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# Molecular mechanisms of irbesartan suppressing atherosclerosis in high cholesterol-diet apolipoprotein E knock-out mice

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**Abstract**

*Objective:* Atherosclerosis is a chronic inflammatory disease in which the renin–angiotensin–aldosterone system plays an important role. Evidence indicate that the angiotensin type 1 receptor blockers can suppress atherogenesis, but the exact mechanisms have not been fully elucidated. The study was undertaken to investigate the potential effects and molecular mechanisms of an angiotensin type 1 receptor blocker irbesartan on atherogenesis in high cholesterol-diet apolipoprotein E knock-out mice.

*Methods and results:* Adult male apolipoprotein E knock-out mice were given normal diet or high cholesterol-diet and randomized to receive no treatment or irbesartan 10 mg $kg^{-1}$ $d^{-1}$ for 12 weeks. The apolipoprotein E knock-out mice with high cholesterol-diet were associated with a marked increase in atherosclerotic lesion area, plasma lipid and angiotensin II levels, as well as the expressions of angiotensin type 1 receptor in the aorta. High cholesterol-diet feeding increases the activity of NADPH oxidase subunits ($p47^{phox}$ and Rac), extracellular signal-regulated kinase 1/2, janus kinase 2, signal transducer and activator of transcription 3, nuclear factor-κB and the expression of tumor necrosis factor-α, interleukin 6, monocyte chemoattactant protein-1 and vascular cell adhesion molecule-1 in the aortas. These changes were suppressed in mice that were treated with irbesartan 10 mg $kg^{-1}$ $d^{-1}$, with no significant change in systolic blood pressure and plasma lipid levels.

*Conclusions:* The results suggest that irbesartan can attenuate atherosclerosis, and this effect is partly related to the inhibition of oxidative stress and inflammatory signal transduction pathways which eventually leads to the decrease in the expression of inflammatory cytokines.


*Keywords:* Atherosclerosis; Hypercholesterolemia; Angiotensin type 1 receptor blocker; Oxidative stress; Inflammation

## 1. Introduction

Atherosclerosis is a chronic inflammatory disease and many factors take part in its process [1]. Evidence indicates that renin–angiotensin system (RAS) plays an important role in the initiation and amplification of atherosclerosis [2]. Angiotensin II (Ang II), which is the main effector hormone of the RAS, directly causes cellular phenotypic changes and cell growth, regulates the gene expression of various kinds of bioactive substances, and activates multiple signal transduction cascades in heart and kidney [3]. Ang II is involved in the physiopathologic process of atherosclerosis through various processes such as endothelial dysfunction, cellular proliferation and inflammation. To a certain extent, Ang II is a pro-inflammatory mediator in nature [4].

Ang II type-1 receptor ($AT_1R$), a G-protein-coupled receptor, mediates most of the physiological and pathophysiological functions of Ang II [5,6]. Acting via $AT_1R$, Ang II enhances the production of reactive oxygen species (ROS)

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through stimulation of nicotinamide-adenine dinucleotide phosphate [NAD(P)H] oxidase in the vascular wall. Increased oxidative stress contributes to endothelial dysfunction and vascular inflammation by stimulating signal transduction pathways such as mitogen-activated protein kinase (MAPK), janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear factor-κB (NF-κB), which upregulate adhesion molecules, cytokines and chemokines [7,8]. There is evidence that $AT_1R$ blockers (ARBs) decrease the atherosclerotic lesion area and the expression of inflammatory cytokines [9–12]. Furthermore, some clinical trials indicated that irbesartan could improve endothelial function [13], inhibit the intravascular oxidative state [14] and suppress the expression of inflammatory markers [15], while the exact molecular mechanism has not been fully elucidated. Therefore, in the present study, we used apolipoprotein E knock-out (ApoE KO) mice to establish atherosclerotic model and explored the potential molecular mechanism of irbesartan on the progression of atherogenesis.

## 2. Materials and methods

ApoE KO mice on a C57BL/6J background, purchased from Jackson Laboratory (Bar Harbor, Maine, USA), were bred and maintained in the Animal Center of Beijing University. This investigation was performed according to the principles of the Experimental Animal Ethics Committee. All animal studies were reviewed and approved by the Animal Study Committee of Tongji Medical College of Huazhong University of Science and Technology.

### 2.1. Animals and treatment

Six-week-old homozygous ApoE KO male mice with a body weight ranging from 18 to 22 g were used in this study. The animals were kept in a room where lighting was controlled (12 h on, 12 h off), and the temperature was kept at 25 °C. After being well-adapted to this environment for a week, the mice were divided into three groups at random: (1) normal-diet group (ND, $n=15$): received a standard diet (mouse food, Oriental Yeast) for 12 weeks; (2) high-cholesterol diet group (HCD, $n=15$): received a high-cholesterol diet (1.25% cholesterol, 10% coconut oil in mouse food) [16] for 12 weeks; (3) high-cholesterol diet plus irbesartan (Sanofi-Synthelabo SA) group (HCD+Irb, $n=15$): received a high-cholesterol diet and irbesartan 10 mg $kg^{-1}$ $d^{-1}$ by gavage for 12 weeks. The water was freely available.

### 2.2. Measurement of blood pressure, lipid level and Ang II

Systolic blood pressure was measured by a kind of non-invasive tail cuff system in conscious mice (EHSY ZH-HX-Z). The plasma total cholesterol and triglyceride concentration were measured by autoanalyzer (Hitachi 917), and plasma concentration of Ang II was measured by radioimmunoassay.

### 2.3. Tissue preparation and atherosclerosis lesions evaluation

To evaluate the atherosclerotic lesions, 3 approaches were used: en face whole, paraffin and frozen histological section analysis. The mice were euthanized at 19 weeks of age; the heart and aorta were removed rapidly after perfusion with PBS. The thoracic and abdominal aorta was quick-frozen in nitrogen for later extraction of protein and RNA. The heart, including the aortic root, was snap-frozen in OCT compound for cryostat section [17]. For en face whole, the whole aorta was dissected free from surrounding tissues, opened longitudinally, and fixed in 4% formaldehyde for 2 h. Then the vessel was rinsed, stained with oil red O solution for 1 h, rinsed again, and pinned to a wax surface. The whole vessel images were collected under the Nikon microscope (NIKON TE2000-U). For morphometric analysis, paraffin sections were used. Hematoxylin staining was performed according to standard protocols. For quantification of the lesion area, approximately 100 serial cross sections (10 μm thick) of the aortic root were prepared according to the method described by Paigen et al. [18] with a slight modification. In brief, atherosclerotic lesions in aortic sinus region were examined at 5 locations, each separated by 120 μm, with the most proximal site starting where the 3 aortic valves first appear. Five serial sections prepared from each location were conventionally stained with oil

Table 1
Changes in plasma lipids, Ang II, $AT_1R$ mRNA of the aorta and blood pressure in high cholesterol-diet apolipoprotein E knock-out mice after treated by irbesartan.

| | ND | HCD | HCD+Irb |
|---|---|---|---|
| Total cholesterol (mg/dl) | 551.35±54.83 | 1650.19±228.19* | 1571.43±206.95* # |
| Triglyceride (mg/dl) | 94.70±3.54 | 184.08±32.75* | 173.46±23.9* # |
| Angiotensin II (pg/ml) | 182.4±17.53 | 285.64±26.07* | 196.27±18.77* # |
| $AT_1R$ mRNA (arbitrary unit) | 1.00±0.09 | 1.87±0.13* | 1.04±0.11* # |
| SBP (mm Hg) | 96.8±1.6 | 98.4±2.2# | 96.3±1.5# |

SBP, systolic blood pressure; Irb, irbesartan 10 mg $kg^{-1}$ $d^{-1}$. Values are the mean±SEM, $n=15$ for each group. *$P<0.01$ vs. the ND group, #$P>0.05$ vs. the HCD group.

red O. The quantification of lesion areas was performed by a single observer blinded to the experimental protocol. All images were captured and analyzed by computer image analysis software (HMIAS 2000 W). The average value for the 5 locations for each animal was used for analysis [19].

### 2.4. Real-time reverse-transcription polymerase chain reaction

Total RNA was harvested from mouse aortas. After reverse transcription reaction, real-time reverse-transcription polymerase chain reaction (PCR) was performed with a

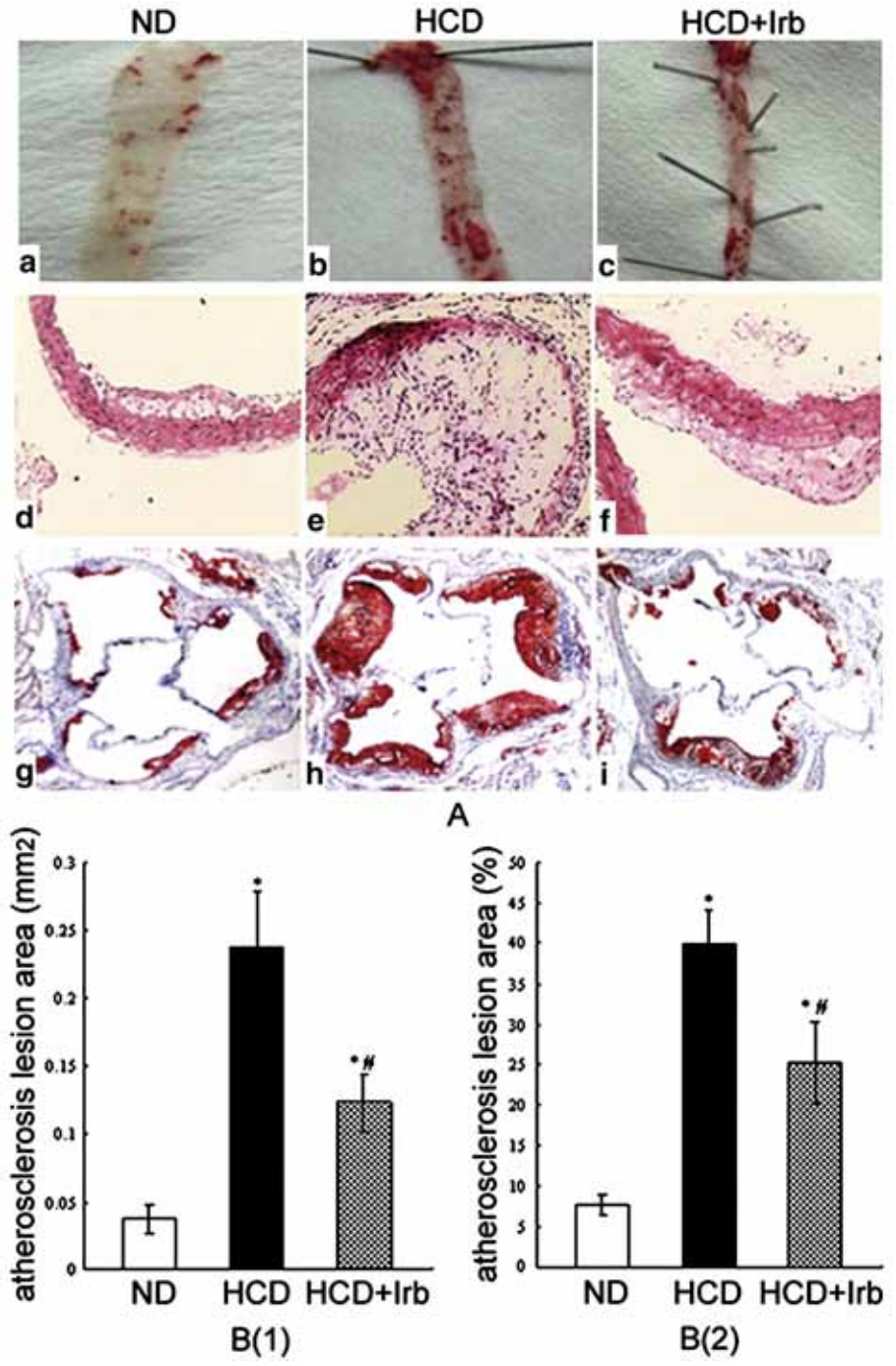



Fig. 1. A. Atherosclerotic lesion in ApoE KO mice. a→c, The whole aorta stained with oil red O. d→f, Cross-sectional section stained with hematoxylin–eosin. Magnification ×100. g→i: Cryostat section of aortic root stained with oil red O. Magnification ×40. a, d, g: ND group; b, e, h: HCD group; c, f, i: HCD+Irb group. B. Atherosclerotic lesion area in aortic root [B (1)] and the percentage of the lesion area/lumina [B (2)]. Each bar represents mean±SEM ($n$=8 per group). Irb, irbesartan 10 mg $kg^{-1}$ $d^{-1}$. *$P$<0.01 vs. the ND group; $^{\#}P$<0.01 vs. the HCD group.

SYBR Green I kit (Biotium, USA). PCR primers are as follows:

$AT_1R$:
forward, 5′-TGAGACCAACTCAACCCAGA-3′;
reverse, 5′-GCATTACATTGCCAGTGTG-3′.
$p47^{phox}$:
forward, 5′-GCCAGATGAAGACAAAGCGAGG-3′;
reverse, 5′-GCAGATACATGGATGGGAAATAGC-3′.
Rac-1:
forward, 5′-CCGTCTTTGACAACTATTCTGCC-3′;
reverse, 5′-TCGC-ACTTCAGGATACCACTTTG-3′.
tumor necrosis factor-α (TNF-α):
forward, 5′-TACTGAACTTCGGGGTGATCGGTC 3′;
reverse, 5′-CAGCCTTGTCCCTTGAAGAGAACC 3′.
interleukin 6 (IL-6):
forward, 5′-AGAGCAATACTGAAACCCTA-3′;
reverse, 5′-CAACATAATTTAGATACCCATC-3′.
monocyte chemoattactant protein-1 (MCP-1):
forward, 5′-CTCACCTGCTGCTACTCAT TCAC-3′;
reverse, 5′-GATTTACGGGTCAACTTCACATTC-3′.
vascular cell adhesion molecule-1 (VCAM-1):
forward, 5′-TGAACCCAAACAGAGGCAGAG-3′;
reverse, 5′-GGTATCCCATCACTTGAG-CAG-3′.
β-actin:
forward, 5′-TGCTGTCCCTGTATGCCTCTG-3′;
reverse, 5′-TTGATGTCACGCACGATTTCCC-3′.

### 2.5. Western blot analysis

Total proteins, membrane extracts, cytoplasmic extracts and nuclear extracts were prepared from pooled arteries (six arteries for each group) [20–22]. The concentrations of proteins were determined by the BCA protein assay (Pierce). Protein samples (20–50 μg) were separated on denaturing 7.5%, 10% or 15% SDS-PAGE, and electro-transferred onto nitrocellulose membranes. After being blocked with 5% nonfat dry milk in TBS with 0.05% Tween 20 (TBS-T) for 1 h at room temperature, membranes were washed three times for 5 min in TBS-T, then incubated at 4 °C overnight with primary antibodies in their blocking solution. These primary antibodies include anti-phospho-$p47^{phox}$, anti-Rac (1:2000, Upstate cell signaling solutions); anti-extracellular signal-regulated kinase 1/2 (ERK1/2), anti-phospho-ERK1/2, anti-JAK2, anti-phospho-JAK2, anti-STAT3, anti-phospho-STAT3 (1:1000, Cell Signal Technology), anti-NF-κB p50, anti-NF-κB p65, anti-inhibitors of κB (IκB), anti-phospho-IκB (p-IκB), anti-TNF-α, anti-IL-6 (1:1000, Santa Cruz Biotechnology), anti-VCAM-1 (1:1000, R&D systems), anti-MCP-1, anti-β-actin (1:1000, Abcam) and anti-histone H3 (1:1000, BioLegend). Membranes were washed three times in TBS-T for 10 min and incubated with appropriate horseradish peroxidase-conjugated secondary antibodies in TBS for 1 h. After another three washes with TBS-T for 10 min, membranes were reacted with the enhanced chemiluminescence system (Pierce) and exposed to films [23,24]. Protein levels were quantified by scanning densitometry using image-analysis systems.

### 2.6. Statistical analysis

Values are expressed as mean±SEM in the text and figures. The data were analyzed by ANOVA. If significance was found, Newman–Keuls test was performed for post-hoc analysis to detect the difference between groups. A probability value of $P<0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. Systolic blood pressure, plasma lipids and Ang II, $AT_1R$ mRNA expression in the aorta

After 12 weeks of the HCD feeding, the plasma total cholesterol and triglyceride levels as well as plasma Ang II and the $AT_1R$ mRNA expression in aortas were markedly increased ($P<0.01$ vs. the ND group, Table 1). Irbesartan 10 mg $kg^{-1}$ $d^{-1}$ had no apparent influence on the systolic blood pressure, plasma total cholesterol and triglyceride levels, but can decrease the plasma Ang II and the $AT_1R$ mRNA expression in the aorta (Table 1).

### 3.2. Effect of irbesartan on atherosclerotic lesion area

The atherosclerotic lesion in the aorta sinus was measured in this study. As shown in Fig. 1, after 12 weeks of HCD feeding, ApoE KO mice displayed a marked increase in atherosclerotic lesion formation in the aorta sinus. The lesion area was significantly reduced by treatment with irbesartan at 10 mg $kg^{-1}$ $d^{-1}$ ($P<0.01$ vs. the HCD group, Fig. 1).

### 3.3. Effect of irbesartan on vascular oxidative stress

Oxidative stress is involved in the mechanism of atherosclerotic lesion formation. Reducing oxidative stress may be a reasonable therapeutic approach to treat cardiovascular diseases. So we measured the expression and activity of the NAD(P)H oxidase subunits in the aorta. Real-time PCR using aortic samples revealed that after a 12-week HCD feeding, the mRNA expressions of the NAD(P)H oxidase subunits $p47^{phox}$ and Rac-1 were highly increased. Irbesartan 10 mg $kg^{-1}$ $d^{-1}$ treatment markedly attenuated this change ($P<0.01$ vs. the HCD group, Fig. 2A). Western blot using pooled aortic samples revealed that Rac-GTP and $p47^{phox}$ in membrane extract were highly increased in HCD group, while irbesartan 10 mg $kg^{-1}$ $d^{-1}$ significantly decreased this change ($P<0.01$ vs. the HCD group, Fig. 2B).

### 3.4. Effect of irbesartan on signal transduction proteins

To examine the signaling mechanism, we focused on the ERK1/2, JAK2, STAT3 and NF-κB activity. ERK1/2 and

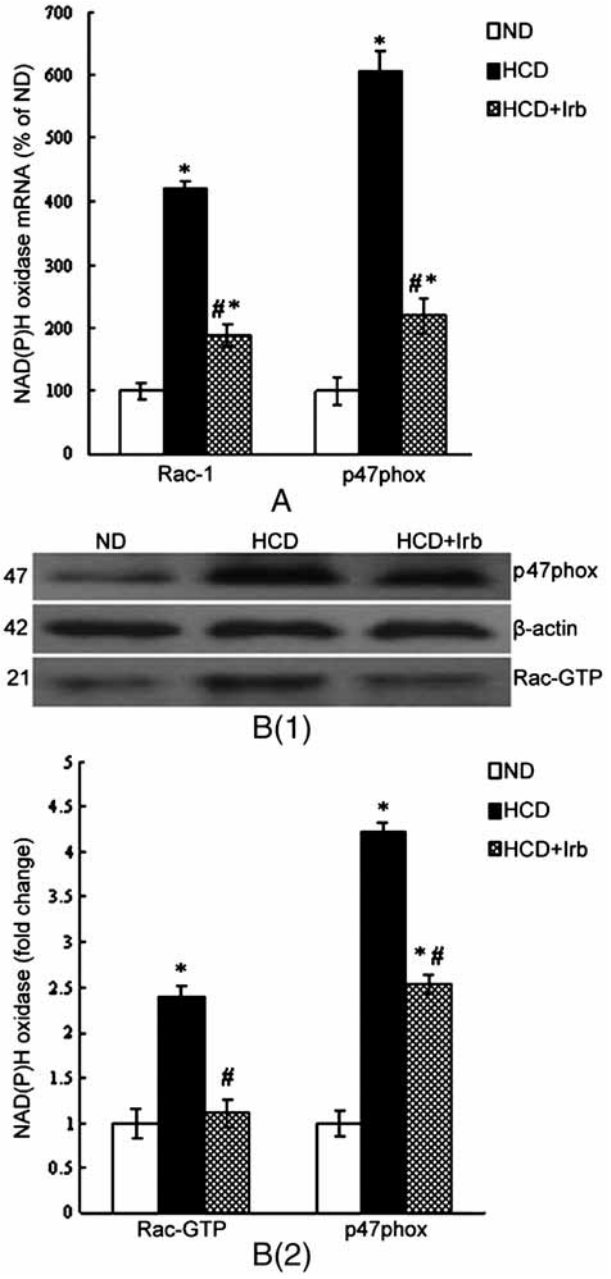



Fig. 2. Expression of $p47^{phox}$ and Rac in the atherosclerotic aorta of ApoE KO mice. A: Levels of mRNA for $p47^{phox}$ and Rac were assayed by quantitative real-time reverse-transcription polymerase chain reaction. Each bar represents mean±SEM ($n$=6 per group). B: The protein levels of $p47^{phox}$ in membrane extract and Rac-GTP were measured by Western blots. B (1): The representative results of Western blots of $p47^{phox}$ and Rac-GTP are shown. B (2): Densitometric measurements of $p47^{phox}$ and Rac-GTP from Western blots. Each bar represents mean±SEM ($n$=3). Irb, irbesartan 10 mg $kg^{-1}$ $d^{-1}$. *$P$<0.01 vs. the ND group; #$P$<0.01 vs. the HCD group.

JAK2/STAT3 signal transduction pathways play important roles in the process of atherosclerosis. In this study, HCD feeding for 12 weeks increased the phosphorylation of ERK1/2, JAK2 and STAT3 without changes in total protein levels of these proteins, whereas irbesartan significantly inhibited activation of ERK1/2, JAK2 and STAT3 (Fig. 3A and B). To assess the effects of irbesartan on the key regulator of atherosclerosis, we measured the expression of NF-κB p65 and p50 in nuclear and cytoplasmic extracts of aortas independently. As shown in Fig. 3, the activation of NF-κB was determined as its expression in nuclear extracts. Irbesartan decreased the expression of NF-κB p65, p50 in nuclear extracts ($P$<0.01 vs. the HCD group, Fig. 3C and D). As we all know, at the cellular level, NF-κB is activated through phosphorylation of I-κB. Phosphorylated I-κB is released from NF-κB/I-κB complex, allowing the translocation of NF-κB molecules into the nucleus. So we also measured the phosphorylation of IκB in total proteins of aortas, and the results showed that irbesartan decreased the phosphorylation of IκB either ($P$<0.01 vs. the HCD group, Fig. 3E).

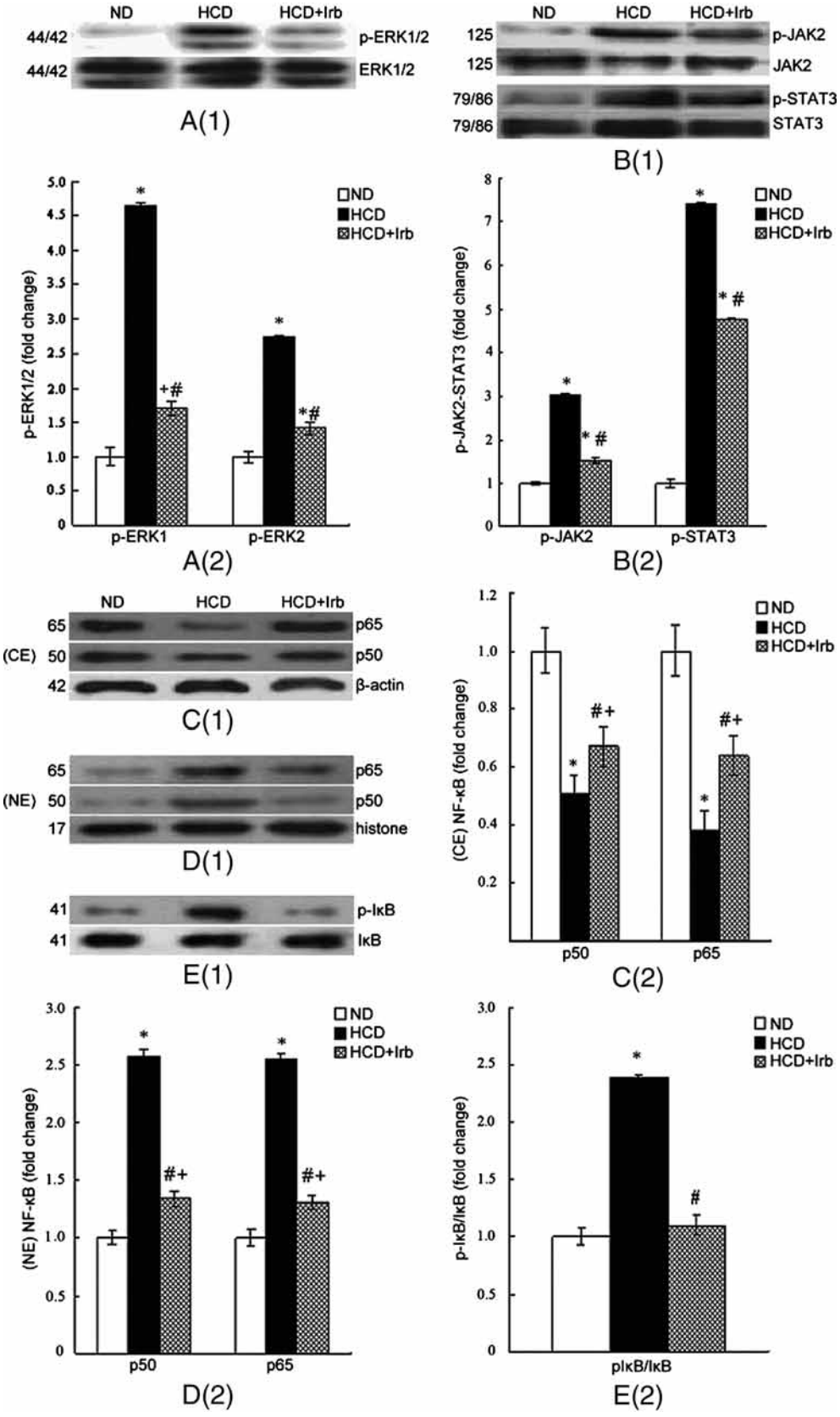
### 3.5. Effect of irbesartan on inflammatory response

Fig. 4A showed the results that the mRNA expressions of TNF-α, IL-6, MCP-1 and VCAM-1 were highly increased by HCD treatment. Administration of irbesartan for 12 weeks inhibited these changes ($P$<0.01 vs. the HCD group, Fig. 4A). In addition, Western blot also demonstrated an increase in TNF-α, IL-6, MCP-1, and VCAM-1 protein expressions of HCD group, which was significantly reduced by treatment of irbesartan ($P$<0.01 vs. the HCD group, Fig. 4B).

## 4. Discussion

In the present study, we demonstrated that irbesartan 10 mg $kg^{-1}$ $d^{-1}$ treatment obviously attenuated oxidative stress and inflammatory signal transduction pathways which led to the decrease in expression of cytokines and reduction of subsequent atherosclerotic plaque formation in ApoE KO mice, and did not apparently influence the blood pressure and plasma lipid levels. These results suggest that irbesartan actually has anti-atherosclerotic effects.

The development of atherosclerosis plaque is a highly regulated and complex process. The epidemiological and experimental studies have indicated that hypercholesterolemia, hypertension, hyperglycemia, hyperhomocysteinemia as well as life style factors are the major risk factors associated with the development of atherosclerosis [25,26]. Recent studies have shown that hypercholesterolemia was associated with increased systemic angiotensinogen, angiotensin peptides, and enhanced sensitivity of the vessel wall to Ang II stimulation, and furthermore, low density lipoprotein (LDL) could markedly augment $AT_1R$ mRNA and protein expression [5,27,28]. Additionally, clinical trials with $AT_1R$ blockers VALIANT (Valsartan in Acute Myocardial infarction) [29], LIFE (Losartan Intervention For Endpoint reduction in hypertension study) [30] and CHARM (Candesartan in Heart Failure Assessment of Reduction in Mortality and morbidity in patients with chronic heart failure) [31]


ND
HCD
HCD+Irb
44/42
p-ERK1/2
44/42
ERK1/2
A(1)
125
p-JAK2
125
JAK2
79/86
p-STAT3
79/86
STAT3
B(1)
p-ERK1/2 (fold change)
p-ERK1
p-ERK2
A(2)
p-JAK2-STAT3 (fold change)
p-JAK2
p-STAT3
B(2)
65
p65
(CE) 50
p50
42
β-actin
C(1)
(NE) 50
histone
17
D(1)
41
p-IκB
IκB
E(1)
(CE) NF-κB (fold change)
C(2)
(NE) NF-κB (fold change)
D(2)
p-IκB/IκB (fold change)
pIκB/IκB
E(2)

have demonstrated that ARBs prevent different risk factors associated with the development of atherosclerosis and decrease the occurrence of cardiovascular events.

Though current studies have already demonstrated that some ARBs decreased oxidative stress [32,33], improved endothelial function [13], inhibited cell proliferation and activated lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) [34], little is known about the exact effect and mechanism of irbesartan on atherosclerosis. In order to further understand the mechanism of irbesartan in reducing the atherosclerotic lesion area, we investigated the signal transduction pathways related to Ang II, and the possible pathways are shown in Fig. 5.

1. Effects of irbesartan on oxidative stress: Ang II is a predominant modulator of the NAD(P)H oxidase, and NAD(P)H oxidase has been reported as a major enzyme in oxidative stress which induces the production of ROS [35]. NAD(P)H oxidase mainly consists of membrane components (nox1, nox4, $p22^{phox}$), cytoplasmic components ($p47^{phox}$, $p67^{phox}$) and the low molecular weight G protein Rac (Rac-1 or Rac-2) [36]. It has been reported that $p47^{phox}$ and Rac play important roles in the activation of NAD(P)H oxidase [22,37]. In ApoE KO mice, hypercholesterolemia activates NAD(P)H oxidase to produce ROS by enhancing the expression of $AT_1R$ [38]. ROS stimulates a series of signal transduction pathways to produce generous cytokines which not only participate in the inflammatory response of vascular wall, but also act as stimulators to activate more signal transduction proteins and produce more inflammatory cytokines conversely. Thus the interaction forms a complex signal transduction network and accelerates the development of atherosclerosis. In the present study, we examined the mRNA expression of $p47^{phox}$ and Rac, protein expression of $p47^{phox}$ in membrane extract and Rac-GTP in the aorta. Consistent with the results of the atherosclerotic lesion size, irbesartan markedly attenuated the expression and activation of $p47^{phox}$ and Rac. This confirmed that irbesartan could decrease the activity of oxidative stress mediated signal transduction via inhibition of $AT_1R$.
2. Effects of irbesartan on the signal transduction proteins: In addition to inducing G protein- and non-G protein-related signaling pathways, Ang II, via $AT_1R$, carries out its functions via MAP kinases, receptor tyrosine kinases and nonreceptor tyrosine kinases. MAPK/ERK, JAK2/STAT3 and NF-κB signal pathways play critical roles in the process of atherosclerosis. For example, MAPK/ERK signal

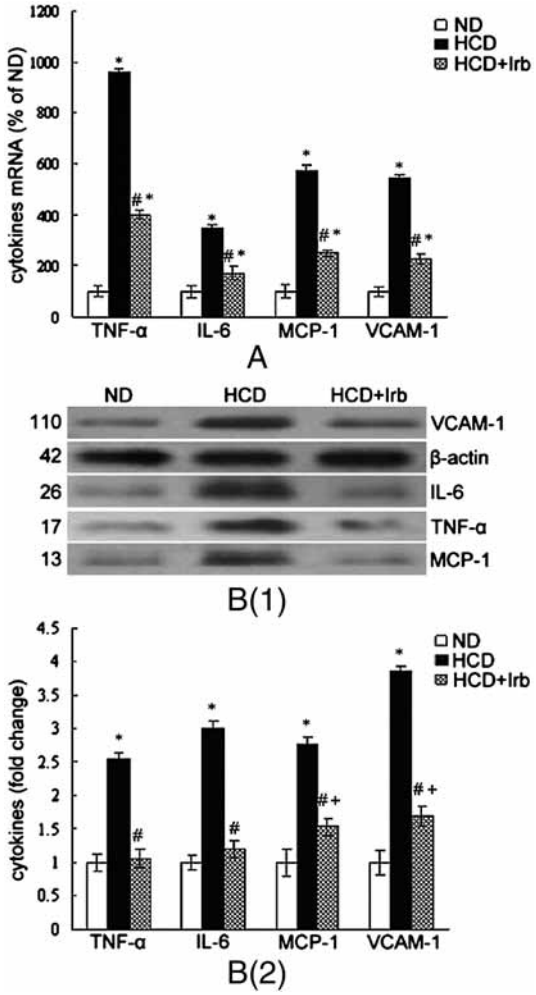



Fig. 4. Expression of TNF-α, IL-6, MCP-1 and VCAM-1 in the atherosclerotic aorta of ApoE KO mice. A: Levels of mRNA for TNF-α, IL-6, MCP-1 and VCAM-1 were assayed by quantitative real-time reverse-transcription polymerase chain reaction. Each bar represents mean ± SEM ($n=6$ per group). B: The protein levels of TNF-α, IL-6, MCP-1 and VCAM-1 were measured by Western blots. B (1): The representative results of Western blots of TNF-α, IL-6, MCP-1 and VCAM-1 are shown. B (2): Densitometric measurements of TNF-α, IL-6, MCP-1 and VCAM-1 from Western blots. Each bar represents mean ± SEM ($n=3$). Irb, irbesartan 10 mg $kg^{-1}$ $d^{-1}$. $^{*}P<0.01$ vs. the ND group; $^{\#}P<0.01$ vs. the HCD group; $^{+}P<0.05$ vs. the ND group.

Fig. 3. Effect of irbesartan on the signal transduction proteins. A: Activation of ERK1/2 in the atherosclerotic aorta. A (1): The representative results of Western blots of p-ERK1/2 and ERK1/2 are shown. A (2): Densitometric measurements of p-ERK1/2 from Western blots. B. Activation of JAK2/STAT3 in the atherosclerotic aorta. B (1): The representative results of Western blots of p-JAK2/JAK2 and p-STAT3/STAT3 are shown. B (2): Densitometric measurements of p-JAK2 and p-STAT3, p-STAT3 and STAT3 from Western blots. C→E. Activation of NF-κB in the atherosclerotic aorta. C: The protein levels of p65 and p50 were measured by Western blots in cytoplasmic extracts. C (1): The representative results of Western blots of p65 and p50 are shown. C (2): Densitometric measurements of p65 and p50 from Western blots. D: The protein levels of p65 and p50 were measured by Western blots in nuclear extracts. D (1): The representative results of Western blots of p65 and p50 are shown. D (2): Densitometric measurements of p65 and p50 from Western blots. E: The protein levels of p-IκB/IκB were measured by Western blots in total protein. E (1): The representative results of Western blots of p-IκB/IκB are shown. E (2): Densitometric measurements of p-IκB/IκB from Western blots. Each bar represents mean ± SEM ($n=3$). Irb, irbesartan 10 mg $kg^{-1}$ $d^{-1}$. CE, cytoplasmic extracts; NE, nuclear extracts. $^{*}P<0.01$ vs. the ND group; $^{\#}P<0.01$ vs. the HCD group; $^{+}P<0.05$ vs. the ND group.

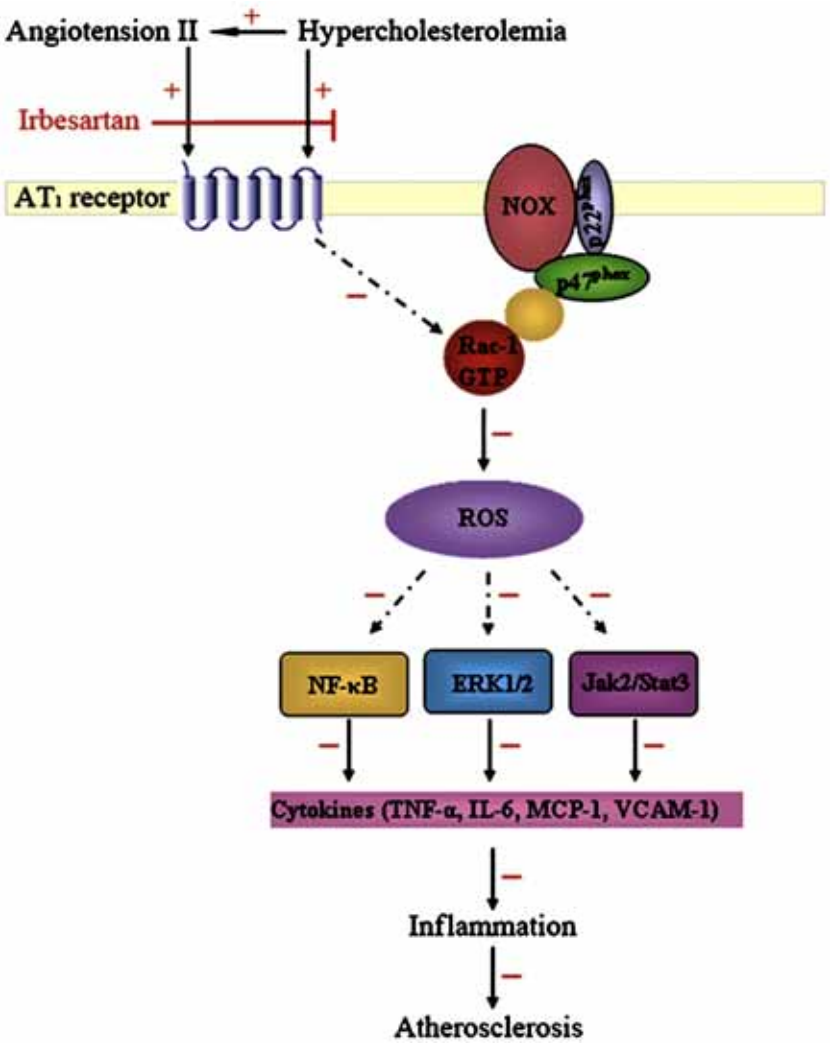



Fig. 5. A model for irbesartan influenced signaling cascades involved in atherosclerosis. NOX, NAD(P)H oxidase; Rac, $p47^{phox}$, $p22^{phox}$, NAD(P)H oxidase subunits; +, increase, −, decrease.

pathway is involved in both inflammatory response and cell proliferation [39,40]; JAK2/STAT3 regulates the expression of IL-6, IL-10 and cell growth [41,42]; NF-κB, which is the key regulator of atherosclerosis, has been regarded as a proatherogenic factor for a long time, mainly because of its regulation of many of the proinflammatory genes linked to atherosclerosis[43,44]. NF-κB may play an important role in guarding the delicate balance of the atherosclerotic process as a direct regulator of proinflammatory and anti-inflammatory genes and as a regulator of cell survival and proliferation [43,45]. The present study showed that irbesartan apparently decreased the activation of ERK1/2, JAK2, STAT3 and NF-κB. So we suggested that irbesartan could suppress the development of atherosclerosis by inhibiting the signal transduction cascades of ERK1/2, JAK2, STAT3 and NF-κB.

3. Effects of irbesartan on the inflammatory cytokines: To investigate the further molecular mechanism effects of irbesartan on atherosclerosis, the study examined the main downstream signaling effectors of ERK1/2, JAK2, STAT3 and NF-κB, such as TNF-α, IL-6, MCP-1 and VCAM-1. Our results showed that irbesartan decreased the expression of TNF-α, IL-6, MCP-1 and VCAM-1. TNF-α is a proinflammatory cytokine which has close relationship with atherogenesis, it not only activates NF-κB, but also induces the production of ROS and mediates other proinflammatory cytokines [46]. IL-6 can induce JAK2/STAT3 activation and aggravate the oxidative stress and endothelial dysfunction by up-regulating the $AT_1R$ [47]. MCP-1 and VCAM-1 are the key factors in the process of angiogenesis. They recruit mononuclear cells and other blood leukocytes to the arterial wall [17,48]. It can be presumed that TNF-α, IL-6, MCP-1 and VCAM-1 play very important roles in the initiation and progression of atherosclerosis. Therefore, we thought that attenuation of the expression of these factors could suppress the atherosclerosis.

However, our study has some limitations. First, we did not compare irbesartan with other drugs. Second, we only

selected one dosage of irbesartan in accordance with the others and did not compare the effects of different dosages.

In summary, our results suggest that an ARB, irbesartan, induces the reduction of atherosclerotic lesion independent of lowering blood pressure and decreasing plasma lipid levels. The mechanisms might be related to the inhibition of $AT_1R$, thus leading to the inhibition of oxidative stress and inflammatory signal transduction pathways which eventually lead to the reduction of inflammatory cytokine expression. Our findings provide some molecular mechanisms of irbesartan in the treatment of atherosclerosis.

## Acknowledgements

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