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Cholecalciferol administration blunts the systemic renin–angiotensin system in essential hypertensives with hypovitaminosis D

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Abstract

Introduction: Vitamin D plasma levels are negatively associated with blood pressure and cardiovascular mortality, and vitamin D supplementation reduces cardiovascular events. Renin–angiotensin system (RAS) suppression may be one of the mechanisms involved. However, there are no interventional prospective studies demonstrating a reduction in circulating RAS components after vitamin D treatment.

Methods: Fifteen consecutive drug-free patients with essential hypertension and hypovitaminosis D underwent therapy with an oral dose of 25000 I.U. of cholecalciferol once a week for two months, while maintaining a constant-salt diet. In basal conditions and at the end of the study, RAS activity (plasma angiotensinogen, renin, PRA, angiotensin II, aldosterone and urinary angiotensinogen) was investigated, in addition to blood pressure and plasma vitamin D levels (25(OH)D).

Results: After cholecalciferol administration, all patients exhibited normalized plasma 25(OH)D values. At the end of the study, a reduction ($p < 0.05$) in plasma renin and aldosterone, and a decrement, although not significant, of PRA and angiotensin II, was observed. No difference was found in plasma and urinary angiotensinogen or blood pressure values.

Conclusions: Our data indicate that in essential hypertensives with hypovitaminosis D, pharmacological correction of vitamin D levels can blunt systemic RAS activity.

Keywords

Hypovitaminosis D, hypertension, renin–angiotensin–aldosterone system, cholecalciferol, cardiovascular risk

Introduction

A number of observations suggest a close relationship between vitamin D and cardiovascular disease in humans. Vitamin D plasma levels inversely correlate with blood pressure[1] and cardiovascular disease and mortality,[2,3] and intervention studies indicate that vitamin D therapy improves cardiovascular risk profile.[4–7] In addition, vitamin D positively influences chronic renal disease progression in patients with diabetic[8,9] and non-diabetic nephropathy.[10] Despite the fact that there is no univocal data obtained from recent meta-analysis on vitamin D supplementation and cardiovascular events,[11] the link between this vitamin and cardiovascular disease is solid.

Suppression of the renin–angiotensin system (RAS) by vitamin D could be involved in this association. Thus animal[12,13] and in vitro[14] studies suggest that vitamin D receptor activation inhibits intra-renal mRNA levels and protein expression of key components of RAS (angiotensinogen, renin, renin receptors and angiotensin II type 1 receptor) independently of calcium metabolism.

In contrast, among the few studies in humans reported in the literature, no suppressive effect on systemic RAS has been found in patients acutely[15] or chronically[8] treated with vitamin D. Very recently, we confirmed this finding in essential hypertensives after short-term calcitriol administration and after long-term cholecalciferol therapy.[16] However, both in our study and in those reported above,[8,15] patients were under treatment with RAS inhibitors. Thus, vitamin D, at the doses utilized, may have been unable to

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suppress the compensatory increment in plasma renin associated with the use of RAS blockers. Another possibility is that the failure of the expected benefit (i.e. RAS inhibition) may be only apparent and may genuinely occur at the local (intra-renal) RAS level without influencing systemic circulation, as recently reported by Vaidya et al.[17] In effect, the regulatory mechanisms of systemic and intra-renal RAS are different and sometimes divergent.[18] Recently, a new sandwich enzyme-linked immunosorbent assay (ELISA) system was developed for plasma and urine angiotensinogen (AGT) determination.[19] From data obtained in hypertensives[20] and in patients with chronic kidney disease,[21,22] it has been hypothesized that urinary AGT could be an index of intra-renal RAS status.

The purpose of the present study was to evaluate whether chronic vitamin D receptor activation in drug-free essential hypertensives influences RAS components, including plasma and urinary AGT.

Patients and methods

Patients

The study was conducted in a tertiary hypertension center (Department of Internal Medicine of Pisa University). Patient enrollment was performed during the autumn-winter period in order to obtain, as far as possible, the most homogeneous vitamin D levels, free from exposure to sunlight.

A group of 26 outpatients with essential hypertension was evaluated; 11 showed plasma vitamin D (25(OH)D) levels above 30 ng/ml and for this reason were excluded from the study. Thus, 15 patients with hypovitaminosis D (25(OH)D < 30 ng/ml) were consecutively enrolled. Exclusion criteria included estimated glomerular filtration rates (eGFR) < 60 ml/min per 1.73 m^2, hypercalcemia, hyperphosphoremia, parathyroid disorders, hepatic insufficiency, obesity (body mass index (BMI) > 30 kg/m^2), diabetes mellitus and chronic granulomatous disease. Patients taking drugs interfering with RAS (angiotensin-converting enzyme (ACE)-inhibitors and angiotensin II type 1 receptor antagonists) and sympathetic nervous system (SNS) (beta-blockers or beta-agonists, clonidine), diuretics and other drugs affecting calcium-phosphorus metabolism were likewise excluded. Such patients were shifted (n=13), when possible, to calcium-antagonists and/or α1-blockers for at least one month prior to the study. All patients were requested to maintain a constant-salt diet (~ 3 g/ of NaCl per day).

The study was approved by the institutional review board and each subject gave informed written consent to the study after a detailed description of the experimental protocol.

Protocol

Cholecalciferol was chosen among vitamin D receptor activators because it is easily transformed, in patients with normal renal function, into the active form, $1,25(OH)_2$ vitamin D; furthermore, it's safer and it has a better compliance (weekly intake), at variance with calcitriol or paricalcitol. At time 0, an oral dose of 25.000 international units (I.U.) once a week (cumulative dose 200.000 I.U.) for eight weeks was administered. This dose regimen was adopted in order to maintain 25(OH)vitamin D levels consistently above 30 ng/ml.[23]

Measurements

In basal conditions and after eight weeks the following parameters were analyzed: 1) blood pressure, in accordance with recent international guidelines;[24] 2) plasma creatinine, calcium, phosphorus, magnesium, sodium, potassium, 25(OH)vitamin D, $1,25(OH)_2$vitamin D and parathyroid hormone (PTH); 3) plasma AGT, aldosterone (ALD), plasma renin activity (PRA), renin and angiotensin II; 4) 24-hour (h) urinary creatinine, albumin, sodium, and a spot urine sample for AGT.

Blood samples for the assay of RAS components were obtained in fasting conditions in the morning (08:00–09:00 a.m.) after at least 2 h of upright posture (i.e. sitting, standing or walking) and with patients seated at the time of blood collection.

Laboratory

Serum concentrations of creatinine, calcium, phosphorus, magnesium, sodium and potassium were analyzed by standard methods. Urinary creatinine and albumin were evaluated with a DCA 2000 Analyzer. The eGFR was calculated by means of the Modification of Diet in Renal Disease (MDRD) formula. Specific radioimmunoassays were used to measure 25(OH)vitamin D and $1,25(OH)_2$ vitamin D (DiaSorin Inc., Stillwater, MN, USA; intra-assay 10.5% and 11.3%, respectively; inter-assay 9.6% and 14.9%, respectively), PRA (DiaSorin, Saluggia, Italy; intra-assay 7.6% and inter-assay 9.1%, normal values (n.v.) 0.2–5.7 ng/ml/h), ALD (DiaSorin, Saluggia, Italy; intra-assay 9.7% and inter-assay 11.5%, n.v. 3.5–30.0 ng/dl)) and renin (CisBIO, Bedford, MA, USA; intra-assay 1.8% and inter-assay 4.0%, n.v. 5.1–59.4 pg/ml). Plasma PTH was evaluated by immunoradiometric assay for quantitative determination of active intact human PTH 1-84 (DiaSorin Inc., Stillwater, MN, USA; intra-assay 2.5% and inter-assay 4.4%, n.v. 13–54 pg/ml), while plasma angiotensin II measurement was performed by ELISA kits (Pantec s.r.l, Torino, Italy; intra-assay 3.1% and inter-assay 4.3%, n.v. 5.5–21.3 pg/ml). Finally, plasma and urinary concentrations of AGT were assayed with human AGT ELISA kits, as previously described (Immuno-Biological Laboratories Co., Ltd.; intra-assay 5.0% and inter-assay 5.3%, n.v. 28–71 μg/ml in plasma and 7.1–35 ng/ml in urine). Since the daily urinary AGT excretion rate is highly correlated with the ratio of

Table 1. Demographic characteristics of the patients. Plasma levels of 25(OH)D, 1,25$(OH)_2$D and PTH and blood pressure values (mean ± SE) before and after (eight weeks) cholecalciferol administration.

Parameters	Time 0	Week 8	*p*
Patients *n*	15	—	—
Gender	8 M (53.3%) 7 F (46.6%)	—	—
Mean age (years) (range)	43.6 (22–71)	—	—
BMI (kg/m^2)	24.6 ± 2.6	—	—
25(OH)D (ng/ml)	18.3 ± 2.8	38.4 ± 3.2	< 0.001
1,25$(OH)_2$D (pg/ml)	22.4 ± 3.6	48.6 ± 2.3	< 0.001
PTH (pg/ml)	47.7 ± 5.5	38.9 ± 6.0	0.13
SBP (mmHg)	137.4 ± 1.8	134.8 ± 2.3	0.23
DBP (mmHg)	81.6 ± 1.8	81.0 ± 1.6	0.73

25(OH)D: plasma vitamin D levels; PTH: parathyroid hormone; M: male; F: female; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2. Serum and urinary parameters (mean ± SE) before and after (eight weeks) cholecalciferol administration.

Parameters	Time 0	Week 8	*p*
Calcium (mg/dl)	9.4 ± 0.12	9.3 ± 0.11	0.23
Phosphorus (mg/dl)	2.6 ± 0.14	2.9 ± 0.17	0.19
Magnesium (mg/dl)	2.0 ± 0.04	2.0 ± 0.04	0.70
Sodium (mEq/l)	141.0 ± 0.45	140.8 ± 0.14	0.43
Potassium (mEq/l)	3.9 ± 0.30	4.1 ± 0.47	0.39
Creatinine (mg/dl)	0.89 ± 0.04	0.85 ± 0.03	0.56
eGFR (MDRD) (ml/min/1.73m^2)	94.4 ± 4.66	97.1 ± 3.76	0.62
Urine sodium (mEq/24 h)	126.7 ± 12.7	125.8 ± 16.1	0.51

eGFR (MDRD): estimated glomerular filtration rate (Modification of Diet in Renal Disease formula).

urinary AGT concentration to urinary creatinine concentration (UAGT/UCr) in humans,[25] we collected spot urine samples to analyze UAGT/UCr.

Statistical analysis

The paired Student *t* test and Wilcoxon test for non-parametric variables (RAS components) were employed for statistical analysis. Correlation analysis (Spearman test) was adopted to assess the relationship between individual variables. Results were expressed as mean±SEM and mean±SD. $P < 0.05$ was considered statistically significant.

Results

Our drug-free essential hypertensives under a controlled-salt diet did not show other comorbidities, except for hypercholesterolemia in two cases (treated with statins) and hyperuricemia in one patient. In basal conditions, eight patients had 25(OH)vitamin D levels < 20 ng/ml (deficiency) and seven patients < 30 ng/ml (insufficiency). After cholecalciferol administration, plasma 25(OH)vitamin D significantly increased ($p < 0.001$) and this parameter became normalized in all cases. Basal PTH levels were normal, with systolic and diastolic blood pressure values proving to be well controlled by the therapy administered at the start of the investigation. These parameters remained unchanged throughout the study (Table 1). Serum calcium, phosphorus, magnesium, sodium, potassium, creatinine, eGFR and urine sodium likewise underwent no change (Table 2).

Despite the constant-salt diet documented by 24-h urinary sodium values, measurement of the systemic RAS components at the end of the study (Figure 1) showed a reduction in plasma renin (13.6 ± 3.4 pg/ml vs 24.0 ± 5.9 pg/ml, $p < 0.05$) and ALD (21.9 ± 3.0 ng/dl vs 31.3 ± 5.5 ng/dl, $p < 0.05$) and a decrease, albeit not significant, in PRA (1.3 ± 0.3 ng/ml/h vs 1.9 ± 0.4 ng/ml/h) and angiotensin II (9.4 ± 1.5 pg/ml vs 13.0 ± 2.5 pg/ml) levels. Cholecalciferol administration tended to increase plasma (42.1 ± 2.1 µg/ml vs 39.0 ± 2.5 µg/ml) and urinary AGT (16.1 ± 2.6 µg/g urinary creatinine vs 14.1 ± 2.3 µg/g urinary creatinine), though not to a significant extent (Figure 2).

No correlation was found between plasma 25(OH)D or 1,25$(OH)_2$D levels and blood pressure, RAS components and PTH values before and after drug administration.

Discussion

Our results indicate that in essential hypertensives with hypovitaminosis D who are not on drugs interfering with

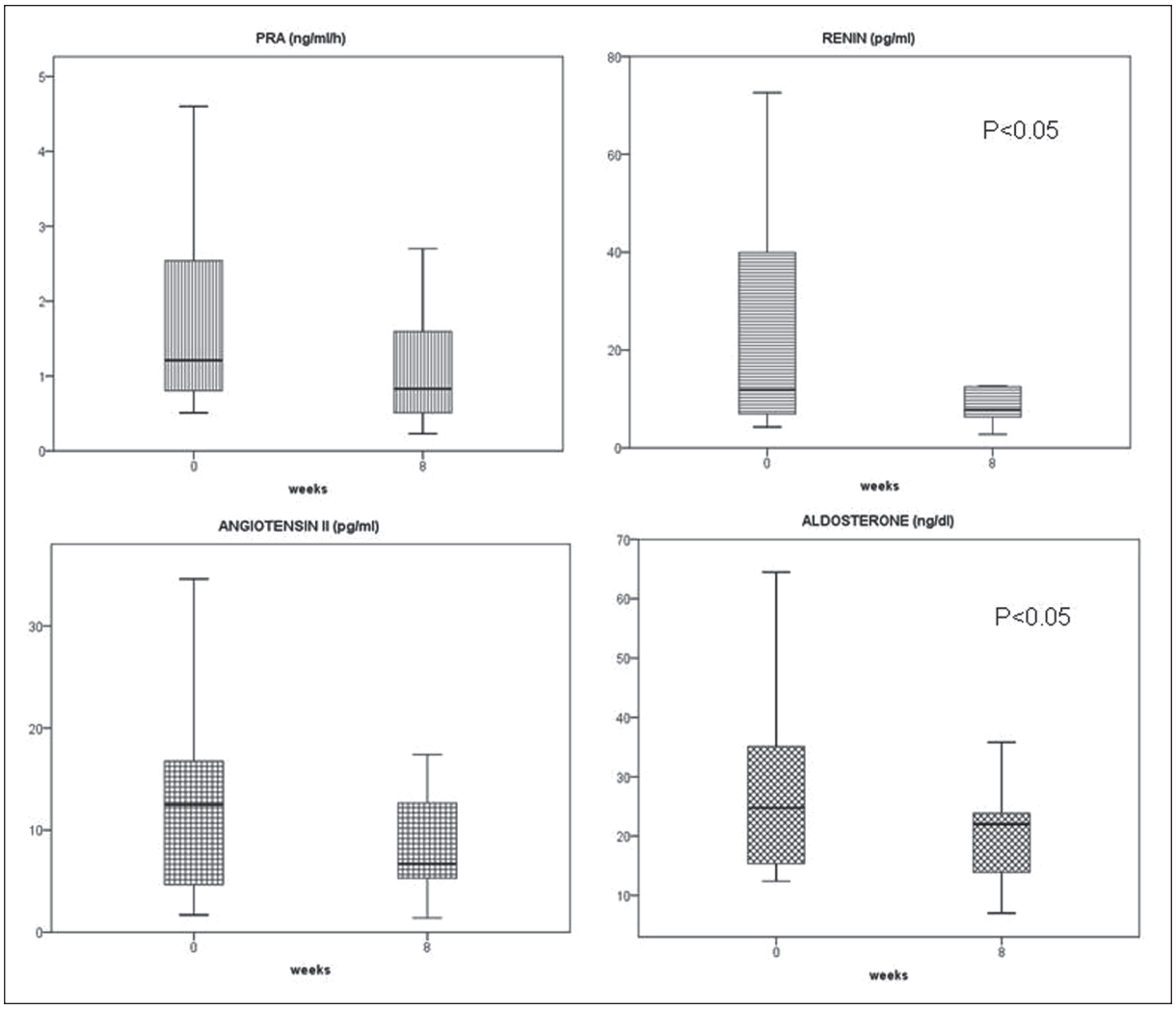

Figure 1. Box plots describing the cholecalciferol effects on plasma renin activity (PRA), renin, angiotensin II (ang II) and aldosterone (ALD).

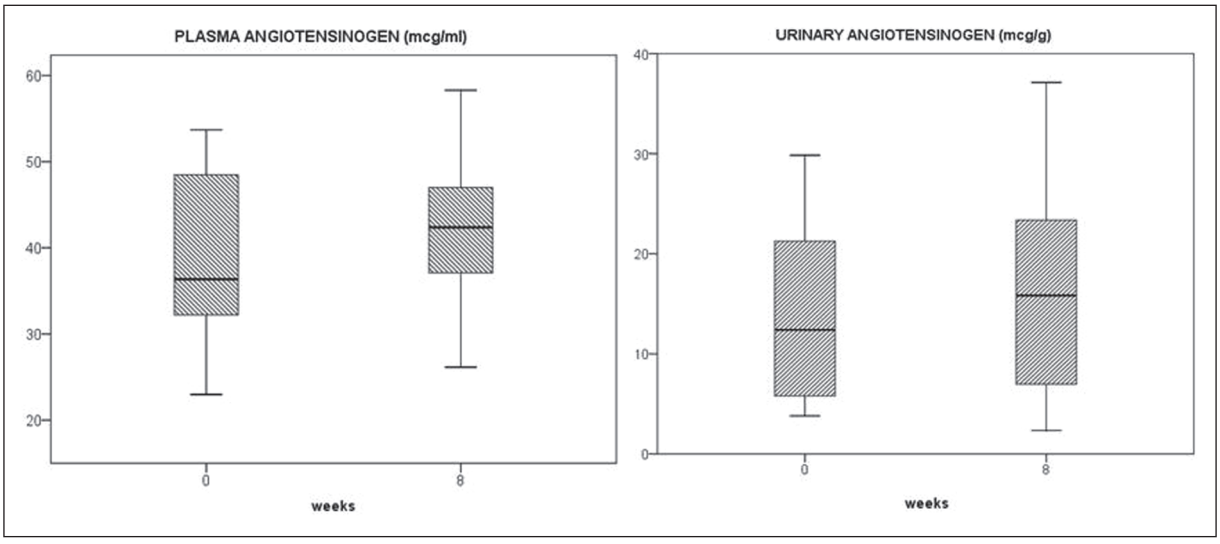

Figure 2. Box plots describing the cholecalciferol effects on plasma and urinary angiotensinogen.

RAS and are under constant-salt intake, cholecalciferol therapy blunted systemic RAS activity. Such findings are in agreement with several animal[12,13] and in vitro[14] studies suggesting that vitamin D inhibits intra-renal mRNA levels and protein expression of several components of RAS, such as AGT, renin, renin receptors and angiotensin II type 1 receptor, independently of calcium metabolism.

To the best of our knowledge there are only three reports in humans exploring this topic. In all three of these studies, no effect on circulation RAS was observed. Sugden et al.[5] and de Zeeuw et al.[8] performed their investigations in diabetic patients, with and without nephropathy, using ergocalciferol and paricalcitol, respectively. Our group designed a study in hypertensive patients after calcitriol or cholecalciferol administration.[16] However, in the above cited studies the patients were under treatment with RAS inhibitors, and vitamin D may have been unable to suppress the compensatory increment in renin associated with use of these drugs. In the present study the patients were drug free or were not under therapy interfering with RAS (ACE-inhibitors and angiotensin II type 1 receptor antagonists) or with SNS (beta-blockers or beta-agonists, clonidine); furthermore, they were not taking diuretics or other drugs affecting calcium-phosphorus metabolism. In addition, their salt intake remained constant during the study, as shown by urinary sodium excretion. Finally, the dose administered was capable of maintaining 25(OH)D levels above 30 ng/ml in all patients, as recommended by international guidelines.[23] In these experimental conditions, all components of circulating RAS decreased in our hypovitaminemic D patients after cholecalciferol administration, a finding that reached statistical significance for plasma renin and ALD. Our data partially agree with a very recent paper showing that a high dose of cholecalciferol in obese hypertensives enhances ALD response to angiotensin II infusion, a finding indicating a suppressive effect on renal-vascular tissue-RAS.[17] However, at variance with our results, Vaidya et al.[17] did not observe any change in circulating RAS components despite a similar experimental design. This finding could be related to the different population studied. Our investigation involved normal-weight Caucasian patients, while the other study recruited mainly black (70%) obese hypertensives. In addition, the duration of cholecalciferol supplementation was clearly shorter than that of the present paper (one month vs two months). Finally, our patients were on a constant-salt diet (124 mEq/24 h) and in upright posture during blood sampling, while in the study by Vaidya et al.,[17] salt intake was more elevated (330 mEq/24 h) and patients were kept supine overnight during blood collection. This very high salt intake, associated with supine blood collection, may have basically suppressed circulating RAS, thus obscuring the vitamin D effect. Taken together, our results corroborate human studies showing an inverse association between PRA,[26,27] plasma renin,[28] and vitamin D levels in normotensives and hypertensives and a negative relation between 25(OH)D levels and circulating angiotensin II in patients with essential hypertension.[29]

It has been hypothesized that intra-renal RAS may contribute to the development or impairment of renal function in some pathological conditions[18] independently of circulating RAS. In effect, the regulatory mechanisms of systemic and intra-renal RAS are different and sometimes divergent,[17] and renal AGT appears to be a key component of this system. Data obtained in hypertensives[30] and in patients with chronic kidney disease[22] suggest that urinary AGT may provide a specific index of intra-renal RAS status. In the present study, we applied a new sandwich ELISA system, developed for urine and plasma AGT determination,[19] in order to investigate the behavior of plasma and urinary angiotensinogen in hypertensives under cholecalciferol therapy. A slight (not significant) increase in AGT levels was observed and, given the sample size, we cannot rule out a priori an effect of vitamin D supplementation on AGT. As we await a more precise evaluation by using prespecified experimental designs, the present data do not allow us to consider urinary AGT as a marker of intra-renal RAS. It more plausibly derives, through a filtration process, from the liver, the natural source of the peptide. Recent observations seem to confirm this hypothesis.[31]

Some limitations to the current study should be mentioned. First, only a small number of subjects were studied; however, they were well selected and free from drugs interfering with RAS. Second, the extent of hypovitaminosis D in our patients and the dose of drug administered may have been unable to fully clarify the effects of cholecalciferol RAS activity.

In conclusion, our data indicate that in essential hypertensive patients with hypovitaminosis D under constant salt intake and free from drugs interfering with RAS, chronic vitamin D receptor stimulation blunts systemic RAS activity.

Conflict of interest

None declared.

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