Effect of acute and chronic vitamin D administration on systemic renin angiotensin system in essential hypertensives and controls

G. Bernini, D. Carrara, A. Bacca, V. Carli, A. Virdis, I. Rugani, E. Duranti, L. Ghiadoni, M. Bernini, and S. Taddei

Department of Internal Medicine, University of Pisa, Pisa, Italy

ABSTRACT. ***Aim:*** **To investigate the systemic renin-angiotensin system (RAS) in essential hypertensives (EH) and controls (C) after short- and long-term vitamin D receptor activation.** ***Design:*** **Ten consecutive EH (under controlled low-salt diet) and 10 C underwent calcitriol administration (0.25 μg bid) for 1 week (Group A). Eighteen consecutive EH under angiotensin II receptor antagonist therapy received a single oral dose of 300,000 IU of cholecalciferol and were followed up for 8 weeks (Group B).** ***Methods:*** **In basal conditions and at the end of the study (1 week in Group A and 8 weeks in Group B), plasma renin activity (PRA), plasma active renin, aldosterone, and angiotensin II were evaluated, as well as blood pressure, plasma 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], and PTH.** ***Results:*** **In Group A, plasma 25(OH)D levels in EH and C were below the normal range, although lower levels were found in the former. No association between basal plasma 25(OH)D or 1,25(OH)$_2$D levels and blood pressure values or RAS components was observed either in the whole group or in the two subgroups. Calcitriol administration did not affect any RAS parameter either in EH or in C. In Group B, cholecalciferol significantly increased 25(OH)D and 1,25(OH)$_2$D levels without interfering with the angiotensin II receptor antagonist-induced increase in RAS components. No correlation was found between plasma 25(OH)D or 1,25(OH)$_2$D levels and blood pressure values or RAS parameters before and after cholecalciferol administration.** ***Conclusions:*** **The present data suggest that, in our experimental conditions, vitamin D receptor activation is unable to influence systemic RAS activity.**

(J. Endocrinol. Invest. 36: 216-220, 2013)

INTRODUCTION

Observational studies have shown that low vitamin D levels bear a relation to high blood pressure values (1) and cardiovascular morbidity and mortality (2, 3) in humans. In addition, vitamin D administration positively influences blood pressure and endothelial function in patients with Type 2 diabetes mellitus (4) and delays chronic renal disease progression in nephropathic (diabetic and non-diabetic) patients (5, 6).

Renin-angiotensin system (RAS) suppression has been hypothesized to be one of the mechanisms explaining these relationships. Thus animal (7, 8) and *in vitro* (7) studies have demonstrated that vitamin D receptor activation blunts intra-renal mRNA levels and protein expression of several RAS components, such as angiotensinogen, renin, renin receptors, and angiotensin II type 1 receptors. In humans, association studies likewise show that plasma renin activity (PRA) and vitamin D are inversely correlated (9-13) in normotensives and hypertensives and that lower 25-hydroxyvitamin D [25(OH)D] levels are associated with higher circulating angiotensin II levels in hypertensives (14). However, among the few intervention studies in humans reported in the literature (4, 15), no suppressive effect on systemic RAS has been found after vitamin D administration.

The aim of the present study was to investigate systemic RAS in essential hypertensives and controls after short-term calcitriol administration and in patients with essential hypertension after long-term cholecalciferol therapy.

PATIENTS AND METHODS

Patients

The study was conducted in the Hypertension Center (Department of Internal Medicine) of Pisa University. Patient enrollment was performed between October and December 2010 and the study ended within March 2011. This cut-off date was chosen to avoid the possibility that exposure to sunlight could modify plasma vitamin D levels. The population study was divided into two groups, each one undergoing a different experimental protocol.

Group A consisted of 10 consecutive essential hypertensive patients (EH) and 10 normotensive controls (C). Exclusion criteria were: estimated glomerular filtration rates <60 ml/min/1.73 m^2, hypercalcemia, hepatic insufficiency, chronic granulomatosis diseases, body mass index (BMI) >30 kg/m^2, diabetes mellitus, and intake of any anti-hypertensive drugs or other drugs affecting calcium-phosphorus metabolism. While C were allowed a salt-free diet, the patients (in-patients) were maintained on a controlled low-salt diet (~3 g/day of NaCl) during the study.

Group B consisted of 18 consecutive EH (8 females, age 47.3±12.5 yr, mean±SD, BMI 25.5±1.02 kg/m^2, mean±SE). Exclusion criteria were the same as in Group A, except for the use of angiotensin II receptor antagonists, which were introduced into the experimental protocol. Patients in this group followed an ordinary salt diet during the study.

The investigation was approved by the institutional review board

Key-words: Calcitriol, cholecalciferol, essential hypertension, hypovitaminosis D, renin angiotensin system.

Correspondence: G. Bernini, Department of Internal Medicine, University of Pisa, Via Roma 67, 56100 Pisa, Italy.

E-mail: giampaolo.bernini@med.unipi.it

Accepted May 29, 2012.

and each subject gave written informed consent to the study after a detailed description of the experimental protocol.

Protocol

Group A

As the group A study lasted 7 days, we preferred to use the active form among vitamin D receptor agonists, such as calcitriol, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. Thus at time 0, an oral dose of 0.25 µg *bid* (Roche, Milano, Italy) was given for 1 week.

Group B

Given that the group B study was a long-term investigation, we preferred to administer cholecalciferol as a bolus at doses able to maintain 25(OH)D levels consistently >30 ng/ml, as advised by international guidelines (16). Thus, at time 0 a single oral dose of 300,000 IU of cholecalciferol (Abiogen Pharma, Pisa, Italy) was given. Since low renin hypertensive patients are frequent among the hypertensive population and this could have disguised the possible inhibitory effect of vitamin D on RAS, we administered angiotensin II receptor antagonists (telmisartan 80 mg once a day) starting from 15 days prior to beginning of the study and continuing throughout the experiment, in order to activate endogenous plasma renin and angiotensin II levels.

Measurements

Group A

In basal conditions and at the end of the study (1 week) the following parameters were analyzed: a) blood pressure in conformity with recent international guidelines (17); b) plasma creatinine, calcium, phosphorus, magnesium, 25(OH)D, 1,25$(OH)_2$D and PTH; c) PRA, plasma active renin, aldosterone, and angiotensin II; d) 24-h urinary creatinine, albumin, and sodium.

Group B

Before angiotensin II receptor antagonist administration (time –15), at time 0 (cholecalciferol assumption), and after 4 and 8 weeks, the following parameters were analyzed: a) blood pressure, b) PRA, plasma active renin, aldosterone, and angiotensin II. Plasma creatinine, calcium, phosphorus, and magnesium were evaluated only at time 0 and at the end of the study, while 25(OH)D, 1,25$(OH)_2$D, and PTH were also assayed after 4-week cholecalciferol administration.

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 (Bayer). Specific radioimmunoassays were used to measure 25(OH)D and 1,25$(OH)_2$D (DiaSorin Inc., Stillwater, MN, USA; intra-assay 10.5 and 11.3%, respectively; inter-assay 9.6 and 14.9%, respectively). PRA (intra-assay 7.6%, inter-assay 9.1%, normal values 0.2-5.7 ng/ml/h), and plasma aldosterone (intra-assay 9.7%, inter-assay 11.5%, normal values 3.5-30.0 ng/dl) were analyzed by DiaSorin (Saluggia, Italy), and active renin (intra-assay 1.8%, inter-assay 4.0%, normal values 5.1-59.4 pg/ml) by CisBIO (Bedford, MA, USA). Plasma PTH was evaluated by an immunoradiometric assay (intra-assay 2.5%, inter-assay 4.4%, normal values 13-54 pg/ml) for quantitative determination of active intact human PTH 1-84 (DiaSorin Inc., Stillwater, MN, USA), and plasma angiotensin II by enzyme-linked immunosorbent assay kit (Pantec s.r.l, Torino, Italy, intra-assay 3.1%, inter-assay 4.3%, normal values 5.5-21.3 pg/ml).

Statistical analysis

Unpaired and paired Student's t-test or one-way analysis of variance (ANOVA), when appropriate, were used for statistical analysis. Linear correlation analysis was adopted to assess the relationship between individual variables. Results were expressed as mean±SEM and mean±SD. $p<0.05$ was considered as statistically significant.

RESULTS

Group A

EH and C were superimposable as regards gender (5 females in EH and 6 in C), age (43.0±11.7 yr, mean±SD, in EH and 41.2±4.4 yr in C), and BMI (26.4±1.0 kg/m^2, mean±SE, in EH and 24.4±1.3 kg/m^2 in C). In basal conditions, no significant difference was found between the two groups with regard to plasma calcium, phosphorus,

Table 1 - *Group A: humoral and hemodynamic parameters (mean±SE) of essential hypertensives (EH) and controls (C) before and after calcitriol administration.*

Parameters	EH (no.=10)		C (no.=10)	
	Before	After	Before	After
Calcium (mg/dl)	9.5±0.07	9.6±0.04	9.4±0.13	9.3±0.11
Phosphorus (mg/dl)	3.2±0.09	3.4±0.08	3.7±0.42	3.8±0.14
Magnesium (mg/dl)	2.1±0.06	2.0±0.05	2.0±0.02	1.96±0.02
Creatinine (mg/dl)	0.86±0.02	0.86±0.04	0.90±0.03	0.91±0.02
Creatinine clearance (ml/min)	99.2±5.2	97.1±4.7	97.6±5.4	98.3±3.8
24-h urinary sodium (mEq/24 h)	131.1±23.7	87.5±18.6**	134.0±16.5	141.0±15.0
Microalbuminuria (mg/24 h)	7.7±0.54	7.8±1.6	9.8±2.9	6.3±0.6
25(OH)D (ng/ml)	12.6±1.6	14.5±2.0	20.1±2.5**	20.4±2.2
1,25$(OH)_2$D (pg/ml)	28.0±2.4	38.9±11.0	35.4±6.5	24.0±3.9
PTH (pg/ml)	38.2±6.3	30.7±5.6	38.2±8.4	35.3±7.1
SBP (mmHg)	145.0±6.1	133.0±5.3*	107.0±5.5§§	110.0±4.9
DBP (mmHg)	90.0±4.5	81.6±4.5*	65.0±3.5§	70.2±2.8

25(OH)D: 25-hydroxyvitamin D; 1,25$(OH)_2$D: 1,25-dihydroxyvitamin D; SBP: systolic blood pressure; DBP: diastolic blood pressure; *$p<0.05$ and **$p<0.02$ *vs* EH before; §$p<0.001$ and §§$p<0.0001$ *vs* EH before.

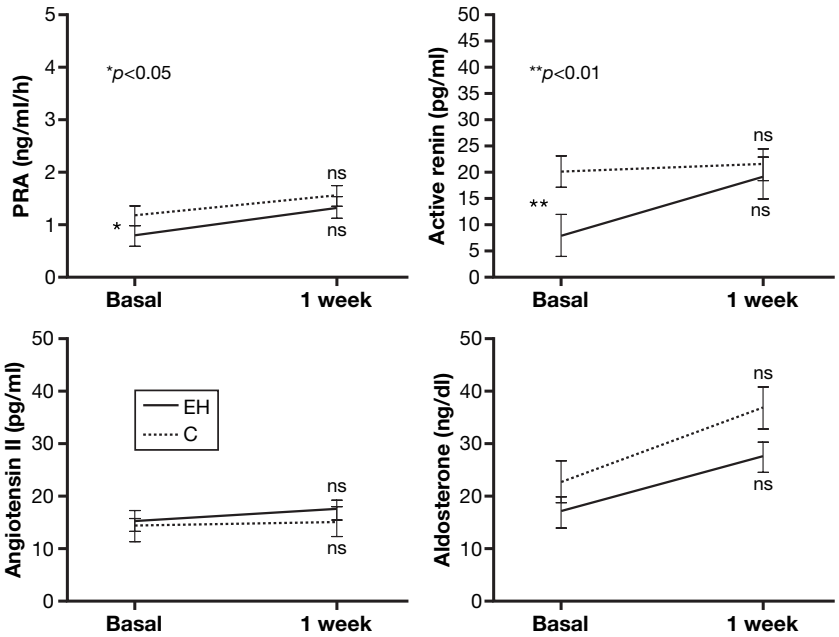

Fig. 1 - *Calcitriol effects on plasma renin activity (PRA), active renin, angiotensin II and aldosterone in essential hypertensives and in controls.*

magnesium, creatinine, creatinine clearance, 24-h urinary sodium, microalbuminuria, and PTH. Interestingly, despite consecutive recruitment of the subjects studied, 25(OH)D levels of both groups were below the normal range reported by the international guidelines (16), although 25(OH)D and 1,25$(OH)_2$D were lower in EH than in C. As expected, blood pressure was higher (p<0.001 or less) in EH than in C (Table 1).

Calcitriol administration did not affect any humoral parameter in either group. At the end of the study, a significant reduction in systolic and diastolic blood pressure was detected in EH, probably due to hospitalization and low-salt diet, as confirmed by a decrement in sodium urinary values (Table 1).

Figure 1 shows that in basal conditions, PRA and active renin were significantly lower in EH than in C, while aldosterone and angiotensin II were similar. Calcitriol administration exerted no suppressive effect on RAS components in either group. In contrast, an increment, though not significant, in PRA, active renin, and aldosterone levels was observed mainly in EH, probably linked to reduction in sodium intake.

Correlation analysis performed in the whole group (patients and controls) and in the two subgroups showed no association between basal plasma 25(OH)D or 1,25$(OH)_2$D levels and blood pressure values, RAS components or PTH values.

Group B

Plasma calcium, phosphorus, magnesium, creatinine, and PTH underwent no modification during the study, while cholecalciferol, as expected, significantly increased 25(OH)D and 1,25$(OH)_2$D levels. Systolic and diastolic blood pressure decreased significantly after angiotensin II antagonist administration, subsequently remaining unmodified throughout the study despite cholecalciferol administration (Table 2).

Table 2 - *Group B: humoral and hemodynamic parameters (mean±SE) prior and at the end of the study.*

Parameters	Essential hypertensives (no.=18)				
	Time –15	Time 0	4 weeks	8 weeks	*p*
Calcium (mg/dl)	-	9.6±0.13	-	9.3±0.12	ns
Phosphorus (mg/dl)	-	2.8±0.15	-	2.9±0.13	ns
Magnesium (mg/dl)	-	2.1±0.01	-	2.0±0.02	ns
Creatinine (mg/dl)	-	0.82±0.04	-	0.84±0.03	ns
25(OH)D (ng/ml)	-	14.9±1.4	38.0±3.0	27.0±2.3	<0.001
1,25$(OH)_2$D (pg/ml)	-	27.7±2.4	41.2±6.0	50.2±11.3	<0.05
PTH (pg/ml)	-	37.3±4.6	34.4±4.3	41.2±3.6	ns
SBP (mmHg)	147.2±3.0	135.0±0.8**	135.0±1.2	132.0±1.3	ns
DBP (mmHg)	93.2±0.9	85.0±1.1*	79.0±1.2	79.0±1.4	ns

25(OH)D: 25-hydroxyvitamin D; 1,25$(OH)_2$D: 1,25-dihydroxyvitamin D; SBP: systolic blood pressure; DBP: diastolic blood pressure; *p<0.05 and **p<0.01 *vs* time –15.

PRA, active renin, and angiotensin II were not suppressed by cholecalciferol during the study. On the contrary, these hormones increased slightly and plasma aldosterone decreased slightly, possibly as a result of angiotensin II receptor blockade (Fig. 2).
No correlation was found between plasma 25(OH)D or $1,25(OH)_2D$ levels and blood pressure values, RAS components or PTH values before and after drug administration. Furthermore, after categorization by vitamin D status on the basis of 25(OH)D and $1,25(OH)_2D$ levels, no significant difference in RAS components and blood pressure was found.

DISCUSSION

Some reports indicate that in normotensives and in hypertensive patients, PRA and vitamin D plasma concentration appear inversely correlated (9-13) and that lower 25(OH)D levels are associated with higher circulating angiotensin II values in hypertensives (14). However, the effects of vitamin D supplementation on systemic RAS are largely lacking in humans.
Our study showed that in consecutively enrolled subjects, the percentage of vitamin D deficiency was very high and that vitamin D levels were lower in EH than in C, a finding in agreement with the literature (1, 18). In addition we observed that in EH and in C with hypovitaminosis D, short-term calcitriol administration did not affect systemic RAS components. The same finding was observed in essential hypertensive patients after long-term cholecalciferol administration given at doses able to restore vitamin D levels. The present data suggest that, in our experimental conditions, vitamin D receptor activation was unable to influence RAS activity, at least on the systemic level.
We are aware of the limitations of our study, in particular with regard to the small number of subjects studied. However, this is a pilot study and, as far as we know, it is the first to explore, as a primary endpoint, RAS activity after vitamin D administration in hypertensive patients. Previous studies (4, 15) on this topic were performed in diabetic patients, with and without nephropathy, and in both cases primary and secondary endpoints differed in RAS component measurement. Accordingly, in the present investigation we focused on the type and doses of drug to be used, follow-up duration, and timing of sampling. Since, in the first protocol, the study had a duration of only 7 days, we preferred to use a biologically active form of vitamin D, such as calcitriol, at customary therapeutic doses. In the second protocol, cholecalciferol was chosen as vitamin D activator, given as a bolus in order to optimize compliance. For this protocol, we used doses capable of maintaining 25(OH)D levels consistently >30 ng/ml, as advised by international guidelines (16) and effectively obtained in our patients. An additional consideration involved endogenous RAS status, which was a critical point in planning the study. Low-renin hypertensive patients are frequent in the hypertensive population, and uncontrolled salt intake (as is known to commonly occur in out-patients) may negatively affect renin secretion. To avoid possible basal RAS suppression which could have obscured the inhibitory effect of vitamin D, our study was designed to ac-

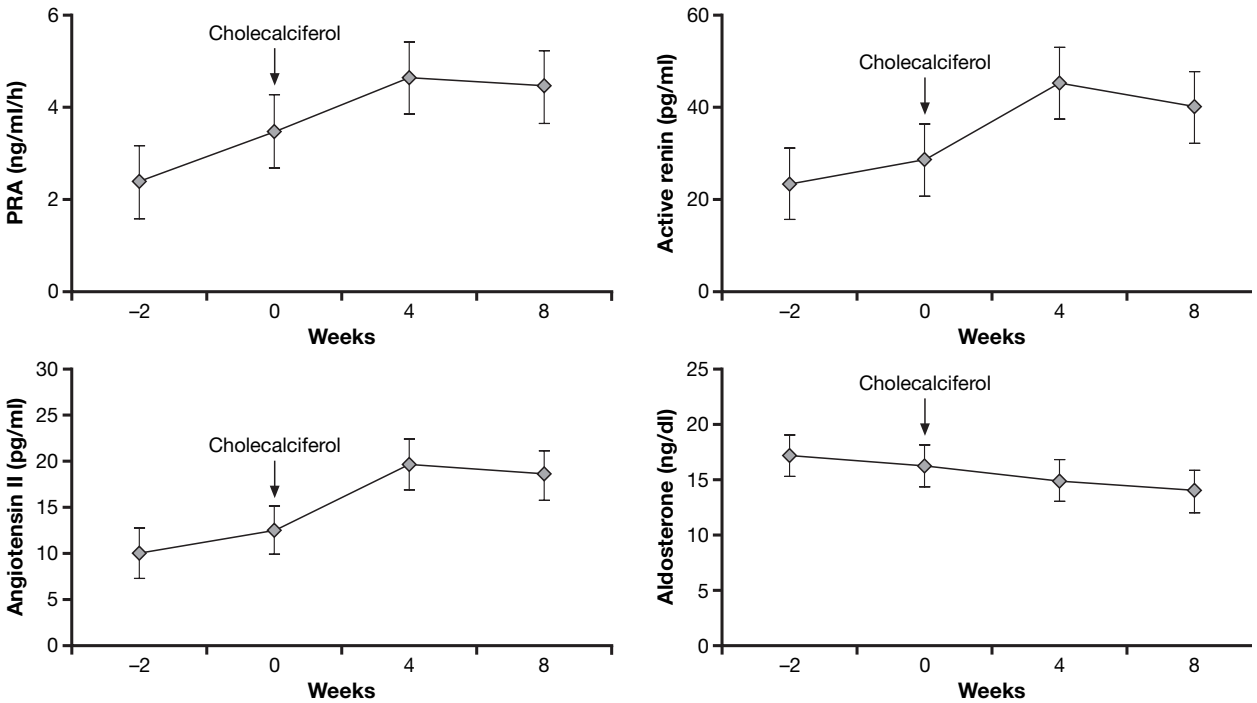

Fig. 2 - *Cholecalciferol effects on plasma renin activity (PRA), active renin, angiotensin II and aldosterone in essential hypertensives treated with angiotensin II antagonists (time –2 weeks).*

tivate endogenous RAS. In the first experimental design, this objective was reached by a well-controlled low-salt diet. In the second protocol, all patients, starting from at least 15 days prior to beginning of the study and continuing throughout the experiment, underwent therapy with angiotensin II receptor antagonists in order to activate endogenous plasma renin and angiotensin II levels. In neither experimental protocol was any significant effect observed after short-term or long-term vitamin D administration. Despite our results, we do not rule out that such an effect could conceivably be induced if a different type, dose, and timing of administration of drugs were adopted, or if a more prolonged study were performed. Our data confirm the findings of Sugden et al. (4) and de Zeeuw et al. (15), whose results, although referring to diabetic patients, showed that despite an improvement in endothelial function (in the former) and a reduction in albuminuria (in the latter), no influence on RAS was observed after vitamin D administration. However, both in our study and in the above-cited papers, patients were under treatment with RAS inhibitors. Thus, vitamin D may be unable to suppress the compensatory increment in renin associated with use of RAS inhibitors. Studies in drug-free subjects could clarify the true role of vitamin D on endogenous RAS. Finally, in this regard another explanation for the discrepancy observed in animals (suppression) (8) *vs* humans (no effect) can be put forward. In animals the focus of study was intra-renal (8), while in humans the investigation focused on systemic RAS both in the present study and in other (4, 15) researches. An increasing and to some extent new body of data indicates that the regulatory mechanisms of systemic and intra-renal RAS are different and sometimes divergent (19). Thus, in humans, the inhibitory action of vitamin D may genuinely occur, but only at local (intra-renal) RAS level without influencing systemic circulation. This would explain why vitamin D receptor knockout mice show overstimulated RAS (20), whereas RAS appears to be normal in humans with corresponding (phenotypically and metabolically) genetic mutation [1,25$(OH)_2$D-resistant rickets] leading to complete or partial target organ resistance to 1,25-hydroxyvitamin D_3 (21). In the animal model, intra-renal RAS was measured, while in humans only peripheral (systemic) RAS was evaluated. Based on these findings we are performing clinical studies to evaluate systemic and intra-renal RAS after vitamin D receptor activation in humans.

In conclusion, our study shows that short-term calcitriol assumption in EH and C with hypovitaminosis D and long-term cholecalciferol administration in EH with hypovitaminosis D exerted no effects on peripheral RAS components. The present data suggest that, at least in our experimental conditions, vitamin D receptor activation was unable to influence circulating RAS activity.

ACKNOWLEDGMENTS

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Declaration of interest

The authors declare that there is no financial or other potential conflict of interest; the authors declare that there is no conflict of interest which could be perceived as compromising the impartiality of the research reported.

REFERENCES

1. Pilz S, Tomaschitz A, Ritz E, Pieber TR. Vitamin D status and arterial hypertension: a systematic review. Nat Rev Cardiol 2009, 6: 621-30.
2. Pilz S, März W, Wellnitz B, et al. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. J Clin Endocrinol Metab 2008, 93: 3927-35.
3. Leu M, Giovannucci E. Vitamin D: epidemiology of cardiovascular risks and events. Best Pract Res Clin Endocrinol Metab 2011, 25: 633-46.
4. Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. Diabet Med 2008, 25: 320-5.
5. Lambers Heerspink HJ, Agarwal R, Coyne DW, et al. The selective vitamin D receptor activator for albuminuria lowering (VITAL) study: study design and baseline characteristics. Am J Nephrol 2009, 30: 280-6.
6. Fishbane S, Chittineni H, Packman M, Dutka P, Ali N, Durie N. Oral paricalcitol in the treatment of patients with CKD and proteinuria: a randomized trial. Am J Kidney Dis 2009, 54: 647-52.
7. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest 2002, 110: 229-38.
8. Freundlich M, Quiroz Y, Zhang Z, et al. Suppression of renin-angiotensin gene expression in the kidney by paricalcitol. Kidney Int 2008, 74: 1394-402.
9. Resnick LM, Muller FB, Laragh JH. Calcium-regulating hormones in essential hypertension. Relation to plasma renin activity and sodium metabolism. Ann Intern Med 1986, 105: 649-54.
10. Burgess ED, Hawkins RG, Watanabe M. Interaction of 1,25-dihydroxyvitamin D and plasma renin activity in high renin essential hypertension. Am J Hypertens 1990, 3: 903-5.
11. Tomaschitz A, Pilz S, Ritz E, et al. Independent association between 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin-angiotensin system: The Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Clin Chim Acta 2010, 411: 1354-60.
12. Vaidya A, Forman JP, Hopkins PN, Seely EW, Williams JS. 25-Hydroxyvitamin D is associated with plasma renin activity and the pressor response to dietary sodium intake in Caucasians. J Renin Angiotensin Aldosterone Syst 2011, 12: 311-9.
13. Vaidya A, Sun B, Forman JP, et al. The Fok1 vitamin D receptor gene polymorphism is associated with plasma renin activity in Caucasians. Clin Endocrinol (Oxf) 2011, 74: 783-90.
14. Forman JP, Williams JS, Fisher ND. Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. Hypertension 2010, 55: 1283-8.
15. de Zeeuw D, Agarwal R, Amdahl M, et al. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. Lancet 2010, 376: 1543-51.
16. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011, 96: 1911-30.
17. Mancia G, De Backer G, Dominiczak A, et al. 2007 ESH-ESC Practice Guidelines for the Management of Arterial Hypertension: ESH-ESC Task Force on the Management of Arterial Hypertension. J Hypertens 2007, 25: 1751-62.
18. Holick MF. Vitamin D deficiency. N Engl J Med 2007, 357: 266-81.
19. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. Pharmacol Rev 2007, 59: 251-87.
20. Zhou C, Lu F, Cao K, Xu D, Goltzman D, Miao D. Calcium-independent and 1,25$(OH)_2D_3$-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice. Kidney Int 2008, 74: 170-9.
21. Tiosano D, Schwartz Y, Braver Y, et al. The renin-angiotensin system, blood pressure, and heart structure in patients with hereditary vitamin D-resistance rickets (HVDRR). J Bone Miner Res 2011, 26: 2252-60.