Clinical Trial Results Synopsis

Name of Sponsor/Company:
Medical University of Graz, Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Austria

Name of Finished Product:
OLEOVIT® D_3 Tropfen (Fresenius Kabi, Austria)

Name of Active Ingredient:
Colecalciferol (vitamin D_3)

Title of Study:
Effects of vitamin D supplementation in healthy women and men on immunological, endocrine and metabolic parameters

Principal Investigator:
Thomas Pieber

Study Centre(s):
This single-centre study was conducted at the outpatient clinic of the Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Austria.

Publication (reference):
Prietl B, Pilz S, Wolf M, Tomaschitz A, Obermayer-Pietsch B, Graninger W, Pieber TR. Vitamin D Supplementation and Regulatory T Cells in Apparently Healthy Subjects: Vitamin D Treatment for Autoimmune Diseases? Isr Med Assoc J 2010;12(3):136-139

Study Period:
The study was conducted in the outpatient clinic of our department from February 2009 to June 2009.

Phase of Development:
Phase IV

Objectives:
The primary objective of the study was to evaluate whether vitamin D supplementation significantly alters the proportion of regulatory T cells (Tregs) of all circulating CD4+ T cells.

The secondary objective of the study was to evaluate whether vitamin D supplementation significantly alters the homeostasis model assessment (HOMA) index, renin, aldosterone, osteoprotegerin, high-density lipoprotein cholesterol and asymmetric dimethylarginine (ADMA) concentrations.

Methodology:
This was an uncontrolled, monocentric trial in healthy participants. The trial consisted of a baseline visit (visit 0) and two follow-up visits, 4 weeks (visit 1) and 8 weeks (visit 2) after the baseline examination. At each study visit blood was drawn after an overnight fast between 7 and 11 AM and a pregnancy test was performed in all female study participants. At visit 0 and 1, all participants who did not meet the exclusion criteria received 140,000 IU vitamin D_3 drops orally (Oleovit D_3®).

For analysis of regulatory T cells (Tregs; $CD4^{+}CD25^{hi}FoxP3^{+}$ cells with low or absent expression of CD127) peripheral blood mononuclear cells were isolated and stored in liquid nitrogen. Thawed cells were stained with anti-CD4 FITC, anti-CD 25 PE-Cy7 and anti-CD127 PE monoclonal antibodies (BD Pharmingen, San Diego, CA, USA). After surface staining, cells were permeabilised for intracellular staining for the transcription factor FOXP3 using anti-FOXP3-Alexa Fluor 647 monoclonal antibodies (BD Pharmigen), followed by cell analysis on a FACSCanto II flow cytometer (BD Biosciences).

Serum vitamin D (25(OH)D) was analysed using an enzyme-linked immunosorbent assay (IDS, Bolden, UK) with an intra- and inter-assay coefficient of variation of 5.6 and 6.4%, respectively. C-reactive protein was measured by the Tina-quant C-Reactive Protein assay (Roche COBAS INTEGRA, Germany). All other laboratory measurements were performed by routine methods.

Number of Subjects

A total of 50 participants have been enrolled in the study and 46 Subjects completed the trial.

Diagnosis and Main Criteria for Inclusion:

Healthy individuals aged at least 18 years, who gave written informed consent were included in the study. Individuals were excluded from the study if any of the following criteria applied: hypercalcaemia (serum calcium >2.65 mmol/L), pregnant or nursing women, any disease that requires medical treatment or participation in other interventional clinical trials.

Test Product, Dose and Mode of Administration:

OLEOVIT® D_3 oral drops, 140,000 IU cholecalciferol, oral administration.

Duration of Treatment:

The trial consisted of a baseline visit and two follow-up visits. The follow-up visits were performed 4.4 ± 0.5 (mean ± SD) weeks (visit 1) and 8.8 ± 1.0 weeks (visit 2) after the baseline examination.

Reference Product, Dose and Mode of Administration:

N.A.

Criteria for Evaluation:

Efficacy:

Primary endpoint of the study was change in the proportion of Tregs of all circulating CD4+ T cells.

Safety:

Safety assessments included adverse events and physical examination.

Statistical Methods:

Descriptive statistics and the Kolmogorov-Smirnov test was used to test for normality of the distribution. 25(OH)D levels followed a skewed distribution and were thus logarithmically transformed before use in parametric procedures.

Pearson correlation analyses of the percentage of Tregs and 25(OH)D values were performed for all study visits. In addition, percentage of Tregs and 25(OH)D values from all study visits were used for a correlation analysis. Paired student's *t*-test was used to test for differences in percentage of Tregs, 25(OH)D and serum calcium between the study visits. Statistical analyses were performed by SPSS version 16.0 (SPSS Inc, Chicago, USA) and a P value below 0.05 was considered statistically significant.

SUMMARY OF RESULTS AND CONCLUSIONS:

Baseline Demographics and Characteristics:

The 50 participants had a mean ± SD age of 31 ± 8 years and 64% were female. The mean body height was 171.7 ± 8.8 cm, mean body weight was 68.8 ± 13.7 kg and mean body mass index was 23.3 ± 4.3 kg/m^2. The mean C-reactive protein level was 2.2 ± 2.7 mg/L and mean serum calcium concentration was 2.4 ± 0.99 mmol/L. At baseline, mean 25(OH)D concentration was 24.1 ± 12.6 ng/mL and 80% of the participants had 25(OH)D levels below 30 ng/mL (indicative for an insufficient vitamin D status).

Subject Disposition:

A total of 50 participants were enrolled into the study and received the study drug. Out of these, 46 participants completed the study. Two participants were excluded after visit 0 (one participated in another interventional trial, one due to previously diagnosed type 1 diabetes mellitus) and two participants were excluded after visit 1 (one was non-compliant with the study protocol, one because of mild asymptomatic hypercalcaemia).

Efficacy Results:

25(OH)D levels increased from 23.9 ± 12.9 ng/mL (mean ± SD) at baseline to 45.9 ± 14.0 ng/mL at visit 1 (P<0.001) and 58.0 ± 15.1 ng/mL at visit 2 (P<0.001). At visit 2, all study subjects had a sufficient vitamin D status (25(OH)D levels >30 ng/mL).

Percentage of Tregs within 20,000 CD4+ cells were 4.8 ± 1.4 at baseline. Compared to baseline values percentage of Tregs were significantly increased at visit 1 (5.9 ± 1.7, P<0.001) and visit 2 (5.6 ± 1.6, P<0.001), but significantly decreased from visit 1 to visit 2 (P=0.011).

Pearson correlation coefficients of percentage of Tregs and 25(OH)D levels were 0.223 (P=0.120) at baseline, 0.092 (P=0.530) at visit 1 and 0.170 (P=0.259) at visit 2. When percentage of Tregs and 25(OH)D levels of all study visits were used for correlation analysis the Pearson correlation coefficient was 0.315 (P<0.001).

C-reactive protein levels did not significantly differ throughout the study period (baseline: 2.3 ± 2.8 mg/L; visit 2: 2.0 ± 1.9 mg/L; visit 2: 2.4 ± 2.7 mg/L). In addition, there was no significant difference between serum calcium levels at baseline compared to visit 1 (2.37 ± 0.09 mmol/L vs 2.36 ± 0.11 mmol/L, P=0.486), however, a significant decrease in serum calcium was observed compared to visit 2 (2.30 ± 0.09 mmol/L, P<0.001).

Safety Results:

No clinically significant adverse events were observed during the study.

Conclusion:

The present study demonstrates that vitamin D supplementation of 140,000 IU at baseline and after 4 weeks is associated with a significant increase in percentage of Tregs in healthy subjects. This finding supports the hypothesis that vitamin D-induced stimulation of Tregs is a possible pathophysiologic mechanism by which vitamin D may prevent autoimmune diseases. The data might therefore serve as a rationale for further placebo-controlled trials to substantiate the beneficial effects of vitamin D supplementation on autoimmunological processes related to dysfunctions of Tregs.

Date of the report: 06 August 2020