosphorus levels directly reflect the dietary phosphate binding ability of the phosphate binders.[2, 6]

The results of in vivo phosphate-binding experiments were analogous at pH 7 to those of the in vitro experiments and were indicated in the dietary effects in the small intestine (Fig. 2). CaCO₃ and PAA-B increased the levels of phosphorus in the feces to 1.42 and 1.34 times those of the controls, respectively (P<0.05 for both). Urinary phosphorus levels decreased when phosphate binder was administered, because superfluous phosphate in vivo was almost all excreted in the urine. Levels of urinary phosphate decreased to 35.4% (P<0.01) and 26.5% (P<0.05) of control levels after administration of CaCO₃ and PAA-B, respectively. On the other hand, Dowex 1X2 had little effect on phosphate binding in vivo.

![Fig. 1 In vitro binding of phosphate as elemental phosphorus absorbed per g CaCO₃ (●), PAA-B (■), and Dowex 1X2 (▲) as a function of pH. Data are the mean without standard errors for four samples.](image1)

![Fig. 2 Changes in fecal and urinary phosphorus levels for rats fed normal phosphate diets with added CaCO₃, PAA-B, or Dowex 1X2. Histograms on left in each group are controls and those on the right are those of animals fed diets with phosphate binders. Data are the mean and SEM (vertical bars) for seven rats each group. * P<0.05, ** P<0.01 vs. the respective controls.](image2)

Although in vitro experiments indicated a potent phosphate-binding capacity for CaCO₃, the ability of CaCO₃ to bind phosphate in in vivo experiments was equal to that of PAA-B (Fig. 2). This difference may be attributable to the incomplete dissociation of CaCO₃ in the stomach. As has been previously pointed out,[2] CaCO₃ administered to treat hyperphosphatemia perhaps dissociates insufficiently in the stomach owing to the
stomach's acidity and the presence of pharmaceuticals. The levels of administered CaCO₃ in the present study may also have been excessive for the dissociation environment of the animals' gastrointestinal tracts, and CaCO₃ may not have dissociated sufficiently to bind the phosphate in the experimental diets.

We next evaluated the effects of the phosphate binders on liquid transport with ¹⁴C-labeled mannitol. Mannitol is little incorporated into the body and resin. We also evaluated the effects of the phosphate binders on the solid transport with ¹⁴C-labeled cellulose, which does not affect gastrointestinal motility in the gastrointestinal tract of mice, [6] because treatment with ion exchange resin principally induces its adverse effects in the gut. Adverse events observed in the gut during treatment with Sevelamer HCl included nausea, constipation, diarrhea, flatulence, or dyspepsia.[3]

In the present study, CaCO₃ did not affect the gastrointestinal transport of liquids and solids in mice (Fig. 3). On the other hand, PAA-B and Dowex 1X2 significantly reduced liquid and solid transport. The application of PAA-B resulted in 74% of the administered cellulose remaining in the mouse stomach. Dowex 1X2 reduced the emptying of gastric contents into the duodenum, a finding that may be attributable to the retention of water and a long stay within the stomach. PAA-B and Dowex 1X2 have the same degree of water content (measured as [wet weight – dry weight]/[dry weight] ≈ 3.4). However, turbidity measurements demonstrated that PAA-B could not be suspended in water and precipitated readily, whereas Dowex 1X2 could be partially suspended (data not shown). These results suggest that the PAA-B remaining in the body of the stomach may perhaps function in the fundus by promoting the storage of solids and delaying their release into the duodenum.

![Fig. 3 Influences of phosphate binders on gastrointestinal transport of liquid (left) and solid (right) in mice. Data represent the mean ± SEM from ten mice. *P<0.05; **P<0.01 vs. the controls](image)

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**References**