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The development and in-vitro evaluation of novel mixed metal hydroxy-carbonate compounds as phosphate binders

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Abstract

The currently available phosphate binders are relatively inefficient and suffer from clinical side-effects of increased absorption of calcium and aluminium and the diarrhoea-inducing effects of magnesium. A new class of compounds based on mixed metal hydroxides has been developed and evaluated for their potential as phosphate binders. The mixed metal hydroxides were prepared using a standard procedure for hydrotalcite ($Al_2Mg_6(OH)_{16}.CO_3.4H_2O$) by substituting Fe^{3+} for Al^{3+} with Mg^{2+} or Ca^{2+} as the divalent metal ion. Phosphate precipitation (binding) was examined at different pH values in aqueous solution and in various food mixtures in comparison with hydrotalcite, $Al(OH)_3$, $CaCO_3$ and $Mg(OH)_2$ on the same weight-to-weight basis. A series of compounds with differing ratios of metal ions (Fe:Mg/Ca 1:2 or 1:3) gave analytically similar ratios to those predicted from the initial amounts added. CTFeCa bound > 90% phosphate in aqueous solution compared with 65% binding with CTFeMg, 85% binding with $Mg(OH)_2$, and less than 30% binding for $CaCO_3$ and $Al(OH)_3$. The mixed metal compounds also bound up to 80% phosphate in various food matrices, which was relatively independent of changes in pH, compared with $Mg(OH)_2$, where binding decreased from 85% at pH 3.0 to 25% at pH 8.0. $Al(OH)_3$ and $CaCO_3$ were relatively ineffective phosphate binders under all the conditions tested. The mixed metal hydroxides compounds show considerable promise as phosphate binders over those currently available and warrant further patient-based in-vivo testing.

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Introduction

Hyperphosphataemia, defined as serum phosphate greater than 1.7 mmol L^{-1}, in patients with end-stage renal failure, is a well recognized complication which may lead to secondary hyperparathyroidism and renal osteodystrophy (Rutherford et al 1977; Ghazali et al 1993; Knochel & Agarwal 1996). Treatment of hyperphosphataemia involves administration of compounds to form insoluble phosphate complexes in the gastrointestinal tract, thus preventing absorption of inorganic phosphate. A number of aluminium-containing compounds have been used in the past as phosphate binders. However, evidence shows that the aluminium absorbed from the gastrointestinal tract after dosing with such compounds is not removed by dialysis (Powell & Thompson 1993). Thus, haemodialysis patients are prone to accumulate aluminium (Davenport & Roberts 1989; Salusky et al 1991), which may cause dialysis encephalopathy and other long-term haematological and neurological problems (Alfrey et al 1976). The current binders of choice are calcium-containing salts, such as calcium carbonate and calcium acetate, which have been used with

varying degrees of success (Llach & Bover 1996). Other studies have reported the use of magnesium hydroxide and the potential of lanthanum salts (Guillot et al 1982; O'Donovan et al 1986; Oe et al 1987; Dewberry et al 1997), and relatively newer complexes such as the polymeric gel (Goldberg et al 1998) and an iron oxide complex (Hergesell & Ritz 1999). Dietary restriction of phosphate has been suggested as an alternative treatment, although patient compliance in the long-term may be a problem due to the unpleasant nature of the diet (Knochel & Agarwal 1996).

There is therefore a need for a high-capacity phosphate binder that can be administered over long time periods without toxicity problems. Here we report in-vitro results comparing the mixed metal hydroxy-carbonate compounds CT100 (a hydrotalcite), CTFeCa and CTFeMg (where CT is a code of Crosfield Co Ltd, Warrington UK), with the conventional phosphate binding compounds $Al(OH)_3$, $Mg(OH)_2$ and $CaCO_3$. The hydrotalcite CT100, ($Al_2Mg_6(OH)_{16}.CO_3.4H_2O$, a double-layered hydroxide) has been previously shown in in-vitro studies to be a potential alternative phosphate binder (Ookubo et al 1992; Rankin 1997). CTFeCa and CTFeMg complexes were synthesized using a procedure established for laboratory hydrotalcite synthesis substituting a ferric source for the aluminium and, in the case of CTFeCa, a calcium source for magnesium.

Materials and Methods

All chemicals were obtained from BDH (Poole, UK). CT100 was obtained from Crosfield UK Ltd (Warrington, UK). MCT PEPTIDE 2+ (a synthetic composite food mixture containing 210 mg phosphorus, 13.8 g protein, 59 g carbohydrate and 18 g fat per 100 g of mixture) was obtained from Scientific Hospital Supplies (Liverpool, UK). Phosphate, iron and calcium were measured using standard Boehringer Mannheim chemistries on a Hitachi 747 analyser. Magnesium was measured using atomic absorption spectrometry (model 2280; Perkin Elmer, High Wycombe, UK). Water aluminium concentrations were measured using a modified fluorescence procedure (Ioannou & Piperaki 1986) on a Perkin Elmer LS2 filter fluorimeter or graphite furnace atomic absorption spectrometry on a Varian Spectra AA-400 (Varian Instruments, Warrington, UK).

Production of the CT compounds

CT compounds were made using a standard laboratory procedure for synthesizing hydrotalcite $Al_2Mg_6(OH)_{16}.CO_3.4H_2O$ (1:3 $metal^{3+}/metal^{2+}$ ratio) (Reichle 1986). CTFeMg compounds contained ferric iron as the $metal^{3+}$ cation and magnesium as the $metal^{2+}$ cation, and CTFeCa contained calcium as the $metal^{2+}$ cation. By changing the ratio of the $metal^{3+}/metal^{2+}$ cations to 1:1, 1:2, 1:3 and 1:5, materials of different composition were produced and in all cases, CO_3^{2-} was the exchangeable anion.

The composition of the reagents for CT compound synthesis was dependent on the final $metal^{3+}/metal^{2+}$ ratio (X) required. $Metal^{3+}$ sulfate (2 mol) and $metal^{2+}$ sulfate (2(X) mol) were dissolved in 4 L de-ionized water. In a separate 4 L, 4(X) mol NaOH and 5 mol Na_2CO_3 were dissolved. Both solutions were pumped using peristaltic pumps into a flask with an overflow at approximately 2 L and constantly mixed. The rate of addition of the solutions was such that the mixed solution had a pH of 10.0–10.5. After discarding the first litre, 3–4 L of overflowing slurry was collected. The slurry was heated to 80°C for 2 h (aged) or left as collected (unaged), vacuum filtered using a Buchner, washed with de-ionized water and re-filtered leaving a wet cake. Compounds were dried by heating the wet cake to constant weight at 50°C and powdered using a mortar and pestle.

X-ray diffraction studies to establish the structural arrangement of the complexes were kindly carried out at Crosfield UK Ltd, using a Siemens X-ray spectrometer model D-5000 (Siemens, Manchester, UK).

Phosphate binding in aqueous solution

Phosphate binding was measured in a 20 mmol L^{-1} KH_2PO_4 solution adjusted to pH 3 with 1 M HCl. Phosphate solutions at pH 7 or pH 8 were produced by mixing appropriate amounts of 20 mmol L^{-1} KH_2PO_4 and 20 mmol L^{-1} K_2HPO_4 solutions. A known weight of binding compound (0.01–1 g) was added to 25 mL 20 mmol L^{-1} phosphate solution, pH 3, pH 5 or pH 7. These were mixed to ensure homogeneity, gently agitated for 30 min at 20°C and centrifuged at 3000 rev min^{-1} for 5 min. Phosphate, pH and, where appropriate, aluminium, calcium and magnesium, were measured in the supernatant following filtration through 0.2-μm Millipore filters.

Phosphate binding in food

Phosphate binding was measured in a breakfast meal consisting of 50 g cornflakes, 250 g semi-skimmed milk, 2 slices wholemeal bread and 5 g Marmite. These were

mixed in a stomacher for 30 min with 100 mL 0.01 M HCl. Slurry (10 mL) was removed and 2 g of phosphate binder added to the bulk slurry. Samples (5 mL) were taken after 30 and 60 min of mixing and immediately centrifuged to remove retained phosphate. The free phosphate was measured in the supernatant.

Binding was also measured in the synthetic diet MCT PEPTIDE 2+ which was mixed to a 20% (w/v) slurry in 0.01 M HCl, conditions chosen to mimic the stomach pH. Separate 0.2 g samples of each binder were mixed with 20 mL food slurry and gently agitated for 30 min. A 10 mL sample was removed, centrifuged for 10 min at 3000 rev min^{-1} and the phosphate in solution measured. To the remaining 10 mL slurry/binder mixture, 10 mL 70 mmol L^{-1} $NaHCO_3$, 70 mmol L^{-1} NaCl and 10 mmol L^{-1} KCl were added, to mimic the pH and ionic concentration of pancreatic bicarbonate secretions. After gentle agitation for 30 min at 20°C, phosphate was measured in the supernatant after centrifugation. Phosphate bound was expressed as a percentage of phosphate in the control, that is, solution without addition of binder. Differences between groups were measured using analysis of variance and Student's *t*-test and a value of $P < 0.05$ was considered significant.

Results

Analysis of the CT compounds

CTFeCa was made with Fe^{3+}/Ca^{2+} ratios of 1:1, 1:2, 1:3 and 1:5. CTFeMg was made with Fe^{3+}/Mg^{2+} ratios of 1:2 and 1:3. X-ray diffraction studies demonstrated the CTFeMg compounds had a distinct double-layer structure, similar to CT100 and typical of hydrotalcites, whereas the CTFeCa compounds were amorphous. Analysis of the metal compositions of the CT compounds after acid hydrolysis demonstrated the actual incorporated $metal^{3+}/metal^{2+}$ ratio (Table 1). For simplicity, these are referred to by the predicted $metal^{3+}/metal^{2+}$ ratios.

Effect of hydrothermal ageing, drying, and $metal^{3+}/metal^{2+}$ ratio on phosphate binding

The effect of ageing and drying the CT compounds on phosphate binding was ascertained by measuring the percentage of phosphate precipitated from 25 mL of a 20 mmol L^{-1} phosphate solution by 0.5 g of compound. For binders in the wet cake form, hydration was accounted for; for example, if a compound contained 50% water, 1 g was used.

Table 1 Predicted and actual $metal^{3+}/metal^{2+}$ ion ratios in CTFeCa and CTFeMg.

Compound	Predicted $metal^{3+}/metal^{2+}$	Actual $metal^{3+}/metal^{2+}$
CTFeCa	1:1	1:1.3
CTFeCa	1:2	1:1.6
CTFeCa	1:3	1:2.6
CTFeCa	1:5	1:1.3
CTFeMg	1:2	1:1.7
CTFeMg	1:3	1:2.3

The poor agreement between analysed and predicted values for CTFeCa 1:5 suggested a compound of an indefinable nature and therefore was not tested further.

At each pH studied, the CTFeMg preparations with Fe^{3+}/Mg^{2+} ratios of 1:2 and 1:3 bound more phosphate in the wet cake form than the dry powder form. There was little difference between the aged and unaged preparations (Table 2). Comparisons of phosphate binding efficiency showed little difference between CTFeMg 1:3 and CTFeMg 1:2 at each pH studied. The CTFeMg 1:3 unaged wet cake preparation bound more phosphate at pH 3 than any other preparation and is subsequently classified as CTFeMg for further experiments.

For the CTFeCa preparations with Fe^{3+}/Ca^{2+} ratios of 1:2 and 1:3, there was no difference in the efficiency of phosphate binding between unaged and aged preparations or the wet cake and dry powder preparations (Table 2) at each pH studied. The further experiments reported were carried out using the CTFeCa 1:3 wet unaged preparation (CTFeCa) for comparison with the wet unaged CTFeMg 1:3 sample.

Phosphate binding by CT compounds, $Al(OH)_3$, $CaCO_3$ and $Mg(OH)_2$ in aqueous solution

Effect of pH

Figure 1 shows the relationship between phosphate binding and solution pH for the compounds CTFeCa, CTFeMg, CT100, $Al(OH)_3$, $CaCO_3$ and $Mg(OH)_2$. Results plotted for the CTFeCa and CTFeMg compounds are the means of four different preparations, each tested twice. The results for the remaining compounds are the means of three separate experiments. The percentage phosphate binding by CTFeCa and CT100 was essentially independent of the solution pH. However, CTFeCa bound at least 90% of the phosphate over the pH range 3–8, compared with CT100, which bound at least 61%. CTFeMg displayed some pH-dependency,

Table 2 Effect of ageing and drying CTFeCa and CTFeMg compounds on their phosphate binding capacity (% phosphate bound) at pH 3 and pH 7 in aqueous solution.

$Metal^{3+}/metal^{2+}$ ratio	CTFeCa*		CTFeMg	
	pH 3	pH 7	pH 3	pH 7
1:2 Unaged wet	98.1±1.18	96.8±2.24	64.0±2.33	55.8±2.85
1:2 Aged wet	97.7±2.23	94.7±4.37	66.2±4.23	62.6±3.53
1:2 Unaged dry	95.7±3.44	88.8±8.57	23.4±3.22†,‡	15.9±2.15†,‡
1:3 Unaged wet	95.2±2.85	92.4±6.72	68.1±7.77	53.4±4.17
1:3 Aged wet	94.3±5.48	91.6±5.89	50.9±3.66	54.8±5.41
1:3 Unaged dry	95.0±3.41	92.9±3.94	32.6±5.11†	27.6±7.13†

The results are the mean±s.d. of triplicate readings on two separate occasions. *$P < 0.01$ compared with CTFeMg compounds. †$P < 0.01$ compared with all other preparations. ‡$P = 0.02$ compared with 1:3 unaged dry.

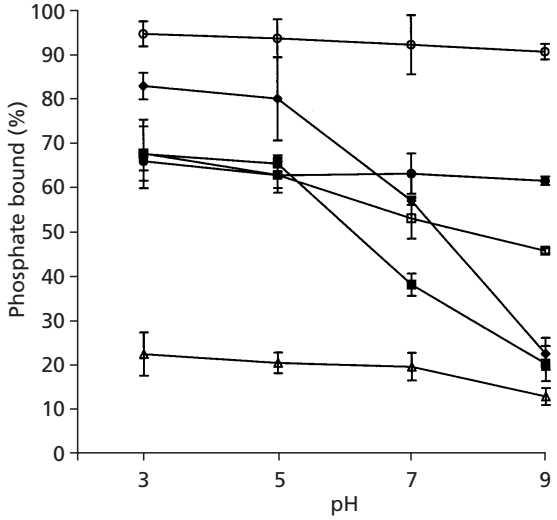

Figure 1 Effect of pH on phosphate binding by CT compounds, $Al(OH)_3$, $CaCO_3$ and $Mg(OH)_2$. Percentage phosphate precipitated from a 20 mmol L^{-1} sodium phosphate solution at pH 3, 5, 7 and 8 by CTFeCa 1:3 unaged wet (○), CTFeMg 1:3 unaged wet (□), CT100 (●), $CaCO_3$ (■), $Al(OH)_3$ (△) and $Mg(OH)_2$ (◆).

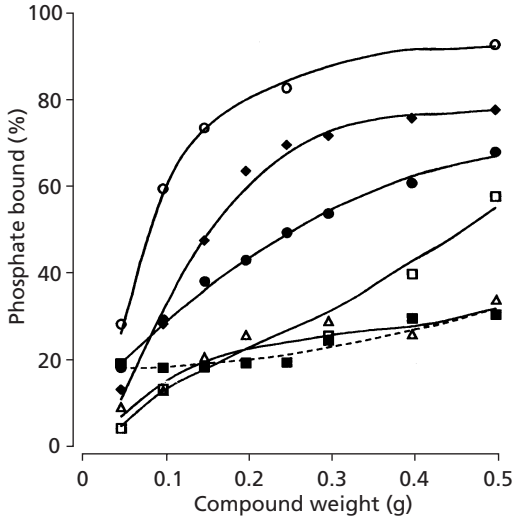

Figure 2 Effect of compound weight on phosphate binding by CT compounds, $Al(OH)_3$, $CaCO_3$ and $Mg(OH)_2$ at pH 3. Percentage phosphate precipitated from a 25 mL 20 mmol L^{-1} sodium phosphate solution pH 3 by 0.05–0.5 g CTFeCa 1:3 unaged wet (○), CTFeMg 1:3 unaged wet (□), CT100 (●), $CaCO_3$ (■), $Al(OH)_3$ (△) and $Mg(OH)_2$ (◆).

binding 65% of the phosphate at pH 3 and 46% at pH 8. Both $CaCO_3$ and $Mg(OH)_2$ were effective binders at pH 3, precipitating 70% and 85% of the phosphate, respectively, but were affected by pH, and at pH 8 bound only 20% and 25% phosphate respectively. $Al(OH)_3$ was relatively ineffective at each pH studied.

Effect of compound weight

Dose–response curves were constructed between 0.05 and 0.5 g compound in phosphate solution pH 3 (Figure 2). CTFeCa was the most efficient phosphate binder. The order of binding of the remaining compounds was $Mg(OH)_2$ > CT100 > CTFeMg > $CaCO_3$ and $Al(OH)_3$ (Figure 2).

The final buffered pH was measured after phosphate binding. $Mg(OH)_2$ buffered the phosphate solution to pH 8–11, CT100 and CTFeMg to pH 7–8, CTFeCa and $CaCO_3$ to pH 5.5–6.5, and $Al(OH)_3$ to pH 3–5.

The amount of iron released into solution after phosphate binding by CTFeMg in aqueous solution was not

Table 3 Soluble magnesium and aluminium from 0.05 to 0.5 g CT100, $Mg(OH)_2$ and $Al(OH)_3$ after phosphate binding at pH 3.

Metal ion release	Weight of CT100 (g)			Weight of $Mg(OH)_2$ (g)			Weight of $Al(OH)_3$ (g)		
	0.05	0.25	0.5	0.05	0.25	0.5	0.05	0.25	0.5
Al^{3+} (μg L^{-1})	66	21	28	–	–	–	41183	3189	144
Mg^{2+} (mmol L^{-1})	2.1	2.8	2.8	4.2	0.12	0.12	–	–	–

Values are the mean of triplicate readings.

greater than 0.42 mmol L^{-1}, and in the case of CTFeCa was below the assay detection limit of 0.001 mmol L^{-1}. The amount of aluminium in solution was measured over a range of doses of $Al(OH)_3$ and CT100, with the highest concentrations, 41183 μg L^{-1} and 66 μg L^{-1}, respectively, being observed at 0.05 g (Table 3). In the case of $Al(OH)_3$, the amount of aluminium in solution decreased significantly as the amount of binder increased. For CT100, although the aluminium in solution decreased as the quantity of CT100 increased, there was no clear relationship between the aluminium in solution and the amount of CT100 used. Similarly, the levels of magnesium in solution were highest (4.2 mmol L^{-1}) after phosphate binding with low quantities (0.05 g) of $Mg(OH)_2$, and decreased with increasing amounts of compound. The magnesium concentration in solution after phosphate binding by $Mg(OH)_2$ ranged from 4.2 to 0.12 mmol L^{-1}, and with CT100, from 2.1 to 2.8 mmol L^{-1}.

Phosphate binding in food

The efficiency of phosphate binding in a food slurry was CTFeMg > CTFeCa > CT100 > $Mg(OH)_2$ > $CaCO_3$ > $Al(OH)_3$. The majority of compounds bound phosphate maximally in the first 30 min of incubation (Table 4), with only $Mg(OH)_2$ and $Al(OH)_3$ requiring longer times to exhibit a slight increase in binding.

The use of CT100 caused food slurry aluminium to rise 5.5- and 148-fold after 30 and 60 min incubation, respectively. With $Al(OH)_3$, the slurry aluminium concentration rose 848-fold after 30 min and 1314-fold after 60 min, compared with the initial concentration. Food slurry magnesium concentrations increased 2.1- and 2.4-fold, and 1.5- and 1.6-fold after 30 and 60 min incubation with $Mg(OH)_2$ and CT100, respectively, compared with the initial measurement. In the case of CTFeMg, only a slight increase was observed even after 60 min incubation (Table 5). Table 6 shows that food slurry iron concentration increased 2.6-fold and 8.1-fold after 60 min treatment with CTFeMg and CTFeCa, respectively. Calcium concentration increased by less than 10% after 60 min incubation with CTFeCa. However, the initial calcium concentration was high due to the nature of the food studied (milk).

The capacity of these compounds to bind phosphate was also assessed in the food medium MCT PEPTIDE 2+. This was solubilized in 0.01 M HCl to mimic the pH of stomach secretions. Dose relationships of phosphate binding in the synthetic food mixture showed that $Mg(OH)_2$, CTFeCa, CTFeMg and CT100 were much more effective than $Al(OH)_3$ and $CaCO_3$ (Figure 3), and the final pH readings after the binding experiments were reasonably similar at pH 8.7, 7.1, 7.1, 6.9, 6.1 and 6.0, respectively. In a separate series of experiments, after addition of the binding compound to the synthetic

Table 4 Time-course of phosphate binding in a breakfast food slurry by CT compounds, $Mg(OH)_2$, $Al(OH)_3$ and $CaCO_3$.

Time (min)	Phosphate bound (%)					
	CTFeCa	CTFeMg	CT100	$Mg(OH)_2$	$Al(OH)_3$	$CaCO_3$
30	53.1 ± 17.1	53.6 ± 7.2	42.5 ± 14.1	8.5 ± 2.1†	2.6 ± 1.3†	14.3 ± 1.4†
60	50.8 ± 12.7	53.4 ± 1.4	38.7 ± 9.8*	17.5 ± 7.7†	8.4 ± 3.5†	12.5 ± 3.5†

The results are the mean ± s.d. of triplicate readings on two separate occasions. *$P < 0.05$ compared with other CT compounds. †$P < 0.01$ compared with CT compounds.

Table 5 Breakfast food slurry aluminium and magnesium concentrations after phosphate binding by CT100, $Al(OH)_3$, $Mg(OH)_2$ and CTFeMg.

Time (min)	Mg^{2+} (mmol L^{-1})			Al^{3+} (μg L^{-1})	
	CT100	CTFeMg	$Mg(OH)_2$	CT100	$Al(OH)_3$
0	5.1	5.04	6.8	183	105
30	7.4	5.9	13.7	1005	8.9×10^4
60	8.2	6.4	16.1	27000	1.38×10^5

Conditions as shown in Figure 4. Values are the mean of triplicate readings.

Table 6 Breakfast food slurry iron and calcium concentrations after phosphate binding by CTFeCa and CTFeMg.

Time (min)	Fe^{3+} (mmol L^{-1})		Ca^{2+} (mmol L^{-1})
	CTFeMg	CTFeCa	CTFeCa
0	0.12 ± 0.04	0.48 ± 0.05	22.43 ± 2.67
30	0.18 ± 0.03	1.55 ± 0.37*	25.05 ± 0.83
60	0.32 ± 0.10	3.88 ± 0.36†	25.74 ± 0.57

The results are the mean ± s.d. of triplicate readings on two separate occasions. *$P < 0.05$ compared with other times for CTFeCa and same time for CTFeMg. †$P < 0.01$ compared with time 0 for CTFeCa and same time for CTFeMg.

food mixture and mixing for 30 min, a sample was removed and phosphate measured in the supernatant after centrifugation. A further sample was removed and diluted 1 : 1 with a bicarbonate solution to model the pH of pancreatic secretions. CT100 bound 71.5 ± 2.0 % (n = 3) of phosphate in the model stomach conditions, and the model intestine conditions (i.e. the addition of bicarbonate) caused some release of the bound phosphate (34 .0 ± 17.0 %, n = 3). CTFeCa and CTFeMg respectively bound 59.3 ± 1.2 % and 55.3 ± 1.8 % of phosphate, and 50.3 ± 4.3 % and 39.9 ± 9.5 %. $Mg(OH)_2$ initially bound 51.6 ± 9.2 % phosphate, and 57.9 ± 19.9 % after bicarbonate addition. Both $Al(OH)_3$ and $CaCO_3$ were ineffective binders in the MCT PEPTIDE 2+ medium with or without the addition of bicarbonate (Figure 4).

The solution iron concentration in the MCT PEPTIDE 2+ medium after incubation with CTFeMg was 17-fold higher than the control for both the initial measurement and after the addition of bicarbonate, whereas the solution iron concentration with CTFeCa was similar to the control before and after addition of

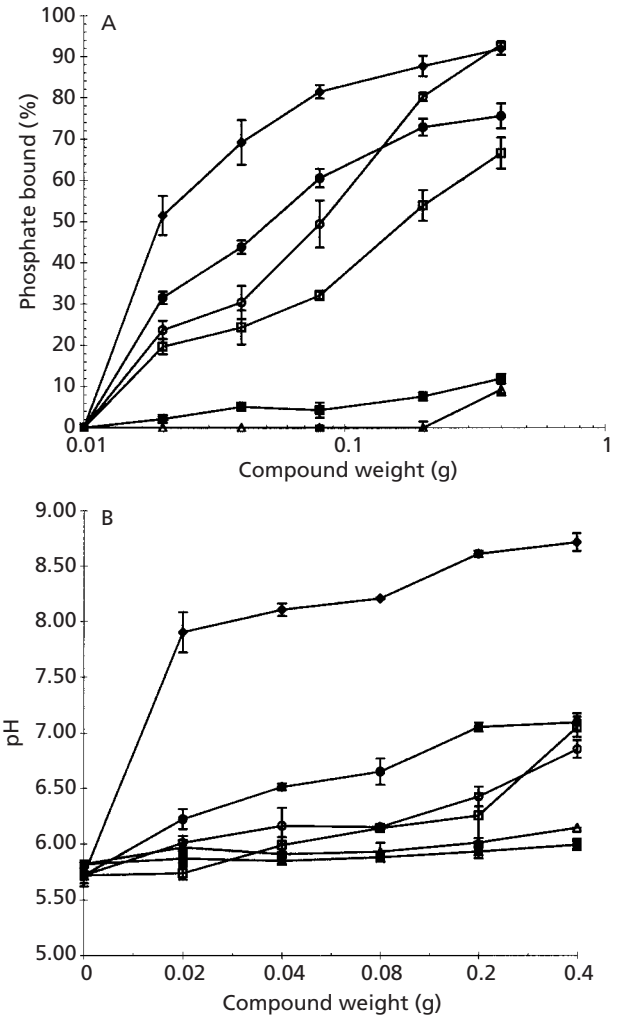

Figure 3 Dose–response relationships with the MCT PEPTIDE 2+ solution before addition of bicarbonate. A. Percentage phosphate precipitated from MCT PEPTIDE 2+ (5 mL). CTFeCa 1 : 3 unaged wet (○), CTFeMg 1 : 3 unaged wet (□), CT100 (●), $CaCO_3$ (■), $Al(OH)_3$ (△) and $Mg(OH)_2$ (◆). B. Final pH for each solution; note the buffering of the initial acid with the food mixture to pH 5.6–5.7.

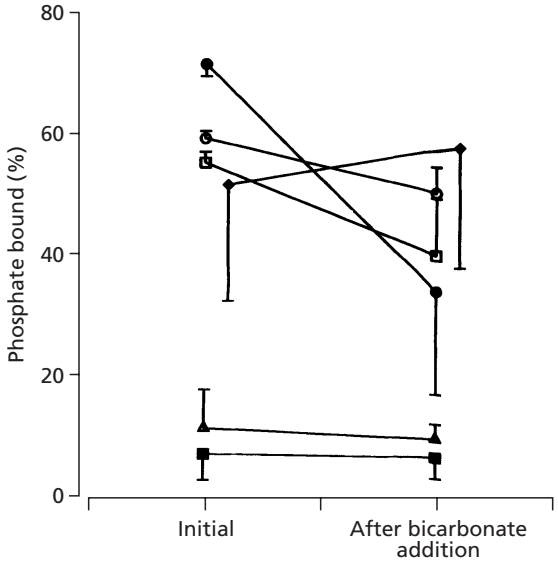

Figure 4 Percentage phosphate bound in MCT PEPTIDE 2+ by CT compounds, $Al(OH)_3$, $CaCO_3$ and $Mg(OH)_2$ after alkalinization by addition of bicarbonate buffer. Percentage phosphate precipitated from 20 mL 20% (w/v) MCT peptide 2+ in 0.01 M HCl by 0.2 g CTFeCa 1:3 unaged wet (○), CTFeMg 1:3 unaged wet (□), CT100 (●), $CaCO_3$ (■), $Al(OH)_3$ (△) and $Mg(OH)_2$ (◆). Values are the mean ± s.d. for four repeat experiments.

bicarbonate. The calcium release by CTFeCa was increased by approximately 2.5-fold, although the initial concentration was low (2.0 mmol L^{-1}).

Discussion

Hyperphosphataemia is problematic in 64% of patients receiving haemodialysis (Ansell & Feest 1997). Phosphate absorption is limited by the ingestion of compounds which retain phosphate in the gastrointestinal lumen. The current compounds of choice, calcium carbonate and calcium acetate, can however cause hypercalcaemia. Additionally, calcium carbonate is an inefficient binder, and can be required in doses of up to 20 g daily to be effective (Schaefer et al 1988). Aluminium hydroxide, the compound of choice for many years, is associated with dialysis dementia, osteomalacia and microcytic anaemia, and consequently is only used in cases where the serum phosphate concentration can not be controlled by other compounds (Cournot-Witmer et al 1979; O'Hare & Murnaghan 1982). Magnesium carbonate has been suggested as a viable alternative (O'Donovan et al 1986), and previous studies have indicated the potential of the hydrotalcite $Al_2Mg_6(OH)_{16}.CO_3.4H_2O$ (CT100) as a phosphate binder (Ookubo et al 1992; Rankin 1997).

This study has confirmed that the hydrotalcite CT100 is an effective phosphate binder in-vitro. However, if used in-vivo, an increased release of aluminium, similar to the amounts measured in the food slurry, would be of concern. Even if only approximately 0.1% of an aluminium load is absorbed this could lead to a substantial burden over time (Powell & Thompson 1993). Urinary aluminium excretions from four normal individuals, taking 6 g CT100 or $Al(OH)_3$ for one day, were over the range of 50 to 150 μg L^{-1} and were approximately 10-fold higher than normal urinary aluminium excretion (5 to 30 μg L^{-1}) (data not published).

Thus, we endeavoured to produce a compound that has the benefits of CT100 (e.g. pH-independent, high-capacity binding) without the associated toxicity concerns (e.g. aluminium release). We developed a series of compounds using the same procedure for the laboratory synthesis of hydrotalcite itself, but with Al^{3+} substituted by Fe^{3+} and the metal^{2+} cation being Ca^{2+} or Mg^{2+}. Studies showed the actual incorporation ratio of metal ions into CTFeCa and CTFeMg compounds broadly correlated with the predicted ratio. Alteration of the metal^{3+}/metal^{2+} ratio did not appreciably alter phosphate binding by either the CTFeCa or the CTFeMg preparations. All the CTFeMg preparations on X-ray diffraction showed structures similar to CT100, suggesting they were double-layered hydroxides. Indeed, this is likely as these compounds with the formula $Fe_2Mg_6(OH)_{16}.CO_3.4H_2O$ occur naturally (Calino 1987). The CTFeCa preparations had amorphous structures on X-ray diffraction analysis, unlike either CTFeMg or CT100. Also we found no reference in the literature to a double-layered hydroxide composed of ferric iron and calcium occurring either naturally or synthetically.

The duodenum and jejunum have the highest phosphate absorption rate in the intestinal tract, although due to the longer transit time of luminal contents, the terminal ileum can contribute substantially to total phosphate uptake (Kayne et al 1993). An ideal binder would therefore be capable of precipitating phosphate across the pH range (pH 1–8) present in the gastrointestinal tract. Additionally, the phosphate bound at low pH in the stomach should not be released on contact with the high-pH duodenal secretions.

In an aqueous phosphate solution, all the CT compounds bound phosphate independently of pH, although CTFeCa was clearly the most effective. $Mg(OH)_2$ was an effective binder, although in-vivo its high-buffering capacity may limit further phosphate

binding if high pH is produced. In-vivo studies with this latter compound have in fact shown it to be an ineffective binder, presumably because it is poorly tolerated (Oe et al 1987; Salusky et al 1991). In both the food slurry and MCT PEPTIDE 2+ experiments, the CT compounds bound more phosphate than $Mg(OH)_2$, $CaCO_3$ and $Al(OH)_3$. After the addition of a bicarbonate solution to model pancreatic secretions, all compounds, with the exception of CT100, retained the majority of the phosphate bound in MCT peptide 2+ under acid conditions. Thus, both CTFeCa and CTFeMg can cause phosphate precipitation in the acid conditions of the stomach and alkali conditions of the jejunum and ileum, thus reducing the amount of phosphate available for absorption.

Solubilization of the compounds was of concern as phosphate binders can be administered in-vivo over long periods of time with possible accumulation of metal ions. There was an increase in the soluble iron (Fe^{3+}) measured in both food slurry and MCT PEPTIDE 2+ with the use of CTFeMg, although not when CTFeCa was used as a binder. The amount of iron released by CTFeMg was relatively low with the MCT PEPTIDE 2+ (0.04 mmol L^{-1} Fe^{3+} above the food concentration of 0.18 mmol L^{-1}). However, as haemodialysis patients are often anaemic and iron supplements are administered, an extra iron intake of this order may be beneficial (Remuzzi & Rossi 1996).

The concentrations of soluble magnesium and calcium were not substantially higher for CTFeMg or CTFeCa in the food slurry or MCT PEPTIDE 2+ when compared with the control. It is unlikely therefore that major toxicity problems would be caused by excessive magnesium or calcium absorption. If necessary, the effect of increased magnesium absorption could be controlled by the use of a low magnesium dialysate while increased calcium ingestion could be of benefit to those renal patients who are hypocalcaemic.

In conclusion, we have developed a number of mixed metal hydroxy-co-precipitates that are effective phosphate binders in-vitro and could have potential in-vivo. These preparations may have limited toxicity and may provide an alternative to the current phosphate binders used to treat patients with chronic renal failure.

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