PROTOCOL NUMBER: HS-18-00737

TITLE: A pilot study evaluating single, high-dose pharmacokinetics/pharmacodynamics of vitamin D3 in CF

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TABLE OF CONTENTS

1.0 BACKGROUND AND HYPOTHESES

Background

Vitamin D is a fat-soluble vitamin that plays an important role in immune modulation in addition to its classical roles in regulation of calcium homeostasis and bone health (1). Vitamin D undergoes two subsequent hydroxylation reactions to form the active metabolite, $1,25(OH)_2D$. 25(OH)D, which is an intermediate inactive metabolite, is the major circulating form of vitamin D, and its total serum concentration is used to assess the overall vitamin D status of an individual. Vitamin D insufficiency is defined as a serum 25(OH)D level between 29 and 20 ng/mL, while vitamin D deficiency is defined as a serum 25(OH)D levels below 20 ng/mL. Identification of vitamin D receptors and vitamin D-metabolizing enzymes (CYP27B1, CYP24A1) in immune cells revealed the immune-modulating activity of vitamin D. There is a plethora of evidence of anti-inflammatory effect for vitamin D through inhibition of pro-inflammatory cytokine expressions (2-4).

Current recommended threshold for vitamin D adequacy has been controversial. According to the Endocrine Society, target 25(OH)D level of 30 ng/mL (or 75 nmol/L) is based on the finding that it is the minimal concentration required for optimal bone health while maximally suppressing parathyroid hormone productions in adults (5-7). However, in vitro study revealed that concentrations greater than 30 ng/mL may be required to have maximal anti-inflammatory effect (8). Clinical consequences of vitamin D inadequacy go well beyond osteoporosis; vitamin D deficiency is associated with increased risks of several diseases, including diabetes, cancer and inflammatory diseases (9-13). Accordingly, several studies demonstrated a positive effect of vitamin D supplementation on inflammatory markers indicating that correcting vitamin D status may serve as an effective anti-inflammatory therapy (14-16).

Cystic Fibrosis and Vitamin D deficiency

Cystic fibrosis (CF) is a lethal autosomal recessive disorder characterized by chronic airway infections, inflammation and obstruction, leading to progressive loss of lung function and ultimately respiratory failure. Vitamin D deficiency is highly prevalent in CF patients, affecting approximately 90% of patients with CF (17, 18). Malabsorption of fat-soluble vitamins, which is a result of exocrine pancreatic enzyme insufficiency, likely play a role in vitamin D deficiency (19). Over 85% of CF patients are affected by pancreatic insufficiency, which causes intestinal malabsorption of nutrients, such as vitamin D (20). In addition to malabsorption, other factors may also contribute to suboptimal vitamin D status, including reduced exposure to sunlight, reduced levels of vitamin D binding proteins and poor adherence to daily dosing (20-22). Inadequate vitamin D level is of particular concern for patients with CF as vitamin D deficiency has been associated with increased risk of respiratory infection and worsened pulmonary function (21).

Study Rationale

Despite the extensive literatures on adverse clinical outcomes associated with vitamin D deficiency and beneficial effects of vitamin D supplementation in the treatment of various inflammatory and infectious diseases , there are currently no proven treatment strategy that effectively achieves and maintains optimal serum vitamin D status in CF patients (23). For the treatment of vitamin D deficiency in CF, CF Foundation currently recommends 2,000 IU daily (24). However, because achieving adequate serum 25(OH)D levels is a challenge in CF, higher doses of vitamin D may be necessary to reach and maintain vitamin D sufficiency (25, 26). A pilot study of vitamin D supplementation in adults with CF experiencing pulmonary exacerbation revealed that high dose of vitamin D (250,000 IU) raised 25(OH)D levels while it did not cause hypercalcemia or significant changes in PTH concentrations (26), which indicates that this mega-dose of vitamin D3 is safe in CF patients. In addition, several studies in non-CF population have demonstrated that high single doses of vitamin D3 did not significantly alter the PTH or calcium levels (27-29). In a retrospective chart

review of pediatric CF patients, majority of patients (79%) rapidly and safely achieved and sustained optimal 25OHD levels over a 12 month period with a single high-dose oral vitamin D3 (300,000 IU – 600,000 IU), also known as stoss therapy (25). However, approximately 20% of patients still could not achieve levels above 30 ng/mL. Wide inter-individual variability in response to vitamin D treatment has been previously reported in non-CF population, suggesting that fixed-dose approach to replacement therapy may not be effective in improving 25(OH)D status in CF (30). Poor oral bioavailability of ergocalciferol has been demonstrated in CF patients (31), which suggests that CF patients may potentially exhibit low absorption of cholecalciferol. Therefore, based on the degree of malabsorption of vitamin D and basal vitamin D status, supplementation should be tailored to individuals. In order to optimize the treatment of deficiency in CF, kinetic disposition of vitamin D must be well understood. However, there are very few data currently available describing the kinetics of both vitamin D and 25-hydroxyvitamin D, and to the investigator's knowledge, no studies have yet characterized the pharmacokinetic disposition of vitamin D in cystic fibrosis. Addressing this issue is crucial in effectively and safely correcting vitamin D deficiency in CF.

Our study hypothesis is that CF patients with vitamin D deficiency exhibit altered pharmacokinetics of vitamin D and 25(OH)D when compared to non-CF subjects with vitamin D insufficiency or deficiency.

2.0 OBJECTIVES AND PURPOSE

2.1 Primary Objective:

Characterize the pharmacokinetics of vitamin D3 and 25(OH)D3 with a single, high-dose of vitamin D3 in adults with CF.

Compare the pharmacokinetics of vitamin D3 and its metabolites between non-cystic fibrosis controls with low vitamin D levels and adults with cystic fibrosis

2.2 Secondary Objective:

Assess the anti-inflammatory effects of high dose vitamin D therapy in adults with CF.

3.0 STUDY DESIGN

This is a pilot study to determine the pharmacokinetics-pharmacodynamics of vitamin D3 in CF patients. Clinically stable CF patients with a history of pancreatic insufficiency (n=6) and matching non-CF subjects (n=6) will be recruited in this study. The presence of pancreatic insufficiency will be based on a known CF genotype associated with pancreatic insufficiency. All subjects will be pre-screened for 25(OH)D status to include those with 25(OH)D levels below 30 ng/mL. The subjects will receive a single oral dose of vitamin D3, known as stoss therapy. The dose of stoss therapy will be based on patient's baseline serum 25-hydroxyvitamin D3 level. For CF patients, the dose will be administered with food and pancreatic enzyme supplement to maintain nutritional status. This dose was chosen because previous studies in pediatric CF patients demonstrated that a large single dose of up to 600,000 IU vitamin D3 raised and maintained sufficient 25(OH)D concentrations over 12 weeks without any signs of adverse events (25, 26).

4.0 DRUG/DEVICE INFORMATION

4.1 Vitamin D3 (Cholecalciferol)

Currently, best approach to treat vitamin D deficiency is vitamin D supplementation. Cholecalciferol has shown to be effective in raising serum 25(OH)D concentrations in adult CF patients (32). Each patient will be given vitamin D3 50,000 IU capsules from BioTech Pharmacal Inc. Vitamin D3 is considered a dietary supplement and therefore is not part of FDA-regulated drugs.

5.0 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Inclusion Criteria (CF) :

Diagnosis of CF based on positive sweat chloride or known CF mutation
Patients with pancreatic insufficiency
Age ≥ 18 years
Serum 25(OH)D concentrations below 30 ng/mL (75 nmol/L)

Inclusion Criteria (non-CF) :

Age ≥ 18 years
Serum 25(OH)D concentrations below 30 ng/mL (75 nmol/L)

5.2 Exclusion Criteria

Pregnancy
History of lung transplant,
Severe anemia (hemoglobin concentration < 7 g/dL),
Liver disease (AST/ALT > 3x ULN), kidney disease (GFR ≤ 40 mL/min), or granulomatous conditions
Patients taking steroids, cholesterol-lowering drug (cholestyramine), weight-loss drugs (Orlistat) , statins, anti-tuberculosis drugs (rifampin and isoniazid), phenobarbital, phenytoin, carbamazepine, immunosuppressant (cyclosporine, tacrolimus) (33, 34)

5.3 Withdrawal Criteria

Subject can be discontinued from the study for any of the following reasons:
Subject's personal reasons
Other significant study related adverse events

6.0 STRATIFICATION/DESCRIPTIVE FACTORS/RANDOMIZATION SCHEME

6.1 Stratification factors: Not applicable

6.2 Descriptive factors. Not applicable

7.0 STUDY AGENT ADMINISTRATION OR INTERVENTION AND TOXICITY MANAGEMENT PLAN

7.1 Patients will be given a single dose, oral capsule of vitamin D3 based on measured 25-(OH) vitamin D level (25).

7.2 Drug studies:

AGENT	DOSE	ROUTE	DAYS	ReRx	NOTES
Cholecalciferol	600,000 IU	PO	1	Once	25-(OH) vitamin D ≤ 25 nmol/L (≤ 10 ng/mL)
Cholecalciferol	500,000 IU	PO	1	Once	25-(OH) vitamin D 25-50 nmol/L (10-20 ng/mL)
Cholecalciferol	300,000 IU	PO	1	Once	25-(OH) vitamin D 50-75 nmol/L (20-30 ng/mL)

7.3 Criteria for removal from treatment

7.31 A patient may always be removed from treatment whenever he/she wishes.

7.4 Ancillary treatments.
Patients will not be allowed to take supplements containing vitamin D3 during the trial.

8.0 ASSESSMENT OF EFFICACY AND SAFETY

8.1 Side effects/Toxicities to be monitored.

8.11 Possible side effects of vitamin D include: hypercalcemia, nausea, appetite, thirst, frequent urination, constipation, vomiting, abdominal pain, muscle weakness, muscle and joint pain, confusion, lethargy and fatigue (14, 35, 36). To ensure safety of high dose vitamin D in CF patients, we will be assessing the patients' calcium, magnesium, phosphate, albumin and alkaline phosphatases levels, at screening and day 7.

8.12 Long-term toxicities to be monitored after completion of therapy. List side effects/toxicities and define levels.
Long-term vitamin D toxicities have not been determined. Single high intramuscular dose (600,000 IU) of vitamin D3 was shown to be safe in patients with osteoporosis (37), and accidental mega-dose of 2,000,000 IU was well

tolerated, with slightly elevated calcium levels in the first two weeks, in two nursing home patients (38), suggesting that high bolus dose of cholecalciferol is safe.

8.2 Dosage change based on toxicity. Not applicable.

8.3 Adverse Event Reporting: Procedures for reporting unexpected and fatal toxicity should be explained.

8.31 Any significant adverse events that occurs during the course of the study will be documented and reported. The types of event to be reported include Type of event to be reported and timing of reports.

8.32 Places for submitting reports: IRB

8.4 Data Monitoring Committee (if applicable): Not Applicable

9.0 CLINICAL AND LABORATORY EVALUATIONS AND STUDY CALENDAR

See Appendix I

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

Primary Outcome Measure
1. Peak plasma concentrations (Cmax)
2. Time taken to reach the maximum concentration (Tmax)
3. Area under the plasma concentration versus time curve (AUC)

Secondary Outcome Measure
1. Levels of serum inflammatory biomarkers
   - Changes in IL-6, IL-8, TNF-α, IL-1β, C-reactive protein

11.0 SPECIAL INSTRUCTIONS:

Blood samples will be taken at baseline (0 h) and at 2, 4, 6, 8 hours and 1, 7, 28, 56 and 70 days after intervention. At each defined time points, 5 mL of venous blood will be collected in serum separator tubes (BD Vacutainer) with clot activator (39) to measure serum vitamin D3 and 25(OH)D3 concentrations. Samples will be handled with care to minimize sunlight exposure during the time of collection. Samples will be immediately placed on ice and stored in aliquots at -80 °C until analysis. Serum levels of vitamin D3 and its metabolites will be determined using LC/MS-MS. Levels of inflammatory biomarkers will also be determined at each defined time points.

12.0 DATA COLLECTION AND MONITORING

Data will be collected on case report forms, which will be retained for the duration of the study. Data will include subject demographics, clinical characteristics, laboratory data, and results of pharmacokinetic analyses. Data will be coded and stored on a password protected electronic database.

13.0 STATISTICAL CONSIDERATIONS

13.1 Pharmacokinetic Analysis

Population pharmacokinetic analysis will be performed using the maximum likelihood estimation via the EM algorithm (MLEM) as implemented in ADAPT 5 (Biomedical Simulations Resource, University of Southern California). Multiple candidate models will be evaluated to identify the model that best describes the observed plasma concentration data for vitamin D3 and 25(OH)D3. The maximum concentration and time to maximum concentration will be determined from the observed data.

Patient demographics, clinical characteristics, and pharmacokinetic parameters will be summarized using descriptive statistics.

Differences in the pharmacokinetic parameters between non-CF controls and CF patients will be determined by non-parametric Wilcoxon-Mann Whitney test.

13.2 Comparison of mean serum and plasma concentrations of inflammatory markers at baseline and 10 weeks post intervention

Mean serum/plasma concentrations of inflammatory biomarkers will be compared between CF and non-CF, as well as within groups. Differences in the mean concentrations of inflammatory markers will be determined by non-parametric Wilcoxon-Mann Whitney test.

13.3 Pharmacodynamic model of vitamin D3

Time course of inflammatory responses to vitamin D3 treatment will be used to develop the pharmacodynamics model of vitamin D3.

14.0 REGISTRATION GUIDELINE

14.1 Subjects will be registered into the study through submission of the research order form, informed consent, and patient bill of rights to the Clinical Trials Office (CTO) at USC.

15.0 BIOHAZARD COMTAINMENT

Not Applicable

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

All institutional and Federal regulations concerning the Informed Consent form will be fulfilled. The study will be conducted in adherence to ICH Good Clinical Practice.

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APPENDICES

Appendix I: Study Calendar

Appendix I

	Screening	Day 0	Day 1	Day 7	Day 28	Day 56	Day 70
Informed Consent	X						
Medical History	X						
Medication History	X						
Height, weight	X						
Vital signs[A]	X	X	X	X	X	X	X
Safety labs[B]	X			X			
Pre-screening of 25OHD status	X						
PO drug administration		X					
Blood Pharmacokinetics (PK)[C]		X	X	X	X	X	X
Blood inflammatory biomarkers		X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X

A. Vital signs to include blood pressure, heart rate, temperature, respiratory rate.
B. Safety labs to include comprehensive metabolic panel
C. All subjects will have a pre dose blood PK levels, 2, 4, 6, 8 hours and 1, 7, 28, 56, 70 days post dose of cholecalciferol. A 5mL serum separator tube will be used to collect the blood sample.