International Heart and Vascular Disease Journal
Volume 5, Number 16, December 2017

Journal of the Cardioprogress Foundation

EXPERIMENTAL STUDY

Role of vitamin D in the development and correction of stress-induced arterial hypertension in rats

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Summary

Objective

To investigate the levels of 25-hydroxyvitamin D (25(OH)D) and effects of additional cholecalciferol intake on endothelial function and blood pressure (BP) in case of chronic stress-induced arterial hypertension (AH) in rats.

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Materials and methods

This study was performed on 136 adult wild type male rats with body weight ranged between 200-250g. Rats were placed in the condition of overpopulation for 4 months that led to development of stress-induced AH in the majority of rats by the end of this period. In order to investigate the role of vitamin D on the mechanisms underlying AH development, rats were administered with cholecalciferol (2500 MU/day) during all the period of overpopulation experiment. We estimated therapeutic effects of cholecalciferol on BP levels and endothelial function that was evaluated using blood levels of nitric oxide and acetylcholine-dependent vasodilatation. As the control we used hypertensive rats who did not consume cholecalciferol and healthy animals.

Results

Development of stress-associated AH was accompanied with suppressed endothelial vascular function that was expressed as reduction of NO concentration in blood and endothelium-dependent vasodilatation after acetylcholine administration. Use of cholecalciferol (2500 MU/day) in hypertensive rats led to reduction of average BP levels, and improved the characteristics of endothelial function and increased NO production.

Conclusion

Long-term administration of cholecalciferol (2500 MU/day) to hypertensive rats leads to normalization of hemodynamic parameters and improves the characteristics of endothelial function. The results of our study demonstrate that cholecalciferol can become an important additional component of antihypertensive therapy, but it requires further detailed studies.

Key words

Arterial hypertension, cholecalciferol, nitric oxide, endothelium-dependent vasodilatation, vitamin D.

Introduction

Numerous studies have demonstrated the link between low concentrations of vitamin D and increased risk of development of arterial hypertension (AH), atherosclerosis, myocardial infarction, metabolic syndrome, diabetes mellitus, several autoimmune and other diseases [1, 2, 3, 4]. Nowadays vitamin D deficiency is considered to be a new risk factor for development of cardiovascular diseases (CVD) that becomes particularly relevant due to their high prevalence of vitamin D hypovitaminosis in the population, up to 60-96% in different age groups [5, 6, 7]. Direct involvement of vitamin D in regulation of vascular homeostasis has been hypothesized because of exploration of the presence of vitamin D receptors (VDR) in the cells of all cardiovascular system (CVS). More than that, many types of cells including vascular smooth muscle myocytes, endothelial cells and cardiomyocytes are capable to produce 1-α hydroxylase enzyme that catalyzes the conversion of 25-hydroxy-vitamin D (25(OH)D) into its more active form 1,25 dihydroxy-vitamin D (1,25(OH)2D), natural ligand of VDR. Thus, the cells of CVS are able to provide vitamin D metabolism and to produce vitamin D active form for their own regulatory mechanisms.

Many studies demonstrated that D (1,25(OH)2D) directly regulates rennin-angiotensin system and endothelial function, inhibits proliferation of vascular smooth muscle cells, reduces the intensity of coagulation [8, 9, 10, 11]. More than that, vitamin D effects can be mediated through its involvement of calcium and phosphate homeostasis, immune/inflammatory response, carbohydrate balance etc. Several animal models of AH development caused by acute vitamin D deficiency have been created [8, 20]. In vitro experiments demonstrated that VDR activation induces NO production in vascular endothelial cells and increases their functional capacity. Apart from it, vitamin D participates in regulation of proliferation, migration and mineralization of vascular smooth muscle cells [1, 7]. At the same time, review articles demonstrate contradictory data on the role of vitamin D in stabilization of hypertensive state [12]. The questions of mechanisms underlying vitamin-D-dependent changes of BP levels and AH development remain open. It's possible that the main explanation of contradictory results of performed studies is that up to now we have no full distinct understanding of mechanisms mediating vitamin D regulation of BP and CVS. Up to nowadays this lack of knowledge prevents us from using vitamin D-containing drugs for CVD prevention and treatment.

The objective of this study was to investigate the levels of 25-hydroxyvitamin D (25(OH)D) and effects of additional cholecalciferol intake on endothelial function and BP in case of chronic stress-induced AH in rats.

Materials and methods

This study was performed on 136 adult wild type male rats with body weight ranged between 200-250 g. Animals were kept in vivarium conditions and received standard meal. All experiments were performed according to the principles of Helsinki agreement on ethical animal experimentation. Hemodynamic parameters (mean blood pressure – mBP, and heart rate (HR)) were registered in awake rats using the equipment for direct BP registration in small animals (PowerLab/400 ML 401, ID Instruments, 2002, Australia) and Chart 4 software with BP sensors (MLT0699, PowerLab, ID Instruments). For this experiment polyethylene catheter was implanted into aorta of rats through the left branch of carotid artery under general nembuthal anesthesia (0,40 mg/kg) [13, 14].

Stress-induced AH in experimental animals has been modeled in the condition of long-term overpopulation [15]. BP and HR measurements were performed according to standard procedure of direct BP registration. Blood of animals was taken in the morning during fasting period. BP levels, 25(OH)D and NO concentrations were evaluated monthly following the development of hypertension in rats.

25(OH)D concentration in blood (ng/mL) was estimated using immunofluorescence analysis and RAT 25OH VITAMIN D TOTAL ELISA kits (Cat. No.: RIS022R).

NO concentration (μg/mL) was evaluated using Griess test and "SF-2000 Bio" spectrophotometer at 583 nm wavelength [16, 17].

Endothelium-dependent vasorelaxation in normotensive and hypertensive rats was investigated under intravenous administration of acetylcholine (0,3 μg/kg, Pharma, Czech Republic) together with estimation of maximal mBP change within the first minute after bolus injection of the drug.

All experimental animals were divided into 4 groups: normotensive rats (NR) who had normal BP, hypertensive rats (HypR) with elevated values of mBP and HR; NR+D3 and HypR+D3 that consisted of normotensive and hypertensive rats who received pharmacological treatment with vitamin D (Colecalciferol, 2500 ME in form of water solution) daily during 4 months. Each group consisted of 8-12 rats.

Statistical analysis of results was done using "Statistica 7.0" software. Data are shown as mean value ± standard error (M±m). Comparative comparison between two groups was assessed using Student's t-test. Pearson's correlation test (r) was used for evaluation of correlation between variables. Characteristic of dynamics (Δ) was quantified as difference between repeated and initial measurements. The null hypothesis was rejected if $p < 0,05$.

Results

Table 1 demonstrates the values of mBP, HR and 25(OH)D concentration in blood of rats after 1, 2, 3 and 4 months of experimental model of stress-induced AH. As it can be seen from the results shown in Table 1, experimental rats started to develop AH after 4 months of chronic stress that was proved by significant increase of mBP and HR comparing with the control group. HypR demonstrated significant decrease of vitamin D concentration by 46% (Δ46±1%; $p<0,05$) (Table 1). NO production has changed by 56% in the same way (Δ56±1%; $p<0,05$) comparing with the control group. So, average NO concentration in blood was 0,17±0,01 μg/mL, that was lower($p=0,04$) than in intact animals who had NO blood concentration in the range of 0,38±0,02 μg/mL (Table 1).

mBP values after acetylcholine administration are present in Figure 1 that demonstrates decrease of mBP by 58±5% in NR after acetylcholine administration and proves intact endothelium-dependent vasodilatation in these animals. There were no statistically significant mBP changes after acetylcholine administration; the dynamics was Δ8±1% that demonstrates the development of endothelial dysfunction during chronic BP elevation (Figure 1).

The results reflecting the influence of pharmacological substitution of vitamin D on studied parameters in the groups NR+D3 and HypR+D3 are pres-

Table 1. **mBP, HR and 25(OH)D values in blood of rats during AH development**

Parameters	Control n=10	Stress-induced AH model			
		1 month n=10	2 months n=10	3 months n=10	4 months n=10
mBP, mm Hg	109±3	115±4	112±5	117±3	149±3*
HR, beats per minute	382±14	397±12	394±11	401±12	444±14*
25(OH)D, ng/mL	19,9±1,1	18,2±0,6	19,0±0,6	18,1±0,5	10,8±0,5ra*
NO, μg/mL	0,38±0,02	0,34±0,01	0,36±0,05	0,29±0,08	0,17±0,01 *

* $p<0,05$ comparing with the control.

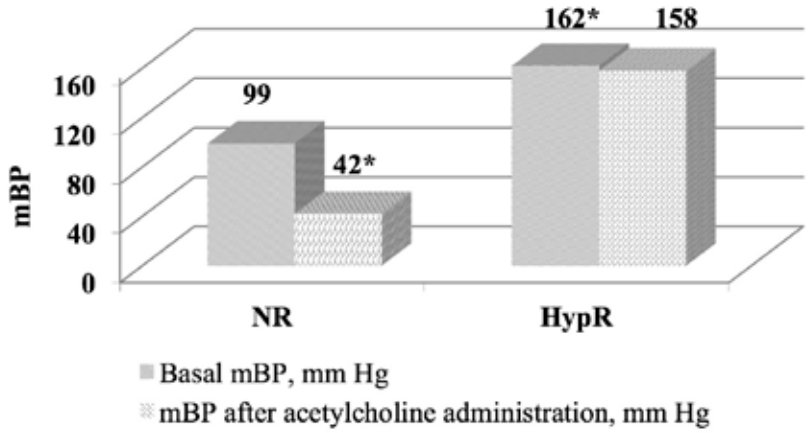

Figure 1. mBP values after acetylcholine administration in NR and HypR groups.
* - p< 0,05 comparing with the control; † - p< 0,05 comparing with basal mBP.

Table 2. **mBP and 25(OH)D and NO blood concentrations in rats receiving cholecalciferol**

Parameters	NR, before drug administration n=10	NR+D3 n=10	HypR before drug administration n=10	HypR+D3 n=8
mBP, mm Hg	109±3	106±3	149±3*	127±3†*
NO, μg/mL	0,38±0,02	0,36±0,03	0,17±0,01*	0,29±0,02†*
25(OH)D, ng/mL	19,9±1,1	20,3±0,7	10,8±0,5*	13,9±0,3†*

* p<0,05 comparing with NR,
† p<0,05 comparing with initial values.

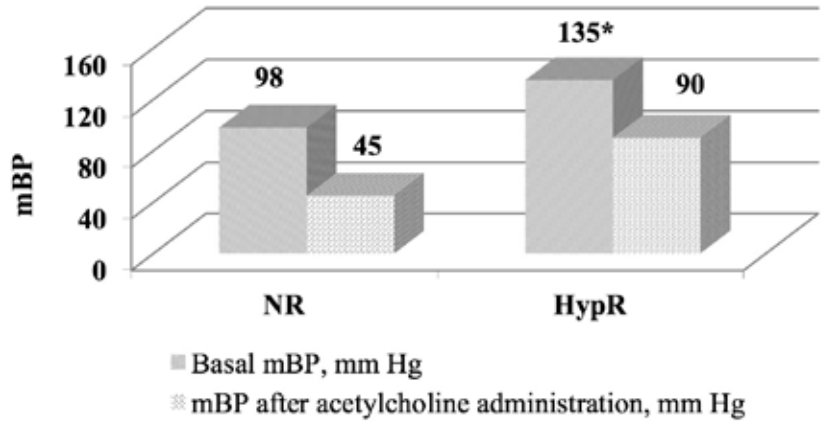

Figure 2. mBP values after acetylcholine administration in NR and HypR groups who received cholecalciferol for 4 months.
* - p< 0,05 comparing with the control; † - p<0,05 comparing with the basal mBP levels.

ent in Table 2, and they demonstrate that long-term (during all period of AH development in rats) daily administration of cholecalciferol 2500 ME had therapeutic effect on mBP, NO and 25(OH)D blood concentrations. Thus, mBP was significantly reduced by 15% in HypR+D3 group (p<0,05), and 25(OH)D and NO blood concentrations were increased by 29% (p<0,05) and 70% (p<0,05), respectively. At the same time, although these parameters have significantly improved, they didn't reach normal values. It is worth to mention that cholecalciferol administration in NR had no effect neither on mBP levels nor on 25(OH)D and NO blood concentrations (Table 2).

Investigation of the effects of long-term administration of cholecalciferol on endothelium-dependent vasodilatation revealed similar results, thus vitamin D pharmacological administration had therapeutic action on hypertensive and not on normotensive rats (Figure 2). As it can be seen on the Figures 1 and 2, vascular sensitivity to acetylcholine did not change in NR, but it significantly improved by 34% (p<0,05) in HypR, and the intensity of this reaction was 4,3 times higher comparing with the group that did not receive cholecalciferol (Figure 2).

Discussion

Results of this study demonstrated that the development of stress-induced AH is accompanied with suppressed endothelial function of vessels that results in reduced NO blood concentrations and impaired endo-

thelium-dependent vasodilatation after acetylcholine administration. These results go along with the existing conception of impairment of endothelial vasodilatation during AH development [17, 18]. Previous clinical and experimental studies demonstrated reduced activity of NO-ergic system and vascular sensitivity to acetylcholine in AH [10, 16, 19, 20-22].

In order to investigate the role of vitamin D in AH development we performed several experiments divided into two stages. During the first step we estimated concentrations and changes of vitamin D levels in blood of adult rats during hypertensive status formation. The results demonstrated that high BP levels corresponding to AH correlate with the development of vitamin D deficiency (Table 1). These data go along with the results of several authors that reported reduction of vitamin D levels in hypertensive animals [1-3, 11, 23-27]. Other AH models like rats spontaneously acquiring AH showed that together with AH development these animals form the deficiency of the main active metabolite of vitamin D production and that it goes along with suppressed endothelium-dependent contraction of aorta due to decreased concentration of free calcium in the cytoplasm of endothelial cells [11].

The second part of experiments aimed to find an answer to the question: Is it possible to substitute pharmacologically vitamin D and to correct its deficiency and associated vascular disorders? To solve this problem, we evaluated such characteristics like mBP, 25(OH)D and NO concentrations in blood of rats during long-term daily administration of cholecalciferol 2500 ME that is supposed to be its most effective dose for additional AH correction according to several studies [1, 2, 3, 20]. Cholecalciferol was additionally administered to rats during 4 months of hypertension formation in these animals. It has been shown that cholecalciferol had no effects on these parameters in NR, whereas HypR demonstrated reduction of mBP and improvement of endothelium-dependent vasorelaxation and increased NO production (Figures 1 and 2).

It goes along with the existent experimental results demonstrating that antihypertensive effects of vitamin D are mediated through improvement of endothelial function (increasing the activity of endothelial NO-synthase, decreasing expression of endothelial adhesion molecules, through anti-inflammatory effects) and suppression of renin-angiotensin-aldosterone system activity, reducing oxidative stress and involvement of several genomic mechanisms. [8, 9, 11, 24, 26, 27].

It is worth to mention that pharmacological replacement of vitamin D deficiency in HypR was not sufficient for efficient AH treatment. Although its therapeutic effects on mBP and characteristics of endothelial vascular function were significant.

Conclusion

Long-term administration of cholecalciferol (2500 MU/day) to hypertensive rats leads to normalization of hemodynamic parameters and improves the characteristics of endothelial function. The results of our study demonstrate that cholecalciferol can become an important additional component of antihypertensive therapy, but it requires further detailed studies.

Conflict of interest: None declared

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