Title of Project: Averting Complications of Proton Pump Inhibitor Therapy by Effervescent Calcium Magnesium Citrate

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Specific Aims: Proton pump inhibitors (PPIs) are widely used for the control of gastric ulcer-gastritis, erosive esophagitis (gastroesophageal reflux disease), peptic ulcer disease (duodenal ulcer), and heartburn. Despite their efficacy, their use has been implicated in possibly causing fragility fractures (osteoporosis), hypomagnesemia (magnesium deficiency) and increased risk of chronic kidney disease (CKD). The current trial represents our ongoing effort to discern whether these complications could be averted by effervescent calcium magnesium citrate (EffCaMgCit).

In a single-dose bioavailability study, we showed previously that provision of calcium and magnesium in a soluble form as EffCaMgCit improved intestinal absorption of calcium and magnesium and suppressed parathyroid function during PPI treatment, compared with calcium carbonate. In a multidosing trial with esomeprazole 40 mg/day for 28 days, EffCaMgCit suppressed parathyroid function and bone turnover, and increased serum and urinary magnesium, compared with placebo. Moreover, EffCaMgCit co-administered with PPI conferred an alkali load, and averted apparent acid load conferred by PPI (when given with placebo).

In the current proposal, we wish to conduct a 2-year treatment trial, directed at obtaining more definitive evidence that EffCaMgCit overcomes all three complications of PPI.

Aim 1. To test the hypothesis that EffCaMgCit would prevent/treat osteoporosis, by suppressing parathyroid function and bone resorption, thereby stabilizing bone mineral density (BMD). The critical endpoint will be overall change in BMD T-Score and Z-Score from baseline to the end of study. Secondary endpoints will be the change in serum PTH and C-terminal telopeptide (CTX).
Aim 2. To test the hypothesis that EffCaMgCit would prevent/treat hypomagnesemia/magnesium deficiency, by providing bioavailable magnesium. The critical endpoint will be the overall change in the fractional excretion of magnesium (FEMg) and free muscle magnesium by MRS from baseline to the end of study. Secondary endpoints will be the change in serum and urinary magnesium.
Aim 3. To test the hypothesis that EffCaMgCit would reduce the risk of CKD during PPI use by averting putative hypomagnesemia/magnesium deficiency and neutralizing acid load. We propose that PPI causes hypomagnesemia/magnesium deficiency and confers an acid load, -factors implicated for incident CKD and its progression. EffCaMgCit is expected to avert incident CKD by providing bioavailable magnesium and alkali load. Critical endpoints will be the overall change in endogenous creatinine clearance, FEMg, free muscle magnesium and net acid excretion, a measure of acid-base status.

Circumstances Leading to This IND: Since 2012, our group has explored the therapeutic potential of EffCaMgCit in overcoming the complications of prolonged PPI use, namely osteoporosis, hypomagnesemia and risk of CKD. (Since adverse symptoms sometimes accompanied hypomagnesemia, the term magnesium deficiency more aptly defines this

complication.) The initial studies with EffCaMgCit were conducted without IND but with the approval of UTSW's IRB, which decreed that calcium magnesium citrate is already available in the marketplace and would not require a special authorization from the FDA to conduct a research study. To make EffCaMgCit, we contracted Sterling Pharmaceutical Services, Inc (Dupo, IL), which followed all the FDA's guidelines for Chemistry, Control and Manufacturing. However, Sterling did not use ingredients with drug master file (DMF) and letter of authorization (LOA).

The first study was a single-dose bioavailability protocol (IRB #082012-030). In healthy subjects, intestinal absorption of calcium and magnesium was estimated from the rise in serum and urinary magnesium following a single oral load of calcium carbonate or EffCaMgCit while taking placebo or omeprazole (OMP) 20 mg bid for 1 week. Calcium absorption from calcium carbonate was much lower on OMP compared with placebo, and magnesium absorption from calcium carbonate was negligible on both OMP and placebo. In contrast, calcium absorption from EffCaMgCit was greater than from calcium carbonate on placebo; it was undiminished by OMP. EffCaMgCit also provided bioavailable Mg on both OMP and placebo. OMP attenuated parathyroid suppression (measured from changes in serum PTH) from calcium carbonate, but it did not affect parathyroid suppression from EffCaMgCit.

The second study was a multidosing protocol (IRB # 112014-057). The effect of EffCaMgCit was compared with placebo in a crossover trial in 22 subjects taking PPI at twice the usual dose (esomeprazole 40 mg/day) for 28 days, representing the recommended treatment regimen for erosive esophagitis-gastritis. While on PPI, EffCaMgCit increased serum calcium, thereby reducing serum PTH and serum CTX, a bone resorption marker; it also raised serum and urinary magnesium.

The preceding trials conducted without IND showed that EffCaMgCit provided bioavailable calcium and magnesium, reduced bone resorption, and increased serum and urinary magnesium in subjects taking PPI. However, we did not obtain evidence that osteoporosis was stabilized or prevented, or that magnesium deficiency was averted. To do so, we would have to show effects on bone mass and tissue content of magnesium. Moreover, the foregoing studies did not address another complication of PPI use - increased risk of CKD. Though theoretically possible, there is so far no evidence that EffCaMgCit prevents incident CKD.

We envision that another trial (a long-term treatment, possibly considered as Phase III) would be required to more firmly establish the value of EffCaMgCit in the prevention/treatment of osteoporosis, magnesium deficiency and CKD during PPI use. We would like to conduct such a trial under IND, using ingredients with DMF and LOA.

We have followed the same steps with another formulation of EffCaMgCit, with the same Mg content but lower Ca to accommodate special need of CKD. After acquiring promising data from single-dose bioavailability and feasibility multidosing study using IRB-approved protocols, we are now conducting a more definitive study under IND (#139613).

Background and Significance: PPIs are effective inhibitors of gastric acid secretion. Thus, they are widely used for the control of gastric ulcer-gastritis, erosive esophagitis (gastroesophageal

reflux disease), peptic ulcer disease (duodenal ulcer), and heartburn. PPIs are among the most commonly used drugs worldwide, with annual sales in the USA alone exceeding $10 billion.

However, several population-based studies have reported increased risk of skeletal fractures during prolonged PPI use (Roux, 2009). Moreover, hypomagnesemia has been reported during PPI treatment (Kuipers, 2009). The symptomatic presentation of some of the cases suggested that magnesium deficiency with tissue depletion of magnesium had occurred. The alert by the FDA of these complications led to their inclusion under Warnings & Precautions in the package inserts of PPIs (FDA, 2011; Tamura, 2012). More recently, PPI use has been associated with increased risk of CKD, defined by estimated glomerular filtration rate (eGFR) of less than 60 ml/min (Wijarnpreecha, 2017; Xie, 2017). These reports led to a news alert by a leading nephrology society (ASN, 2017).

For each complication, we shall review studies conducted to date on how EffCaMgCit might be useful in overcoming these disturbances. We shall then cite additional studies needed to obtain further validation.

To discuss complication of osteoporosis first, a plausible explanation is the induction by PPI of hypo- or achlorhydria that impairs the solubility of calcium salts and hence the calcium bioavailability. The consequent stimulation of parathyroid function would cause bone loss leading eventually to fragility fractures. To prove this scheme, the inventors conducted a multi-dosing trial, in which the effect of EffCaMgCit was compared with placebo in a crossover trial in 22 subjects taking PPI at twice the usual dose (esomeprazole 40 mg/day) for 28 days, representing the recommended treatment format for erosive esophagitis-gastritis. Subjects took a dose of EffCaMgCit (containing 19 meq Ca and 10 meq Mg) or placebo twice per day, along with esomeprazole 40 mg per day.

Recognizing short $t_{1/2}$ of EffCaMgCit, a venous blood sample was obtained at 4 hours after the morning dose of EffCaMgCit or placebo after 2 weeks and 4 weeks of treatment. Serum calcium was significantly higher after EffCaMgCit than placebo, with virtually every subject showing a higher value (Fig. 1). In the Placebo group (while on esomeprazole), a low or marginally low serum calcium (< 9 mg/dL) was encountered in 23% of subjects at 2 weeks and in 18% of subjects at 4 weeks. After EffCaMgCit (with esomeprazole), none of the patients had low or marginally low serum calcium. Thus, not only did EffCaMgCit raise serum calcium, but it also corrected low or marginally low serum calcium; EffCaMgCit *treated* hypocalcemia or marginal hypocalcemia from PPI therapy.

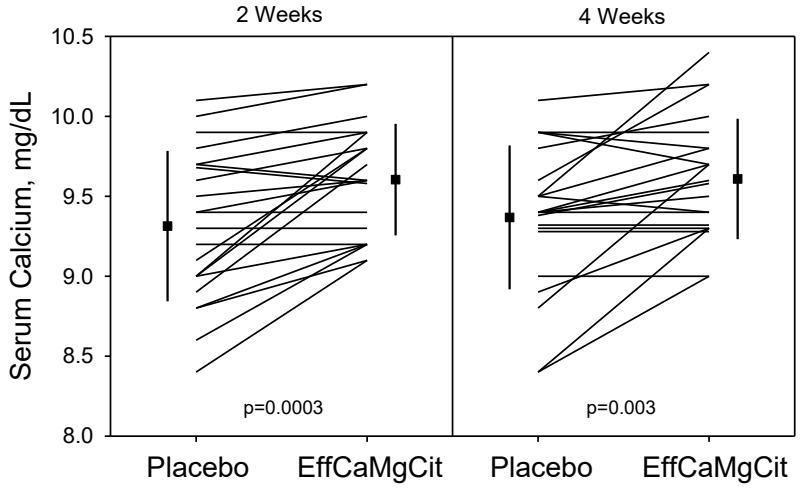

Fig. 1. Comparison of serum calcium between Placebo and EffCaMgCit after 2 weeks and 4 weeks of treatment along with PPI. Solid square and vertical line indicate mean ± SD. Data from the completed multidosing trial with EffCaMgCit in 22 subjects.

In the same multi-dosing trial, serum PTH was significantly lower after 2 weeks and 4 weeks of treatment with EffCaMgCit (plus esomeprazole) compared with placebo (plus esomeprazole) (Fig. 2). Prevalence of high serum PTH (> 65 pg/ml) was much lower during 2 weeks and 4 weeks of treatment with EffCaMgCit (plus esomeprazole) compared with placebo (plus esomeprazole). Thus, EffCaMgCit *treated* hypocalcemia from esomeprazole. While on placebo (esomeprazole alone), a higher number of subjects had high serum PTH at 2 weeks (n = 15) and 4 weeks (n = 10), indicating that some subjects acquired secondary hyperparathyroidism on esomeprazole. On EffCaMgCit (plus esomeprazole), fewer subjects had high serum PTH at 2 weeks (n = 4) and at 4 weeks (n = 3). Thus, EffCaMgCit *prevented* development of secondary hyperparathyroidism from PPI.

At both 2 weeks and 4 weeks of treatment, serum CTX was significantly lower on EffCaMgCit (plus esomeprazole) compared with placebo (plus esomeprazole). Six subjects had high serum

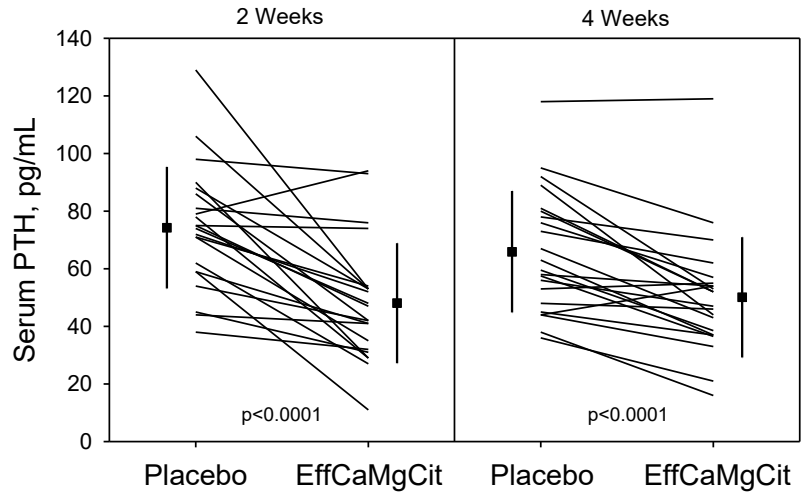

Fig. 2. Comparison of serum PTH between Placebo and EffCaMgCit after 2 weeks and 4 weeks of treatment along with PPI. Solid square and vertical line indicate mean ± SD. Data from the completed multidosing trial with EffCaMgCit in 22 subjects.

CTX at both 2 weeks and 4 weeks of placebo (on esomeprazole)(Fig. 3). On EffCaMgCit (plus

esomeprazole), high serum CTX was found in none of the subjects at 2 weeks and only one subject at 4 weeks. Thus, EffCaMgCit *treated* excessive bone loss from PPI.

CTX is a degradation product of bone collagen; it is released into the circulation when bone is resorbed by osteoclasts. Serum CTX is an excellent biochemical marker for bone resorption. Serum CTX is used commonly to evaluate response to anti-osteoporosis drugs. However, CTX is a static measure of bone resorption; it does not reflect the amount of bone mass. BMD, a measure of bone mass, is strongly correlated inversely with the rate of fractures. BMD has been used to define osteoporosis from the degree of decline from the peak value. In the proposed trial, we plan to measure BMD in L2-L4, femoral neck and distal radius.

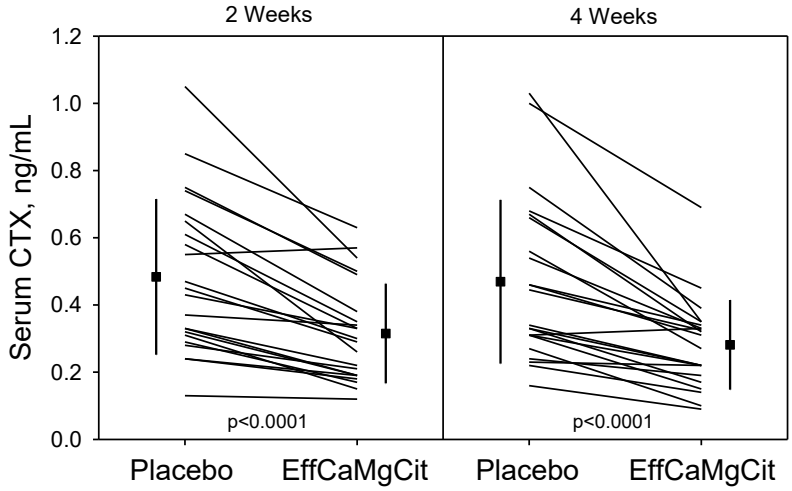

Fig. 3. Comparison of serum CTX between Placebo and EffCaMgCit after 2 weeks and 4 weeks of treatment along with PPI. Solid square and vertical line indicate mean ± SD. Data from the completed multidosing trial with EffCaMgCit in 22 subjects.

We shall now discuss the second complication of PPI. In the FDA's Adverse Event Reporting System in 2012, the odds ratio of hypomagnesemia was 2.76 between omeprazole use and non-omeprazole use (Tamura, 2012). The risk increased with longer duration of PPI use.

A prominent cause for the development of hypomagnesemia which we ourselves championed is the induction of hypo- or achlorhydria by PPI that impairs the solubility and hence the bioavailability of magnesium salts (Kuipers, 2009). PPI might also inhibit magnesium absorption by impairing passive absorption of magnesium (Thongon, 2011) or by promoting bicarbonate secretion (Mertz-Nielson, 1996).

By delivering magnesium in a soluble form, EffCaMgCit should overcome the low solubility of magnesium salts resulting from PPI-induced inhibition of gastric acid secretion or promotion of bicarbonate secretion; it might also accommodate inhibition of passive magnesium absorption by PPI. In our aforementioned multi-dosing study with esomeprazole 40 mg per day, serum magnesium (obtained at 4 hours after the morning dose) was significantly higher after EffCaMgCit than after placebo (Fig. 4). At baseline (before starting esomeprazole), 9% of subjects had hypomagnesemia or marginal hypomagnesemia (< 2 mg/dL). During treatment with Placebo with esomeprazole, hypomagnesemia or marginal hypomagnesemia was found in a higher percentage of subjects (14% at 2 weeks, and 23% at 4 weeks). During treatment with EffCaMgCit with esomeprazole, no one had hypomagnesemia or marginal hypomagnesemia. Thus, EffCaMgCit *treated* or *prevented* hypomagnesemia or marginal hypomagnesemia from PPI therapy.

We acknowledge that hypomagnesemia is an inadequate gauge of magnesium deficiency, reflective of depletion of magnesium stores in tissues. It is magnesium deficiency that is

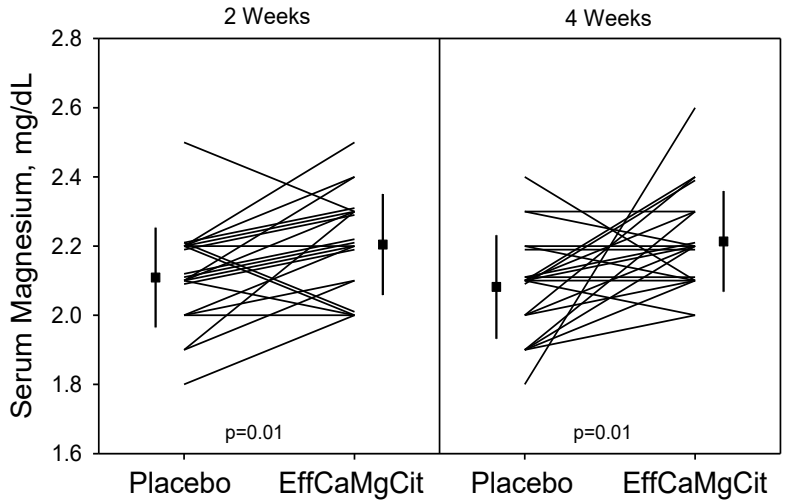

Fig. 4. Comparison of serum Mg between Placebo and EffCaMgCit after 2 weeks and 4 weeks of treatment along with PPI. Solid square and vertical line indicate mean ± SD. Data from the completed multidosing trial with EffCaMgCit in 22 subjects.

responsible for neuromuscular disturbances, arrhythmia, cardiovascular death (Sakaguchi, 2014) and deterioration of renal function (van Laecke, 2013). Serum magnesium may be normal in some cases of magnesium deficiency. FEMg is a good biomarker of systemic Mg deficiency as it exploits the renal response to defend body Mg deficit. In addition, FEMg helps distinguish renal versus extrarenal losses of magnesium (Elisaf, 1997), although the likelihood of PPI inducing renal Mg wasting is very low. Free muscle magnesium might also be an excellent marker of intracellular magnesium deficiency (Irish, 1997). In the proposed trial, we plan to measure FEMg and free muscle magnesium by MRS, as well as serum magnesium.

We shall now discuss the last complication of PPI use, - increased risk of CKD. The evidence for this complication is based largely on observational, association studies. PPI use has been associated with increased incidence of CKD (eGFR < 60 ml/min, representing Stage III or higher), from a meta-analysis of published trials (Wijarnpreecha, 2017) and from a large VA database (Xie, 2017). The fact that PPIs cause acute kidney injury via acute interstitial nephritis is

unequivocal, with a 3-fold risk in PPI users versus non-users. The increased risk for CKD is slightly lower (2-fold). The underlying pathology is not known (Moledinoa 2016, Toth-Manikowsli 2017). It is conceivable that PPI-induced CKD may be due to chronic interstitial nephritis.

A detailed review of the literature and our own data suggests a causal role of hypomagnesemia/magnesium deficiency and acid load from PPI for the increased risk of CKD. Both factors are potentially correctible by EffCaMgCit.

Hypomagnesemia alone has been associated with impaired renal function (Sakaguchi, 2015; van Laecke, 2013). Magnesium deficiency has been associated with inflammation and magnesium supplementation has anti-inflammatory activities (Nielsen 2018). It is possible that the magnesium wasting can worsen chronic interstitial nephritis in chronic PPI users. In patients on hemodialysis, those on PPI have a reduced survival rate (Ago, 2016). Excessive acid load is associated with renal impairment (Banerjee, 2015), and progression of CKD slows while on alkali therapy in small clinical trials (Phisitkul, 2010; de Brito-Ashurst, 2009; Mahajan, 2010). It is commonly believed that PPI does not deliver an acid load, since the reduced proton secretion in the upper bowel by PPI is compensated by a commensurate fall in bicarbonate secretion by the pancreas (Cameron, 2012). However, a contrary evidence has appeared, showing that PPI promotes duodenal bicarbonate secretion (Mertz-Nielson, 1996). If enhanced duodenal bicarbonate secretion in fact exceeds the reduction in gastric acid secretion, PPI will impart an acid load.

Our data from the multidosing trial indicate that PPI under certain circumstances might deliver an acid load (Fig. 5). 24-h urinary citrate and ammonium were determined at Baseline and after PPI + Placebo or PPI + EfffCaMgCit. On PPI alone (with Placebo), urinary citrate was significantly lower than at Baseline, indicative of the kidneys' response to a systemic acid load. In subjects on

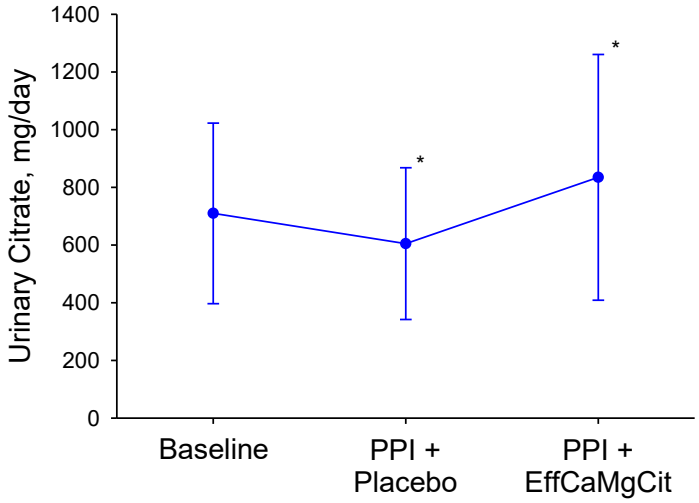

Fig. 5. 24-h urinary citrate during Baseline, PPI + Placebo, and PPI + EffCaMgCit. Data from the completed multidosing trial in 22 subjects. * $p < 0.05$ vs Baseline.

PPI with EffCaMgCit, urinary citrate was significantly higher than at Baseline.

EfffCaMgCit. On PPI alone (with Placebo), urinary citrate was significantly lower than at Baseline, indicative of the kidneys' response to a systemic acid load. In subjects on PPI with EffCaMgCit, urinary citrate was significantly higher than at Baseline.

On PPI alone (with Placebo), urinary ammonium was marginally higher than at Baseline, supportive of marginal delivery of acid load (Fig. 6). On PPI with EffCaMgCit, urinary ammonium was significantly lower than at Baseline. Thus, PPI might not only cause hypomagnesemia/magnesium deficiency, but it might also deliver an acid load. Both factors might contribute to renal impairment. Increased ammoniagenesis might also contribute to renal tubular injury by interacting with complement (Nath, 1985). EffCaMgCit should correct magnesium deficiency, acid load, and enhanced ammoniagenesis, thereby averting incident CKD.

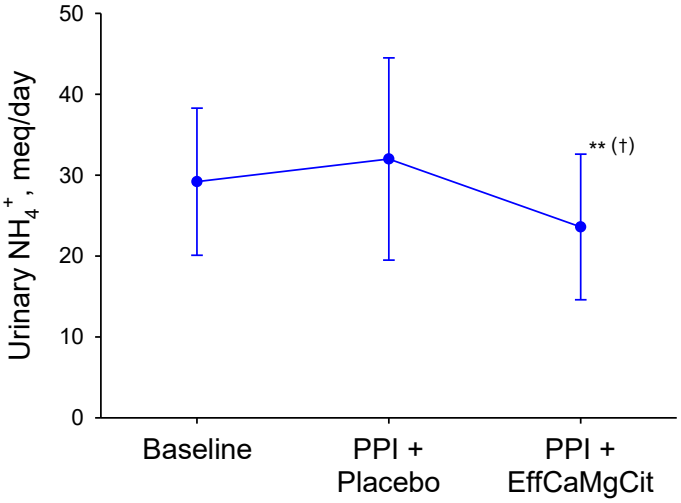

Fig. 6. Urinary ammonium during Baseline, PPI + Placebo, and PPI + EffCaMgCit. Data from the completed multidosing trial in 22 subjects. ** $p < 0.01$ from Baseline; (†) $p < 0.001$ between PPI + Placebo vs. PPI + EffCaMgCit. P between Baseline and PPI + Placebo was 0.18.

Our preliminary data from the multidosing trial indicated that PPI alone (with placebo) tended to increase, whereas EffCaMgCit (with PPI) tended to reduce serum FGF23. The difference between the two treatment phases was significant at 4 weeks of treatment. If verified, FGF23 might be another factor involved in the PPI-induced incident CKD, which is ameliorated by EffCaMgCit. In conclusion, we expect that:

- EffCaMgCit should prevent/treat osteoporosis from PPI use.
- PPI produces hypomagnesemia/magnesium deficiency and EffCaMgCit should abrogate this disturbance.
- By delivering bioavailable Mg and alkali load, EffCaMgCit should retard or block renal impairment and progression of CKD during PPI therapy.

The goals of the proposed trial are to conduct a two-year treatment trial instead of 4- week treatment, to:

- Measure BMD (in addition to bone turnover markers), to show effectiveness of EffCaMgCit in prevention or treatment of osteoporosis.

- Obtain other measures of Mg status (FEMg and free muscle Mg), besides serum Mg, to demonstrate maintenance of Mg sufficiency or reversal of Mg deficiency by EffCaMgCit during PPI therapy.
- Evaluate acid-base status (NAE, urinary citrate and ammonium), to show that acid load from PPI is abrogated by alkali load delivered by EffCaMgCit.
- Measure renal function (endogenous creatinine clearance and eGFR), besides serum creatinine, to show that EffCaMgCit stabilizes or inhibits deterioration of renal function.

Research Design and Methods:

Materials

EffCaMgCit and Placebo will be purchased by CMMCR from Sterling Pharmaceutical Services (Dupo, IL) as research drugs. EffCaMgCit and Placebo will be contained in sachets of identical appearance, identified by different lot numbers. To provide adequate blinding, each medication sachet will be labelled with the study name, IRB number, principal investigator's name, expiration date and identification number of the study subject. Labels will be applied to the appropriate medication sachets once the subject has been randomized and assigned to a treatment group. Labelling of the sachets will be done by personnel who are not engaged in patient care.

Each sachet of EffCaMgCit will contain 19 meq or 380 mg calcium, 10 meq (122 mg) magnesium, and 50 meq total citrate. Each sachet of Placebo will contain microcrystalline cellulose, but no calcium, magnesium or citrate. Both are designed to be added to 6 oz water for 1-2 minutes, to be dissolved/suspended before swallowing. Each sachet of EffCaMgCit or placebo will contain 400 units of vitamin D.

Conflict of Interest Statement

UT Southwestern so far has not yet secured a pharmaceutical partner for EffCaMgCit. Despite lack of industrial sponsorship, the investigators feel that the proposed study is sufficiently meritorious scientifically to be pursued by them. Thus, this protocol is investigator-initiated and research-driven, and not industry-sponsored. The costs of the study will be borne by the Biotechnology Program of CMMCR. CMMCR will purchase test medications from Sterling Pharmaceutical Services.

Study Subjects

Ambulatory adult subjects (> 21 years of age) of either gender of any ethnicity will be recruited by advertisement at UTSW campus and Parkland Health & Hospital System as well as utilizing the UT Southwestern Clinical Research Information System (utCRIS) which pulls data from the UT Southwestern electronic medical record system. To be eligible for the study, subjects must have taken PPI (omeprazole or equivalent ≥ 20 mg/day, ≥ three times per week, for at least 2 months) and expected to continue at a similar dosage in the foreseeable future. utCRIS will be used to search for subjects who have a known condition which justifies long term use of PPIs, such as GERD and NERD requiring long-term management, Barrett's esophagus, Zollinger-Ellison syndrome, and anti-platelet therapy[Scarpignato 2016 BMC 14:179]. utCRIS will also be used to identify subjects who have unsuccessfully attempted discontinuation of PPIs. Patients

who are required to take calcium by their primary physician are excluded from the study. Also excluded from the study will be those with end-stage renal failure on dialysis, hypercalcemia, hypophosphatemia (serum P < 2.5 mg/dL), hypertension stage 2 or higher, diabetes Type II with HbA1C ≥ 7%, and treatment with adrenocorticosteroids, diuretics, non-steroidal anti-inflammatory agents (≥ 2 doses per week), regular dose of magnesium supplements, bisphosphonate, teriparatide, denosumab or selective estrogen receptor modulators. Stage 1 hypertension (with systolic blood pressure <140 and diastolic <90) and controlled diabetes mellitus Type II with HbA1C less than 7% will be allowed. Inclusion/exclusion of other drugs or conditions will be considered on an individual basis. A careful record will be made for the type and dose of all medications.

We plan to recruit 70 subjects at the primary site at UTSW and Parkland Health & Hospital System. We expect that 20% of subjects might have eGFR <65 ml/min at entry.

Outline of Study

Screening. Before enrollment into the trial, potential candidates will undergo screening, whereby a venous blood sample will be obtained for complete metabolic panel (including calcium, phosphorus, and creatinine), HbA1c, Mg and complete blood count (with differential). A pregnancy test will be done if applicable. Ambulatory blood pressure will be obtained. Those who meet the entry-exclusion criteria will be selected for consideration into the study.

Randomization. Eligible patients will be randomized into two equal groups, stratified according to age (> or ≤ 50 years) and, dose (> or ≤ 20 mg three times/week). After baseline evaluation, one group (EffCaMgCit Group) will take EffCaMgCit, and the other group (Placebo Group) will take microcrystalline cellulose suspension for two years (Fig. 7). Both groups will take PPI at the prevailing dose. Both drugs will be taken 1 sachet each just before or along with breakfast and dinner. Thus, patients in the EffCaMgCit group will receive 38 meq (760 mg) Ca, 20 meq (365 mg) Mg, and 100 meq total citrate per day. Those in the Placebo group will not take any calcium, magnesium or citrate.

Diet and customary drugs. Customary *ad lib* diet will be taken throughout the study except for instructed breakfast on testing days. Customary drugs will be continued, except those specified under exclusions. It is expected that most patients at entry will be taking vitamin D. Each sachet of EffCaMgCit or placebo contains 400 units of vitamin D. During the trial, additional vitamin D judged to be needed by their physicians will be allowed.

Baseline evaluation (0 month, Table 1). Patients will stop their customary calcium or magnesium supplements on enrollment, and they will omit them throughout the study. After one week of stopping above, they will return to the clinic after eating a regular breakfast meal (instructed, low in calcium and magnesium). Two-4 hours later, a venous blood sample will be obtained. Patients will complete side effect questionnaire with the aid of a research nurse or study coordinator (Table 1).

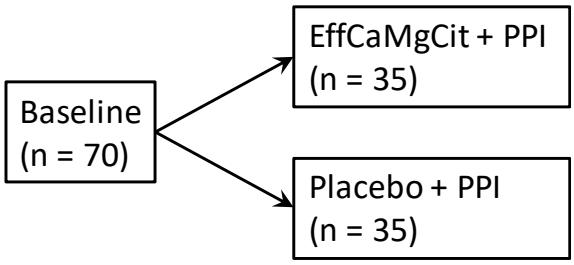

Fig. 7. Scheme of study.

Test drug treatment. After baseline evaluation, patients will then take EffCaMgCit or Placebo 1 sachet bid (1 with breakfast and 1 sachet with dinner) for two years, by adding contents of sachet to 6 oz water and drinking the solution. They will also take omeprazole (or similar PPI) delayed release upon awakening at a similar dose as prior to entry into the trial. Study participants will not be switched to a new PPI, a new dose, or a new delivery method solely for the purposes of the study. The tolerance to taking the medication will be evaluated by using the questionnaire (Table 1).

Table 1. Side Effect Questionnaire

Adverse Symptoms: (Indicate frequencies of none, < 2/wk, 2-7/wk or > 7/wk. Indicate severities of none, mild, moderate or severe.)

	Frequency				Severity				Notes
	None	<2/wk	2-7/wk	>7/wk	None	Mild	Moderate	Severe	
Vomiting	❑	❑	❑	❑	❑	❑	❑	❑	_____
Nausea	❑	❑	❑	❑	❑	❑	❑	❑	_____
Belching	❑	❑	❑	❑	❑	❑	❑	❑	_____
Diarrhea	❑	❑	❑	❑	❑	❑	❑	❑	_____
Loose BM	❑	❑	❑	❑	❑	❑	❑	❑	_____
Constipation	❑	❑	❑	❑	❑	❑	❑	❑	_____
Pain/Cramps	❑	❑	❑	❑	❑	❑	❑	❑	_____
Melena	❑	❑	❑	❑	❑	❑	❑	❑	_____
Dyspepsia	❑	❑	❑	❑	❑	❑	❑	❑	_____
Anorexia	❑	❑	❑	❑	❑	❑	❑	❑	_____
Dysphagia	❑	❑	❑	❑	❑	❑	❑	❑	_____
Muscle Weakness	❑	❑	❑	❑	❑	❑	❑	❑	_____
Muscle Cramps	❑	❑	❑	❑	❑	❑	❑	❑	_____
Arrythmia	❑	❑	❑	❑	❑	❑	❑	❑	_____
Distaste	❑	❑	❑	❑	❑	❑	❑	❑	_____
Other	❑	❑	❑	❑	❑	❑	❑	❑	_____

Follow-up visits. Patients will be followed in an ambulatory setting every 3 months (Table 2). At each visit, a venous blood sample will be collected 2-4 hours after breakfast and/or test drug. The meal will be a standard breakfast, low in calcium and magnesium. Patients will be instructed to collect 24-h urine under oil while kept cold in an ice chest or refrigerator the day preceding the visit and bring the sample to the clinic on the day of the visit. The side effect questionnaire will be completed. At Baseline and each follow-up visit, blood pressure will be measured after subjects sit quietly for at least 10 minutes. At Baseline, 12 months and 24 months of treatment, BMD of L2-L4 vertebrae, femoral neck and radial shaft, and free muscle Mg by MRS will be obtained. At Baseline, 12 months and 24 months, spinal fracture assessment will be determined by DEXA.

Table 2. Follow-up Visits

	0	3	6	9	12	15	18	21	24
Venous blood post-meal & load	✓	✓	✓	✓	✓	✓	✓	✓	✓
24 h urine	✓	✓	✓	✓	✓	✓	✓	✓	✓
Bone mineral density	✓				✓				✓
MRS, free muscle Mg	✓				✓				✓
Side effect questionnaire Blood pressure	✓	✓	✓	✓	✓	✓	✓	✓	✓

Laboratory Tests. A venous blood sample obtained at each visit will be analyzed for metabolic panel (including calcium, and creatinine), magnesium, PTH, CTX, BSAP (bone specific alkaline phosphatase, a marker of bone formation), FGF23, Klotho, IL6 and isoprostane (Fig. 3). At 0, 12 and 24 months, HbA1c will be obtained. 24-h urine samples will be measured for stone risk profile (including Ca, Mg, P, Na, K, sulfate, uric acid, citrate, ammonium, creatinine and pH), pCO_2, and markers for tubular damage (N-acetyl-β-D-glucosaminidase (NAG), microglobulin, and albumin). Titratable acid (TA) will be measured and FEMg calculated. BMD will be measured by dual photon absorptiometry. Free muscle Mg will be obtained in a calf muscle by MRS.

Calculations. Net acid excretion will be calculated using the formula: NAE = (titratable acidity + ammonium) – (citrate – bicarbonate). Endogenous creatinine clearance will be obtained by using 24-h urinary creatinine and post-meal/load venous blood sample ((uCr, mg/24hr) / (sCr, mg/dL * 14.4)) as well as using the Cockcroft and Gault formula ([(140 - age) x TBW] / (Scr x 72) (x 0.85 for females)). FEMg is the ratio of magnesium clearance and creatinine clearance, using 24-h urinary magnesium and creatinine and corresponding serum magnesium and creatinine obtained post meal/load.

MRS for free muscle magnesium will be performed at UTSW's Advanced Imaging Research Center. Intracellular Mg will be measured in a calf muscle, by using 31P magnetic resonance spectroscopy, based on the formula: [Mg] – kd(10.796-D)/(D-8.251), in which kd is the dissociation constant for MgATP complex (=50 μM) and D is the chemical shift difference between alpha- and beta-ATP 31P signals observed in 31P MR spectrum.

Metabolic panel will be obtained from Quest. All other tests will be performed or analyzed by CMMCR's laboratory. Serum for inflammatory marker (such as IL6 or isoprostane) will be saved frozen. Urine samples will be saved frozen for NAG, macroglobulin and albumin (Mahajan, 2010). Upon study completion, the saved samples will be analyzed for specified tests if warranted.

Table 3. Laboratory Tests

	0	3	6	9	12	15	18	21	24
Serum Ca, PTH CTX, BSAP, IL6	✓	✓	✓	✓	✓	✓	✓	✓	✓
BMD	✓				✓				✓
Serum Mg FEMg	✓	✓	✓	✓	✓	✓	✓	✓	✓
Free muscle Mg	✓				✓				✓
24 h urinary citrate NH_4, TA, NAE, NAG	✓	✓	✓	✓	✓	✓	✓	✓	✓
Serum Cr End Cr Cl	✓	✓	✓	✓	✓	✓	✓	✓	✓

Expectations

Skeletal status

- In the EffCaMgCit + PPI group, BMD would slightly increase or be stabilized, and serum PTH and CTX would decline,
- In the Placebo + PPI group (PPI alone), BMD would slightly decline, and serum PTH and CTX would slightly increase.

Magnesium status

- In the EffCaMgCit + PPI group, FEMg, free muscle Mg and serum Mg would be increased from Baseline.
- In the Placebo + PPI group (PPI alone), FEMg, free muscle Mg and serum Mg would be slightly decreased from Baseline.

Acid-base balance

- In the EffCaMgCit + PPI group, urinary citrate would be increased, and urinary NAE and ammonium would be decreased from Baseline.
- In the Placebo + PPI group (PPI alone), urinary citrate would be reduced, and urinary NAE and ammonium would be increased from Baseline.

Renal function

- In the EffCaMgCit + PPI group, endogenous creatinine clearance and eGFR would be unchanged or slightly increased from Baseline.
- In the Placebo + PPI group (PPI alone), endogenous creatinine clearance and eGFR would be decreased from Baseline. One possible explanation for the decline in eGFR by PPI might be PPI-induced interstitial fibrosis, which continues to a chronic condition with continued PPI use. There is a possibility that EffCaMgCit may alleviate interstitial fibrosis,

by overcoming acid load, FGF23 excess, and inflammation worsened by magnesium deficiency.

Potential Side Effects
EffCaMgCit might cause loose bowel movements or diarrhea. Threshold magnesium dose for diarrhea is 40 meq/day; we will be using 20 meq/day. In general, even among few who initially develop diarrhea, the gastrointestinal tolerance improves with continued treatment.

Very rarely, patients might develop hypophosphatemia (serum P < 2.5 mg/dL) from excessive intestinal phosphate binding by Ca and/or Mg contained in EffCaMgCit. Rare patients might develop hypercalcemia from oral calcium load during both treatments. If a patient presents with either a serum P < 2.5 mg/dL or grade $\geq$ 2 hypercalcemia, as defined as analbumin-corrected serum Ca > 11.5 mg/dL, in two consecutive follow-up visits, they will be withdrawn from the study.

Statistical Considerations

Sample size calculations.

Aim 1. To the best of our knowledge, there is no prospective report on the serial change in BMD during PPI. Hence, we used CTX to calculate sample size (Table 2). In the completed crossover 4 week trial, CTX was 0.47 ± 0.24 in the placebo + PPI, and 0.28 ± 0.13 in the EffCaMgCit + PPI. Assuming power of 0.8, 54 subjects would be needed.

Aim 2. For magnesium status, the aforementioned trial revealed serum magnesium of 2.08 ± 0.15 in placebo + PPI, and 2.21 ± 0.15 in EffCaMgCit + PPI (Table 2). With power of 0.8, 34 subjects would be needed.

The same trial also revealed FEMg of 0.034 ± 0.013 for placebo + PPI, and 0.052 ± 0.018 for EffCaMgCit + PPI (Table 2). For a power of 0.8, 34 subjects would be needed.

Thus, 34-54 patients would be required. We plan to enroll 70 patients to accommodate 20-30% attrition.

Table 2. Sample Size Calculations

	Placebo + PPI		EffCaMgCit + PPI			
Variable	Mean	SD	Mean	SD	Power	N
Bone CTX	0.47	0.24	0.28	0.13	0.8	54
Mg Status						
sMg	2.08	0.15	2.21	0.15	0.8	34
FEMg	0.034	0.013	0.052	0.018	0.8	34
eGFR Decline	14.5	18	0	18	0.8	54

Aim 3. We were unable to locate a publication which showed actual change in eGFR during PPI treatment; no histological description of the lesion has been reported. However, retrospective analysis of large database are available. Xie (2018) reported that 15% of subjects had incident CKD with eGFR less than 60 ml/min after 2 years of PPI therapy, and 12% in those not on PPI. The following assumptions were made. (a) eGFR was 95 ml/min before PPI therapy. (b) 15% had eGFR of 55 ml/min after 2 years of PPI treatment. The remaining 85% of subjects had a decline from 95 ml/min to 85. Thus, estimated decline in eGFR would be 14.5 ml/min over 2 years. (c) The SD of eGFR was assumed to be 25%, the same as in our trial among stone formers. (e) EffCaMgCit + PPI would maintain endogenous creatinine clearance and eGFR unchanged (0±18) during 2 years of treatment, but Placebo + PPI would produce a decline of 14±18%. Setting power at 0.8, 27 subjects in each group would be needed to detect a decline in eGFR of 14.5%. In addition to Xie, two other papers described similar increase in risk of CKD in PPI users (Lazarus 2016, Peng 2016)

Randomization. Allocation to EffCaMgCit or Placebo treatment will be determined with a stratified blocked randomization scheme, stratified according to age (> or ≤ 50 years) and, dose (> or ≤ 20 mg three times/week), and programmed using SAS Proc Plan.

Statistical analysis. The statistical mean difference between PPI + Placebo and PPI + EffCaMgCit for variables (serum PTH, CTX and BMD T-Score; serum Mg, FEMg, and free muscle Mg; urinary citrate, ammonium and NAE; serum creatinine and endogenous creatinine clearance) will be assessed by mixed-effects model repeated measures analysis. For subjects 50 years or older, the T-Score will be utilized. Those with T-Score < -2.5 will be excluded from the study. For subjects < 50 years, the Z-Score will be utilized. Those with Z-Score < -2 will be excluded from the study. The effect of duration of treatment (particularly at 1 year and 2 years) will be evaluated by including fixed effects factors for time in the repeated measures model; the study participant will be modeled as a random effect. For skewed variables, data transformations or nonparametric tests will be implemented as appropriate. Statistical analyses will be performed with SAS software (SAS Institute, Cary, NC).

Data Safety and Monitoring:

The Research Personnel and PI now meet weekly to review and discuss patient safety, study data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, protocol adherence and other factors that affect study outcome. They will continue to monitor and ensure the safety of the patient, quality of the data, and success of the study.

Interim analysis. An intent to treat analysis will be performed. Participants with at least one post-randomization study visit will be included. Interim analysis might be performed at midpoint of the study (about one year of treatment). If a decision is made to conduct it, the type I error rate will be controlled using a Lan-Demets alpha-spending function with O-Brien-Fleming boundaries. The interim analysis will be performed by the study statistician for efficacy and reassessment of sample size; the sample size may be modified or the interim look can result in no action. To ensure trial integrity, efficacy results will not be disclosed to study investigators or personnel who are in contact with the participants.

A two-sided alpha <5% will be considered significant for all analyses. Power analysis assumptions will be reassessed and the adjustment for multiple looks will be based on O'Brien-Fleming boundaries.

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