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Randomized control trials

Effect of cholecalciferol on vitamin D-regulatory proteins in monocytes and on inflammatory markers in dialysis patients: A randomized controlled trial

Marion Schneider Meireles [a], Maria Ayako Kamimura [a], Maria Aparecida Dalboni [b], José Tarcísio Giffoni de Carvalho [b], Danilo Takashi Aoike [b], Lilian Cuppari [a, b, *]

[a] The Nutrition Graduation Program, Federal University of São Paulo, Brazil
[b] Department of Medicine, Division of Nephrology, Federal University of São Paulo, Brazil

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SUMMARY

Background & aims: Hypovitaminosis D and inflammation are highly prevalent among patients undergoing dialysis, and the association of both conditions with worse survival has been well recognized. Although a potential role for vitamin D in the immune system has been suggested, the effect of the treatment of hypovitaminosis D on the modulation of the inflammatory response remains unclear. The aim of this study was to investigate the effect of the restoration of the vitamin D status on the expression of vitamin D-regulatory proteins in monocytes and on circulating inflammatory markers in dialysis patients.

Methods: In this randomized double-blind placebo-controlled 12-week trial, 38 patients on dialysis with serum 25-hydroxyvitamin D [25(OH)D] <20 ng/mL were randomized either to the cholecalciferol group ($n = 20$; 50,000 IU of cholecalciferol twice weekly) or to the control group ($n = 18$; 50 drops of a placebo solution twice weekly). The expression of vitamin D receptor (VDR), CYP27B1, CYP24A1 and interleukin-6 (IL-6) in monocytes was determined by flow cytometry. Serum concentrations of 25(OH)D, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) were measured.
The trial is registered at ClinicalTrials.gov #NCT01974245.

Results: After 12 weeks, the serum 25(OH)D increased from 14.3 ± 4.7 ng/mL to 43.1 ± 11.0 ng/mL ($p < 0.05$) in the cholecalciferol group and did not change in the control group (13.9 ± 4.2 ng/mL to 13.5 ± 4.3 ng/mL; $p = 0.56$). In monocytes, while CYP27B1 expression and VDR expression increased in the cholecalciferol group ($p < 0.05$), CYP27B1 expression did not change, and VDR expression decreased in the control group ($p < 0.05$). There were no changes in IL-6 and CYP24A1 expression in both groups. Serum concentration of IL-6 and CRP decreased from 8.1 ± 6.6 pg/mL to 4.6 ± 4.1 pg/mL ($p < 0.05$) and from 0.50 (0.10–1.27) mg/dL to 0.28 (0.09–0.62) mg/dL ($p < 0.05$), respectively only in the cholecalciferol group. Assessed overtime, the treatment group differences in 25(OH) D, PTH, CRP and IL-6, CYP27B1 and VDR remained significant.

Conclusions: Restoration of vitamin D status of patients undergoing dialysis promoted upregulation of CYP27B1 and VDR expression in monocytes and a decrease in circulating inflammatory markers.

1. Introduction

Hypovitaminosis D and disorders of vitamin D metabolism are common among patients with chronic kidney disease (CKD) [1,2]. The impaired conversion of 25-hydroxyvitamin D [25(OH)D] to 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], due to decreased renal 1-α hydroxylase enzyme (CYP27B1) activity, contributes to the development of bone and mineral disorders highly prevalent in this

* Corresponding author. Federal University of São Paulo, Rua Pedro de Toledo, 282, Vila Clementino, São Paulo, SP 04039-000, Brazil. Tel.: +55 1159048499; fax: +55 1155721862.
E-mail addresses: mariondschneider@gmail.com (M.S. Meireles), m.kamimura@uol.com.br (M.A. Kamimura), dalboni@nefro.epm.br (M.A. Dalboni), tarcisiogiffoni@yahoo.com.br (J.T. Giffoni de Carvalho), aoike@ig.com.br (D.T. Aoike), lcuppari@uol.com.br (L. Cuppari).

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population of patients [3]. More recently, it has been demonstrated that in addition to tubular renal cells, CYP27B1 is present in numerous extrarenal sites, allowing the local production of $1,25(OH)_2D$, which binds to its receptor (VDR) triggering autocrine and paracrine responses [4]. It has been estimated that approximately 85% of serum 25(OH)D is used as a substrate for the extrarenal synthesis of $1,25(OH)_2D$ [5]. Therefore, the availability of 25(OH)D is of paramount importance to produce effective responses. The pleiotropic effects of vitamin D have been demonstrated in numerous organs and cells, including prostate, mammary, colon, pancreatic β-cells, keratinocytes and immune cells [6]. Particularly in the immune system, the $1,25(OH)_2D$ synthesized in monocytes has been proposed to have immunomodulatory properties through the inhibition of the proinflammatory cytokine production [7].

A chronic inflammatory state and a low 25(OH)D serum concentration are highly prevalent among patients with CKD, and both conditions are associated with poor outcomes especially due to cardiovascular events [8–10]. Although multiple causes involve an elevated inflammatory response in these patients, low serum concentration of 25(OH)D has been associated with elevated markers of inflammation [11]. Therefore, the repletion of nutritional vitamin D might represent a safe and low-cost therapeutic approach to minimize inflammation in patients with CKD. Currently, randomized clinical trials investigating the impact of the correction of hypovitaminosis D on inflammation in dialysis patients are scarce.

Therefore, in the present study we tested the hypothesis that restoration of vitamin D status would modulate the expression of vitamin D regulatory proteins in monocytes and decrease circulating markers of inflammation of patients undergoing dialysis.

2. Materials and methods

2.1. Subjects

Patients undergoing hemodialysis (HD) and peritoneal dialysis (PD) from single dialysis unit of the Oswaldo Ramos Foundation (São Paulo, Brazil), aged between 18 and 80 years, with a dialysis vintage of at least 3 months and serum 25(OH)D <20 ng/mL, were invited to participate in the present study. The exclusion criteria were the use of any vitamin D compound, glucocorticoids, or immunosuppressors or history of liver failure, intestinal malabsorption, malignancy, autoimmune disease, active infection, positive HIV, peritonitis in the last month or elevated serum ionized calcium (>1.40 mmol/L).

As shown in Fig. 1, from September 2012 to May 2014, 348 patients were screened (194 HD/154 PD). Among these, 112 patients were excluded due to the use of vitamin D compounds, 77 were excluded because the serum 25(OH)D was greater than 20 ng/mL, and 104 patients did not meet other inclusion criteria. Therefore, 55 patients were randomized. Seventeen patients were lost during follow-up due to hospitalization (n = 8), death (n = 2) or lack of compliance with the study protocol (n = 7). Therefore, the present study was completed with a total of 38 patients (23 HD/15 DP). Patients undergoing HD were on conventional HD for 4 h, 3 times a week, using bicarbonate-buffered dialysate and polysulfone dialyzer membranes. The majority of PD patients (87%) were treated by automated PD using a glucose-based solution. The baseline demographic, clinical, laboratory and nutritional characteristics of the patients lost during follow-up were similar to those of the patients in the group that completed the study (data not shown).

The study was approved by the Human Investigation Review Committee of the Federal University of São Paulo and was conducted in accordance with the Declaration of Helsinki. Written consent was obtained from each participant. The trial is registered at ClinicalTrials.gov #NCT01974245.

2.2. Study design and protocol

The present study was a 12-week randomized, double blind, placebo-controlled clinical trial. Participants and researchers were blinded. The patients were assigned to cholecalciferol or control groups after a blocked randomization procedure by using a random block of 4 participants. An independent researcher generated a computerized random list and the allocation sequence was concealed in closed box. A pharmacist was responsible for label and number all containers according to the random schedule. The patients in the cholecalciferol group received 50,000 IU of cholecalciferol (1000 IU/drop; Magister Pharmacy, São Paulo, SP, Brazil) twice a week, while the control group received a placebo solution and were instructed to take 50 drops twice a week. The pharmaceutical presentations of the placebo and cholecalciferol were identical. To minimize the effects of vitamin D synthesis in the skin, all patients received and were instructed to wear sunscreen (SPF 30) during the study period. To evaluate compliance, the patients were requested to bring the containers to the follow-up visits. It was expected that the supplied volume would be finished in 6 weeks, when the patient received another container to complete the study. In addition, during the monthly visits and through phone calls, the patients were encouraged to follow the treatment.

2.3. Data collection

Demographic and clinical data were collected from medical records. Body weight and height were measured, and the body mass index (BMI) was calculated. Patients underwent nutritional evaluation using the 7-point Subjective Global Assessment (SGA) and were classified as malnourished when the SGA score was below five. Blood samples were collected after eight hours of fasting from all participants at baseline and after 12 weeks. Serum levels of 25(OH)D (chemiluminescence immunoassay, Abbott Architect assay, Germany), high-sensitivity C-reactive protein (immunoturbidimetric assay, Beckman Coulter Biomedical, Ireland), intact parathyroid hormone (chemiluminescence immunoassay, reference values: 10–69 pg/mL), alkaline phosphatase (calorimetric method, reference values: 35–104 U/L), phosphorus (colorimetric method, reference values: 2.3–4.3 mg/dL), ionized calcium (ion-selective electrode *method*, reference values: 1.11–1.40 mmol/L), albumin (bromocresol green), urea (enzymatic method) and creatinine (kinetic Jaffe colorimetric method) were measured. The serum concentrations of high-sensitivity interleukin-6 (IL-6), high-sensitivity tumor necrosis factor-α (TNF-α) and intact fibroblast growth factor 23 (FGF-23) were measured using the enzyme linked immune sorbent assay technique (R&D Systems, Minneapolis, MN, USA). Respective inter- and intra-assay coefficients of variation were 8.4% and 5.3% (TNF-α), 7.7% and 7.4% (IL-6) and 4.1% and 5.9% (FGF-23).

2.4. Flow cytometry

The expression of vitamin D receptor (VDR), 1-α hydroxylase enzyme (CYP27B1), 24-hydroxylase enzyme (CYP24A1) and interleukin-6 (IL-6) in monocytes was determined by flow cytometry.

An aliquot of 100 μL of heparinized whole blood was incubated with 10 μL of PE-conjugated anti-human CD14 antibody (BD Biosciences, San Diego, CA, USA) for 15 min in the dark at room temperature (according to the manufacturer's instructions) to characterize the monocytes. Subsequently, the cells were further

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