Atherosclerosis 237 (2014) 739–747

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Renin inhibition reduces atherosclerotic plaque neovessel formation and regresses advanced atherosclerotic plaques

Hongxian Wu [a], Xian Wu Cheng [a, b, d, *], Lina Hu [b], Chang-Ning Hao [a], Mutsuharu Hayashi [a], Kyosuke Takeshita [a], Mohammad Shoaib Hamrah [a], Guo-Ping Shi [c], Masafumi Kuzuya [b], Toyoaki Murohara [a, *]

[a] Department of Cardiology, Nagoya University Graduate School of Medicine, Nagoya, Japan
[b] Department of Community Healthcare & Geriatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan
[c] Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, United States
[d] Department of Cardiology, Yanbian University Hospital, Yanji, China

ARTICLE INFO

Article history:
Received 16 May 2014
Received in revised form
20 October 2014
Accepted 27 October 2014
Available online 30 October 2014

Keywords:
Aliskiren
Angiotensin II
Toll-like receptor 2
Cathepsin S
Atherosclerosis

ABSTRACT

Objective: The interaction between the renin-angiotensin system and toll-like receptors (TLRs) in the pathogenesis of advanced atherosclerotic plaques is not well understood. We studied the effects of the renin inhibitor aliskiren on the progression of advanced atherosclerotic plaque in apolipoprotein E-deficient (ApoE$^{-/-}$) mice with a special focus on plaque neovessel formation. **Methods and results**: Four-wk-old ApoE$^{-/-}$ mice were fed a high-fat diet for 8 wks, and the mice were randomly assigned to one of three groups and administered a vehicle, hydralazine, or aliskiren for an additional 12 wks. Aliskiren reduced the atherosclerotic plaque area and plaque neovessel density. It increased the plaque collagen and elastin contents, and reduced plasma angiotensin II levels and plaque macrophage infiltration and cathepsin S (CatS) protein. Aliskiren also decreased the levels of AT1R, gp91phox, TLR2, monocyte chemotactic protein-1, and CatS mRNAs in the aortic roots. Hydralazine had no beneficial vascular effects, although its administration resulted in the same degree of blood pressure reduction as aliskiren. CatS deficiency mimicked the aliskiren-mediated vasculoprotective effect in the ApoE$^{-/-}$ mice, but aliskiren showed no further benefits in ApoE$^{-/-}$ CatS$^{-/-}$ mice. *In vitro*, TLR2 silencing reduced CatS expression induced by angiotensin II. Moreover, aliskiren or the inhibition of CatS impaired the endothelial cell angiogenic action *in vitro* or/and *ex vivo*. **Conclusion**: Renin inhibition appears to inhibit advanced plaque neovessel formation in ApoE$^{-/-}$ mice and to decrease the vascular inflammatory action and extracellular matrix degradation, partly by reducing AT1R/TLR2-mediated CatS activation and activity, thus regressing advanced atherosclerosis.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Increasing evidence shows that the renin-angiotensin system (RAS) play a pivotal role in the pathogenesis of atherosclerosis-based cardiovascular disease by stimulating a series of coordinated cellular and molecular events in atherosclerotic lesions [1]. Angiotensin II (Ang II) is a major bioactive component of the RAS and has been demonstrated to be a crucial mediator for atherosclerotic lesion development by inducing the production of reactive oxygen species and stimulating the expression of adhesion molecules and chemokines through the activation of the angiotensin II type 1 receptor (AT1R), thereby leading to endothelial dysfunction, the accumulation of inflammatory cells, lipid deposition, and the proliferation of vascular smooth muscle cells (SMCs) [1]. Aliskiren, a direct renin inhibitor which reduces the formation of angiotensin I from angiotensinogen, suppresses Ang II biosynthesis at the first step of the RAS [2]. A few experimental studies have reported the anti-atherosclerotic effects of aliskiren at the early stage of atherosclerosis [2,3]. However, the vasculoprotective action of aliskiren's renin inhibition on advanced atherosclerosis and its underlying mechanisms are not yet understood.

Recent advances in immunologic studies suggest that the toll-like receptor (TLR) system plays an important role in the pathogenesis of atherosclerosis [4]. Human atherosclerotic arteries

* Corresponding authors. Department of Cardiology, Nagoya University Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8550, Japan.
E-mail addresses: xianwu@med.nagoya-u.ac.jp (X.W. Cheng), murohara@med.nagoya-u.ac.jp (T. Murohara).

http://dx.doi.org/10.1016/j.atherosclerosis.2014.10.098
0021-9150/© 2014 Elsevier Ireland Ltd. All rights reserved.

showed increased expressions of TLR-1, -2, and -4, compared to normal arteries [5]. The targeted deletion of TLR2 or TLR4 [6,7] or their downstream adaptor protein MyD88 [6] inhibited atherosclerotic lesion formation in apolipoprotein E-deficient ($ApoE^{-/-}$) mice. TLR2 has been shown to play an important role in monocyte activation and in stimulating the release of inflammatory chemokines and cytokines, which are crucial processes in the progression of atherogenesis [5]. Several lines of evidence indicate that RAS activation induces vascular inflammation through a TLRs-dependent signaling pathway [8]; however, the precise mechanisms of the RAS and the TLR signaling pathways in the progression of atherosclerosis remain unknown.

Neovascularization has been associated with advanced atherosclerotic plaque growth in diet-induced animal models [8,9]. Pharmacological inhibition of angiogenesis with endostatin and angiopoietin-2 overexpression has been reported to lead to a reduction of plaque neovascularization and growth [9]. Conversely, a number of experimental studies have shown that the stimulation of angiogenesis accelerates the progression of atherosclerotic plaque [10]. Angiogenic action stimulation has been closely linked to the Ang II/AT1R signaling pathway and protease activation (including matrix metalloproteinases (MMPs) and cysteinyl cathepsins) [8,11–13]. Cysteine protease cathepsins have also been implicated in the angiogenesis of pathophysiological conditions [14,15]. Several previous studies showed that cathepsin S (CatS) contributes to wound repair- or tumor growth-related angiogenesis [16,17]. However, the role of Ang II signaling in cathepsin activation-induced plaque neovessel formation and the mechanisms underlying the vasculoprotective action of the upstream inhibition of the RAS remain unclear, especially in atherogenesis at the advanced stage. To address these issues, we examined the effect of aliskiren-mediated RAS inhibition on the pathogenesis of advanced atherosclerosis in $ApoE^{-/-}$ mice, with a special focus on the plaque neovessel formation associated with TLR-dependent inflammation and cathepsin activation.

2. Materials and methods

2.1. Animals and treatment

All of the animal studies were conducted in accord with the animal care guidelines of the Nagoya University Graduate School of Medicine. Male $ApoE^{-/-}$ mice (C57BL/6 genetic background) were purchased from the Japan SLC (Hamamatsu, Japan). The animals were maintained in a 22 °C room with a 12-h light/dark cycle and received drinking water ad libitum. For the experiments, 4-wk-old male mice were fed a Western-type diet [18] containing 21.00% fat from lard and 0.15% cholesterol for 8 wks, and the mice were randomly assigned to one of three groups and administered vehicle (control, $n = 8$), hydralazine (25 mg mg/kg per day, in drinking water, Sigma–Aldrich; $n = 8$) or aliskiren (25 mg/kg per day via a subcutaneous [SC] mini-pump, Novartis, Basel, Switzerland; $n = 8$) for an additional 12 wks. In order to exclude the influence of mini-pump implantation, the control group and hydralazine group mice also underwent to a mini-pump implantation loaded with saline. Systolic blood pressure (BP) and heart rate (HR) were determined by using a tail-cuff pressure analysis system (Softron BP-98A, Tokyo) in conscious mice. Three reliable recordings were taken and used for the determination of systolic BP and HR.

In separate experiments, cathepsin S-deficient ($CatS^{-/-}$) mice (C57BL/6 background) were crossed with $ApoE^{-/-}$ mice (C57BL/6 background) to generate $ApoE^{-/-}$ $CatS^{-/-}$ mice. Male mice from both genetic backgrounds ($n = 6$ for each group) consumed an atherogenic Western-type diet [18] from 10 wks of age for 20 wks to allow the development of atherosclerotic lesions until sacrifice.

In another, separate experiment, aliskiren (25 mg/kg daily, by mini-pump; $n = 4$) or vehicle (saline; $n = 4$) was administered to $ApoE^{-/-}$ $CatS^{-/-}$ mice from 18 wks to 30 wks by supplementation of the Western-type diet (from the age of 10 wks).

2.2. Tissue collection and processing

Mice were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg), and blood was collected from the cardiac apex for analysis. For histological and immunohistochemical staining, the hearts with aortic roots were dissected and immersed in fixative for 24 h (4 °C) and embedded in paraffin. For the biological analysis, aortic roots were stored in liquid nitrogen and RNAlater® solution (Life Technologies, Frederick, MD).

The cross-sections of the aortic root were analyzed according to the modified method of Matsumoto et al. [19] with a small modification. Each heart was cut in a plane between the lower tips of the right and left atria. The upper portion was embedded in paraffin. The aortic root was then sectioned (4 μm) serially at 5-μm intervals from the appearance of the aortic valve to the ascending aorta until the valve cusps were no longer visible. Five cross-sections in each aortic tissue were quantified for neointima and media, and the results for each mouse were averaged.

2.3. Histological and immunohistochemical staining and morphometry

Paraffin sections (4 μm) from the aortic roots were deparaffinized in xylene, rehydrated in decreasing alcohol solutions, and stained routinely with hematoxylin-eosin (H&E) staining, Elastica van Gieson staining (EVG) for elastin, and Picrosirius Red (PSR) staining for collagen as described [18]. We used the corresponding sections on separate slides for the immunohistochemical staining against macrophages (Mac-3; 1:100, BD Pharmingen, San Diego, CA) and CD31 (1:100, BD Pharmingen), CatS (M-19) (1:50, Santa Cruz Biotechnology, Santa Cruz, CA), α-smooth muscle cell actin (ASMA; 1:100, Sigma–Aldrich), and against monocyte chemotactic protein-1 (MCP-1; 1:500, Novus Biologicals, Littleton, CO). The positive areas for each stain were analyzed with BZ8000 analysis software (Keyence, Tokyo, Japan) or MetaMorph imaging analysis software (Molecular Devices, Sunnyvale, CA). Five cross-sections of vessels in each aorta were quantified and averaged for each animal. We set a threshold to automatically compute the positive areas for each stain and then computed the ratio of the positive area to the intimal area.

2.4. Human umbilical vein endothelial cells (HUVECs) culture and stimulations

HUVECs were purchased from Clonetics (San Diego, CA) and cultured in endothelial basal medium (EBM)-2 (Lonza, Walkersville, MD) plus 10% fetal bovine serum (FBS) and endothelial growth medium (EGM)-2 SingleQuots™ (Clonetics) in a humidified atmosphere of 5% CO_2 and 95% air. After being cultured in serum-free EBM-2 for 24 h, the HUVECs were used for the following experiments. The cells were treated with and without Ang II (100 nmol/L, Sigma–Aldrich) for 12 h and then subjected to quantitative real-time polymerase chain reaction (PCR) for the examination of TLR gene expression. To explore the mechanism of Ang II-induced CatS expression, we pretreated HUVECs with or without several inhibitors, including the AT1R antagonist olmesartan (10 μmol/L), the NADPH oxidase inhibitor apocynin (100 μmol/L), and the phosphatidylinositol-3-kinase (PI3K) inhibitor LY294002 (10 μmol/L, Calbiochem, EMD Chemicals, San Diego, CA). We performed five independent experiments in triplicate for each cell culture assay.

Download English Version:

https://daneshyari.com/en/article/5945287

Download Persian Version:

https://daneshyari.com/article/5945287

Daneshyari.com