Biochemical and Biophysical Research Communications 470 (2016) 845–850

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Icariin inhibits atherosclerosis progress in Apoe null mice by downregulating CX3CR1 in macrophage

Yao Wang [a], Yun-Shan Wang [b], Shu-Liang Song [b], Hao Liang [b], Ai-Guo Ji [a, b, *]

[a] School of Pharmaceutical Sciences, Shandong University, Jinan, China
[b] Weihai International Biotechnology Research and Development Centre, Shandong University, Weihai, China

ARTICLE INFO

Article history:
Received 14 January 2016
Accepted 20 January 2016
Available online 21 January 2016

Keywords:
Icariin
Atherosclerosis
CX3CR1
Apoe null mice

ABSTRACT

Horny Goat Weed is a commonly used in Chinese herbal medicine. And it is used in multiple kinds of diseases including cardiovascular diseases. Icariin is the major component isolated from Horny Goat Weed. It is reported to have lipid-lowering effect. In atherosclerosis, icariin attenuate the enhanced prothrombotic state independently of its lipid-lowering effects. However, its detail mechanism is remaining unclear. This study aimed to investigate the effect and mechanism of icariin on atherosclerosis. We performed gene expression profiling on icariin treated LPS-stimulated RAW264.7 and its control cells. Microarray analyses identified a list of genes significantly differentially expressed after icariin treated including downregulation of CX3CR1. Apoe null mice were assigned into 3 groups: control group, diet with 30 mg/kg/d icariin and diet with 60 mg/kg/d icariin. The results showed that icariin treatment significantly reduced lesion area and macrophage infiltration. Also icariin reduced CX3CR1 and CX3CL1 protein levels in the artery wall. In conclusion, icariin could be a potential anti-atherosclerosis agent by downregulating the expression of CX3CR1.

1. Introduction

Cardiovascular diseases are now a serious threat to health worldwide [1]. Evidence suggests atherosclerosis is a chronic inflammatory disease and also the fundament of other cardiovascular diseases. Oxidative stress, inflammation, and endothelial dysfunction contribute to the development of atherosclerosis. The essential steps in the development of atherosclerosis include recruitment and adhesion of monocytes in the circulation system to endothelium, migration of adhered monocytes to the intima, differentiation into macrophages, endocytosis of modified LDLs and formation of foam cells. Thus the lesions initiate and advance to higher stage.

CX3C chemokine receptor 1 (CX3CR1) as the receptor binding the chemokine CX3CL1, involves in the adhesion and migration of monocytes and macrophages. Soluble CX3CL1 potently chemoattracts T cells and monocytes, while the cell-bound chemokine promotes strong adhesion of leukocytes to activated endothelial cells, where it is primarily expressed [2–6]. CX3CL1 and CX3CR1 have been identified in human atherosclerotic plaques [7–9]. CX3CR1 knockout mice showed decreased atherosclerotic lesion formation [10,11]. The CX3CR1-CX3CL1 interaction plays a direct and critical role in monocyte recruitment and atherosclerotic lesion development.

Yin Yang Huo or Horny Goat Weed, is a commonly used Chinese herbal medicine. And it is used in multiple kinds of diseases including cancer, bone loss and cardiovascular diseases. Icariin is the major component isolated from Yin Yang Huo. It is reported to have anti-oxidative effect [12,13] and lipid-lowering effect [14]. It has been demonstrated that icariin can protects erythrocytes against free-radical-induced peroxidation [12], and protect DNA from radical-induced oxidative damage [13]. In atherosclerosis, icariin may also attenuate the enhanced prothrombotic state independently of its lipid-lowering effects [14]. Icariin has ability of inhibiting the adhesion of monocyte to HUVECs [15]. In this study, we invested the function of icariin in downregulation of CX3CR1 and attenuation of atherosclerosis.

2. Materials and methods

2.1. Agents and antibodies

Icariin was purchased from Sigma–Aldrich Co. (Shanghai, China). Antibodies used include anti-monocyte and macrophage

* Corresponding author. School of Pharmaceutical Sciences, Shandong University, Jinan, China.
E-mail address: jiaiguo@sdu.edu.cn (A.-G. Ji).

http://dx.doi.org/10.1016/j.bbrc.2016.01.118
0006-291X/

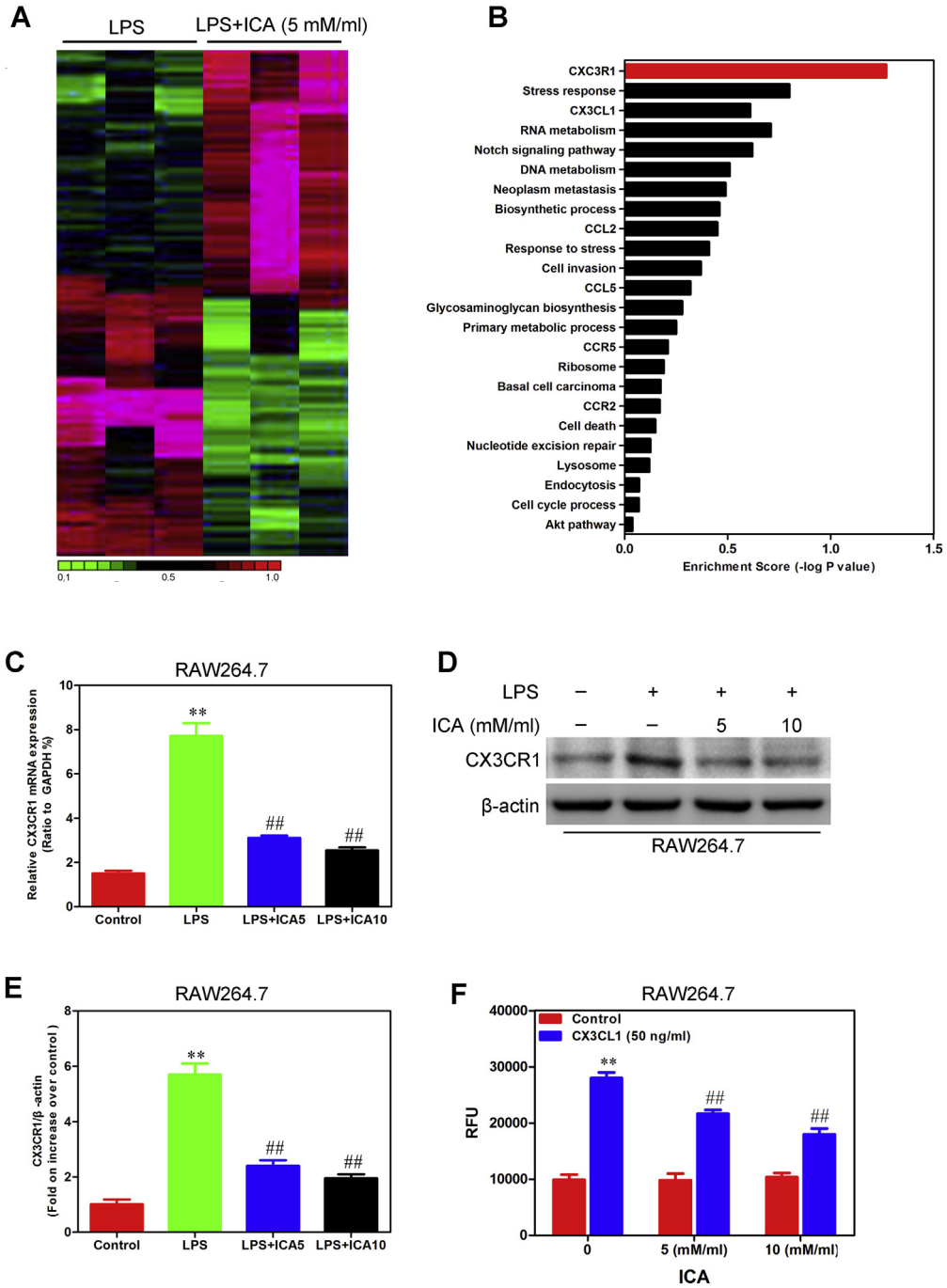

Fig. 1. Gene expression profiling results. (A, B) Microarray analyses identified a list of genes significantly differentially expressed after icariin treated including downregulation of CX3CR1. Expression of CX3CR1 in the cells with icariin treated was further evaluated by qRT-PCR (C) and western blot (D, E). (F)Icariin inhibited the chemotaxis of stimulated RAW264.7 towards soluble CX3CL1 (50 ng/ml) in a dose-dependent manner ($n = 3$, $^{**}p < 0.01$, $\#\#p < 0.01$).

antibody (MOMA-2) for IHC, anti- CX3CR1, anti- CX3CL1 and anti-β-actin for Western blot.

2.2. Animals and diet

Apoe null mice, male, 8-week-old, were purchased from Vital River (Beijing, China) and housed under specific pathogen-free conditions. All animals were used in accordance to institutional guidelines and experiments were approved by the Use Committee for Animal Care. All the mice were fed a high-cholesterol diet for 20 weeks. And at the first day of the 10th week, the mice were separated into 3 groups and 10 in each group: control group, diet with 30 mg/kg/d icariin and diet with 60 mg/kg/d icariin. The treatments lasted for 10 weeks.

2.3. Cell culture

The macrophage cell line, RAW264.7 was purchased from Shanghai institute for biological sciences, Chinese academy of sciences institute of cell resource center (Shanghai, China). Cell culture