Relative *in vitro* Efficacy of the Phosphate Binders Lanthanum Carbonate and Sevelamer Hydrochloride

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ABSTRACT: The high tablet burden and poor compliance associated with phosphate-binding drugs has led to a search for more potent agents. *In vitro*-binding studies were performed on the recently introduced binder, lanthanum carbonate (LC; Fosrenol®), to compare its phosphate-binding affinity with sevelamer hydrochloride (SH; Renagel™). Langmuir equilibrium binding affinities (K_1) for LC and SH were established using different phosphorus (5–100 mM) and binder (134–670 mg per 50 mL) concentrations at pH 3–7, with or without salts of bile acids present (30 mM). At all pH levels, LC had a higher binding affinity for phosphate than SH. For LC, K_1 was 6.1 ± 1.0 mM^{-1} and was independent of pH. For SH, K_1 was pH dependent, being 1.5 ± 0.8 mM^{-1} at pH 5–7 and 0.025 ± 0.002 mM^{-1} at pH 3, that is, >200 times lower than for LC. In the presence of 30 mM bile salts, SH lost 50% of its phosphate, whereas no displacement of phosphate occurred for LC. These findings indicate that LC binds phosphate more effectively than SH across the pH range encountered in the gastrointestinal tract, and has a lower propensity for bound phosphate to be displaced by competing anions in the intestine. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 96:2818–2827, 2007

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INTRODUCTION

The kidney is the primary organ involved in phosphorus homeostasis. In the normal individual, the serum phosphorus concentration is maintained within the range of 2.2–4.4 mg/dL primarily by regulation of urinary phosphorus excretion, and modulation of renal 1,25-dihydroxy vitamin D synthesis to increase or decrease active intestinal phosphorus absorption.[1] In early kidney failure, these processes adapt to maintain serum concentrations in the desirable range, but with advanced disease, systemic phosphorus retention ensues with serious health consequences. Hyperphosphataemia in end-stage renal disease (ESRD) is associated with secondary hyperparathyroidism, metabolic bone disease (renal osteodystrophy), cardiovascular calcification, and an increased risk of death, particularly cardiovascular death.[2–6] While control of dietary phosphorus intake can be effective in reducing phosphorus retention, this approach carries a risk of malnutrition owing to a consequential decrease in protein intake.[7,8] Most dialysis patients, therefore, require a phosphate-binding drug to trap dietary phosphate in the gastrointestinal tract and reduce absorption into the systemic circulation. While phosphate-binding agents have been available for several years, they are associated

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with disadvantages, and the key need for newer agents is an improved therapeutic profile.

Aluminum is widely recognized as the most effective phosphate-binding drug but its use is now generally restricted to ESRD patients who are uncontrolled on other agents, owing to concerns about its toxicity, including dialysis encephalopathy,[9,10] osteomalacia,[11,12] and microcytic anaemia.[13] Calcium-based binders largely replaced aluminum in the 1980s and 1990s and have been the mainstay of treatment for many years.[14] However, large doses are needed with each meal leading to concerns about elevated calcium load, hypercalcaemia, and an increased potential for vascular calcification.[6,15,16] For this reason, current Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines recommend restricting the total dose of elemental calcium provided by calcium binders to less than 1500 mg/day in Stage 5 chronic kidney disease (CKD) patients.[17] The introduction of sevelamer hydrochloride (SH; Renagel™) a cationic hydrogel of cross-linked poly(allylamine hydrochloride) that does not contain aluminum or calcium (third-generation binder) was a significant advance, enabling reduction of serum phosphate without adding to the patient's calcium load; however, its efficacy is reported to be lower compared with calcium acetate.[18] Issues of low potency, high tablet burden, and poor compliance, therefore remain, and control of serum phosphorus, calcium, and parathyroid hormone to KDOQI targets is achieved in only a minority of ESRD patients.[19]

Lanthanum carbonate (LC; $La_2(CO_3)_3.4–5H_2O$; Fosrenol®) is a newer, third-generation non-aluminum, noncalcium phosphate binder, approved for the control of hyperphosphataemia in ESRD.[20,21] It has been launched with the potential of higher phosphate-binding potency and a reduced tablet burden for dialysis patients.[22] While higher potency alone is not generally considered a significant therapeutic advance, there has been a clear medical need for more effective phosphate-binding drugs, with current agents being consumed in high gram doses, delivered in multiple tablets with each meal. About 35% of hemodialysis patients require 10 or more drug treatments per day, while 5% require 15 or more treatments.[23] As some treatments are administered as multiple tablets, the daily pill burden is substantial. This may be one reason why up to 50% of hemodialysis patients do not adhere to essential treatment regimens, resulting in an increased risk of adverse outcomes including death, compared to the compliant population.[24]

To date, no clinical trials have directly compared the efficacy of these two available third-generation binders, and published studies on the individual binders have involved treatment to a defined target serum phosphorus concentration, making it difficult to compare relative efficacy using hyperphosphataemia as an endpoint. Our study is the first to directly compare the ability of LC and SH to bind phosphate under a variety of *in vitro* conditions that model the environment in the gastrointestinal tract. The results support the higher potency of LC relative to SH and suggest this may be due to the maintenance of a high phosphate-binding affinity across the physiologically relevant pH range in the gastrointestinal tract. Unlike existing agents, lanthanum is able to bind phosphate effectively in the acidic environment of the stomach and proximal small intestine before it is absorbed, as well as in more distal regions.

METHODS

Materials

LC was obtained from Shire Pharmaceutical Development Ltd (Basingstoke, UK) and SH (403 mg capsules, Genzyme Corporation, Cambridge, MA) from a commercial source. Bile acids (sodium salts) and other chemicals were obtained from Sigma–Aldrich Company Ltd, Poole, UK.

Apparatus

The binding of phosphate by SH or LC was studied at constant pH. In a typical experiment, a calculated amount of the solid binder was added to a stirred solution containing a known concentration of phosphate (and salts of bile acids) in water. The pH was maintained automatically throughout by addition of 1.0 M hydrochloric acid solution or 0.2 M ammonia solution using a Metrohm 718 Tritino pH-Stat apparatus (Metrohm, Switzerland). The temperature in the specialist Metrohm apparatus thermostated reaction vessel was maintained at 37°C using a Grant GD120 thermostat. The electrodes used to maintain the pH were calibrated at pH 4 and 7 using a buffer (Hydrion™). Water was distilled immediately before use. The phosphorus content of all batches of reagents and solutions was

determined and found to be negligible (typical phosphorus concentrations of reagent solutions before the addition of $(NH_4)H_2PO_4$ were: distilled water < 0.003 mM; 0.1 M HCl solution = 0.1 mM; 0.1 M ammonia solution = 0.17 mM).

Phosphorus concentration was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) at the Centre for Analytical Sciences, University of Sheffield, UK. The method was established over a linear calibration range of 10 ng/mL to 10 mg/mL. Performance was monitored using quality control samples, assayed with each batch of study samples, with accuracy and precision falling within 5%.

Data Analysis

The Langmuir equation[25] was used to calculate the phosphate-binding affinities of LC and SH in these systems. The Langmuir relationship mathematically describes the adsorption (hereafter referred to as binding) of an adsorbate to an adsorbent in a two-phase (heterogeneous) system. Principally, the Langmuir relationship has been applied to the adsorption of a gas to a solid. The Langmuir relationship has also been applied to the binding of phosphate to SH,[26] and we are adopting the approach developed in this earlier work. In our case, the adsorbate is phosphate ion in solution to the solid adsorbent (SH or LC).

The phosphate-binding affinities of LC and SH were determined graphically using the Langmuir equation shown in Equation (1). It is important to be clear from the beginning that, unless stated otherwise, throughout this paper "phosphate" refers to the total phosphate in solution. Depending on the pH of the solution, this "phosphate" can be in the form of PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, or H_3PO_4. We will return to discuss which form binds to LC and SH in a later section.

$$\frac{[\text{phosphate}]_{\text{unbound}}}{[\text{phosphate}]_{\text{bound}}/\text{mass binder}} = \frac{1}{K_1 K_2} + \frac{[\text{phosphate}]_{\text{unbound}}}{K_2} \quad (1)$$

Under all experimental conditions reported in this paper, a plot of {$[\text{phosphate}]_{\text{unbound}}/[\text{phosphate}]_{\text{bound}}$/mass binder} against $[\text{phosphate}]_{\text{unbound}}$ was a good straight line. The data were analyzed using a straight line fit by Excel® and from the fit, $1/K_1K_2$ is the intercept and $1/K_2$ is the slope (Figure 1 and Figure 2). In the Langmuir equation, K_1 is the Langmuir equilibrium constant, which is a measure of the affinity of phosphate for the binder, and K_2 is the Langmuir capacity equilibrium constant of the binder and is a measure of the maximum amount of phosphate that can be bound per unit mass of binder.

Phosphate-Binding Affinities of LC and SH at Constant pH

The following procedure was employed to measure the amount of phosphate bound by either LC or SH. A known concentration of phosphate (5, 10, 30, 50, or 100 mM) as $(NH_4)H_2PO_4$ in aqueous solution (volume = 50 mL) was introduced into the

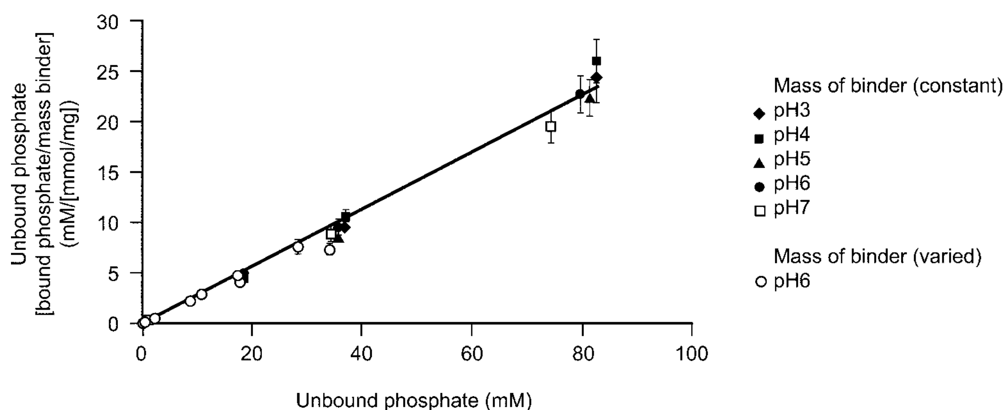

Figure 1. Langmuir graph for the binding of phosphate to lanthanum carbonate, showing that the binding is independent of pH. Data points correspond to the following experiments: (i) mass of binder (kept constant) = 134 mg (pH 3–7); (ii) mass of binder (varied) = 134–670 mg (pH 6).

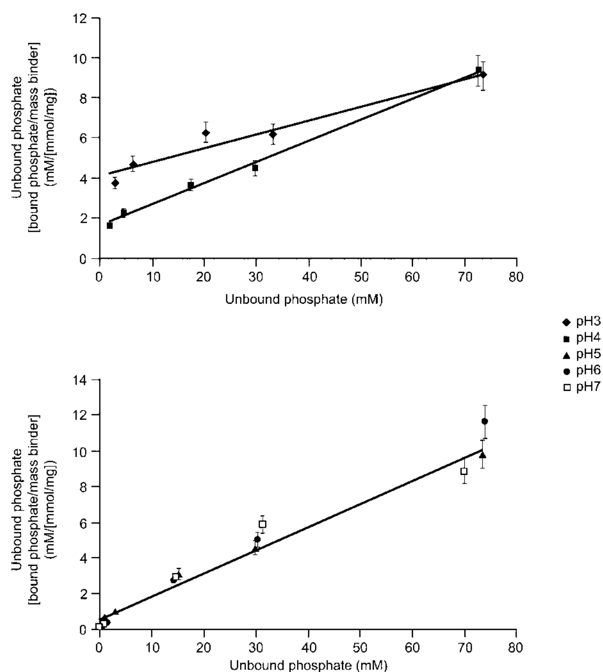

Figure 2. Langmuir graphs for the binding of phosphate to sevelamer hydrochloride at 37°C, showing the effect of pH. Mass of binder = 134 mg.

pH-Stat vessel. Subsequently, a known mass of the binder (SH or LC; 134, 239, 403, 536, or 670 mg) was added to the thermostated phosphate solution, and the mixture titrated to the desired pH (pH 3, 4, 5, 6, or 7).

Samples (0.5 mL) of the mixture were removed at known times, using a polypropylene/polyethylene syringe, transferred to a polypropylene tube by filtration through Anotop 0.2 μm Whatman filters, and the phosphorus content determined. From the results, the concentration of phosphate in solution ($[\text{phosphate}]_{\text{unbound}}$) was calculated. The amount of bound phosphate ($[\text{phosphate}]_{\text{bound}}$) was determined by difference using the mass balance equation shown in Equation (2), where $[\text{phosphate}]_{\text{total}}$ is the total concentration of phosphate added to the system. All experiments were replicated, and agreement between the results was good. When experiments were repeated, analyses of the amounts of free phosphate present in solution gave results that differed by only 2–8%. This degree of reproducibility is reflected in the calculated results by the y-error bars shown in Figures 1 and 2.

$$[\text{phosphate}]_{\text{bound}} = [\text{phosphate}]_{\text{total}} - [\text{phosphate}]_{\text{unbound}} \quad (2)$$

Stability of the Binder–Phosphate Adduct

To measure the stability of the binder–phosphate adduct, the lanthanum–phosphate and sevelamer–phosphate complexes were prepared *in situ* and their stabilities followed over the course of 4 h by monitoring the concentration of unbound phosphate present in the solution. The binder–phosphate complexes were prepared by suspending the binder (134 mg) in a known concentration of phosphate (5 mM). The pH was adjusted to pH 3, 5, 6, or 7 and the mixture left for 3 h to come to equilibrium using the pH-Stat apparatus. Samples (0.5 mL) were taken every 20 min for 4 h, filtered and analyzed for phosphorus as described above.

Binding of Phosphate to LC and SH in the Presence of Bile Acids

A solution of phosphate (5, 10, 15, 20, or 40 mM) was introduced into the pH-Stat vessel. When the temperature of the phosphate solution had stabilized, a known quantity of binder (134 mg) was added to the solution to form the binder–phosphate complex *in situ*. The solution was titrated to pH 3 to simulate postprandial gastric conditions. Phosphate binders are administered with or immediately after food to ensure adequate contact and mixing with dietary phosphate; consequently a gastric pH below pH 3 is unlikely to be encountered with this class of drug. Conditions were then altered to simulate the duodenal environment. The mixture was titrated to pH 5 or 6, stirred for a further hour and another sample taken. A known concentration of bile acids (3, 10, or 30 mM) was added. Samples were taken every 10–30 min for the next 4 h, filtered and analyzed for phosphorus, as described above.

The bile acid mixture was chosen to be representative of that reported to be present in human bile (total concentration 30 mM), and comprised: glycocholic acid (9 mM), glycochenodeoxycholic acid (9 mM), glycodeoxycholic acid (4.5 mM), taurocholic acid (3 mM), taurochenodeoxycholic acid (3 mM), and taurodeoxycholic acid (1.5 mM).[27–30] Lower bile acid concentrations were prepared by dilution of the stock mixture.

RESULTS AND DISCUSSION

Phosphate-Binding Affinity

The phosphate-binding affinities of LC and SH were determined under identical conditions

(composition of solution, pH, and temperature). The concentration of phosphate was varied in the range of 5–100 mM, and the amounts of the binder varied in the range of 134–670 mg. The effect of pH was studied over a range of 3–7. All experiments were performed at least in duplicate, and reproducible results were obtained in all studies. In all cases, linear Langmuir plots were obtained. Figure 1 shows the plot for LC, and Figure 2 shows the plots for SH. It is to be noted that the data presented in Figures 1 and 2 show some scatter about the line. This scatter is acceptable considering the wide range of phosphate concentrations, amounts of binder, and pH range over which all the data were collected.

For LC, the Langmuir constants $K_1^{La} = 6.1 \pm 1.0$ mM^{-1} and $K_2^{La} = 3.7 \pm 0.1$ mmol mg^{-1} were independent of pH in the range of 3–7, as shown in Figure 1. The reliability of these estimates is supported by agreement between: (i) studies where the amount of binder was kept constant whilst the phosphate concentration was varied and (ii) studies in which the amount of binder was varied and the concentration of phosphate was kept constant. Both sets of data are shown in Figure 1.

In contrast to the results with LC, the phosphate-binding affinity of SH showed a marked dependence on pH. It is evident from inspection of the Langmuir plots shown in Figure 2 that, although the graphs are linear, the slope and intercept of the lines vary with pH. Thus, at pH 3, $K_1^{Se} = 0.025 \pm 0.002$ mM^{-1} and $K_2^{Se} = 10.5 \pm 0.5$ mmol mg^{-1}, and at pH 5–7, $K_1^{Se} = 1.5 \pm 0.8$ mM^{-1} with $K_2^{Se} = 6.0 \pm 1.0$ mmol mg^{-1}. The relationships between pH and K_1 or K_2 for both LC and SH are shown in Figure 3. At all pH values, the binding affinity of LC was appreciably higher than that of SH, the difference being largest at pH 3, where the K_1^{La}/K_1^{Se} ratio was 244. As calcium carbonate and calcium acetate have been shown to be ineffective at pH 3,[22] the ability to bind phosphate strongly in the acidic environment of the stomach and upper small intestine, prior to the phosphate being optimally available for absorption throughout the intestine, may represent an advantage of LC over other currently used agents. Studies of ^{32}P-phosphorus absorption in ESRD patients indicate that almost all dietary phosphorus is absorbed within 3 h, suggesting the upper small intestine to be the most important absorption site.[31] The highest fractional rate of absorption occurred 1 h after ingestion, indicating that phosphorus is absorbed most rapidly in the very upper reaches of the small intestine, where active vitamin D-dependent transport is present. At these locations, the environment is usually acidic, and LC would therefore be expected to bind phosphate more effectively than SH. This provides a possible explanation for the greater phosphate-binding efficacy of LC seen *in vivo* in animal renal failure models compared with SH and calcium carbonate.[22]

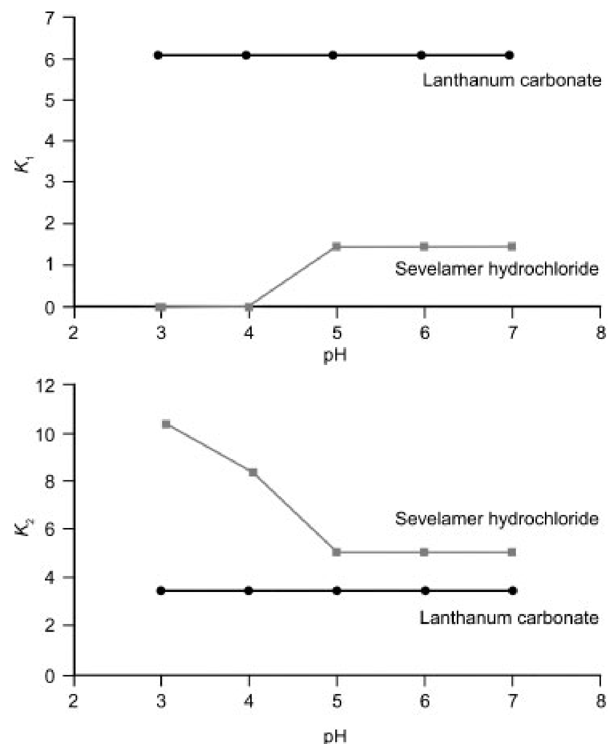

Figure 3. Comparisons of the variations of the Langmuir constants K_1 (top) and K_2 (bottom) with pH for lanthanum carbonate and sevelamer hydrochloride.

The pH dependence of phosphate binding by SH has been reported previously. Our results with SH are in good agreement with those obtained by Swearingen et al.[26] The previous study showed that K_1^{Se} for SH decreased appreciably at pH 4. This was attributed by the authors to the nature of phosphate in solution. In the pH range studied (pH 4–8), no H_3PO_4 (p$K_a = 2.1$) or PO_4^{3-} ions are present, the major phosphate species being $H_2PO_4^-$ (p$K_a = 7.2$) and HPO_4^{2-} (p$K_a = 12.4$). The proportion of the di-anion increases with increasing pH in this range. It was argued that the lower K_1 at pH 4 was due to the predominance of $H_2PO_4^-$, and that the binding affinity was defined principally by the charge on the anion. Consequently, it

was argued, $H_2PO_4^-$ binds more weakly than HPO_4^{2-}. The nonlinear nature of the Langmuir plots observed by the authors, which was most notable at low pH, was attributed to the detection of both $H_2PO_4^-$ and HPO_4^{2-} ions binding to SH.

If the change in K_1^{Se} shown in Figure 3 was attributable to selective binding of $H_2PO_4^-$, or HPO_4^{2-} to SH we would expect the change to occur at a pH which reflected the pK_a of these species. In fact the change occurs in the range pH 5–6, significantly different from the pK_as of either $H_2PO_4^-$ or HPO_4^{2-}. Although there are only limited data, the results shown in Figure 3 are not indicative of the protonation state of the phosphate being a major factor influencing the binding affinities for phosphate to SH. In particular, K_1^{Se} increases from pH 3 to 5 and then remains constant until, at least, pH 7. If SH were selectively binding $H_2PO_4^-$ or HPO_4^{2-} it would have been expected that K_1^{Se} would increase over the whole of this pH range.

The principal mechanism for binding of phosphate to SH is an ion-exchange process, in which phosphate replaces chloride ion and is held to the polymeric, cationic binder by electrostatic attraction to the $–CH_2–NH_3^+$ residues of SH. Of course, other mechanisms such as encapsulation of the phosphate within the polymer could also contribute to the binding. An alternative explanation for the quite abrupt change in K_1^{Se} with pH shown in Figure 3 might be an alteration in the gross structure of the SH polymer in the range pH 5–6.

We further investigated the pH dependence of SH binding at pH 3 or 4. The Langmuir plot for the data collected at pH 3 is shown in Figure 2. The data define a reasonable linear relationship. The only sign of any deviations from the linear behavior is at very low concentrations of phosphate, where there may be insufficient phosphate present to achieve the capacity of the binder. Under these conditions the amount of free phosphate is low and consequently the error is large.

The binding of phosphate by LC is independent of pH over the range 3–7, indicating that, if present, any differences in the affinities of $H_2PO_4^-$ and HPO_4^{2-} are small. The mechanism by which phosphate binds to LC is unclear, but it seems likely that it is fundamentally different to that of SH. The structure of $La_2(CO_3)_3.4–5H_2O$ involves a ten-coordinate La with both monodentate and bidentate carbonate ligands. It seems likely that when phosphate binds and replaces carbonate, the phosphate is also coordinated to the lanthanum, possibly in a variety of different coordination modes.

The very different nature of the binding of phosphate by the two binders precludes any detailed comparison of the difference in the values of K_2 for SH and LC. Furthermore, the *in vivo* relevance of the K_2 values is limited, as they reflect binding capacity in the absence of competing anions, a situation that will not exist in the complex chemical environment of the gastrointestinal tract lumen.

Comparison of the Stabilities of the Phosphate-Binder Complexes

The lanthanum–phosphate complex was stable at pH 3 and pH 6 over 4 h, with no detectable release of phosphate. At pH 6, the sevelamer–phosphate complex was stable over 4 h. At pH 3, the sevelamer–phosphate complex released about 2% of its phosphate over the course of 4 h.

Effect of Salts of Bile Acids on Phosphate-Binding Affinity

The binding of bile acids to SH has been previously described in a study that involved the incubation of SH with bile acids and determining the amount of free bile acids remaining in solution using high performance liquid chromatography (HPLC).[32] The principal conclusions from this study were: (i) there was cooperativity in the binding of the bile acid; (ii) in the presence of oleic acid, the binding affinity for bile acid decreased by a factor of 2; and (iii) the presence of oleic acid suppressed the release of bile acid from SH. This sequestration of bile acids by SH is believed to be the mechanism by which it lowers serum lipid concentrations,[32] as for other ion-exchange resins such as cholestyramine.

As our interest was primarily to investigate the extent to which bile acids can influence phosphate-binding efficacy, we studied the binding of salts of bile acids (bile salts) in competition with phosphate bound to the binder. This is physiologically relevant as it models what happens *in vivo* under conditions where both phosphate and bile acid are present and could be adsorbed by the binder. By monitoring the release of the phosphate, we have also been able to measure the kinetics of phosphate displacement from the sevelamer–phosphate complex in the presence of bile salts. The results of these kinetic studies

indicate that bile salts and phosphate cannot bind simultaneously to SH. Furthermore, once phosphate was bound to LC, bile salts could not displace the phosphate under any condition we employed (high concentrations of bile salts or protracted times).

Bile is principally water (97%) with bile acids accounting for approximately 50% of the solid components. Chemically, bile acids are carboxylic acids with a cyclopentanoperhydrophenanthrene nucleus and a branched side chain of 3–9 carbon atoms. There are few references to the actual concentration of bile acids in the duodenum, and therefore, reported concentrations in bile were used.[27–30]

The lanthanum–phosphate complex, prepared *in situ* before the bile salts were added, was stable over the course of at least the next 5 h at pH 5 and 6. No phosphate was released or adsorbed (Figure 4). The lanthanum–phosphate complex

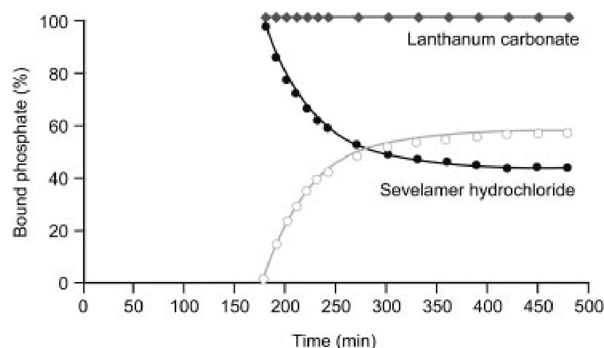

Figure 4. Typical time course for the reactions of sevelamer–phosphate or lanthanum–phosphate complex with bile salts (30 mM) in the presence of phosphate (5 mM) at pH 6 and 37°C. The horizontal line and data points (◆) show that the lanthanum–phosphate complex is unperturbed by the addition of bile acids. The curves show the effect of bile salts on the sevelamer–phosphate complex. The data points associated with the decrease in bound phosphate in the sevelamer–phosphate complex following addition of the bile acids (●) are fitted to the expression $[\text{phosphate}]_t = 43.4 + (54.6)\exp(-0.021t)$ [see Eq. (3)]. The data points associated with the increase in unbound phosphate (○) are fitted to the expression $[\text{phosphate}]_t = 56.6 - (54.6)\exp(-0.021t)$ [see Eq. (3)]. The errors associated with determining the rate constants by this curve-fitting method are $\pm$ 0.002 min^{-1}. Thus, the fit to the data shown in this figure is good using $k_{\text{obs}} = 0.021\ \text{min}^{-1}$, but a fit using $k_{\text{obs}} = 0.023$ or $0.019\ \text{min}^{-1}$ shows appreciable departure from the experimental data points.

was sufficiently stable (phosphate bound sufficiently tightly) that the bile salts could not replace the phosphate.

The time course for binding of phosphate to SH in the presence of bile salts was investigated at pH 6. The sevelamer–phosphate complex was prepared *in situ* by mixing phosphate (5, 10, 15, 20, or 40 mM) and SH. Upon addition of bile salts (3, 10, or 30 mM), the amount of free phosphate in the solution was monitored over the course of the next 5 h. When the bile acid concentration was 3 mM, the sevelamer–phosphate complex was stable, and no change in the phosphate present in solution could be detected. When the concentration of bile salts was 10 mM, a small loss of phosphate occurred from the complex. When the concentration of bile acid was 30 mM (the approximate concentration of bile acids in bile), appreciable loss of phosphate from the sevelamer–phosphate complex was observed as shown in Figure 4.

The data in Figure 4 show: (i) the increase in the concentration of phosphate in solution owing to the release of phosphate from the sevelamer–phosphate adduct and (for completeness) (ii) the decrease in phosphate bound to SH. The data in the two curves are related to one another by the relationship shown in Equation (2).

The addition of bile salts to the equilibrium mixture containing the sevelamer–phosphate complex leads to the formation of a new equilibrium mixture, in which bile salts compete with phosphate for binding to SH. At long times (after about 500 min), the new equilibrium position has been reached. Analysis of the data from these experiments yields: (i) the Langmuir constants (K_1^{SeB} and K_2^{SeB}) for phosphate binding to SH in the presence of 30 mM bile salts and (ii) the kinetics for the attainment of the new equilibrium position in the presence of 30 mM bile salts.

Analysis of the amount of phosphate bound at the end of the reaction in the presence of 30 mM bile salts yielded a 13-fold reduction in binding affinity with $K_1^{\text{SeB}} = 0.11 \pm 0.1\ \text{mM}^{-1}$ and an approximate halving of binding capacity with $K_2^{\text{SeB}} = 3.5 \pm 0.3\ \text{mmol mg}^{-1}$. It is worth noting that when the phosphate concentration was >60 mM, the data were poorly reproducible. There was a great deal of scatter in the data, and no systematic trend could be discerned. The reasons for this behavior are not clear.

The experimental data shown in Figure 4 were fitted using the exponential function shown in Equation (3), and the curves in Figure 4 were

drawn using Excel®. Details of the curve fitting procedure are presented in the legend of Figure 4. The good fit to the data obtained using Equation (3) indicates that the reaction exhibits a first-order dependence on the concentration of the sevelamer–phosphate complex.[33] In Equation (3), $[\text{phosphate}]_t$ is the concentration of phosphate at time (t), $[\text{phosphate}]_{\text{inf}}$ is the concentration of phosphate at the end of the reaction, when the new equilibrium conditions have been attained, and $\Delta[\text{phosphate}]$ is the change in the phosphate concentration during the reaction, $\Delta[\text{phosphate}] = [\text{phosphate}]_{t=0} - [\text{phosphate}]_{t=\text{inf}}$. Equation (3) describes a reaction in which there is an increase in the concentration of unbound phosphate during the reaction (i.e., the release of phosphate from the sevelamer–phosphate complex). See the legend to Figure 4 for the corresponding equation that describes the decrease in the amount of bound phosphate.

$$[\text{phosphate}]_t = [\text{phosphate}]_{\text{inf}} - (\Delta[\text{phosphate}])\exp(-k_{\text{obs}}t) \quad (3)$$

At a particular concentration of phosphate, the exponential fit to the curve yields the observed rate constant (k_{obs}). In a series of experiments where the concentration of bile acid was kept constant ([bile acid] = 30 mM), the phosphate concentration was varied systematically and the corresponding values of k_{obs} were determined. Although there are only limited data, Figure 5 shows that k_{obs} decreases slightly, but appreciably, as the concentration of phosphate increases.

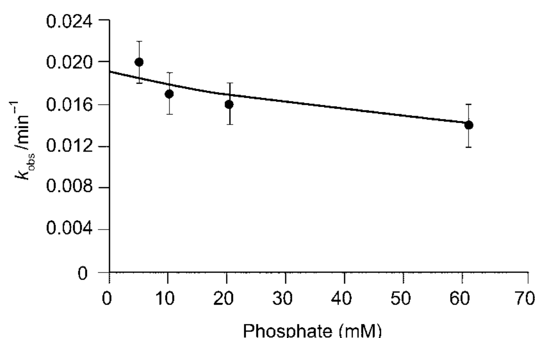

Figure 5. Effect of phosphate on the rate of the reaction of the sevelamer–phosphate complex with bile salts. The curve drawn is that defined by Equation (5) and the parameters described in the text. The y-error bars shown in this figure show the error in determining the rate constants (± 0.002 min^{-1}) from the curve-fitting procedure described in the legend to Figure 4.

That increasing the concentration of phosphate inhibits the reaction is not consistent with simple synchronous processes described by a single equilibrium in which bile salt displaces bound phosphate in the forward reaction (k_{a}) and phosphate displaces bound bile acid in the reverse reaction (k_{r}). Such a single step process would be associated with the rate law shown in Equation (4), in which the rate would exhibit a first-order dependence on the concentration of phosphate but (crucially) is not inhibited by phosphate.

$$\text{Rate} = \{k_{\text{a}}[\text{bile acid}] + k_{\text{r}}[\text{phosphate}]\} \times [\text{sevelamer}] \quad (4)$$

The inhibition of the rate of the reaction by increasing phosphate concentrations is consistent with the mechanism shown in Figure 6, which shows two coupled equilibria.[34] In the first equilibrium reaction, phosphate dissociates from the sevelamer–phosphate complex. In the second equilibrium, the bile salts bind to the site on the sevelamer left vacant by the dissociation of the phosphate.

In the absence of bile salts (the conditions under which the sevelamer–phosphate complex is prepared), the first equilibrium is established. A consequence of the mechanism is that the binding of phosphate to SH is a dynamic process in which phosphate dissociates and binds to the binder all the time, as shown in the initial equilibrium in Figure 6. Upon addition of bile salts, the initial equilibrium is perturbed as the bile salts compete with phosphate for binding to free SH. The general rate law for a mechanism involving two coupled equilibria has been defined, and for the specific reaction we are studying the rate law is that shown in Equation (5).

$$\text{Rate} = \left\{\frac{k_1 k_2[\text{bile acid}]}{k_1 + k_{-1}[\text{phosphate}]} + k_{-2}\right\} \times [\text{sevelamer}] \quad (5)$$

Thus, at a constant concentration of bile salts, increasing the concentration of phosphate inhibits the rate at which the system reaches equilibrium owing to competition between phosphate and bile salts for binding sites on SH.

Analysis of the kinetic data in Figure 5 gives: $k_{-1}/k_1 = 11 \pm 2$ mM; $k_2[\text{bile acid}] = 1.2 \times 10^{-2}$ min^{-1}; and $k_{-2} = 7 \times 10^{-3}$ min^{-1}. The kinetic results are consistent with values of $K_1^{\text{SeB}} = 0.11 \pm 0.1$ mM^{-1} obtained from the Langmuir analysis. Inspection of the mechanism shown in Figure 6 shows that for

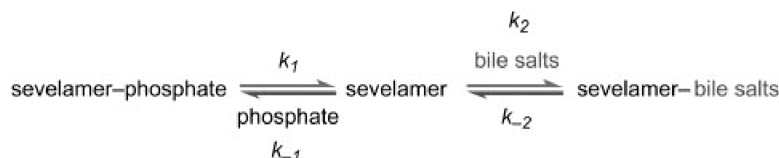

Figure 6. Scheme for the equilibrium binding of phosphate and bile salts to sevelamer hydrochloride, in which phosphate dissociates before binding of bile salts.

the initial equilibrium, $k_1/k_{-1} = K_1 = K_1^{\mathrm{SeB}}$. From the kinetic analysis, we can calculate $K_1^{\mathrm{SeB}} = 0.09 \pm 0.02\ \mathrm{mM}^{-1}$. This value is in excellent agreement with the value of K_1^{SeB} ($0.11 \pm 0.1\ \mathrm{mM}^{-1}$) established from the Langmuir analysis.

An important feature of the mechanism is that, for any particular site on SH, bile salts cannot displace phosphate (or indeed phosphate displace bile salts) by a synchronous process. Rather, the binding of any substrate to the site involves a dissociation mechanism in which any bound substrate must dissociate before any other substrate can bind.

CONCLUSIONS

The studies presented in this paper allow us, for the first time, to compare the efficacies of LC (Fosrenol®) and SH (Renagel™) to bind phosphate under a variety of conditions (pH and in the presence of bile salts) that model physiological conditions in the gastrointestinal tract. LC had significantly higher phosphate-binding affinity compared with SH; the difference was particularly marked at low pH. The phosphate-binding affinity of LC was independent of pH, whilst that of SH decreased at low pH. Consequently, the phosphate-binding affinity of LC was >200-fold higher at gastric pH (pH 3) and 4-fold higher at intestinal pH (pH 5–7).

The presence of a physiological concentration of bile acids was unable to displace phosphate from the lanthanum–phosphate complex, whereas the addition of bile acids to the sevelamer–phosphate complex leads to a 13-fold reduction in phosphate-binding affinity and the release of phosphate for possible absorption.

The results presented here provide possible explanations for the greater phosphate-binding efficacy observed with LC in animal renal failure models, and indicate the importance of trapping dietary phosphate early in the acidic milieu of the stomach and duodenum before phosphate can be absorbed in the small intestine. The bile acid sequestrant action of sevelamer reduces its phosphate-binding affinity in the presence of bile acids, which may cause some bound phosphate to be released and made available for absorption in the intestine.

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