Original Article

Vitamin D Attenuates Cardiac Hypertrophy in Rats through mRNA Regulation of Interleukin-6 and Its Receptor

Ahad Nikkholgh[1], Fatemeh Tavakoli[1], Nasrin Alborzi[2], Fatemeh Araste[3]

[1]Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shahid Sadoughi University of Medical Sciences, [2]Department of Physiology, School of Medicine, Shahid Sadoughi University of Medical Sciences, [3]Department of Medical Biotechnology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Abstract

Context: Interleukin-6 (IL-6), a pro-inflammatory cytokine, plays an important role in the pathogenesis of myocardial hypertrophy. By integrating its membrane receptor complex (gp80), IL-6 activates the signal guidance components (gp130) and activates the hypertrophic signaling pathways. There is some evidence that 1,25 dihydroxyvitamin D exerts antihypertrophic effects, but the cellular and molecular mechanisms are not fully understood. The aim of this study was to evaluate the effect of calcitriol on the level of IL-6 and its receptor components in hypertrophied rat heart. **Subjects and Methods:** Male rats were divided into control, hypertrophy, Vitamin D + hypertrophy, and propylene glycol + hypertrophy groups. The groups receiving Vitamin D and propylene glycol were treated 2 weeks before induction of hypertrophy and 2 weeks after hypertrophy. Myocardial hypertrophy was induced by abdominal aortic stenosis. Mean arterial blood pressure was measured by cannulation of the left carotid artery, and expression of genes was determined by reverse transcription-polymerase chain reaction. **Results:** Blood pressure and heart-to-body weight ratio increased in hypertrophic groups compared to the control group ($P < 0.01$), but Vitamin D administration decreased these parameters ($P < 0.05$). Abdominal aortic stenosis increased IL-6 expression levels ($P < 0.001$) and Vitamin-D decreased IL-6 mRNA levels ($P < 0.01$). The expression of gp80 in the hypertrophic group increased compared to the control group ($P < 0.05$), but Vitamin D did not affect the expression of receptor subunits genes. **Conclusions:** The data from this study suggest a possible mechanism for the antihypertrophic effects of Vitamin D through the regulation of inflammatory responses during hypertrophy. Thus, Vitamin D can reduce IL-6 expression levels, thereby reducing hypertrophy.

Keywords: 1,25 Dihydroxyvitamin D, gp130, gp80, interleukin-6, myocardial hypertrophy

Introduction

Cardiovascular diseases (CVD), the leading cause of disease burden in the world, are common and have poor survival. Prevalent cases of total CVD nearly doubled from 271 million in 1990 to 523 million in 2019.[1] Cardiac hypertrophy is not only a single disease but also a pathological process of many forms of CVD, such as hypertension, ischemic diseases, congestive heart failure, and valvular diseases.[2] Pathological cardiac hypertrophy is characterized by the thickening of the heart muscle, a reduction in the size of the chambers of the heart, and a reduced capacity of the heart to pump blood to the organs around the body.[3,4] At the cellular level, pathological hypertrophy is typically associated with increased cell death (apoptosis and necrosis).[5] Cell hypertrophy is the increase in the size of cells, which also leads to an increase in the size of the organ. In other words, no new cells are formed in hypertrophy, and only the cells become larger and increase in volume due to increased synthesis of organs and building proteins. Microscopic findings define cardiac hypertrophy as thickening of the inner wall of the ventricle and/or septum.[6] Cardiac hypertrophy is one of the main and adaptable responses of heart cells to biological stresses such as mechanical traction and release of neurohormonal factors. This increase in volume enables myocytes to increase their output and improve cardiac

Received: 20-September-2023 **Revised:** 15-November-2023
Accepted: 23-November-2023 **Published:** 26-March-2024

Access this article online

Quick Response Code:

Website: http://journals.lww.com/RCVM

DOI: 10.4103/rcm.rcm_60_23

Address for correspondence: Dr. Fatemeh Tavakoli, Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
E-mail: f.tavakoli@ssu.ac.ir

How to cite this article: Nikkholgh A, Tavakoli F, Alborzi N, Araste F. Vitamin D attenuates cardiac hypertrophy in rats through mRNA regulation of interleukin-6 and its receptor. Res Cardiovasc Med 2023;12:123-8.

pumping performance as a compensatory response. Although this is initially a compensatory response that unsustainably causes biomechanical stress, if the stimulus becomes chronic, prolonged hypertrophy becomes a pathological heart disease that can lead to heart failure.[5]

Inflammation is a key component in the myocardial remodeling that occurs in response to different cardiac damages. Interleukin-6 (IL-6), an inflammatory cytokine with multiple effects in various cells and organs, is produced by cardiac myocytes and has been associated with cardiovascular pathologies. Meléndez *et al*. have publicized that IL-6 infusion produced concentric hypertrophy in rats. Increased levels of IL-6 correlate with the severity of heart failure and are strongly prognostic of 1-year mortality.[7,8] IL-6 is made of four α-chain nanostructures and exerts its action through an interaction with a cell surface receptor (IL-6R) which comprises two subunits, an 80 kDa ligand-binding subunit glycoprotein 80 of 468 amino acids (gp80/IL-6Rα) and a 130 kDa signal-transducing protein glycoprotein 130 of 896 amino acids residues (gp130/IL6Rβ).[9] Dimerization of gp80 with gp130 induces transactivation and autophosphorylation of Janus kinases (Jak) that phosphorylates signal transducer and activator of transcription 3 (STAT3). gp130 is the main subunit of multifunctional receptors for the IL-6 family of cytokines.[10,11] According to Hirota *et al*.'s study, persistent activation of gp130, as a signal-transducing component of IL-6 receptor caused myocardial hypertrophy in mice.[12]

Vitamin D, a fat-soluble vitamin, is naturally present in a few foods, added to others, and available as a dietary supplement. It is also made endogenously when ultraviolet (UV) rays from sunlight strike the skin and elicit Vitamin D synthesis.[13] Vitamin D plays an essential role in the regulation of body function comprising the cardiovascular system. A number of studies and clinical analyses have shown a close association between Vitamin D deficiency and risk of CVD. Insufficiency of Vitamin D is a global health issue that afflicts a wide-ranging population with a myriad of acute and chronic illnesses involving heart and circulatory disorder which may be a prominent reason for death.[14,15]

Despite the well-known role of IL-6 in the pathogenesis of myocardial hypertrophy, as well as the proposed cardioprotective effects of Vitamin D, there has been no report for the effect of this vitamin on IL-6 and its receptor components in hypertrophied myocardial tissue. Therefore, in this study, we investigated this issue on rats with abdominal aortic stenosis (myocardial hypertrophy model). Our main aim was to determine the effects of 1,25-dihydroxyvitamin D on the IL-6 and its receptor components' transcription levels in rat hypertrophied hearts.

Subjects and Methods

Materials

Ketamine was provided from Alfasan, The Netherlands). Isopropyl alcohol and propylene glycol were purchased from Merck Chemical Company (Darmstadt, Germany). Vitamin D was obtained from Darupakhsh (Tehran, Iran). cDNA synthesis kit, RNX PLUS, Mastermix cybergreen, and red mastermix were purchased from Parstous (Tehran, Iran), Cinnagen (Tehran, Iran), Yektatajhiz (Tehran, Iran), and Ampliqon (Stenhuggervej, Denmark), respectively.

Animals

Male Wistar rats weighing 170–220 g, obtained from a single breeding colony, quarantined for 1 week before use in the animal house of the Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences. The animals were kept under controlled environmental conditions of temperature (25°C), relative humidity of 50%–55%, and 12 h light/dark cycle. Rats had free access to standard laboratory food and water *ad libitum*.

Induction of cardiac hypertrophy in rats

Animals were anesthetized with intraperitoneal injection of a mixture of ketamine (70 mg/kg) and xylazine (10 mg/kg). After shaving the left side of the animal, betadine/alcohol wiping was used to disinfect the surgical area, and when the animal was unresponsive to toe-pinch, an incision was used to expose the abdominal aortic artery and to isolate the vessel. The aorta was constricted by a silk thread at 0.6 mm. Sham operation was carried out in age-matched control rats in the same way with the animals operated but without aorta banding. Then, we sutured the skin, and tetracycline antibiotic was sprayed on the sutures. The rats were retained on a heating pad while recovering from anesthesia. Postoperative care was continued using analgesia and hemodynamic monitoring for 24 h. Each animal was kept in a separate cage.

Experimental protocol and groups

The animals were divided into four groups, six in each: control (Sham), hypertrophy, hypertrophy + Vitamin D (H + D), and hypertrophy + propylene glycol (H + P).

Rats in H + D group received intraperitoneal injection of Vitamin D at a dose of 0.1 μg/kg/day[16] 2 weeks before surgery and 2 weeks after surgery. Rats in H + P group just received propylene glycol as the solvent of Vitamin D. The animals of age-matched control and hypertrophy group were given the same volume of normal saline at the same time.

Blood pressure measurement

At the end of the experiment, before tissue collection, rats were anesthetized and to determine blood pressure changes, systolic and diastolic pressure was recorded by cannulating the left carotid artery using a transducer device.

Tissues collecting for reverse transcription-polymerase chain reaction

After weighing, the rats were euthanized with a pentobarbital overdose, then the hearts were explanted and weighted and left ventricle tissues were homogenized for molecular studies (reverse transcription-polymerase chain reaction [RT-PCR]).

Gene expression analysis by real-time polymerase chain reaction

Total RNA was extracted from left ventricle tissues using RNX-Plus kit (CinnaGen, Iran), according to the manufacturer's instruction. RNA concentrations and purity were determined through spectrophotometer methods (Eppendorf BioPhotometer, Germany).

cDNA synthesis was performed according to the instructions in the Parstous kit (Tehran, Iran). Reverse transcription was performed using H-MMuLV Reverse transcriptase enzyme. After optimizing the reaction, the prepared cDNAs of the tested groups were used to perform real-time PCR according to the conditions mentioned in Table 1. The glyceraldehyde 3-phosphate dehydrogenase gene was used as endogenous reference gene. The sequences of forward and reverse primers are shown in Table 2.

Data analysis

Rotor-Gene Q Series Software was used to analyze real-time PCR data. Comparative quantification analysis was used to analyze the raw data, and the Ct values were measured for each of the studied genes in different groups. Next, to analyze the expression information of the target genes, we analyzed the Ct values from the real-time PCR results by $2^{-\Delta\Delta ct}$ method. Final data, presented as mean ± S.E.M., were compared using one-way analysis of variance (ANOVA). Where a significant difference was detected by ANOVA, the treated groups were compared with the control one or with each other using Dunnett and Tukey posttest, respectively. All statistical analyses were performed with GraphPad Prism software (GraphPad software, Inc, Boston, USA).

Results

Effect of Vitamin D on blood pressure level following myocardial hypertrophy

Table 3 shows the systolic and diastolic blood pressure in the different tested groups. The results indicate that in the hypertrophy group, systolic and diastolic blood pressure increased significantly compared to the control group ($P < 0.0001$). In the H + D group, systolic and diastolic blood pressure decreased significantly compared to the hypertrophy group ($P < 0.0001$).

Heart-to-body weight ratio

Results indicated that myocardial hypertrophy due to abdominal aortic stenosis significantly increased the heart-to-body weight ratio (4.12 ± 0.975) compared to the control group (3.224 ± 0.119; $P < 0.01$), but Vitamin D treatment prevented the increase of heart-to-body weight ratio [Figure 1].

Interleukin-6 gene expression in different groups

Based on obtained data, although myocardial hypertrophy, significantly increased the expression levels of IL-6 compared to the control group (48 ± 5.9%, $P < 0.001$), administration of Vitamin D in rats with myocardial hypertrophy significantly decreased the expression level of IL-6 compared to hypertrophic and hypertrophic plus propylene glycol groups ($P < 0.01$) [Figure 2].

gp80 and gp130 gene expression in different groups

As shown in Figure 3, the gp80 gene expression in the hypertrophy group (55 ± 13%) and hypertrophy plus propylene glycol group increased significantly ($P < 0.05$) compared to the control group. While in the hypertrophy plus Vitamin D treatment group, the level of gp80 expression did not show a significant difference in comparison with the control group [Figure 3].

The levels of gp130 gene expression were not significantly different between the experimental groups [Figure 4].

Discussion

Abdominal aortic stenosis is a classic model for analyzing heart damage caused by aortic artery occlusion. Elevated blood pressure is a recognized risk factor for CVDs. Some

Table 1: Real-time reverse transcription-polymerase chain reaction thermocycling conditions

Description	Time	Temperature (°C)			Cycle
Initial denaturation	3 min		95		1x
Template denaturation	15 s		95		45x
Primer annealing	30 s	*IL*-6 56	*gp*130 60	*gp*80 61	45x
Primer elongation	30 s		72		45x
Final elongation	5 min		72		1x

IL-6: Interleukin-6

Table 2: Polymerase chain reaction primers for real-time reverse transcription-polymerase chain reaction analysis

Primer name	Sequence (5'–3')
IL-6 – forword	TTGCCTTCTTGGGACTGATG
IL-6 – reverse	GCCATTGCACAACTCTTTTC
gp130 – forword	CCGTCAGTGCAAGTGTTCTCA
gp130 – reverse	CACTATCCACCAGCTGCAGGT
gp80 – forword	GCCCAGCATCAATGTGTCATC
gp80 – reverse	TCCTCCTTCCCTCGGACCT
GAPDH – forword	AACGACCCCTTCATTGAC
GAPDH – reverse	TCCACGACATACTCAGCAC

IL-6: Interleukin-6

Table 3: The effect of Vitamin D on blood pressure levels following myocardial hypertrophy

Groups	Systolic pressure (mmHg)	Diastolic pressure (mmHg)
Control	110.5±2.266	70.5±2.212
Hypertrophy	146.9±2.052****	101.7±1.658****
H + D	123.1±3.011###	78±2.093###
H + P	146±2.129****	103.2±5.510****

****P<0.0001 compared to the control group and ###P<0.0001 compared to the hypertrophy and H + P groups. Data are shown as mean±SEM. SEM: Standard error of mean

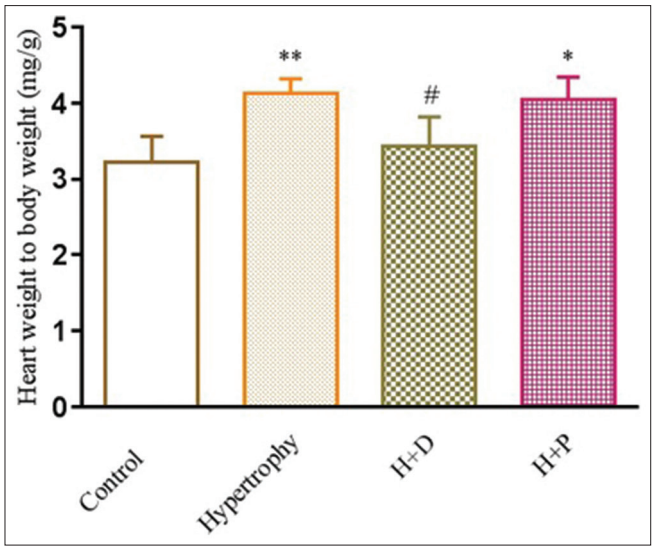

Figure 1: The effect of Vitamin D on the ratio of heart-to-body weight following myocardial hypertrophy due to abdominal aortic stenosis. Heart-to-body weight ratios were measured in the groups: control, hypertrophy, hypertrophy + Vitamin D, and hypertrophy + propylene glycol (H + P). Propylene glycol was considered solvent of Vitamin D. The data are displayed as Mean ± SE, $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control group, and $^{\#}P < 0.05$ compared to the hypertrophic and H + P groups. H + D: Hypertrophy + Vitamin D, H + P: Hypertrophy + propylene glycol

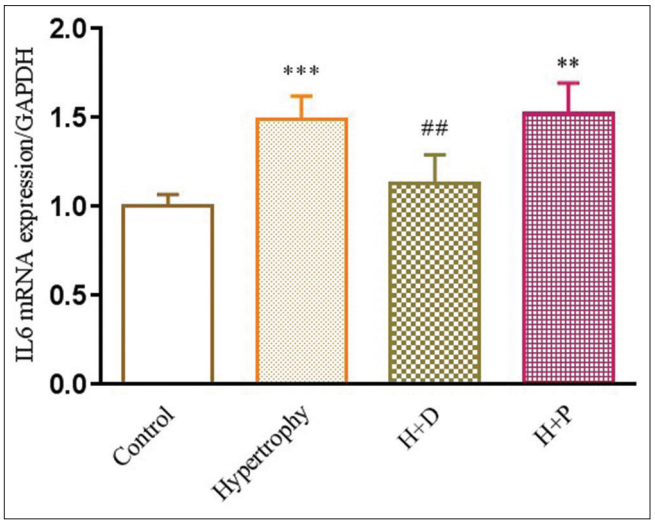

Figure 2: The effect of Vitamin D on interleukin-6 mRNA levels following myocardial hypertrophy due to abdominal aortic stenosis. mRNA levels were measured in groups: control, hypertrophy, hypertrophy + Vitamin D, and hypertrophy + propylene glycol. The data are displayed as mean ± SE, propylene glycol was considered solvent of Vitamin D. $^{***}P < 0.001$, $^{**}P < 0.01$ compared to the control group and $^{\#\#}P < 0.01$ compared to the hypertrophic and H + P groups. H + D: Hypertrophy + Vitamin D, H + P: Hypertrophy + propylene glycol

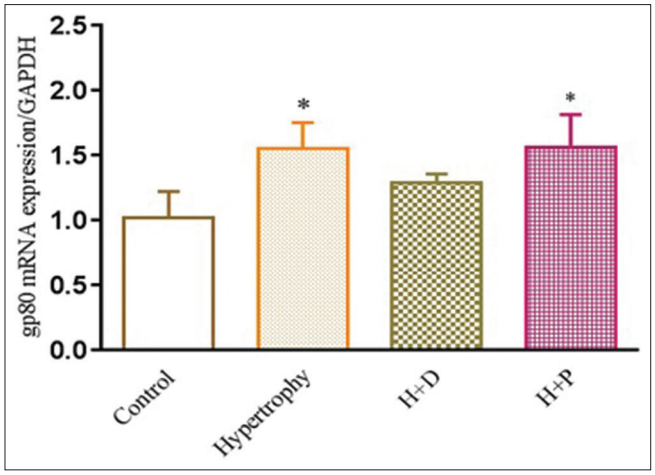

Figure 3: The effect of Vitamin D on the level of gp80 mRNA following myocardial hypertrophy due to abdominal aortic stenosis. mRNA levels were measured in groups: control, hypertrophy, hypertrophy + Vitamin D, and hypertrophy + propylene glycol. Propylene glycol was considered solvent of Vitamin D. The data are displayed as mean ± SE. No significant difference was observed between the data. H + D: Hypertrophy + Vitamin D, H + P: Hypertrophy + propylene glycol

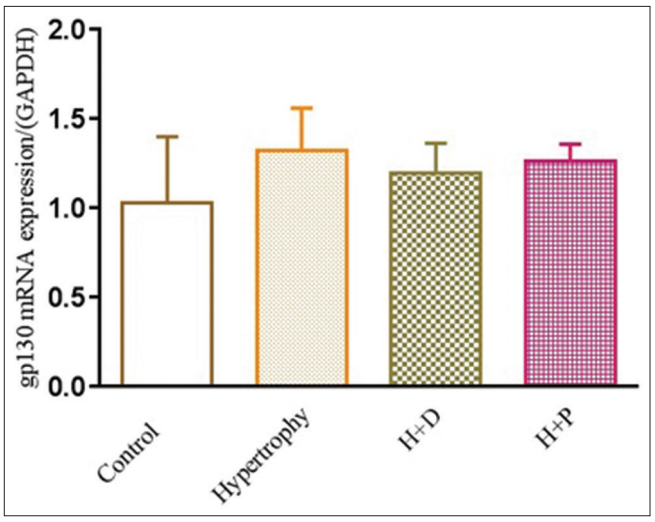

Figure 4: The effect of Vitamin D on the level of gp130 mRNA following myocardial hypertrophy due to abdominal aortic stenosis. mRNA levels were measured in groups: control, hypertrophy, hypertrophy + Vitamin D, and hypertrophy + propylene glycol. Propylene glycol was considered solvent of Vitamin D. The data are displayed as mean ± SE $^{*}P < 0.05$ compared to the control group. H + D: Hypertrophy + Vitamin D, H + P: Hypertrophy + propylene glycol

experimental evidence suggest that hypertension may promote endothelial expression of cytokines and stimulate inflammation, subsequent inflammatory responses play a pivotal role in the pathogenesis of atherosclerosis and cardiac hypertrophy. Elevated levels of IL-6, an important stimulant of the acute phase response and of intercellular adhesion molecule-1 (sICAM-1), are associated with future risk of cardiovascular death.[17]

One of the most important factors that have attracted the scientists' attention in terms of its protective effects on the cardiovascular system is Vitamin D which is made as a prohormone in the skin under the influence of UV light and is also absorbed from the gastrointestinal tract. Both sources should be activated in the liver to 25(OH) D and in the kidney to 1,25-dihydroxyvitamin D (1,25(OH)$_2$D). 25-Hydroxyvitamin

D deficiency has been recognized as a potential CVD risk factor.[18] Although data from laboratory, ecologic, and epidemiologic studies propose a protective effect of Vitamin D against CVDs, the exact mechanism of Vitamin D in the prevention of CVDs is not fully understood.

In recent decades, several compounds have been considered through the involvement of various pathways in the treatment of cardiac hypertrophy. These include Vitamin D. Safari *et al*. showed that administration of Vitamin D for 3 weeks reduced the heart-to-body weight ratio and blood pressure and myocardial hypertrophy markers in rats following the abdominal aortic banding.[19] According to Chen and Gardner study, Vitamin D was effective in reducing hypertrophy in mice, possibly by suppressing modulatory calcineurin inhibitor protein 1.[20] Many clinical and laboratory studies have reported the involvement of IL-6 in various aspects of cardiac hypertrophy and heart failure. A study of the effects of hypertension on inflammatory markers such as IL-6 and sICAM-1 in 508 apparently healthy men indicated that increased blood pressure might be a stimulus for inflammation and data showed significant graded relationships between blood pressure and levels of sICAM-1 as well as IL-6.[17] Mir *et al*. analyzed the mechanism by which IL-6 modulates cardiac hypertrophy in an *in vitro* and an *in vivo* model; they concluded that STAT3 is the major downstream signaling molecule during IL-6-mediated collagen gene upregulation during hypertrophy.[21]

To discover whether the concentration of circulating IL-6, the major proinflammatory cytokine could be altered by decreasing blood pressure in hypertensive subjects, Vázquez-Oliva *et al*. treated 17 hypertensive never-treated patients with irbesartan, 150–300 mg/day for 3 months and measured serum IL-6 at 0 and 12 weeks. Their results showed that the treatment of hypertension lowers circulating IL-6 in young hypertensive patients.[22] Various studies have revealed that cytokines of the IL-6 family induce cardiomyocyte hypertrophy and prevent their apoptosis. In 2003, Ancey *et al*. investigated the capability of human atrial cardiac cells to present the gp130 receptor subunit and to evaluate its functionality. According to their results, tissue fibroblasts or primary culture fibroblasts express gp130, but the signal in cardiomyocytes is weaker. Using of gp130 agonist antibody in cardiac cells culture increased expression of atrial natriuretic peptide and β myosin heavy chain (β-MHC) in cardiomyocytes. This process could involve STAT3 phosphorylation. Conclusively, gp130 activation in human cardiac cells leads to cardiomyocyte hypertrophy.[23]

In the present study, to investigate the effect of Vitamin D on inflammatory factors, expression of IL-6, gp130, and gp80 genes in hypertrophied tissues by abdominal aortic stenosis was evaluated. The results showed that treatment with Vitamin D by inhibiting the expression of IL-6 gene leads to improved cardiac hypertrophy. Our study revealed that Vitamin D could alter blood pressure and heart-to-body weight ratio in cardiac hypertrophied rats caused by abdominal aortic stenosis.

To our knowledge, this is the first study to assess the level of IL-6 in hypertrophy due to abdominal aortic stenosis and showed that the levels of mRNA IL-6 and mRNA gp80 increased after hypertrophy. However, the mRNA level of gp130 has not changed. It can be assumed that abdominal aortic stenosis could not increase the level of gp130 receptor during 4 weeks, but maybe if the period of aortic stenosis was a little longer, an increase in the level of gp130 expression would also be observed or probably the different producing pathways of gp130 and gp80 caused different effects of Vitamin D on these two receptors.

Although a wide range of drugs are used to treat myocardial hypertrophy, the prevalence of heart failure indicates the inability to treat the disease. Several studies have been reported on the effect of Vitamin D on cardiac hypertrophy, but the effect of this drug on inflammatory factors in hypertrophy caused by abdominal aortic stenosis has not been determined.

In an observational study in 2017, dietary intake was assessed in 79 outpatients with chronic stable heart failure using a validated food frequency questionnaire. Blood concentrations of a number of micronutrients, including Vitamin D, were measured in fasting blood samples, drawn at the time of food frequency questionnaire completion. Finally, it became obvious that Vitamin D deficiency was common in the patients with heart failure.[24] In Shedeed study, administration of Vitamin D oral drops for 12 weeks in infants with heart failure, could reduce the level of IL-6 and PTH, and led to significant improvement of HF score; thus, it can be concluded that Vitamin D supplementation has pronounced benefits as an anti-inflammatory agent in infants with CHF.[25] In our study, treatment with Vitamin D for 4 weeks could attenuate IL-6 expression levels. However, Vitamin D could not affect the transcription level of IL-6 receptor components. An increase in the dose of Vitamin D or an increase in the duration of treatment may affect the expression levels of IL-6 receptor components, but in this study, the dose of 0.1 μg/kg for 4 weeks failed to affect IL-6 receptor transcription levels, and it only showed its anti-inflammatory and cardioprotective effects through the reduction of IL-6 expression, without affecting the receptor.

Conclusion

The data in this study suggest a possible mechanism for the antihypertensive effects of Vitamin D through the regulation of inflammatory responses during cardiac hypertrophy. In this way, Vitamin D can reduce the level of IL-6 expression, thereby reducing hypertrophy.

Ethical consideration

This study was conducted under the consideration of medical ethics committee of Shahid Sadoughi university of Medical Sciences with the registration number of: IR.SSU.MEDICINE.REC.1396.324.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, *et al.* Global burden of cardiovascular diseases and risk factors, 1990-2019: Update from the GBD 2019 study. J Am Coll Cardiol 2020;76:2982-3021.
2. Liu Y, Chen X, Zhang HG. Editorial: Cardiac hypertrophy: From compensation to decompensation and pharmacological interventions. Front Pharmacol 2021;12:665936.
3. Kavazis AN. Pathological versus Physiological cardiac hypertrophy. J Physiol 2015;593:3767.
4. Frey N, Katus HA, Olson EN, Hill JA. Hypertrophy of the heart: A new therapeutic target? Circulation 2004;109:1580-9.
5. Bernardo BC, Weeks KL, Pretorius L, McMullen JR. Molecular distinction between physiological and pathological cardiac hypertrophy: Experimental findings and therapeutic strategies. Pharmacol Ther 2010;128:191-227.
6. van Empel VP, De Windt LJ. Myocyte hypertrophy and apoptosis: A balancing act. Cardiovasc Res 2004;63:487-99.
7. Meléndez GC, McLarty JL, Levick SP, Du Y, Janicki JS, Brower GL. Interleukin 6 mediates myocardial fibrosis, concentric hypertrophy, and diastolic dysfunction in rats. Hypertension 2010;56:225-31.
8. Zhao L, Cheng G, Jin R, Afzal MR, Samanta A, Xuan YT, *et al.* Deletion of interleukin-6 attenuates pressure overload-induced left ventricular hypertrophy and dysfunction. Circ Res 2016;118:1918-29.
9. Trovato M, Sciacchitano S, Facciolà A, Valenti A, Visalli G, Di Pietro A. Interleukin-6 signalling as a valuable cornerstone for molecular medicine (review). Int J Mol Med 2021;47:107.
10. Remeniuk B, King T, Sukhtankar D, Nippert A, Li N, Li F, *et al.* Disease modifying actions of interleukin-6 blockade in a rat model of bone cancer pain. Pain 2018;159:684-98.
11. Kawagishi H, Nakada T, Numaga-Tomita T, Larrañaga M, Guo A, Song LS, *et al.* Cytokine receptor gp130 promotes postnatal proliferation of cardiomyocytes required for the normal functional development of the heart. Am J Physiol Heart Circ Physiol 2022;323:H103-20.
12. Hirota H, Yoshida K, Kishimoto T, Taga T. Continuous activation of gp130, a signal-transducing receptor component for interleukin 6-related cytokines, causes myocardial hypertrophy in mice. Proc Natl Acad Sci U S A 1995;92:4862-6.
13. Ross AC, Taylor CL, Yaktine AL, Del Valle HB. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Washington, DC: The National Academies Press; 2011.
14. El-Bassiouny NA, Helmy MW, Hassan MA, Khedr GA. The cardioprotective effect of Vitamin D in breast cancer patients receiving adjuvant doxorubicin based chemotherapy. Clin Breast Cancer 2022;22:359-66.
15. Holick MF. The Vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Rev Endocr Metab Disord 2017;18:153-65.
16. Moradi A, Maroofi A, Hemati M, Hashemzade T, Alborzi N, Safari F. Inhibition of GTPase Rac1 expression by Vitamin D mitigates pressure overload-induced cardiac hypertrophy. Int J Cardiol Heart Vasc 2021;37:100922.
17. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. Hypertension 2001;38:399-403.
18. Danik JS, Manson JE. Vitamin D and cardiovascular disease. Curr Treat Options Cardiovasc Med 2012;14:414-24.
19. Safari F, Fazeli M, Dehghani M, Naghedi A, Jahanifar F, Safari F. 1, 25 dihydroxyvitamin D3 protects the heart against pressure overload-induced hypertrophy without affecting sirt1 MRNA level. IJML 2015;2:208-17.
20. Chen S, Gardner DG. Liganded Vitamin D receptor displays anti-hypertrophic activity in the murine heart. J Steroid Biochem Mol Biol 2013;136:150-5.
21. Mir SA, Chatterjee A, Mitra A, Pathak K, Mahata SK, Sarkar S. Inhibition of signal transducer and activator of transcription 3 (STAT3) attenuates interleukin-6 (IL-6)-induced collagen synthesis and resultant hypertrophy in rat heart. J Biol Chem 2012;287:2666-77.
22. Vázquez-Oliva G, Fernández-Real JM, Zamora A, Vilaseca M, Badimón L. Lowering of blood pressure leads to decreased circulating interleukin-6 in hypertensive subjects. J Hum Hypertens 2005;19:457-62.
23. Ancey C, Menet E, Corbi P, Fredj S, Garcia M, Rücker-Martin C, *et al.* Human cardiomyocyte hypertrophy induced *in vitro* by gp130 stimulation. Cardiovasc Res 2003;59:78-85.
24. McKeag NA, McKinley MC, Harbinson MT, McGinty A, Neville CE, Woodside JV, *et al.* Dietary micronutrient intake and micronutrient status in patients with chronic stable heart failure: An observational study. J Cardiovasc Nurs 2017;32:148-55.
25. Shedeed SA. Vitamin D supplementation in infants with chronic congestive heart failure. Pediatr Cardiol 2012;33:713-9.