Study Protocol

7.1. Title of Project: Amelioration of Chlorthalidone-Induced Hypokalemia and Metabolic Disturbances by Potassium-Magnesium Citrate (KMgCit) Powder

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In a just completed study (IND 116,208; IRB Study #: STU 072012-001), potassium magnesium citrate (KMgCit) powder did not alter blood pressure in patients with pre- and Stage I hypertension, while potassium chloride (KCl) powder lowered it. However, KMgCit powder reduced urinary 8-isoprostane serum FGF23. Chlorthalidone (CTD), a thiazide diuretic widely used for treatment of hypertension, produces certain metabolic disturbances associated with Type II diabetes and metabolic syndrome. In this "protocol amendment: new protocol", these circumstances led us to explore whether KMgCit powder co-administered with CTD would correct hypokalemia, and ameliorate metabolic disturbances of CTD therapy among patients with Stage I hypertension. We shall examine the effect of CTD alone, CTD with KMgCit powder or CTD with KCl powder in a parallel design.

1. Specific Aims:

Aim 1. To compare the effect of KMgCit powder and KCl powder on correction or prevention of CTD-induced hypokalemia.

Aim 2. To examine CTD-induced changes in renin-angiotensin aldosterone (RAA) system, oxidative stress, and insulin sensitivity between KMgCit powder and KCl powder supplementation.

Aim 3. To evaluate hepatic fat accumulation by MRS.

Aim 4. To compare the Mg status from measurement of muscle Mg by MRS between CTD + KMgCit powder and CTD + KCl powder.

It is expected that: (a) KMgCit powder would be equally effective in correcting/preventing CTD-induced hypokalemia as KCl powder, without incurring hypochloremic metabolic alkalosis. (b) Compared to KCl powder plus CTD, KMgCit powder added to CTD would reveal lower serum aldosterone, urinary isoprostanes, serum glucose, and HOMA-IR and serum FGF23. (c) Hepatic fat content would increase on CTD plus KCl powder, but would decline on CTD + KMgCit powder. (d) Muscle Mg would increase on KMgCit powder +

CTD, but would be decreased on KCl powder + CTD. It is hypothesized that CTD produces various metabolic disturbances that interact with each other or act independently to alter the propensity for Type II diabetes and metabolic syndrome, and that KMgCit powder ameliorates these disturbances or propensity. This protocol will put a special focus on testing a scheme that links Mg depletion from CTD to hepatic fat accumulation and insulin resistance.

2. Background:

The idea for this study came from a just completed trial (IND 116,208 KMgCit Powder; IRB #STU 072012-001). We examined whether KMgCit powder could lower blood pressure in patients with pre- and Stage I hypertension, by providing three components of the "DASH" diet (potassium, magnesium and alkali) that have been shown to lower blood pressure (Sacks, 2001). KMgCit powder was compared with potassium citrate (KCit) powder, KCl powder and placebo. KMgCit powder was shown to be ineffective in lowering blood pressure.

However, the above study disclosed that KMgCit powder might have other usefulness, since it seemed to ameliorate some of the metabolic disturbances associated with CTD.

Metabolic disturbances of CTD

CTD is a thiazide (TZ) type diuretic advocated by many guidelines for the treatment of hypertension (NICE 2011), owing to its longer half-life and greater antihypertensive efficacy at the clinically recommended doses than other thiazide diuretics (Vongpatanasin, 2015). Despite these favorable properties and increasing popularity, CTD and other TZ diuretics are known to cause various metabolic disturbances, such as hypokalemia, activation of renin-angiotensin system and sympathetic nervous system (RAA-SNS) (Menon, 2009), oxidative stress (Ribeiro, 2013; Reungjui, 2007), dyslipidemia (Eriksson, 2008; Price, 2013), increased FGF23 synthesis (Pathare, 2012), insulin resistance (Xie, 2005; Price, 2013; Raheja, 2012; Menon, 2009), and Mg depletion (Sheehan, 1982; Hollifield, 1987).

Mechanism for various metabolic disturbances of CTD/TZ ultimately contributing to the development of type II diabetes and metabolic syndrome are unknown. The traditional explanation implicating hypokalemia has been questioned.

CTD/TZ-induced potassium depletion and hypokalemia are thought to be the main cause of hyperglycemia by impairing insulin secretion. However, serum insulin increases rather than decreases with TZ diuretics (Carter 2008, Raheja 2012). The maintenance of normokalemia during CTD administration by K supplementation failed to prevent CTD-induced increase in fasting plasma glucose and HOMA-IR, an index of insulin resistance (Raheja, 2012; Menon, 2009). CTD is known to induce a sustained activation of RAA-SNS in

hypertensive patients (Menon 2009, Raheja 2012). This neurohormonal activation is independent of serum potassium as it is unaffected by K supplementation (Menon 2009, Raheja 2012). Thiazide diuretic has been shown to increase renal oxidative stress, which cannot be accounted for by the presence of hypokalemia alone (Reungjui, 2007).

Various metabolic disturbances of CTD/TZ might be pathogenetically linked, involving a pathway independent of hypokalemia. Some metabolic disturbance might directly cause insulin resistance and Type II diabetes. Other factors might do so indirectly by affecting other metabolic disturbances.

Activation of RAA system has also been implicated in the pathogenesis of insulin resistance by inhibiting insulin signaling pathway in the adipocytes and skeletal muscle (Wada, 2009; Hitomi, 2007), resulting in impaired insulin mediated glucose uptake (Luther, 2011; Selvaraj, 2009). The deleterious effect of RAA system activation is mediated at least in part by increased oxidative stress (Sowers, 2009). Activation of the SNS system may further promote insulin resistance by reducing skeletal muscle glucose uptake both by flow-dependent (Jamerson, 1993; Sartori, 1999) and flow-independent (Lembo, 1994; Navegantes, 2003) mechanisms.

Mg depletion from CTD/TZ may cause renal potassium loss and refractory hypokalemia (Whang, 1977). Magnesium supplementation alone without potassium ameliorated TZ-induced hypokalemia (Ruml, 1999). Activation of RAA system by CTD or TZ may contribute to Mg depletion. Inverse correlation between plasma renin activity and serum Mg has been demonstrated in a cross sectional study of patients with untreated essential hypertension (Resnick, 1983). In normal volunteers, serum aldosterone at baseline and after angiotensin II infusion was significantly higher on Mg-deficient diet than on Mg-replete diet (Nadler, 1993). Co-infusion of Mg with angiotensin II attenuated the rise in serum aldosterone, suggesting direct inhibitory effect of Mg on the RAA system.

KMgCit powder as a countermeasure for metabolic disturbances of CTD

Effective strategy in preventing the metabolic disturbances of CTD/TZ has not been fully developed.

KCl supplementation is ineffective. Correction of hypokalemia by KCl did not reverse CTD-induced fasting hyperglycemia and increased HOMA-IR (Raheja, 2012; Menon, 2009). CTD-induced activation of RAA-SNS was unaffected by KCl supplementation (Menon 2009, Raheja 2012). In TZ-treated patients with hypertension, KCl supplementation did not reduce serum aldosterone (Kaplan, 1985).

KMgCit powder possesses distinct properties apart from KCl powder in overcoming deleterious metabolic disturbances of CTD therapy. Considerable data on physiological-clinical action of this drug have been obtained by this

investigative group using a tablet formulation (under IND 32,284 & 36,276; NDA 20-964), as summarized below.

Physiological properties of KMgCit. This preparation was shown to confer equivalent potassium bioavailability as potassium chloride, similar magnesium bioavailability as magnesium citrate, and greater alkali load than potassium citrate (Koenig, 1991). In patients with calcium nephrolithiasis, KMgCit significantly increased urinary pH and citrate compared to placebo. It was more effective than potassium citrate in inhibiting the crystallization of uric acid and calcium oxalate in urine (Pak, 1992).

Some of the orally administered citrate may appear in urine by escaping hepatic metabolism *in vivo*. Serum citrate concentration increased significantly within 30 minutes after a single oral dose of citric acid and remained significantly elevated for 3 hours after citric acid load. Commensurate with this change, urinary citrate excretion peaked at 2 hours and gradually decreased during the next 2 hours after a citric acid load (Sakhaee, 1992).

KMgCit was equally effective as potassium chloride in correcting HCTZ-induced hypokalemia. In addition, KMgCit, but not KCl, produced a small but significant increase in serum magnesium concentration by delivering a magnesium load, and it conferred alkalinizing and citraturic actions (Pak, 2000).

The above actions of KMgCit – potassium and magnesium load, citraturic action, and alkali load – might have a role in mitigating metabolic disturbances of CTD, as discussed below.

Prevention of TZ-induced hypokalemia by KMgCit from provision of K and Mg load. In healthy subjects taking HCTZ, the addition of KMgCit corrected/prevented hypokalemia (Ruml, 1999; Wuermser, 2000), without producing metabolic alkalosis (Odvina, 2006).

Potential inhibition of RAA-SNS activation by KMgCit powder from provision of Mg. Pilot data from our laboratory indicated that magnesium supplementation attenuated sympathetic mediated vasoconstriction during muscle contraction in spontaneously hypertensive rats. Normally, exercise triggers parallel increases in sympathetic nervous system to the resting and exercising skeletal muscle. In the resting muscle, sympathetic activation triggers alpha-adrenergic vasoconstriction, thereby redirecting blood flow to the metabolically active muscles. In the working muscles, sympathetic vasoconstriction is markedly blunted by local vasodilator metabolites, particularly nitric oxide. This phenomenon, known as functional sympatholysis, is thought to be a protective mechanism to optimize blood flow to the working muscle (Remensnyder JP, 1962). Our study indicated that functional sympatholysis is impaired in hypertensive patients, which may contribute to exercise intolerance (Vongpatanasin 2011). The impairment in functional sympatholysis in

hypertension was shown to be related to increased skeletal muscle oxidative stress that inactivates NO (Zhoa 2006).

In our study in spontaneously hypertensive rats, dietary Mg supplementation blunted sympathetic mediated vasoconstriction (A, right) compared with Mg deficient diet (A, left), and reduced muscle oxidative stress both in contracting muscle and resting muscle (B) (Fig. 1).

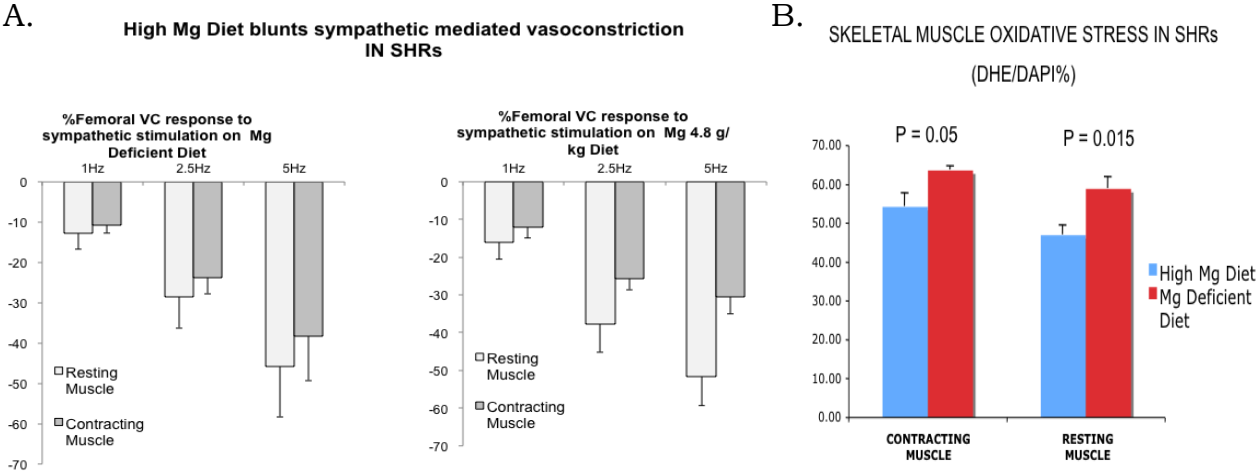

Fig. 1: A. percent change in vascular conductance (VC) in the femoral artery in response to lumbar sympathetic nerve stimulation (1, 2.5, and 5 Hz) at rest (white bars) and during muscle contraction (grey bars) in spontaneously hypertensive rats (SHRs) treated with Mg deficient diet (left, 30 mg/kg) vs. high Mg diet (4.8 grams/kg, right) for 12 weeks. Sympathetic mediated reduction in vascular conductance is attenuated in the SHRs treated with high Mg diet compared to Mg deficient diet, B. Summary data showing increased oxidative stress as evidenced by increased ethidium fluorescence (DHE/DAPI ratio) in resting and contracting muscles of SHRs treated with Mg deficient diet compared to high Mg diet.

Potential inhibition of oxidative stress by KMgCit powder from provision of citric acid, alkali and magnesium. A high dose of citric acid (1–2 g/kg) reduced brain lipid peroxidation and inflammation, liver damage, and DNA fragmentation in mice exposed to lipopolysaccharide, suggesting a protective role of citric acid in endotoxin-induced oxidative stress (Abdel-Salam, 2014).

Supporting an inhibitory role of alkali, a calcium/potassium salt of hydroxycitric acid (rich in alkali) extracted from the dried fruit rind of the plant *Garcinia cambogia* reduced inflammation, oxidative stress, and insulin resistance in obese Zucker rats (Asghar, 2007). In the Dietary Approaches to Stop Hypertension (DASH/SRD) trial, a diet rich in alkali and magnesium and low in sodium was shown to reduce urinary F2-isoprostanes by 31% in hypertensive patients with heart failure with preserved ejection fraction, suggesting decreased oxidative stress (Hummel, 2012). Consumption of black currant juice was shown to reduce plasma F2-isoprostanes and improve endothelial function in individuals with habitually low dietary intake of fruits

and vegetables (Khan, 2014). Induction of metabolic acidosis with oral ammonium chloride for 5 days in normal volunteers increased leucine appearance from body protein and led to leucine oxidation, suggesting increased protein degradation and amino acid oxidation (Reaich, 1992). It can be inferred that alkali load by KMgCit powder would have an opposite effect.

As evidence of its protective role, Mg was shown to ameliorate creatine kinase-MB elevation, oxidative stress, lactate accumulation, and pyruvate reduction, as well as preserve creatinine phosphate, adenine nucleotides and Na(+),K(+)-ATPase activity, in mice with lipopolysaccharide-induced cardiotoxicity (Ahmed, 2012). Dietary magnesium restriction increased ischemic/reperfusion injury in the isolated rat hearts. Dietary Mg deficiency was shown to promote cardiomyopathy, *in situ* cardiac dysfunction, and myocardial intolerance to secondary stresses. Significant protection against most of these Mg deficiency-mediated events has been observed with interventions that modulate neuronal substance P release or its bioactivity, and with several antioxidants (vitamin E, probucol, epicaptopril, *d*-propranolol) (Kramer, 2009).

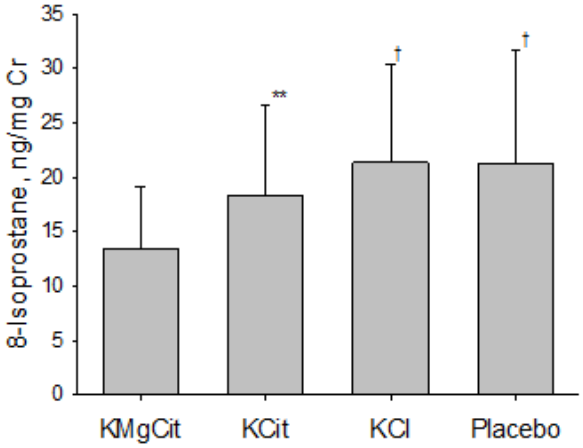

Fig. 2. Spot urinary 8-isoprostane following 4 weeks of treatment with KMgCit powder, KCit powder, KCl powder or placebo. ** $p < 0.05$ and † < 0.001 from the KMgCit powder. $p = 0.11$ between KCit powder and placebo.

We obtained the most direct evidence of KMgCit powder's inhibition of oxidative stress in a just completed trial (original IND 116,208). Thirty patients with pre- or Stage I hypertension underwent a crossover trial, whereby they took KMgCit powder, KCit powder, KCl powder or placebo for 4 weeks. The potassium content during the K salt phases was 20 meq bid. In the KMgCit powder phase, patients also took 10 meq Mg bid. A spot urine sample was obtained at the end of 4 weeks of treatment of each phase. Urinary 8-isoprostane was significantly lower during the KMgCit powder phase than during other phases (Fig. 2).

Potential amelioration of dyslipidemia by KMgCit powder via Mg load. Mg supplementation with bittern, a natural $MgCl_2$ solution from sea or salt lake water, reduced the appearance in serum of lipid components after a fat load in normal volunteers (Kishimoto, 2010). The concentrations of apo-B48, remnant-like particle cholesterol, and nonesterified fatty acid were significantly lower at 2 hours after the fat-with-Mg meal compared with the fat-only meal. Thus, Mg supplementation by KMgCit powder could inhibit fat absorption and improve postprandial hyperlipidemia.

In rabbits fed a high cholesterol diet (1% or 2%), oral magnesium aspartate hydrochloride: (i) lowered serum cholesterol and triglyceride in normal (25-35%) as well as atherosclerotic (20-40%) animals, and (ii) attenuated the atherosclerotic process markedly. In addition, dietary deficiency of Mg augmented atherogenesis markedly and stimulated (or activated) macrophages in the reticuloendothelial system (Altura, 1990; Smith 2013). In rats, Mg deficient diet reduced post-heparin lipase activity and hepatic lipase, resulting in increased serum triglycerides and triglyceride content of chylomicrons, very low density lipoproteins, low density lipoproteins and high density lipoproteins (Rayssiguier, 1991).

Potential suppression of FGF23 synthesis by KMgCit powder from alkali and Mg load. In mouse calvarial and osteoblast preparations, a culture medium mimicking metabolic alkalosis inhibited FGF23 protein and mRNA (Krieger, 2012). Mice exposed to Mg-deficient diet showed a marked increase in serum FGF23 (van Angelen, 2013).

Potential amelioration of insulin resistance and type II diabetes by KMgCit powder from alkali and Mg load. In a randomized crossover study in normal volunteers, Mg deficient diet reduced insulin sensitivity (estimated from intravenous glucose tolerance test), compared to Mg repleted diet (Nadler 1993). In a double-blind, placebo-controlled study in prediabetic subjects, KCit (delivering alkali load) but not KCl reduced insulin resistance as revealed by HOMA-IR (Conen, 2015). In a cross-sectional study, Type 2 diabetes mellitus was associated with lower urinary pH and higher net acid excretion, independent of age, body weight, creatinine clearance, and dietary factors (Cameron 2006). Several populational studies have shown an association between dietary acid load and insulin resistance as well as the risk of type 2 diabetes mellitus (Akter 2015 and Fagherazzi 2014). In the National Health and Nutrition Examination Surveys involving healthy US population, low serum bicarbonate and high anion gap were independently associated with increased plasma fasting insulin and triglyceride, suggesting increased risk of insulin resistance (Farwell 2008). These studies inferred potential protective action of KMgCit powder from alkali load.

Several studies suggested a protective role of Mg. In a case control study of 163 women and 131 men, hypomagnesemia (serum magnesium ≤1.8 mg/dL) was independently associated with incident metabolic syndrome. Additional adjustment by serum C-reactive protein showed that metabolic syndrome remained associated with hypomagnesemia (OR 1.4; 95% CI 1.1-5.9) but not with oxidative stress determined by serum malondialdehyde concentration (OR 1.1; 95% CI 0.9-5.9) (Guerrero-Romero, 2006). In a prospective observational study with median follow up of 16 years, high magnesium intake was a significant protective factor for the incidence of Type 2 diabetes, especially among subjects with insulin resistance, low-grade inflammation and a drinking habit (Hata, 2013).

In rats subjected to magnesium-deficient diet, hypomagnesemia had a deleterious effect on glucose metabolism by impairing both insulin secretion and action. Mg deficient rats displayed 45% reduction in the glucose-stimulated insulin secretion. Mg restriction produced defective tyrosine kinase activity of insulin receptors, leading to decreased phosphorylation of the beta-subunit of the insulin receptor (Suarez, 1995).

Potential Mg repletion by KMgCit powder from provision of bioavailable Mg. By providing bioavailable Mg, KMgCit powder should prevent/ameliorate CTD-induced hypomagnesemia or Mg depletion. In prior studies with a tablet formulation of KMgCit, 30 healthy subjects developed hypomagnesemia (serum Mg < 1.8 mg/dL) on HCTZ 50 mg/day alone for 2-3 weeks (Pak, 2000). When KMgCit was added to HCTZ treatment at a dose of 42 meq K and 21 meq Mg/day, serum Mg increased to the normal range (Fig. 3).

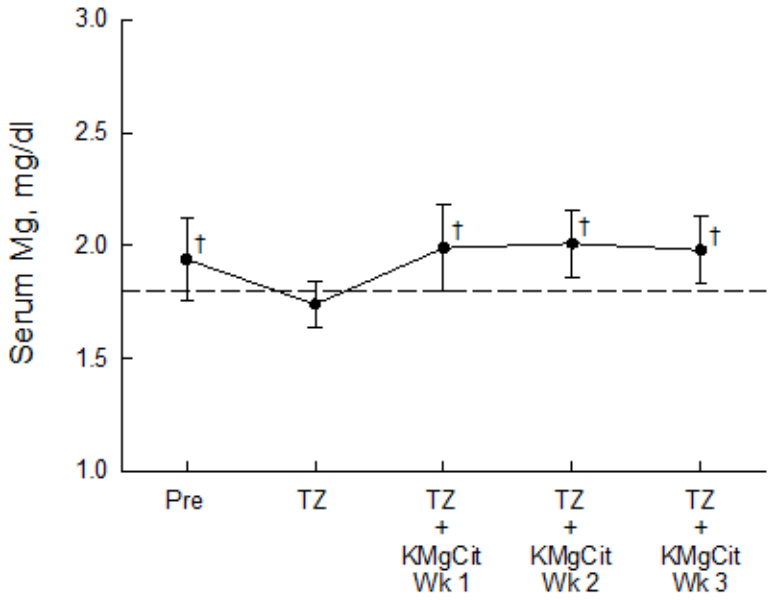

Fig. 3. Effect of KMgCit on serum Mg concentration among 30 subjects who developed hypomagnesemia alone on HCTZ. KMgCit was added to hydrochlorothiazide (TZ) therapy for three weeks. † = $p < 0.001$.

Among 131 subjects who took HCTZ, the percentage of subjects with hypomagnesemia progressively declined when KMgCit was added (Fig. 4, closed circles). However, the percentage of subjects with hypomagnesemia increased when KCl or potassium citrate was added to HCTZ treatment (open circles).

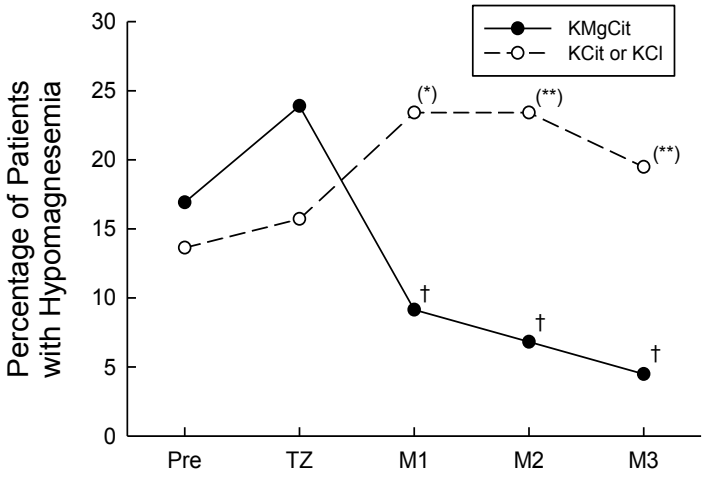

Fig. 4. The percentage of subjects with hypomagnesemia during addition of KMgCit or KCit/KCl to ongoing HCTZ therapy. * $p < 0.05$, ** $p < 0.01$ and † $p < 0.001$. Open symbols indicate within group comparisons, and symbols enclosed in parenthesis indicate between group comparisons. M1, M2 and M3 indicate 1, 2 or 3 weeks of combined treatment.

Summary

In summary, CTD may produce various metabolic disturbances, including hypokalemia, activation of RAA-SNS, oxidative stress, dyslipidemia, FGF23 synthesis, insulin resistance, and magnesium depletion (Fig. 5). These factors act solely or interact with each other to contribute to the development of Type II diabetes and metabolic syndrome. KMgCit powder can potentially overcome all of these metabolic disturbances. On the other hand, the action of KCl powder might be limited to correction of hypokalemia.

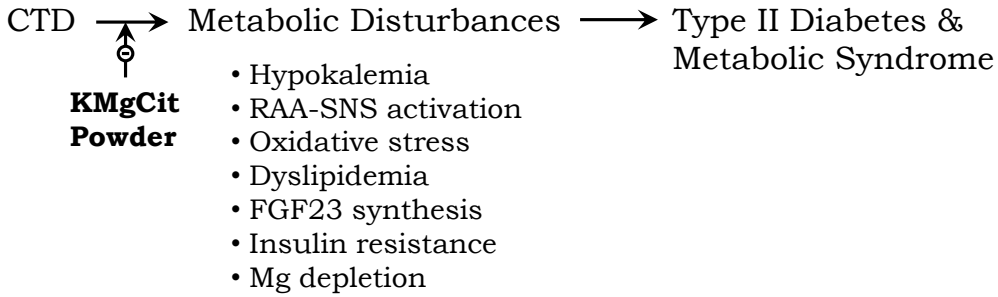

Fig. 5. Scheme for CTD-induced metabolic disturbances that could lead to Type II diabetes and metabolic syndrome. These disturbances might be blocked by KMgCit powder.

A special focus of this protocol will be on the CTD-induced Mg depletion (Fig. 6). CTD is well known to cause Mg depletion, hepatic steatosis and Type II diabetes-metabolic syndrome. We speculate that Mg depletion is responsible for hepatic fat deposition, which then produces insulin resistance. Co-administration of KMgCit powder would avert Mg depletion, block hepatic fat deposition by restoring normal Mg status and direct intestinal binding of fat, thereby ameliorating insulin resistance. To test this hypothesis, we shall quantitate muscle Mg status and hepatic fat content by MRS.

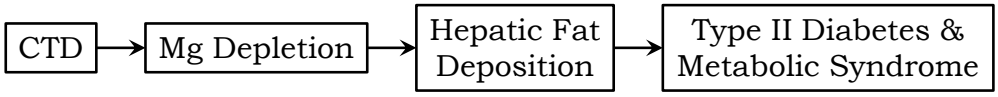

Fig. 6. Hypothesis for the central role of Mg depletion and hepatic fat deposition in the development of CTD-induced metabolic disturbances.

3. Significance:

Thiazide type diuretics are widely used as a first line treatment of hypertension in a very large number of subjects (James 2014, Vongpatanasin 2014). While this class of drugs is effective in lowering blood pressure, its use is complicated by various metabolic disturbances, many of which are features of metabolic syndrome induced or worsened by CTD. Some of these metabolic complications already coexist with hypertension and their exacerbation by TZ diuretics may not be readily recognized. KMgCit powder potentially represents a novel modality to not only prevent CD/TZ-induced hypokalemia, but also inhibit or ameliorate metabolic disturbances. This simple replacement of inorganic solutes can potentially allow CTD/TZ to exert its full antihypertensive efficacy without the negative drawback of worsening metabolic syndrome counteracting the benefits of blood pressure control.

4. Preliminary Data/Past Work:

This investigative group has already obtained preliminary data or published relevant work on all four aims of this proposal. Using a tablet formulation of KMgCit, the group has delineated physiological action of this compound in detail and some of its clinical effects. Safety of usage was also shown. Some data to be shown below were derived from original IND with KMgCit powder or in experimental animals.

Effect of CTD on inflammation. In our pilot data in 16 subjects with uncomplicated stage I hypertension, serum highly sensitive C-reactive protein (hsCRP) rose significantly from 3.2 (1.0 – 4.6; median, 25th – 75th percentile) at baseline to 4.8 (2.6 – 9.8) during CTD ($p < 0.05$) (Fig. 7). In contrast, spironolactone (Spiro) had no effect on hsCRP levels in the same subjects.

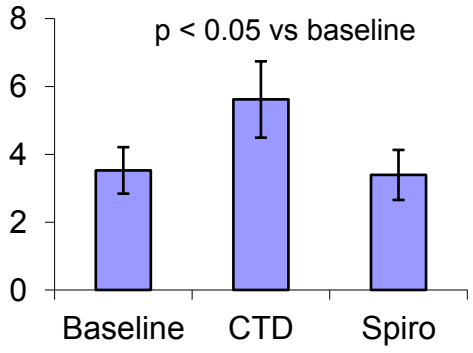

Fig. 7. Summary data showing changes in serum hsCRP (mcg/ml) in 16 untreated hypertensive subjects at baseline, after chlorthalidone treatment for 12 weeks, and after spironolactone treatment for 12 weeks.

Correction/prevention of TZ-induced hypokalemia (Wuermser, 2000). After treatment with HCTZ 50 mg/day alone for 2-3 weeks, 30 patients took KMgCit tab 42 meq K and 21 meq Mg daily, while 30 subjects received KCl 42 meq/day, along with HCTZ for 3 weeks (Fig. 8). Serum potassium declined on HCTZ alone. It rose to the normal range on both KMgCit and KCl to an equivalent degree.

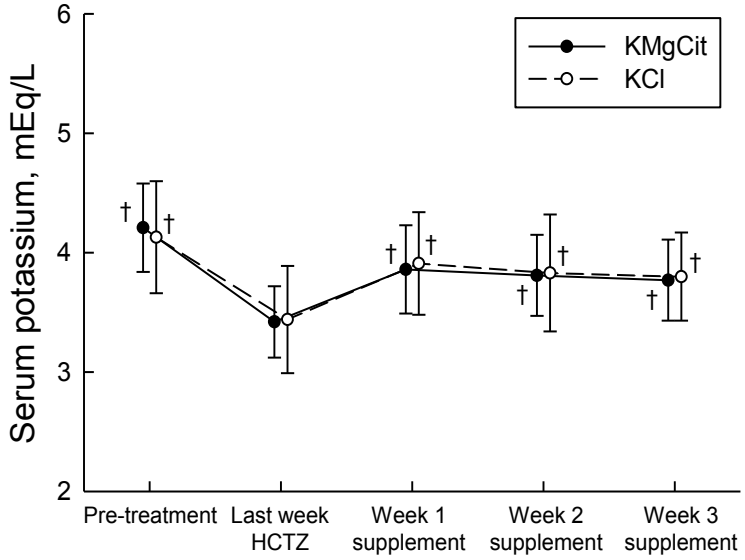

Fig. 8. Effect of KMgCit and KCl on serum potassium. † represents $p < 0.001$ from the last week of HCTZ alone.

Inhibition of RAA-SNS activation. In spontaneous hypertensive rats, high Mg diet blunted sympathetic mediated vasoconstriction (see Fig. 1).

Effect of KMgCit powder on oxidative stress. Urinary 8-isoprostane significantly declined on KMgCit powder. See Fig. 2.

Effect of KMgCit powder on serum triglyceride. In a retrospective analysis of data from a prior study (Odvina, 2006), 8 patients took HCTZ 50 mg/day with KMgCit tab 40 meq K and 20 meq Mg/day for 6 months. 10 patients took HCTZ 50 mg/day with KCl tab 40 meq K/day. Serum triglyceride declined by -32 ± 41 mg/dL from 1 month to 5 months of treatment in the KMgCit group, and 0 ± 31 in the KCl group ($p = 0.09$). Unfortunately, no baseline or 6 month data were available.

Effect of KMgCit powder on serum FGF23. In 30 patients with pre- and Stage I hypertension participating in a crossover randomized trial, serum FGF23 was significantly lower when they were taking KMgCit powder than KCl powder (46.1 +/- 16.7 Rel U/ml vs. 53.1 +/- 22.2 Rel U/ml, $p=0.05$).

Prevention or correction of thiazide-induced hypomagnesemia. See Fig. 3 & Fig. 4.

5. Methods of Procedure:

Patients to be studied

Studies will be conducted only in patients with treated or untreated stage I hypertension without concomitant cardiovascular diseases, chronic kidney diseases, or compelling indication for treatment with ACE inhibitor, beta blockers, or ARB. Patients will be adult men or women (> 21 years of age) of any ethnicity. Study is planned in 72 patients to account for potential dropout rate of 20%.

Excluded will be patients with

- diabetes mellitus,
- Renal impairment (serum creatinine > 1.4 mg/dL),
- Any heart diseases such as congestive heart failure, sustained arrhythmia, or coronary heart disease,
- Chronic regular NSAID use,
- Allergy to thiazide diuretics,
- Gastro-esophageal reflux disease (GERD) requiring treatment with acid reducing agents or antacid more than once a week,
- Esophageal-gastric ulcer or history of gastrointestinal bleeding,
- Chronic diarrhea, vomiting,
- Excessive sweating,
- Unprovoked hypokalemia (serum K < 3.5 mmol/L) or hyperkalemia (serum K > 5.3 mmol/L),
- Abnormal liver function test (AST or ALT above upper limit of normal range),
- Subjects on any potassium supplement on a regular basis for any reason, such as patients with primary aldosteronism,
- Pregnancy,
- History of major depression, bipolar disorder, or schizophrenia,
- History of substance abuse,
- Gout,
- Metabolic alkalosis, with serum bicarbonate > 32 meq/L,
- Severe dietary salt restriction, less than1/2 spoonful or 50 meq sodium/day.

Study design

The patients will participate in a three-phase study in a parallel design (Fig. 9).

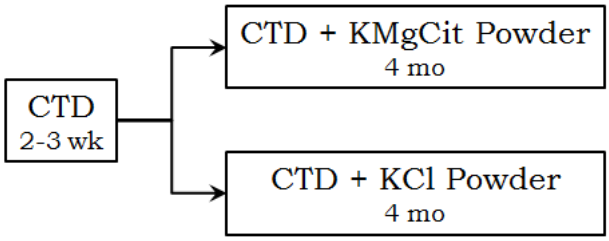

Fig. 9. Study scheme.

After baseline evaluation (Phase 1, without CTD), patients will take CTD alone for 2-3 weeks (Phase 2, CTD alone). They will then be randomized to two equal groups to take KMgCit powder or KCl powder along with CTD for 4 months (Phase 3).

Test drugs

KCl powder, made by and purchased from Sterling Pharmaceutical Services LLC, will contain 20 meq K as potassium chloride per sachet. KMgCit powder, also made by Sterling, will contain 20 meq K, 10 meq Mg, and 30 meq citrate, per sachet. These preparations will be prepared by the same method, and their composition will be the same, as those used in the completed trial (original IND 116,208).

The sachets of KCl and KMgCit powder will be identical in appearance. During Phase 3, patients will dissolve the contents of each sachet of KCl or KMgCit powder in 250 ml water and ingest it with breakfast and again with dinner, to deliver 40 meq K per day. In the group taking KMgCit powder, patients will also receive Mg 20 meq and citrate 60 meq per day.

A taste enhancer and sucralose will be added to improve tolerance of the test medications. Each sachet will be marked by a code, the identity of which will be known only to an independent study monitor. Each sachet will be labeled: Study drug # --; CTD-Induced Hypokalemia; Investigator: W. Vongpatanasin, M.D.

Chlorthalidone, 25 mg generic tablets, will be purchased from a local pharmacy.

Conflict of interest statement

UT Southwestern had licensed KMgCit powder to Althos Pharma. Unfortunately, Althos Pharma is no longer operational due to financial difficulties. Accordingly, all the licensing agreements between UTSW and Althos Pharma, including one for KMgCit powder, have been terminated. (Tracy Roberson, licensing officer of UTSW's Office of Technology Development, can be consulted for verification.) No relationship now exists between Althos Pharma and UTSW or investigators.

Despite lack of industrial sponsorship, the investigators are convinced that the high scientific merit of the proposal mandates its pursuit. Thus, this protocol is investigator-initiated and research-driven, and not industry-sponsored. The initial costs of the study will be borne by the biotechnology program of CMMCR. CMMCR will purchase test medications from Sterling Pharmaceutical Services.

Outline of the study (Table 1)

Baseline (Phase 1, time zero). At enrollment, various tests/procedures as outlined in Table 1 will be obtained. Patients who are on antihypertensive medications will be asked to stop them.

CTD Alone (Phase 2). Patients will take CTD 25 mg/day. Two weeks later, they will return with a 24-h urine collection. Venipuncture and other procedures as outlined in Table 1 will be performed. Subjects who develop hypokalemia (serum K < 3.5 mEq/L) will immediately enter Phase 2. The remaining patients will continue on CTD for another week. On return at 3 weeks, all the tests/procedures will be performed in all patients.

Table 1. Study Outline

Weeks/months	0	2/3w	Rx	1m	2m	3m	4m
Treatment							
CTD	----------	----------	----------	----------	----------	----------	----------
KMgCit/KCl			----------	----------	----------	----------	----------
Office blood pressure	√	√		√	√	√	√
Serum tests	√	√			√		√
Urine tests	√	√			√		√
Side effects QN	√			√	√	√	√
Diet history & BWt	√			√	√	√	√
Sachet count				√	√	√	√
CTD pill count		√		√	√	√	√
Fecal occult blood	√				√		√
Muscle Mg MRS	√						√
Hepatic fat MRS	√						√

Some patients may require only 2 weeks of CTD, instead of 3 weeks. Thus, the complete study duration might be shorter by 1 week.

If patients develop symptomatic hypotension (blood pressure ≤ 90/60 mmHg) after CTD treatment for 2 weeks, the dose of CTD will be reduced to 12.5 mg daily temporarily. Blood pressure will be repeated 1 week after dose reduction. If BP is increased above 120/80 mmHg, the dose of CTD will be increased to 25 mg daily. Few patients who cannot later tolerate a higher dose of 25 mg/day will be withdrawn from the trial. If patients develop (BP ≥ 130/80mmHg) after CTD treatment for 2 weeks, amlodipine 5 mg daily will be added to the regimen for blood pressure control. Blood pressure will be repeated in 1 week.

CTD + KMgCit/KCl (Phase 3). Patients who developed hypokalemia at 2 weeks of CTD will enter Phase 3. Those who completed three weeks of treatment with CTD alone will enter Phase 3 whether or not they develop hypokalemia. All patients will continue to take CTD 25 mg/day. All subjects will be randomized to receive KMgCit powder or KCl powder at a dose of 40 meq of K daily for 4 months. Various tests will be performed at times indicated in Table 1.

Tests

Office blood pressure. During clinic visits at baseline, at 2 and/or 3 weeks of CTD alone, at monthly intervals during combined treatment with CTD and KMgCit or KCl powder, the research nurse will obtain blood pressure by using the clinic's oscillometric device (Welch Allyn, Vital Signs, WA). At each visit, three blood pressure measurements will be made after subjects sit quietly for at least 10 minutes. The first reading will be discarded. The 2nd and 3rd measurements will be averaged.

Serum tests. At baseline, 2 and/or 3 weeks after CTD alone, and at 2 and 4 months of combined CTD and K salt treatment, a fasting venous blood sample will be obtained for electrolytes (potassium, sodium, chloride and carbon dioxide), magnesium, creatinine, calcium, phosphorus, albumin, triglyceride, cholesterol, LDL, HDL, and glucose (by Quest Laboratory). Serum PTH, insulin, 8-isoprotanes, hsCRP, FGF23 and Klotho will be measured (by the Mineral Metabolism Laboratory, Moe's Laboratory or O'Brien Center). At same times, a sample will be frozen for aldosterone (to be determined later when warranted by the results). Lymphocytes will be harvested from EDTA-treated blood sample and stored in RNALater for WNK1 mRNA (to be determined later when warranted in the laboratory of C.-L. Huang).

Urine Tests. A 24-h urine sample will be collected under oil at baseline, 2 and/or 3 weeks of CTD alone, 2 months and 4 months of combined treatment. Samples will be analyzed for stone risk factors (including potassium, pH, citrate, magnesium, sodium, calcium, phosphorus, sulfate, chloride, creatinine, and total volume), and net acid excretion by the Mineral Metabolism Laboratory. Samples will also be analyzed for 8-isoprostanes (by Moe's laboratory) and frozen for aldosterone (to be determined later).

At same times, a fresh spot urine sample will be obtained for creatinine (by Mineral Metabolism Laboratory), and frozen for WNK1 protein and mRNA, and exosomes (to be determined in the laboratories of Huang and Moe if warranted). WNK1 might be a mediator of changes in renal (sodium handling) and vascular (resistance) determinants of blood pressure that might ensue from varying potassium intake. Exosomes are shed membranes enclosing cytoplasm from the kidney shown to contain mRNA The measurement will allow us to assess levels of proteins and signaling molecules involved in the renal mineral transport.

Side effect questionnaire. At baseline, 2 and/or 3 weeks after CTD, and at monthly visits of combined treatments, the research nurse will complete the side effect questionnaire by interviewing the patients (Table 2). This history permits computation of "gastrointestinal symptom score", a quantitative estimate of frequency and severity of gastrointestinal side effects (Ruml, 1999).

Other tests. At each follow-up visit, the research nurse (D. Arbique) will collect the test medications and count the number of unused packets or pills, in order to estimate compliance of patients in taking medications. At each visit, the research nurse will obtain history of unusual dietary intakes. Body weight will be measured. Smear of stool brought in by patients will be tested for occult blood.

Muscle Mg by MRS. Intracellular muscle Mg will be obtained by magnetic resonance spectroscopy in the Department of Radiology.

Hepatic fat by MRS. Hepatic TG content will be evaluated using a1.5 Tesla Gyroscan Achieva whole body clinical system (Philips Medical Systems, Cleveland, USA) equipped with software for localized spectroscopy as described (Price, 2013). In short, high-resolution morphological images are collected to serve as a "roadmap" for selection of a testing volume of 27 cc within the upper right hepatic lobe. All spectra are collected using PRESS sequence (Point RESolved Spectroscopy) for spatial localization and the signal acquisition with the following data acquisition parameters: Te = 27 ms, Tr = 3 s. All data are collected without water suppression. Areas of resonances from protons in water molecules and in methylenes of fatty acid chains will be evaluated with line-fit procedure using a commercial software (NUTS-ACORNNMR, Freemont, CA).

Subjects with claustrophobia or metal devices precluding MRI, will not have MRI completed as part of this study.

Table 2. Side Effect Questionnaire

Gastrointestinal 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	❑	❑	❑	❑	❑	❑	❑	❑	_____
Pain/Cramps	❑	❑	❑	❑	❑	❑	❑	❑	_____
Melena	❑	❑	❑	❑	❑	❑	❑	❑	_____
Dyspepsia	❑	❑	❑	❑	❑	❑	❑	❑	_____
Anorexia	❑	❑	❑	❑	❑	❑	❑	❑	_____
Dysphagia	❑	❑	❑	❑	❑	❑	❑	❑	_____
Dysgeusia	❑	❑	❑	❑	❑	❑	❑	❑	_____
Other	❑	❑	❑	❑	❑	❑	❑	❑	_____

6. Statistical Analysis

Expectations (Table 3)

Table 3. Anticipated Findings

	CTD	CTD + KMgCit	CTD + KCl
Ks	↓	=	=
Isoprostanes	↑	↓	↑
Serum FGF23	=	↓	=
HOMA-IR	↑	↓	↑
Aldosterone	↑	↓	↑
Muscle Mg		↑	↓
Hepatic fat		↓	↑

- On CTD alone, serum K would decline, reaching below normal in most patients. On both KMgCit powder and KCl powder along with CTD, serum K would increase to the normal limits. Similar levels of serum K would be observed during KMgCit powder vs. KCl powder.
- Office blood pressure would decline during CTD alone. It would remain low on combined KMgCit powder or KCl powder with CTD.
- It is expected that serum and urinary isoprostanes (marker of oxidative stress) would increase on CTD alone, decrease on combined KMgCit powder + CTD and remain unchanged on KCl powder + CTD.
- We anticipate that serum FGF23 would increase on CTD alone, decrease on addition of KMgCit powder but not KCl powder.
- Fasting plasma glucose and insulin would be higher during CTD when compared to baseline. KMgCit powder would attenuate the increase in plasma glucose and insulin, whereas KCl powder would not. HOMA-IR would show changes in the same direction.
- Serum aldosterone (marker of renin-angiotensin system) would increase on CTD; the addition of KMgCit powder would attenuate this rise, but KCl powder would not.
- The gastrointestinal symptom score during CTD alone will be similar to baseline. On combined treatment, gastrointestinal symptom score would be higher for KCl powder than KCit powder, with a larger percentage of patients complaining of dysgeusia on KCl powder.
- At 3 weeks, 24-hour urinary potassium on KMgCit powder and KCl powder would be higher than CTD alone by about 35-40 meq/day. Urinary pH and citrate would be higher on KMgCit powder than CTD alone or KCl powder.
- Free muscle Mg would increase on KMgCit powder but decline on KCl powder.
- Hepatic fat would increase on KCl powder + CTD, but decline or remain unchanged on KMgCit + CTD.

Sample Size Calculations

From our previous CTD studies, the mean increase in fasting serum glucose at CTD dose of 25 mg/day plus KCl supplementation to keep serum K constant was 10 mg/dL. The within subject SD of serum glucose was between 2.0-8.5. Assuming SD = 8.5, power of 0.8 and α of 0.05, 60 patients (30 randomized to KCl powder and 30 to KMgCit powder) would be needed to detect a potential reduction in serum glucose of 6 mg/dL by KMgCit. Assuming SD of 5, power of 0.80 and α of 0.05, 60 patients would be needed to detect a potential reduction in serum glucose of 4 mg/dL by KMgCit. To account for a dropout of 20%, we plan to recruit 72 subjects for the study.

Statistical Methods

Descriptive statistics and 95% confidence intervals will be used to summarize blood pressure and other responses for the three phases. Data from the three phases will be compared by using the repeated measures analysis of variance models. Significant differences between the three phases will be further analyzed by comparison of pairs of phases using contrasts constructed from the ANOVA models. By employing Bonferroni inequality to adjust for multiple testing, various comparisons between phases will be made. In addition, the existence of a trend in the differences of various parameters between phases will be sought (Perlman, 2002). Interim analysis will be performed at 50% completion of the trial.

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