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Amlodipine and atorvastatin improve ventricular hypertrophy and diastolic function via inhibiting TNF-α, IL-1β and NF-κB inflammatory cytokine networks in elderly spontaneously hypertensive rats

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ABSTRACT

This study aimed to examine the effects of amlodipine and atorvastatin alone or in combination on the regulation of inflammatory cytokines and the underlying mechanisms in elderly spontaneously hypertensive (SH) rats. The level of serum hs-CRP was detected with ELISA. The serum TNF-α and IL-1β levels were assessed by radioimmunity assay (RIA). Cardiac inflammatory cell infiltration was observed by HE staining. The protein levels of TNF-α, IL-1β, of NF-κB P65 and IκBα were detected by immunoblotting. The intracellular localization of NF-κB p65 was observed using immunohistochemistry. Amlodipine or atorvastatin obviously ameliorated the myocardial inflammatory cell infiltration in SH rats, which was further improved by combinatorial treatment with amlodipine and atorvastatin. Either amlodipine or atorvastatin decreased plasma IL-1β content in SH rats, but there was no significant difference when compared with untreated SH rats. However, the combination of amlodipine and atorvastatin significantly decreased plasma IL-1β level in SH rats. Moreover, amlodipine or atorvastatin intervention significantly reduced myocardial TNF-α and IL-1β protein levels in SH rats, which was further suppressed by the combination of amlodipine and atorvastatin. In addition, amlodipine or atorvastatin inhibited the activity of NF-κB signaling in SH rats, which was further suppressed by combinatorial treatment. Furthermore, amlodipine or atorvastatin restored the activity of IκB-α in SH rats, which was enhanced by combinatorial treatment. Our results demonstrated amlodipine and atorvastatin improved ventricular hypertrophy and diastolic function possibly through the intervention of TNF-α, IL-1β, NF-κB/IκB inflammatory cytokine network. Our study suggests that amlodipine combined with atorvastatin may have additive effect on inhibiting inflammatory response.

1. Introduction

Accumulating evidence suggests that inflammation and hypertension mutually enhance, and hypertension is a chronic low-level inflammation (low-grade inflammation), also known as subclinical inflammation [1,2]. Inflammatory response not only involves in the development of hypertension but also is an important risk factor for organ damages of hypertensive targets [3]. Similar to ischemic heart disease and immune inflammatory cardiomyopathy, during the process of left ventricular remodeling in hypertension, inflammation and myocardial fibrosis coexist in the same lesion sites, suggesting an inherent functional link between them. Previous studies from a variety of rat hypertension models have found that inflammatory cells such as macrophages, lymphocytes and fibroblasts coexist in myocardial fibrosis area, which are accompanied with enhanced expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and MCP-1 [4–8]. These facts suggest a critical role of inflammation in the pathologenesis of hypertension.

Cardiac cells include myocytes, cardiac fibroblasts, endothelial cells and smooth muscle cells. In response to mechanical stress or neurohormonal stimulation, cardiac cells and infiltrated interstitial macrophages and mast cells produce and release pro-inflammatory cytokines, including TNF-α and IL-1β [5–7,9]. TNF-α and IL-1β bind and activate their corresponding cell surface receptors to induce myocardial hypertrophy and initiate inflammatory response via activation of NF-κB/IκB signal pathway. NF-κB/IκB signaling not only plays an important role in myocardial

Abbreviations: TNF-α, tumor necrosis factor-alpha; IL-1β, Interleukin-1β; Hs-CRP, high sensitive C reactive protein; NF-κb, nuclear factor-kappa B; IκB, inhibitor-kappa B; MMP, matrix metalloproteinase; DEPC, diethypyocarbonate.

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hypertrophy [10], but also promotes inflammation by enhancing the transcription and translation of numerous inflammatory cytokines during immune and inflammatory responses [11]. Inflammatory cytokines have been shown to promote the hypertrophy, apoptosis and interstitial fibrosis of cardiac myocyte [12,13]. Further, pro-inflammatory cytokines TNF-α and IL-1β are both upstream regulators and downstream targets of NF-κB/IκB signal pathway, and form an inflammatory cytokines network that play an important role in the process of left ventricular remodeling and heart failure. Thus, targeting TNF-α/IL-1β-NF-κB-mediated inflammatory cytokine network is a promising strategy for the prevention and treatment of hypertension.

Amlodipine, a long-acting dihydropyridine calcium channel blocker (CCB), has been shown to have direct anti-inflammatory effects on the vascular system. Amlodipine can directly inhibit LPS-induced production of TNF-α and IL-1β and other proinflammatory cytokines by vascular smooth muscle cells [14]. Moreover, amlodipine inhibits the expression of MCP-1 and ICAM-1 in the atherosclerotic plaque of apoE gene knockout mice [15]. However, it remains unclear whether amlodipine can inhibit inflammatory response in myocardial tissues.

Statins, HMG-COA reductase inhibitors, are potent inhibitors of cholesterol biosynthesis. Statins have lipid-lowering as well as excellent anti-inflammatory effects via multiple mechanisms. Statins can reduce plasma CRP and ICAM levels and inhibit the expression of MCP-1 and IL-1β. Moreover, statins inhibits the activation of NF-κB signaling thereby reducing AngII-induced inflammatory responses. In addition, statins reduces the recruitment and infiltration of inflammatory cells including macrophages and T lymphocytes [16,17]. Accumulating evidence has shown that co-administration of amlodipine and statins has additive beneficial effects on the inhibition of atherosclerosis through inhibition of proinflammatory cytokine expression. In addition, in the ASCOT-LLA study, low-dose atorvastatin (10 mg/day) in combination with amlodipine was shown to significantly reduce stroke and some cardiac end points in patients with hypertension and average cholesterol levels. These results suggest a possible synergetic or additive beneficial effect of combined amlodipine and atorvastatin on advanced cardiac hypertrophy by suppressing inflammatory responses.

In the present study, to examine the effects of amlodipine and atorvastatin alone or in combination on the regulation of inflammatory cytokines and the underlying pharmacological mechanisms, we used 36-week-old spontaneously hypertensive (SH) rat as a model of hypertensive cardiac hypertrophy complicated with early-stage diastolic dysfunction.

2. Materials and methods

2.1. Reagents

Atorvastatin (brand name: Lipitor) and amlodipine (brand name: Norvasc) were from Pfizer and dissolved in distilled water; TNF-α and IL-1β Immunoassay kits were from PLA General Hospital Radiation Technology RIA Institute (Beijing, China); hs-CRP ELISA kit was from RapidBio System (Tucson, AZ, USA). Goat anti-TNF-α, rabbit anti-IL-1β, rabbit anti-NF-κB p65, and rabbit anti-IκB-α polyclonal antibodies were obtained from Santa Cruz Biothechnology (Santa Cruz, CA, USA). The animal experiments were approved by the Animal Ethical Committee of Hebei medical university.

2.2. Animals

Sixteen-week old male WKY rats (body weight 332 ± 18 g) were purchased from the Chinese Academy of Sciences Shanghai Experimental Animal Center (Certificate No. SCXK2003-0003). Sixteen-week old male spontaneously hypertensive rats (SH, body weight 325 ± 20 g) were purchased from Beijing Wei Tong Lihua Experimental Animal Center (Certificate No. SCXK2007-2001). WKY and SH rats were housed in a clean level environment with light/dark cycle of 12/12 h and relative humidity of 50%–60% at the ambient temperature (22–25 °C). Rats in 4–5 per cage were free to food and water.

2.3. Treatment of animals

Rats of 36-week old were randomly divided into the following 5 groups. WKY control group (n = 14) and SH control group (n = 18) were given the same volume of distilled water as treatment group; SH + amlodipine group (SH + AM, n = 16) were given 10 mg/kg/d amlodipine orally; SH + atorvastatin group (SH + AT, n = 14) were given 10 mg/kg/d atorvastatin orally; SH + amlodipine + atorvastatin group (SH + AM + AT, n = 18) were given 10 mg/kg/d amlodipine and 10 mg/kg/d atorvastatin orally. Rats were given drug daily and weighted once per week. The amount of drug was changed according to body weight. The selection of drug dose was based on previous studies [7,8] and our preliminary experiments. Briefly, we treated rats with 3 mg/kg/d or 10 mg/kg/d amlodipine, and found treatment with 3 mg/kg/d amlodipine did not result in apparaent antihypertensive effect and reduction in myocardial hypertrophy and improvement in cardiac function. In contrast, 10 mg/kg/d amlodipine effectively reduced blood pressure and improved cardiac hypertrophy and diastolic function. Previous studies demonstrated the plasma concentrations of statins in rats treated with 10 mg/kg/d had similar range of plasma concentrations of statins in human with human clinical dose (20mg–80 mg) [9,10]. Accordingly, in this study, we chose 10 mg/kg/d amlodipine and 10 mg/kg/d atorvastatin to treat rats.

2.4. Immunohistochemical analysis

Paraffin-fixed cardiac tissue sections of 6 μM were deparaffinized twice with xylol for 15 min, and rehydrated with graded alcohol. After blocking endogenous peroxidase with 3% methanol-hydrogen peroxide for 10 min, the slides were subjected to antigen retrieval for 5 min in a pressure cooker using sodium citrate buffer (pH 6.0), containing 0.1 M citric acid and 0.1 M sodium citrate in distilled water. After cooling to room temperature, sections were washed twice in PBS. Non-specific binding was blocked by incubating the sections with 10% normal rat serum. Then the slides were incubated with the primary antibodies for NF-κBp65 or IκB-α at 4 °C overnight. The next day, the sections were washed with PBS and incubated with secondary antibody for 30 min. After washing three times with PBS, the sections were visualized using the ABC substrate buffer for 5 min. Tissue sections were counterstained with hematoxylin, and dehydrated in an ascending series of ethanol (85–100%). After xylol treatment, sections were mounted. Positive expression in the nucleus or cytoplasm was stained brown or yellow. Quantitative analysis of target protein expression was assessed using the average value of integrated optical density.

2.5. Hematoxylin & eosin (HE) staining

HE staining of tissue sections was performed in routine histology lab. Using Image-Pro plus image analysis software (American Media cybernotis) was used for quantitative analysis of cardiomyocyte cross-sectional area. The shape factor of cardiomyocytes was counted according to the formula: shape factor = $4 \times 3.14 \times (\text{area}/\text{perimeter}^2)$. The mean area of cardiomyocytes