Reduction of dietary phosphorus absorption by phosphorus binders. A theoretical, in vitro, and in vivo study.

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Research Article

Antacids used to decrease phosphorus absorption in patients with renal failure may be toxic. To find more efficient or less toxic binders, a three-part study was conducted. First, theoretical calculations showed that phosphorus binding occurs in the following order of avidity: Al3+ greater than H+ greater than Ca2+ greater than Mg2+. In the presence of acid (as in the stomach), aluminum can therefore bind phosphorus better than calcium or magnesium. Second, in vitro studies showed that the time required to reach equilibrium varied from 10 min to 3 wk among different compounds, depending upon solubility in acid and neutral solutions. Third, the relative order of effectiveness of binders in vivo was accurately predicted from theoretical and in vitro results; specifically, calcium acetate and aluminum carbonate gel were superior to calcium carbonate or calcium citrate in inhibiting dietary phosphorus absorption in normal subjects. We concluded that: (a) inhibition of phosphorus absorption by binders involves a complex interplay between chemical reactions and ion transport processes in the stomach and small intestine; (b) theoretical and in vitro studies can identify potentially better in vivo phosphorus binders; and (c) calcium acetate, not previously used for medical purposes, is approximately as efficient as aluminum carbonate gel and more efficient as a phosphorus binder than other currently used calcium salts.

Reduction of Dietary Phosphorus Absorption by Phosphorus Binders

A Theoretical, In Vitro, and In Vivo Study

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Abstract

Antacids used to decrease phosphorus absorption in patients with renal failure may be toxic. To find more efficient or less toxic binders, a three-part study was conducted. First, theoretical calculations showed that phosphorus binding occurs in the following order of avidity: $Al^{3+} > H^+ > Ca^{2+} > Mg^{2+}$. In the presence of acid (as in the stomach), aluminum can therefore bind phosphorus better than calcium or magnesium. Second, in vitro studies showed that the time required to reach equilibrium varied from 10 min to 3 wk among different compounds, depending upon solubility in acid and neutral solutions. Third, the relative order of effectiveness of binders in vivo was accurately predicted from theoretical and in vitro results; specifically, calcium acetate and aluminum carbonate gel were superior to calcium carbonate or calcium citrate in inhibiting dietary phosphorus absorption in normal subjects. We concluded that: (*a*) inhibition of phosphorus absorption by binders involves a complex interplay between chemical reactions and ion transport processes in the stomach and small intestine; (*b*) theoretical and in vitro studies can identify potentially better in vivo phosphorus binders; and (*c*) calcium acetate, not previously used for medical purposes, is approximately as efficient as aluminum carbonate gel and more efficient as a phosphorus binder than other currently used calcium salts.

Introduction

In chronic renal failure, phosphorus retention plays a major role in the development of secondary hyperparathyroidism and osteodystrophy (1–6). To prevent phosphorus retention, various aluminum-containing antacids have been used to bind phosphorus within the gastrointestinal tract and thus prevent its absorption. Unfortunately, long-term use of aluminum compounds by patients with chronic renal failure is associated with risk of serious aluminum toxicity (7–12). This has prompted the search for safer phosphorus binders. Calcium carbonate and calcium citrate are two such candidates (13–22); however, large doses are often required, and hypercalcemia is therefore a potential complication.

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In an attempt to understand the process of phosphorus binding and to find more efficient binder(s) with less toxicity, a three-part study was carried out. First, we calculated the binding that theoretically would occur at equilibrium when different binders and phosphorus are mixed in ratios similar to those used clinically. Second, we performed in vitro experiments to evaluate the time required for twelve different compounds to reach equilibrium. And third, since conditions in the gut may differ from some conditions in vitro, we measured the extent to which several selected binders inhibit dietary phosphorus absorption in normal people.

Methods

Definition of phosphorus binding. Phosphorus binding is either a chemical reaction between dietary phosphorus and cation of the binder compound, resulting in the formation of insoluble and hence unabsorbable phosphate compounds (23), adsorption of phosphorus ions on the surface of binder particles (24, 25), or a combination of both processes (26). The operational definition of phosphorus binding varies in the three parts of this study. In the theoretical calculations, binding is defined as formation of insoluble solid phosphate(s). For the in vitro experiments, the amount of phosphorus that did not pass through a millipore filter was regarded as bound by the binder. For the in vivo experiments, binding was defined as reduction in gastrointestinal phosphorus absorption when the binder was ingested with a test meal.

Relative amounts of binder and phosphorus. For most of the theoretical calculations and in vitro experiments, we used 75 meq of binder cation (i.e., 1,500 mg of calcium, 675 mg of aluminum, or 900 mg of magnesium) and 320 mg of elemental phosphorus (equal to 10–31 meq of phosphate depending upon pH) in a volume of 600 ml. These amounts are identical to the dose of binder, phosphorus content, and volume of a test meal in a recent in vivo study (27). For the in vivo part of the study, 50 meq of binder and 345 mg (11–33 meq) of meal phosphorus were used. This reduced binder dose was used in the in vivo study because higher doses are not likely to be used clinically on a chronic basis for fear of toxicity. To better compare the in vivo and in vitro results, the binders used in the in vivo studies were also studied at the lower dose in vitro.

Binders studied. In the theoretical and in vitro parts of the study the following binders were studied: calcium chloride, calcium acetate, calcium lactate, calcium gluconate, calcium citrate, calcium carbonate, aluminum chloride, aluminum hydroxide powder, magnesium hydroxide (all reagent grade), aluminum hydroxide gel (Amphojel; Wyeth Laboratories, Philadelphia, PA), aluminum carbonate gel (Basaljel suspension; Wyeth Laboratories), and sucralfate (α-D-glucopyranoside, α-D-fuctofuranosyl-octakis-(hydrogen sulfate), aluminum complex, Carafate; Marion Laboratories, Kansas City, MO). In the in vivo part of the study, the following binders were studied: calcium carbonate, calcium citrate, calcium acetate, and aluminum carbonate gel (Basaljel suspension).

Part I: Theoretical phosphorus binding at equilibrium. From knowledge of equilibrium constant expressions (28) for the various chemical reactions involved, we calculated the binding that theoretically would

occur at equilibrium at pH range of 3 to 8. The binding reaction was the precipitation reaction of either PO_4^{3-} or HPO_4^{2-} with binder cation. Binding at equilibrium was estimated by calculating the total amount of phosphate that could exist in a saturated solution of the binder cation-phosphate precipitate in the presence of the excess binder at the particular pH of interest. The binding reaction is the formation of the insoluble phosphate(s): $aB + bP \rightleftarrows B_aP_b(s)$ (where B = binder cation, P = PO_4^{3-} or HPO_4^{2-}, s = solid or precipitate form, a = mol of B, b = mol of P). The concentration at equilibrium was assumed to be governed by the solubility product constant, $K_{sp} = [B]^a[P]^b$, where [] denotes molar concentration of the saturated solution and K_{sp} is the solubility product constant for the reaction. Total phosphate concentration was obtained by simultaneous solution of the solubility product constant expressions and the equilibrium constant expressions governing the relative amounts of inorganic phosphate species (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}). In cases where the binder cation formed soluble complexes with other species in solution, such as citrate, these equilibrium constants were also considered in the system of equations solved for determining total phosphate. For these calculations, the effect of ionic strength was ignored and activity coefficients were assumed to be unity. Percent binding was calculated as precipitated phosphate divided by total phosphate times 100.

The binding of phosphorus by calcium chloride at pH 4 will be taken as an example. The initial total phosphate concentration was 0.0172 M (or 320 mg/600 ml) and that of Ca^{2+} was 0.0625 M (or 1,500 mg/600 ml). From the equilibrium constant expressions (28) governing the hydrogen-phosphate equilibria, the concentration of various forms of phosphate at pH 4 was calculated. At this pH the dominant form of phosphate is $H_2PO_4^-$. Using these concentrations and the solubility product constant expression (28) for the precipitation of $CaHPO_4$ and $Ca_3(PO_4)_2$, it was determined that only $CaHPO_4$ would precipitate.

The following reactions occur at equilibrium:

$$CaHPO_4(s) \rightleftarrows Ca^{2+} + HPO_4^{2-}, K_{sp} = 4 \times 10^{-7} \quad (1)$$

$$HPO_4^{2-} + H^+ \rightleftarrows H_2PO_4^-, K = 1.47 \times 10^7 \quad (2)$$

Combining Eqs. 1 and 2:

$$CaHPO_4(s) + H^+ \rightleftarrows Ca^{2+} + H_2PO_4^-, K = 5.9, \text{ or} \quad (3)$$

$$\frac{[Ca^{2+}]\,[H_2PO_4^-]}{[H^+]} = 5.9 \quad (4)$$

If the K_{sp} for $CaHPO_4$ were zero, all the phosphate (0.0172 M) would precipitate as $CaHPO_4$, and there would be (0.0625 − 0.0172) M of calcium in solution. Because the K_{sp} is nonzero, an additional amount, x mol of calcium and phosphate, would be in solution. Hence at equilibrium: $[H_2PO_4^-] = x$ and $[Ca^{2+}] = (0.0625 - 0.0172) + x$. Substituting these values in Eq. 4, and solving: $x = 0.0105$ M, at pH = 4, thus phosphate in precipitate = $0.0172 - x = 0.0172 - 0.0105 = 0.0067$ M. Thus, percent binding = phosphate in precipitate/total phosphate × 100 = 0.0067/0.0172 × 100 = 38.9%.

Part II: In vitro phosphorus binding. 1.43 g of $NaH_2PO_4 \cdot H_2O$ (320 mg of elemental phosphorus) was dissolved in 570 ml of deionized water. The binder was dissolved (or suspended, in case of insoluble compounds) in deionized water to a volume of 30 ml. The binder solution or suspension was added to the phosphorus solution to give a final volume of 600 ml. For each binder study, the phosphorus solutions were titrated, by addition of concentrated HCl or NaOH, to four different initial pH levels: 4, 5, 6, and 7. As there was a drift in pH over time, the solutions were retitrated to their initial pH immediately after addition of the binder solution and 1 and 24 h thereafter. When experiments continued for longer periods the solutions were also retitrated to their initial pH after 4 d and at 1, 2, and 3 wk. During titrations the mixture was stirred with a magnetic stirrer at ~ 100 rpm for ~ 1 min. Then the beakers containing the solutions were kept covered with plastic wrap in a shaker bath at 37°C, shaking at ~ 20 cycles per minute. With calcium carbonate, magnesium hydroxide and aluminum carbonate gel, pH drift was large; hence for these compounds additional experiments were done at pH 4 and 5 with an autotitrator. In these experiments, the beakers were kept at room temperature, and mixtures were stirred with a magnetic stirrer at ~ 100 rpm. This stirring rate was chosen because in vitro antacid activity at such low stirring rates correlates well with in vivo antacid activity in the stomach (29). Samples for phosphorus assay were taken just before titrations to the initial pH and at other time intervals, as shown in Results. The samples were centrifuged at 3,000 rpm for 30 min. The supernatant was filtered sequentially through filter paper (#50; Whatman, Inc., Clifton, NJ) and then through a 0.2-μm filter (Millipore Corp., Medford, MA) before analysis. In preliminary experiments, the filtration process had no effect on phosphorus concentration of solutions with known phosphorus concentration. Phosphorus was assayed by the method of Fiske and Subbarow (30).

The decrease in phosphorus concentration, from the original concentration in the phosphorus solution to that of the filtrate, represented the bound phosphorus. This was expressed as percent of the total phosphorus present in the original solution. The experiments were stopped when either 100% phosphorus binding was achieved, or no more than 5% increase in binding was observed over a 6–7-d period of further incubation.

To assess the reproducibility of the method, three compounds were tested for phosphorus binding at 1 h on seven occasions each. For aluminum hydroxide gel the results were within 1%, for calcium acetate within 7% and for calcium carbonate within 10% of one another. Calcium carbonate was also tested seven times at 24 h; the results agreed within 3%. Other experiments were done in duplicate and mean results are presented.

Part III: In vitro/in vivo correlations. In this part of the study, four compounds, calcium acetate, calcium carbonate, calcium citrate, and aluminum carbonate gel, were tested in vitro as well as in vivo. The in vitro experiments were done in the same way as in part II, except that the amount of the binder used was 50 meq and the samples for phosphorus assay were taken at 1, 4, and 10 h; these times correspond to the approximate residence time in stomach, the time available for absorption in the small intestine and the maximum time available for phosphorus binding in the in vivo studies (see below), respectively.

In the in vivo studies, net phosphorus and calcium absorption was measured in 10 healthy human volunteers (ages 24–32) by a single-day balance method described below. Informed consent was obtained from the subjects and the project was approved by the Institutional Review Board for Human Protection at Baylor University Medical Center. Each subject was studied on separate test days when a meal was ingested with (*a*) placebo, (*b*) calcium acetate, (*c*) calcium carbonate, (*d*) calcium citrate, or (*e*) aluminum carbonate gel. On a sixth test day no meal, placebo, or binder was ingested (the fast). The order of various test days was randomized.

The meal consisted of 80 g of ground sirloin steak seasoned with salt and pepper, 30 g of Swiss cheese, 100 g of French fried potatoes, and 250 ml of water containing 10 g of polyethylene glycol (PEG) as a nonabsorbable marker. For each experiment, two meals were prepared, one to be fed to the subject and the other to be analyzed for phosphorus and calcium content. The duplicate meals contained, on average, 345±4 mg of phosphorus and 214±2 mg of calcium. The phosphorus content of the meal corresponds to the recommended daily allowance of 1,000 mg, if three meals were taken daily.

The total dose of the binder was 50 meq of calcium or aluminum, i.e., 1,000 mg of elemental calcium (2.52 g of calcium carbonate, 4.33 g of calcium acetate, and 4.74 g of calcium citrate), and 450 mg of elemental aluminum (17.0 g of aluminum carbonate gel). The calcium salts were given in gelatin capsules and aluminum carbonate gel was given as a liquid suspension. For each experiment two sets of doses were prepared, one to be administered to the subject and the other to be analyzed for calcium or aluminum content.

Net phosphorus and calcium absorption was measured by a method described in detail and validated previously (31, 27). The subject entered the laboratory after an 8-h fast. The entire gastrointesti-

nal tract was cleansed by lavage with a poorly absorbed solution. 4 h after the lavage was completed, the subject ingested one half of the total dose of placebo or binder with 100 ml of deionized water. Then the subject ate the meal. Immediately thereafter, the second half of the dose of placebo or binder was ingested with 100 ml of water. 10 h after starting the meal, a second lavage was started. This removed unabsorbed material from the gut. Calcium and phosphorus content of the rectal effluent was measured.

Absorption was calculated according to the following equation: Net phosphorus absorption = phosphorus intake − (effluent phosphorus after placebo/binder − effluent phosphorus after fast).

Phosphorus intake is equal to the phosphorus content of the duplicate meal. Calcium absorption is calculated similarly except that calcium intake is the sum of the calcium content of the duplicate meal and the binder.

Test meal and rectal effluent were analyzed for phosphorus by the method of Fiske and Subbarow (30) and for calcium by atomic absorption spectroscopy. PEG was analyzed by the method of Hyden (32).

Results

Part I: Theoretical phosphorus binding at equilibrium. Fig. 1 shows calculated binding at equilibrium at different pH levels for aluminum, calcium, and magnesium compounds. At equilibrium the aluminum compounds bind 100% of the phosphorus in the pH range 3.5–7.5. Above pH 7.5, the binding drops (to 96% at pH 8) due to precipitation of $Al(OH)_3$. For calcium compounds (except calcium citrate) and for magnesium compounds the binding is ~ 100% at pH levels > 5.5 and 6.0, respectively. Binding drops to 0% at pH 3.5 for calcium compounds (except calcium citrate for which the binding drops to 0% at pH 4.5) and to 0% at pH 4.0 for magnesium compounds. This happens because at low pH, where hydrogen ion concentration is high, H^+ competes for phosphorus more effectively than calcium or magnesium. (By contrast, aluminum competes effectively with H^+ so that binding is 100% even at low pH.) As shown in Fig. 1, < pH 5.5 calcium binds phosphorus more effectively than magnesium. Thus, < pH 6 the theoretical order of avidity for reaction with phosphorus (excluding calcium citrate) is $Al^{3+} > H^+ > Ca^{2+} > Mg^{2+}$.

Citrate, in contrast to anions of other calcium compounds used in our experiments, forms soluble complexes with calcium (33); this reduces the availability of calcium for reaction with phosphorus. This is particularly evident at low pH. With rising pH, the phosphate species change from $H_2PO_4^-$ to HPO_4^{2-} to PO_4^{3-}. The latter species have much smaller solubility product constants with calcium (28) and can thus effectively compete with citrate; therefore, phosphorus binding is higher in the higher pH range.

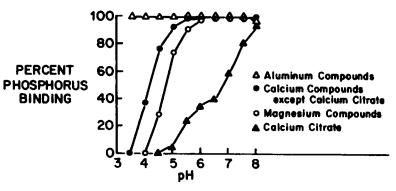

Figure 1. Theoretically calculated phosphorus binding at equilibrium. This is shown as a function of pH for the compounds used in this study, when the total initial concentration of phosphorus is 320 mg/600 ml (0.0172 M), that of calcium 1,500 mg/600 ml, that of magnesium 900 mg/600 ml (0.0625 M), and that of aluminum 675 mg/600 ml (0.0417 M). For calculations of binding with calcium citrate, concentration of ionized calcium was assumed to be 0.01 M, based on the measured amount of dissolved calcium in these experiments, and on equilibrium constants of calcium-citrate complex formation (33).

Part II: In vitro phosphorus binding (Figs. 2 and 3). Results for calcium acetate at 1 and 24 h are similar to each other and to the theoretically calculated binding at equilibrium, indicating that equilibrium is quickly approached (equilibrium values shown by interrupted line). Calcium chloride, calcium lactate, and calcium gluconate show a similar pH effect although the latter two compounds approach equilibrium more slowly. Additional experiments done with calcium chloride to assess the rapidity of reaction at pH 7 showed 99% binding at 10 min. Calcium carbonate binds, depending upon pH, only 10–25% (of total amount of phosphorus added) at 1 h, 8–80% at 4 h and 6–93% at 24 h. At 1 wk, the binding almost reaches theoretical equilibrium values at all pH levels tested. At 4 and 24 h, the binding is much closer to equilibrium values at lower pH (4–5.5) as compared with higher pH (6.5–7.5). Calcium citrate binds less phosphorus than other calcium salts tested. In the lower pH range it binds very little phosphorus (as expected from calculated equilibrium values); > pH 6.5 binding rises gradually to 53% by 1 wk, thus approaching the calculated equilibrium value.

Using Bonferroni multiple comparisons (34) to compare phosphorus binding by different compounds at one hour, the following statistically significant ($P < 0.05$) differences can be shown: calcium acetate, calcium chloride > calcium lactate, calcium gluconate > calcium carbonate > calcium citrate.

Aluminum chloride binds virtually 100% of phosphorus within 1 h, showing that equilibrium is established quickly (Fig. 3). In contrast, aluminum hydroxide powder binds very little phosphorus at 1 h and there is no increase in binding up to 1 wk. Results for aluminum hydroxide gel and aluminum carbonate gel are similar to each other. They bind ~ 40–65% phosphorus at 1 h, binding being greater in lower pH range. A progressive increase in phosphorus binding with time is seen, approaching the calculated equilibrium value of 100% between 24 h and 1 wk at pH 4–5 and in 3 wk at pH 6–7. The binding with sucralfate approaches theoretical equilibrium values at 1 wk.

As shown in Fig. 3, magnesium hydroxide, a poorly soluble compound, approaches theoretical equilibrium values only after 2 wk.

Part III: In vitro/in vivo correlation. Fig. 4 shows the in vitro phosphorus binding at 1, 4, and 10 h with 50 meq amount of the four binders used in the in vivo studies; the amount of phosphorus used in these experiments was 320 mg. The results are qualitatively similar to those in part II (Figs. 2 and 3).

Table I shows the individual and mean data for phosphorus absorption on the various test days. PEG recoveries on different test days were 98–100% as shown in the footnote to Table I. As compared with the placebo, all binders reduced phosphorus absorption significantly ($P < 0.001$ by analysis of variance). By Fisher's least significant difference test for multiple comparisons (35), the difference between aluminum carbonate gel and calcium acetate is not statistically significant, and phosphorus absorption with these two compounds is significantly less than that with calcium carbonate and calcium citrate ($P < 0.01$ in all instances); the difference between the latter two compounds is not statistically significant.

Calcium absorption after ingestion of placebo and the three calcium salts is shown in Table II. Calcium absorption on the test day in which an aluminum binder was ingested was similar to that with placebo (data not shown). As compared

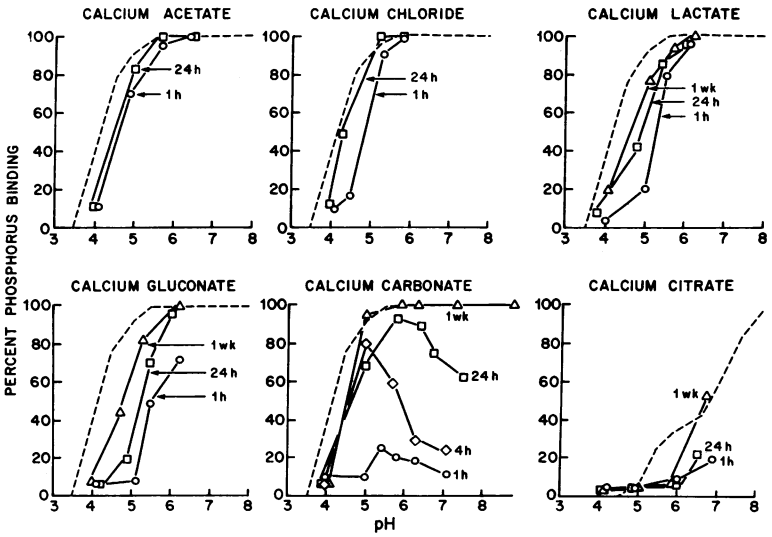

Figure 2. Observed in vitro phosphorus binding for calcium compounds, shown as a function of pH, at different times. Interrupted lines represent theoretically calculated equilibrium values.

with placebo, calcium absorption was significantly higher with calcium salts ($P < 0.001$ by analysis of variance). By Fisher's least significant difference test for multiple comparisons, calcium absorption from calcium acetate was significantly less than that from calcium carbonate ($P < 0.05$) and from calcium citrate ($P < 0.05$); the difference between the latter two compounds was not statistically significant.

After ingestion of the three calcium salts, mean values for calcium absorption and inhibition of phosphorus absorption were inversely correlated ($r = -0.997$, $P < 0.005$). Thus, the calcium salt that best inhibited phosphorus absorption (calcium acetate) was associated with the least calcium absorption.

Discussion

Binding at equilibrium. The results of the calculations based on equilibrium constants show that binding at equilibrium depends upon the binder used, pH, and presence of competing anions. In the absence of competing anions, aluminum compounds bind better than calcium compounds that in turn bind better than magnesium compounds (Fig. 1). These differences between different binders are due to their different inherent abilities to react with phosphorus, as expressed by the equilibrium constants for the various reactions. The extent to which aluminum compounds are better than calcium and magnesium compounds is dependent on pH; at low pH the difference is striking, whereas at high pH it is negligible. The dramatic fall in binding by calcium and magnesium compounds at low pH is mainly due to the fact that high concentrations of H^+ effectively compete with calcium and magnesium for phosphorus. Aluminum, on the other hand, can effectively compete with H^+ for phosphorus and thus is just as effective at low as at high pH.

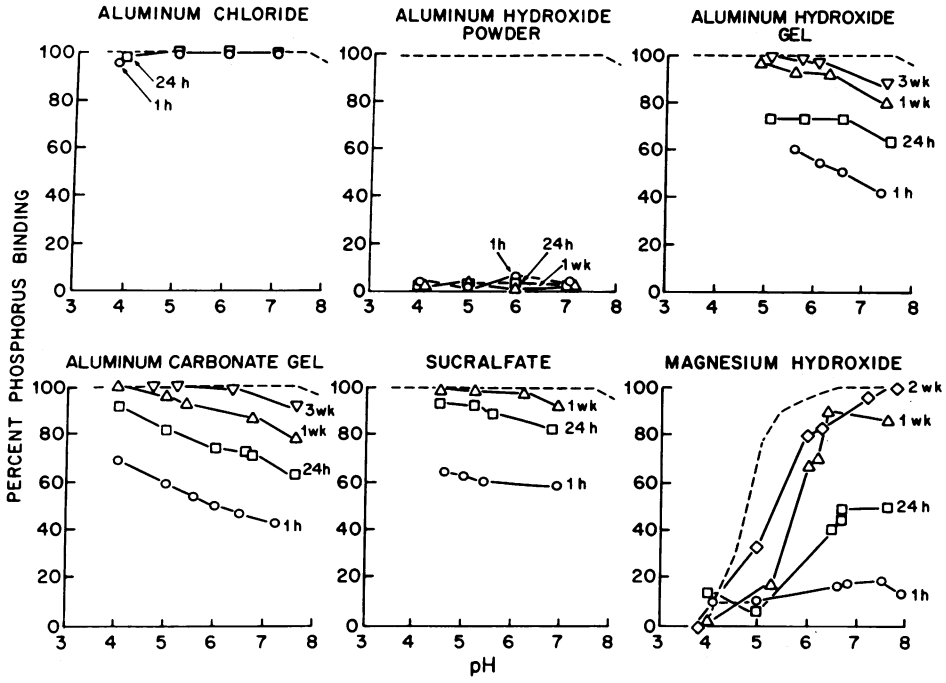

Figure 3. Observed in vitro phosphorus binding for aluminum and magnesium compounds, shown as a function of pH, at different times. Interrupted lines represent theoretically calculated equilibrium values.

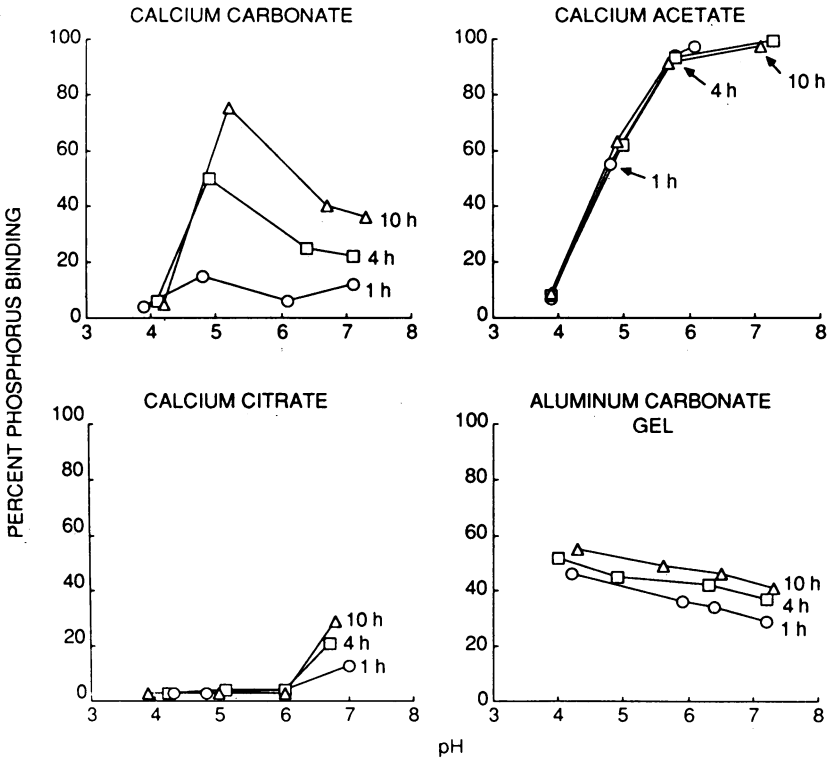

Figure 4. Observed in vitro phosphorus binding at 1, 4 and 10 h with 50 meq amount of the four binders also studied in vivo.

The presence of anions that compete with phosphorus for the binder cation decreases phosphorus binding. For instance, citrate ion forms a strong complex with calcium (33), thereby making calcium unavailable to react with phosphorus. Considering the equilibrium constants of citrate ions with calcium we calculated that calcium citrate would bind much less than other calcium compounds used (Fig. 1) and this was verified in vitro (Fig. 2). This reaction of binder cation and competing anion is also affected by $[H^+]$. In the case of calcium citrate there is greater complex formation at higher pH in the absence of phosphorus (33). Nevertheless, the phosphate species at high pH has a smaller solubility product constant with calcium as compared to citrate; thus the binding increases at high pH in spite of greater calcium citrate complex formation (Figs. 1 and 2).

In all cases except aluminum hydroxide powder, we found good general agreement between equilibrium values calculated on theoretical grounds and presumed equilibrium values

*Table I. Net Phosphorus Absorption**

	Fast	Placebo			Calcium acetate			Calcium carbonate			Calcium citrate			Aluminum carbonate gel		
Subjects	Effl. phos.	Meal phos.‡	Effl. phos.	Net abs.	Meal phos.‡	Effl. phos.	Net abs.	Meal phos.‡	Effl. phos.	Net abs.	Meal phos.‡	Effl. phos.	Net abs.	Meal phos.‡	Effl. phos.	Net abs.
								mg								
1	87	358	170	275	340	271	156	344	187	244	346	228	205	356	315	128
2	58	343	114	287	353	359	52	352	272	138	346	225	179	344	449	−47
3	73	353	186	240	358	386	45	328	285	116	319	268	124	359	381	51
4	50	323	147	226	325	346	29	319	271	98	354	254	150	352	384	18
5	64	336	116	284	345	223	186	360	266	158	322	176	210	351	367	48
6	52	338	105	285	340	318	74	339	243	148	362	232	182	357	318	91
7	60	357	151	266	362	306	116	352	280	132	345	270	135	362	306	116
8	96	344	159	281	347	355	88	345	288	153	345	285	156	333	319	110
9	54	346	194	206	324	353	25	366	296	124	352	290	116	330	353	31
10	85	322	123	284	352	320	117	363	245	203	359	194	250	327	348	64
Mean	68	342	147	263	345	324	89	347	263	151	345	242	171	347	354	61
±SEM	±5	±4	±10	±9	±4	±15	±17	±5	±10	±14	±4	±12	±13	±4	±14	±17

* Net phosphorus absorption = Meal phosphorus − (effluent phosphorus after placebo/binder − effluent phosphorus after fast). ‡ Phosphorus content of duplicate meal. PEG recoveries (mean±SEM) on each test day are as follows: fast 100±1%, placebo 99±1%, calcium acetate 99±1%, calcium carbonate 98±2%, calcium citrate 100±1%, aluminum carbonate gel 100±2%.

Table II. Net Calcium Absorption from Placebo and Three Calcium Salts*

Subjects	Fast	Placebo			Calcium acetate			Calcium carbonate			Calcium citrate		
	Effluent calcium	Calcium intake‡	Effluent calcium	Net abs.	Calcium intake‡	Effluent calcium	Net abs.	Calcium intake‡	Effluent calcium	Net abs.	Calcium intake‡	Effluent calcium	Net abs.
							mg						
1	23	200	226	−3	1,235	1,002	256	1,203	922	304	1,183	969	237
2	28	219	181	66	1,209	1,028	209	1,213	948	293	1,191	910	309
3	38	215	225	28	1,272	1,133	177	1,201	901	338	1,187	1,080	145
4	37	211	212	36	1,235	1,109	163	1,199	1,021	215	1,201	1,006	232
5	14	214	262	−34	1,232	1,191	55	1,215	1,052	177	1,178	1,076	116
6	45	208	189	64	1,182	1,014	213	1,190	992	243	1,194	899	340
7	15	215	154	76	1,142	956	201	1,199	1,139	75	1,158	1,002	171
8	73	217	153	137	1,247	1,138	182	1,181	1,015	239	1,253	1,061	265
9	48	221	297	−28	1,255	1,094	209	1,220	1,020	248	1,205	942	311
10	51	217	193	75	1,221	1,035	237	1,203	876	378	1,222	768	505
Mean	37	214	209	42	1,223	1,070	190	1,202	989	251	1,197	971	263
±SEM	±6	±2	±14	±17	±12	±23	±17	±4	±25	±27	±8	±31	±36

* Net Ca absorption = Ca intake − (effluent Ca after placebo/Ca salt − effluent Ca after fast). ‡ Calcium content of duplicate meal and duplicate dose of calcium salt.

achieved in vitro. Aluminum hydroxide powder bound far less phosphorus than theoretical equilibrium values (Fig. 3); this probably can be attributed to its extreme insolubility and slow dissolution (36). If enough time was allowed, equilibrium values would probably be achieved with aluminum hydroxide powder, since aluminum hydroxide in the form of a gel did approach theoretical equilibrium values in 1–3 wk (Fig. 3).

Rate of establishment of equilibrium in vitro. The rate at which equilibrium is established depends upon the rate at which the binders dissolve and the rate of the precipitation reaction between ionized binder cation and phosphorus. Because the latter is virtually instantaneous for the compounds involved, the rate of dissolution of binders controls the rate at which equilibrium is established. The rate at which binder dissolves in a given medium depends mainly upon water solubility, pH, amount of binder, the rate of stirring, and temperature.

Freely water-soluble compounds dissolve readily thus making all the binder available for reaction. In the case of poorly soluble compounds, however, only a small amount slowly gets dissolved. As this small amount of dissolved binder reacts with phosphorus, the concentration of dissolved binder falls, which in turn allows further dissolution. This process continues until equilibrium is reached. It follows that freely soluble compounds would reach equilibrium quickly, whereas poorly soluble compounds would take a longer time. It is therefore not surprising that highly soluble calcium chloride reached equilibrium within 10 min and calcium acetate and aluminum chloride (Figs. 2 and 3) reached equilibrium within 1 h (the earliest time tested), whereas poorly soluble calcium carbonate, calcium citrate and magnesium hydroxide (Figs. 2 and 3) took 1–2 wk to approach equilibrium at pH 7 (where concentration of H^+ is not enough to enhance solubility).

The pH of the medium affects solubility of the binders. Calcium carbonate, for example, is much more soluble at low pH; thus at pH 5 equilibrium was approached at 4 h, whereas at pH 7 equilibrium was reached only at 1 week (Fig. 2). A similar effect was seen with aluminum carbonate gel where at pH 4 equilibrium value was reached between 24 h and 1 wk, whereas at pH 7 equilibrium value was approached only at 3 wk. At pH 2–3, aluminum carbonate gel would probably approach equilibrium even earlier than 24 h, although we did not perform in vitro experiments at such high levels of acidity.

Correlation of in vitro and in vivo phosphorus binding. To reduce dietary phosphorus absorption, a binder must mix with food and precipitate or adsorb meal phosphorus before phosphorus and the binder are absorbed by the small intestine. The mixing of food phosphorus and the binder can occur in the stomach and upper small intestine as food phosphorus is readily solubilized in the upper gastrointestinal tract (37). Because most of phosphorus is believed to be absorbed by the small intestine (38), and since most of ingested food passes through the stomach and small intestine in 4–6 h (39), the binding reaction needs to occur within this time period if phosphorus absorption is to be prevented. Binding that requires > 4–6 h to develop would probably occur in the colon and therefore is apt to have relatively little effect on absorption of dietary phosphorus.

Our in vitro studies revealed that calcium acetate can bind phosphorus better than aluminum carbonate gel at neutral pH such as found in small intestine, whereas aluminum carbonate gel can bind better than calcium acetate at low pH levels found in stomach. The in vivo studies showed the two binders to be about equally effective. This suggests that ability of aluminum carbonate gel to bind phosphorus in the stomach and the ability of calcium acetate to bind phosphorus more readily in the small intestine are of similar importance and result in similar total in vivo binding by these two compounds.

Our theoretical and in vitro results revealed that calcium acetate has advantages over calcium carbonate or calcium citrate as a phosphorus binder. When these three calcium salts were administered with a meal to normal people, calcium acetate inhibited phosphorus absorption to a greater extent than the other calcium salts. Theoretical and in vitro data thus

predicted the relative effectiveness of these calcium salts as in vivo binders of dietary phosphorus.

Phosphorus absorption with placebo was 263 mg, compared with 89 mg with 50 meq calcium acetate (Table I). This represents an impressive reduction in phosphorus absorption, but that 89 mg was absorbed indicates that phosphorus binding was incomplete. It is interesting to compare this result in the gastrointestinal tract with what happened in vitro (Fig. 4). At pH 6–7 (corresponding to the pH of the jejunum and ileum), 50 meq of calcium acetate is expected to bind ~ 98% of the 320 mg phosphorus in 1 h, leaving 6 mg unbound. Calcium acetate thus inhibited food phosphorus absorption in the intestine less well than it bound phosphorus in a beaker. Several possible explanations may be offered to explain this difference, including incomplete mixing of food phosphorus and binder calcium, absorption of phosphorus and/or calcium in the small bowel before binding could occur, the slightly acid environment of the duodenum (40), which would reduce binding but not phosphorus absorption, and partial conversion of soluble calcium into poorly soluble salts in the small intestine.

At pH 6–7 in vitro, calcium acetate was far superior to calcium carbonate as a phosphorus binder (Fig. 4). For example, after 4 h at ~ pH 6 calcium carbonate bound ~ 30% (96 mg) of phosphorus in the beaker compared with 98% binding (314 mg) by calcium acetate (Fig. 4). Calcium acetate was also superior to calcium carbonate at inhibiting phosphorus absorption in vivo but the difference was not as great (see Table I). The most likely explanation for this observation is partial conversion of calcium carbonate by gastric acid to calcium chloride ($CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2$), which can then bind phosphorus as the pH rises in the small intestine.

In vitro phosphorus binding by calcium citrate was poor compared with other calcium salts, including calcium carbonate. However, calcium citrate was only slightly less effective than calcium carbonate in vivo. One possible explanation for this finding is intestinal absorption of citrate, thus minimizing complex formation between calcium and citrate that would otherwise have prevented phosphorus binding.

As compared to calcium carbonate and calcium citrate, calcium acetate bound more phosphorus but less calcium was absorbed from it. There can be two explanations for this observation. First, less calcium could have been absorbed from calcium acetate and hence more was available in the intestine to bind phosphorus. Second, more calcium from calcium acetate could have reacted with food phosphorus and hence less was available to be absorbed. The first explanation is probably not correct, because in a recent study, calcium absorption was observed to be similar from these calcium salts when ingested without concomitant ingestion of food (41); hence, the second explanation is more likely to be correct. As is evident from Table I, calcium acetate bound 62 mg (151 minus 89) more phosphorus as compared with calcium carbonate. This amount of phosphorus is equal to 3.6 meq (assuming the valence of phosphorus in the gut to be 1.8 as suggested by Lennon et al. [42]), and should be bound by 3.6 meq or 72 mg of calcium. Thus with calcium acetate 72 mg less calcium was presumably available to be absorbed as compared with calcium carbonate. As is evident from Table II calcium absorption from calcium acetate was 61 mg (or 3.1 meq) lower as compared with calcium carbonate, a value in close agreement with that expected from the degree of phosphorus binding by these two compounds.

For clinical purposes, one would like for a phosphorus binder to bind as much phosphorus as possible, and for residual binder to be absorbed as little as possible (in order to avoid toxicity). We found that calcium acetate bound 1.04±0.11 mg phosphorus/mg of calcium absorbed (calculated from data for individual subjects, Tables I and II), which is significantly better than calcium carbonate (0.57±0.15, $P < 0.05$) and calcium citrate (0.43±0.07, $P < 0.001$). Thus, by this method of analysis, calcium acetate has about a twofold advantage over the other calcium salts.[1]

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