International Journal of Cardiology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Inhibition of interleukin-1 suppresses angiotensin II-induced aortic inflammation and aneurysm formation

Kikuo Isoda [a,*], Koji Akita [a], Kenichi Kitamura [a], Yayoi Sato-Okabayashi [a], Tomoyasu Kadoguchi [a], Sarasa Isobe [b], Fumie Ohtomo [a], Motoaki Sano [b], Kazunori Shimada [a], Yoichiro Iwakura [c], Hiroyuki Daida [a]

[a] Department of Cardiovascular Medicine, Juntendo University Graduate School of Medicine, Hongo, Bunkyo-ku, Tokyo, Japan
[b] Division of Cardiology, Keio University, Shinanomachi, Shinjuku-ku, Tokyo, Japan
[c] Research Institute for Biomedical Sciences, Tokyo University of Science, Noda, Chiba, Japan

ARTICLE INFO

Article history:
Received 1 May 2018
Received in revised form 15 May 2018
Accepted 21 May 2018
Available online xxxx

Keywords:
IL-1 receptor antagonist
Anti-IL-1β antibody
Inflammation
Angiotensin II
Hypertension
Aneurysm

ABSTRACT

Background: Angiotensin II (Ang II) activates components of the inflammatory cascade, which promotes hypertension and development of abdominal aortic aneurysm (AAA). This study aimed to elucidate the effects of an IL-1 receptor antagonist (IL-1Ra) and an anti-IL-1β antibody (01BSUR) on Ang II-induced AAA.
Methods and results: Male wild-type (WT) and IL-1Ra-deficient (IL-1Ra$^{-/-}$) mice were infused with Ang II (1000 ng/kg/min) using subcutaneous osmotic pumps for 28 days. Fourteen days post-infusion, both systolic blood pressure (SBP) (Ang II-treated IL-1Ra$^{-/-}$:149 ± 2 vs. Ang II-treated WT:126 ± 3 mm Hg, $p < 0.001$) and abdominal aortic width (0.94 ± 0.09 vs. 0.49 ± 0.03 mm, $p < 0.001$) were significantly higher in IL-1Ra$^{-/-}$ mice than in WT mice. Because 28-day infusion with Ang II in IL-1Ra$^{-/-}$ mice significantly increased the occurrence of fatal aortic rupture (89% vs. 6%, $p < 0.0001$), both types of mice were infused with Ang II for only 14 days, and histological analyses were performed at 28 days. Interestingly, AAA increased more significantly in IL-1Ra$^{-/-}$ mice than in WT mice ($p < 0.001$), although SBP did not differ at 28 days in IL-1Ra$^{-/-}$ and WT mice (117 ± 4 vs. 115 ± 3 mm Hg, $p = 0.71$ (after cessation of Ang II infusion)). Histological analyses showed numerous inflammatory cells around the abdominal aorta in IL-1Ra$^{-/-}$ mice, but not in WT mice. Finally, compared with IgG2a treatment, treatment with 01BSUR decreased Ang II-induced AAA in IL-1Ra$^{-/-}$ mice.
Conclusions: The present study demonstrates that inhibition of IL-1β significantly suppresses AAA formation after Ang II infusion, suggesting that suppression of IL-1β may provide an additional strategy to protect against AAA in hypertensive patients.

1. Introduction

Hypertensive patients with abdominal aortic aneurysm (AAA) are at increased risk of death [1]. AAA is associated with a poor quality of life and extensive health care costs. Therefore, identifying novel therapeutics that prevent AAA in hypertensive patients is crucial.

Hypertension (HT) triggers a sterile inflammatory reaction, and inflammation contributes to atherosclerosis and AAA [2]. Proinflammatory cytokines, such as interleukin (IL)-1β [3,4] and IL-6 [5,6], increase as HT becomes more severe. However, the precise mechanism by which inflammation leads to AAA is poorly understood.

Angiotensin II (Ang II), the main effector of the renin-angiotensin system, increases arterial pressure and activates components of the inflammatory cascade, which promotes HT, and vascular damage [7,8]. Both IL-1α and IL-1β are potent proinflammatory cytokines that act on endothelial cells and smooth muscle cells (SMCs) in vascular homeostasis [9]. IL-1α and IL-1β induce expression of surface leukocyte adhesion molecules in endothelial cells, proliferation of SMCs, and secretion of other cytokines and chemokines from endothelial cells, SMCs, and macrophages [9].

IL-1 receptor antagonist (IL-1Ra), which is produced by endothelial cells, SMCs and macrophages, negatively regulates the signaling of IL-1α and IL-1β, binding and blocking the functional receptor without activation [9]. The balance between IL-1 and IL-1Ra significantly affects host responses to inflammation. Thus, IL-1Ra plays an anti-inflammatory role in acute and chronic inflammation [10]. Because inflammation is a key component in the pathogenesis of cardiovascular disease, IL-1Ra may contribute to the modulation of Ang II-induced aortic inflammation.

The use of a pharmacological IL-1β inhibitor might be a possible method for management of AAA. The anti-mouse IL-1β antibody (01BSUR (Novartis)) is an IgG2a/k monoclonal antibody with a half-life of 14 days [11]. 01BSUR has a very high affinity for mouse IL-1β (dissociation constant (K_D) = 302 pM). 01BSUR is also effective against mouse IL-1β in vivo and can prevent the onset of disease in collagen-induced arthritis [12]. Given the efficacy of 01BSUR in the inflammation

* Corresponding author at: Department of Cardiovascular Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.
E-mail address: kisoda@juntendo.ac.jp (K. Isoda).

https://doi.org/10.1016/j.ijcard.2018.05.072
0167-5273/

models, we were interested in evaluating the effect of this antibody on AAA.

This study aimed to elucidate the effect of IL-1Ra on Ang II-induced AAA. Whether pharmacological interference with IL-1 signaling by administration of 01BSUR could reduce Ang II-induced AAA in a mice model was also investigated.

2. Materials and methods

Materials and methods are available in the Supplementary material.

3. Results

3.1. IL-1Ra deficiency promotes proinflammatory cytokines and matrix metalloproteinases production after Ang II stimulation

Real-time PCR of abdominal aorta in IL-1Ra-deficient (IL-1Ra$^{-/-}$) mice revealed significantly increased mRNA levels of IL-6 (43.7-fold, $p < 0.001$), TNF-α (5.8-fold, $p < 0.01$), and matrix metalloproteinase (MMP)-9 (55.8-fold, $p < 0.001$) compared with WT mice at 14 days after Ang II infusion (Supplemental Fig. 2A–C).

We performed zymography to confirm whether MMP-2 and MMP-9 were expressed in the aorta of either IL-1Ra$^{-/-}$ or WT mice. The amount of active MMP-2 increased by 2.3-fold ($p < 0.01$) in aortas from Ang II-treated IL-1Ra$^{-/-}$ mice compared with Ang II-treated WT mice. Active MMP-9 also increased in aortas from Ang II-treated IL-1Ra$^{-/-}$ mice (6.6-fold; $p < 0.001$) (Supplemental Fig. 2D–E). These findings indicate that IL-1Ra deficiency might promote MMP-2 and MMP-9 production after Ang II stimulation. The abdominal aortic width in IL-1Ra$^{-/-}$ mice increased more significantly compared with WT mice (0.944 ± 0.253 vs. 0.49 ± 0.08 mm, $p < 0.05$).

3.2. IL-1Ra deficiency promotes AAA formation after Ang II stimulation

Next, we tried to investigate prominent AAA formation in both mouse types at 28 days after Ang II infusion. However, Ang II infusion for 28 days into IL-1Ra$^{-/-}$ mice led to a significantly increased occurrence of fatal aortic rupture (89% vs. 6%, $p < 0.01$). Then, WT and IL-1Ra$^{-/-}$mice were infused with Ang II for only 14 days, and histological analyses were performed at 28 days after operation. We monitored blood pressure and heart rate (HR) in both mouse types during the 28-day infusion. At baseline, SBP was similar between groups (IL-1Ra$^{-/-}$ mice: 104 ± 2.5 ($n = 16$) vs. WT mice: 106 ± 1.9 mm Hg ($n = 16$)). In both IL-1Ra$^{-/-}$ and WT mice, SBP increased significantly by day 3, peaked by day 5, and remained continuously high through the end of the 14-day Ang II infusion (Fig. 1A left). We determined that genetic deletion of IL-1Ra in mice significantly increased Ang II-induced HT. In both IL-1Ra$^{-/-}$ and WT mice, SBP began to decrease by day 17, reached baseline by day 21, and continuously maintained baseline SBP through day 28 (Fig. 1A left). Furthermore, we found that genetic deletion of IL-1Ra significantly decreased HR following Ang II infusion (days 5–14) (Fig. 1A right).

Interestingly, although the SBP and HR of IL-1Ra$^{-/-}$ ($n = 8$) and WT ($n = 8$) mice did not differ at 28 days after operation (117 ± 4 vs. 115 ± 3 mm Hg, $p = 0.71$; 601 ± 15 vs. 635 ± 16 bpm, $p = 0.75$) (Fig. 1A), the abdominal aortic width in IL-1Ra$^{-/-}$ mice increased more significantly compared with WT mice (1.47 ± 0.14 vs. 0.59 ± 0.02 mm, $p < 0.001$) (Fig. 1B). Macropathological analysis showed infrarenal AAA in IL-1Ra$^{-/-}$ mice, but not in WT mice (Fig. 1C). The micro-enhanced CT also demonstrated infrarenal AAA without dissection in IL-1Ra$^{-/-}$ mice (Fig. 1C). On the other hands, the abdominal aortic width was similar between groups with no Ang II treatment 28 days after operation (IL-1Ra$^{-/-}$ mice: 0.42 ± 0.01 ($n = 8$) vs. WT mice: 0.43 ± 0.02 mm ($n = 8$)).

3.3. IL-1Ra deficiency promotes aortic inflammation after Ang II stimulation

At baseline, analysis of aortas from IL-1Ra$^{-/-}$ mice indicated that they appeared to be identical to those of WT mice, as determined by histological studies (not shown). We next investigated the aortas from both mouse types 14 days after operation. Histological analyses revealed there were numerous granulocytes (Supplemental Fig. 3A, B (right), and H), (but not macrophages (Supplemental Fig. 3B (left)), around the abdominal aorta in IL-1Ra$^{-/-}$ mice, but not in WT mice (Supplemental Fig. 3D–E). We also detected IL-6 protein expression in the intima, SMCs and inflammatory cells in both mice groups (Supplemental Fig. 4A). However, the percent positive area of IL-6 was much larger in IL-1Ra$^{-/-}$ mice than in WT mice (Supplemental Fig. 4B). Furthermore, we observed TNF-α protein expression in the intima, and inflammatory cells found in the adventitia, and quantitative analysis also revealed TNF-α protein expression increased significantly in IL-1Ra$^{-/-}$ mice compared to WT mice 14 days after Ang II infusion (Supplemental Fig. 4A and C).

At 28 days after operation, there were numerous inflammatory cells (Fig. 2A), that consisted of both macrophages (Fig. 2B (upper left) and E (left)) and granulocytes (Fig. 2B (upper right) and E (middle)) around the abdominal aorta in IL-1Ra$^{-/-}$ mice, but not in WT mice (Fig. 2C–E). Furthermore, we detected destruction of the elastic lamina (Fig. 2A) and decreased SMCs in the abdominal aorta of IL-1Ra$^{-/-}$ mice (Fig. 2B (lower panels) and E (right)), but not in WT mice (Fig. 2C and E (right)). These findings suggest that IL-1Ra deficiency promotes Ang II-induced aneurysmal formation by continuing aortic chronic inflammation after the cessation of Ang II infusion, because we detected many macrophages around the abdominal aorta in IL-1Ra$^{-/-}$ mice, but not in WT mice.

3.4. Treatment with an anti-IL-1β antibody suppressed Ang II-induced HT and aortic dilatation

01BSUR treatment decreased Ang II-induced HT (Fig. 3A) at 14 days, and aortic width (Fig. 3C) at 28 days in IL-1Ra$^{-/-}$ mice compared with IgG2a treatment.

Interestingly, 01BSUR also decreased Ang II-induced HT in WT mice compared with IgG2a treatment (Fig. 3B). Because the abdominal aortic width in Ang II-treated WT mice did not increase compared with control WT mice, 01BSUR treatment did not significantly change aortic width at 28 days in WT mice compared with IgG2a-treated WT mice (Fig. 3C).

3.5. Treatment with an anti-IL-1β antibody suppressed Ang II-induced aortic inflammation

Real-time PCR of the abdominal aorta in IL-1Ra$^{-/-}$ mice showed that treatment with 01BSUR abolished the significant differences in mRNA levels of IL-6, TNF-α, and MMP-9 compared with IgG2a-treated WT mice at 14 days after Ang II infusion (data not shown).

Histological analyses revealed that 01BSUR treatment decreased inflammatory cells around the aorta (Fig. 4C–E) compared with IgG2a treatment (Fig. 4A–B and E) in IL-1Ra$^{-/-}$ mice at 28 days after operation. 01BSUR treatment also prevented destruction of the elastic lamina (Fig. 4C) and degeneration of SMCs in the abdominal aorta of IL-1Ra$^{-/-}$ mice (Fig. 4D (lower panels) and E (right)) compared with control IgG2a (Fig. 4A–B (lower panels) and E (right)). These findings suggest that 01BSUR suppresses Ang II-induced aortic inflammation and aneurysm development.

4. Discussion

The current study assessed the effect of inhibition of IL-1signaling on AAA in an Ang II-induced HT mouse model. A previous report demonstrated that low dose Ang II (400 ng/kg/min) could induce alterations of small resistance arteries in structure, mechanics, and extracellular

Please cite this article as: K. Isoda, et al., Inhibition of interleukin-1 suppresses angiotensin II-induced aortic inflammation and aneurysm formation, Int J Cardiol (2017), https://doi.org/10.1016/j.ijcard.2018.05.072