International Immunopharmacology 40 (2016) 211–218

Contents lists available at ScienceDirect

International Immunopharmacology

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

Nicotinic acid inhibits NLRP3 inflammasome activation via SIRT1 in vascular endothelial cells

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ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form 11 August 2016
Accepted 2 September 2016
Available online xxxx

Keywords:
Nicotinic acid
NLRP3 inflammasome
SIRT1
ROS

ABSTRACT

Emerging evidences indicated that NLRP3 inflammasome initiates inflammatory response involved in cardiovascular disease. Nicotinic acid (NA) has been known to possess potential anti-inflammatory property. The aim of this study was to investigate the effect of NA on the activation of NLRP3 inflammasome and the underlying mechanisms. It was found that lipopolysaccharide (LPS) and adenosine triphosphate (ATP) triggered the activation of NLRP3 inflammasome in human umbilical vein endothelial cells (HUVECs). NA inhibited NLRP3 inflammasome activation and subsequent caspase-1 cleavage as well as interleukin (IL)-1β secretion. Moreover, NA administration up-regulated SIRT1 expression in HUVECs stimulated with LPS plus ATP. Importantly, knockdown of SIRT1 reversed the inhibitory effect of NA on the activation of NLRP3 inflammasome. Further study revealed that NA also decreased the generation of reactive oxygen species (ROS) in HUVECs. In addition, NA inhibited NLRP3 inflammasome activation partly through suppression of ROS. Taken together, these findings indicate that NA is able to regulate the activation of NLRP3 inflammasome in HUVECs, which may be partly mediated by SIRT1 and ROS.

1. Introduction

The NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome is a multi-protein complex that consists of NLRP3, caspase-1 and apoptosis-associated speck-like protein containing a CARD (ASC) [1]. The aberrant activation of NLRP3 inflammasome is implicated in the pathogenesis of a wide range of diseases, including cardiovascular disorders [2–4]. Rosuvastatin has been reported to alleviate diabetic cardiomyopathy by inhibiting NLRP3 inflammasome in rats with type 2 diabetes [5]. Another study suggested that fenofibrate negatively regulates caspase-1 activation and interleukin (IL)-1β maturation [6], the hallmarks of inflammasome activation [7]. It is generally accepted that nicotinic acid (NA) has been a widely used lipid-modifying drug to prevent cardiovascular disease [8]. Recently, we found that NA inhibited the expression of monocyte chemotactic protein-1 (MCP-1) and C-reactive protein (CRP) in the arteries of rabbits with a perivascular carotid collar [9]. While extensive research is dedicated to the effect of lipid regulating agents on NLRP3 inflammasome, little is known about the effect of NA on NLRP3 inflammasome activation and the underlying mechanism.

Sirtuin 1 (SIRT1), a class III histone deacetylase, plays an important role in a variety of cellular processes such as apoptosis, senescence and metabolism [10,11]. In addition to mediating these processes, a report indicated that SIRT1 inhibited the radiation-induced the activation of NLRP3 inflammasome in mesenchymal stem cells [12]. In our previous studies, we found that SIRT1 exerts potential anti-inflammatory effect in vascular endothelial cells (ECs) and adipocytes [13,14]. Moreover, our recent study demonstrated that dietary supplementation of NA could up-regulate the expression of SIRT1 in rabbits [9]. However, whether the regulation of NA on the activation of NLRP3 inflammasome is mediated by SIRT1 remains unknown.

In the present study, we investigated the potential role of NA on NLRP3 inflammasome activation and SIRT1 expression in ECs, and examined whether SIRT1 was involved in the inhibition of NA against NLRP3 inflammasome induction.

Abbreviations: ASC, apoptosis-associated speck-like protein containing a CARD; ATP, adenosine triphosphate; CRP, C-reactive protein; ECs, endothelial cells; HUVECs, human umbilical vein endothelial cells; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; NA, nicotinic acid; NAC, *N*-acetyl-L-cysteine; NLRP3, NOD-like receptor family, pyrin domain-containing 3; ROS, reactive oxygen species; SIRT1, Sirtuin 1.

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http://dx.doi.org/10.1016/j.intimp.2016.09.003
1567-5769/

2. Materials and methods

2.1. Reagents and antibodies

NA, lipopolysaccharide (LPS), adenosine triphosphate (ATP) and reactive oxygen species (ROS) scavenger *N*-acetyl-L-cysteine (NAC) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Antibodies against caspase-1 and ASC were supplied by Cell Signaling Technology (Beverly, MA, USA). The following antibodies: rabbit anti-SIRT1, β-actin, and goat anti-rabbit IgG-HRP as well as IgG-FITC were ordered from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibodies against NLRP3 and IL-1β were obtained from Abcam (Cambridge, MA, UK). Lipofectamine 2000 transfection reagent was from Invitrogen (Carlsbad, CA, USA). Protease inhibitor cocktail was purchased from Roche (Mannheim, Germany). A human IL-1β ELISA assay kit was product of R&D Systems (Minneapolis, MN, USA). All other reagents were of high grade of purity available.

2.2. Cell culture and treatment

Human umbilical vein ECs (HUVECs) were cultured as described previously [9,15]. In Brief, HUVECs were cultured in M199 medium supplemented with 15% fetal bovine serum (FBS), 3 ng/ml β-EC growth factor, 4 U/ml heparin, and 100 U/ml penicillin–streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. When HUVECs were grown to confluence, culture medium was replaced with serum-free medium for 24 h incubation prior to experimental use.

In order to demonstrate the effects of NA on SIRT1 expression, NLRP3 inflammasome activation and ROS production in ECs, HUVECs were divided into five groups: control, LPS + ATP, and LPS + ATP + NA (0.25, 0.5, and 1 mM) groups. The cells were pretreated with NA for 1 h, and then stimulated with LPS (0.5 μg/ml) for 3.5 h, followed by incubation with ATP (5 mM) for 30 min. In the mechanism experiments, the SIRT1 transfected HUVECs were pretreated with NA (1 mM for 1 h), and then stimulated with LPS (0.5 μg/ml for 3.5 h) and ATP (5 mM for 30 min). To determine the effect of ROS scavenger NAC on the inhibitory role of NA on NLRP3 inflammasome, HUVECs were also divided into five groups: control, LPS + ATP, LPS + ATP + NA, LPS + ATP + NA + NAC, and LPS + ATP + NAC groups. HUVECs were pretreated with NAC (10 mM) for 1 h prior to stimulation with NA (1 mM) for 1 h, and then stimulated with LPS (0.5 μg/ml for 3.5 h) and ATP (5 mM for 30 min).

2.3. Immunofluorescence staining

HUVECs were fixed in 4% paraformaldehyde, permeabilized with 0.3% Triton X-100 and blocked with normal goat serum. The cells were incubated overnight with anti-SIRT1 antibody (1:50) at 4 °C. Then a goat anti-rabbit IgG-FITC antibody (1:100) was used as a secondary antibody. The nucleus was stained with DAPI and imaged under an Olympus fluorescent microscope and then quantified using ImageJ software (National Institutes of Health, Bethesda, Maryland).

2.4. RNA interference

ECs were transfected with synthesized small interfering RNA (siRNA) targeting human SIRT1 and a scrambled siRNA (GenePharma Corporation, Shanghai, China). The scrambled siRNA was used as a negative control (NC siRNA). The siRNA and lipofectamine 2000 were separately diluted in serum-free medium and incubated at room temperature for 5 min. The two solutions were mixed softly, incubated for further 20 min, and then were added to the cells. The cells were transfected with the complexes for 48 h before the experiments.

2.5. Western blotting

The cells were washed in PBS and total protein was extracted with RIPA lysis buffer containing protease inhibitor cocktail. Protein samples were separated on SDS–PAGE gels and transferred onto a polyvinylidene difluoride membrane. The membranes were blocked with 5% nonfat dry milk, and incubated with the following primary antibodies: anti-human SIRT1 (1:200), NLRP3 (1:4000), ASC (1:1000),

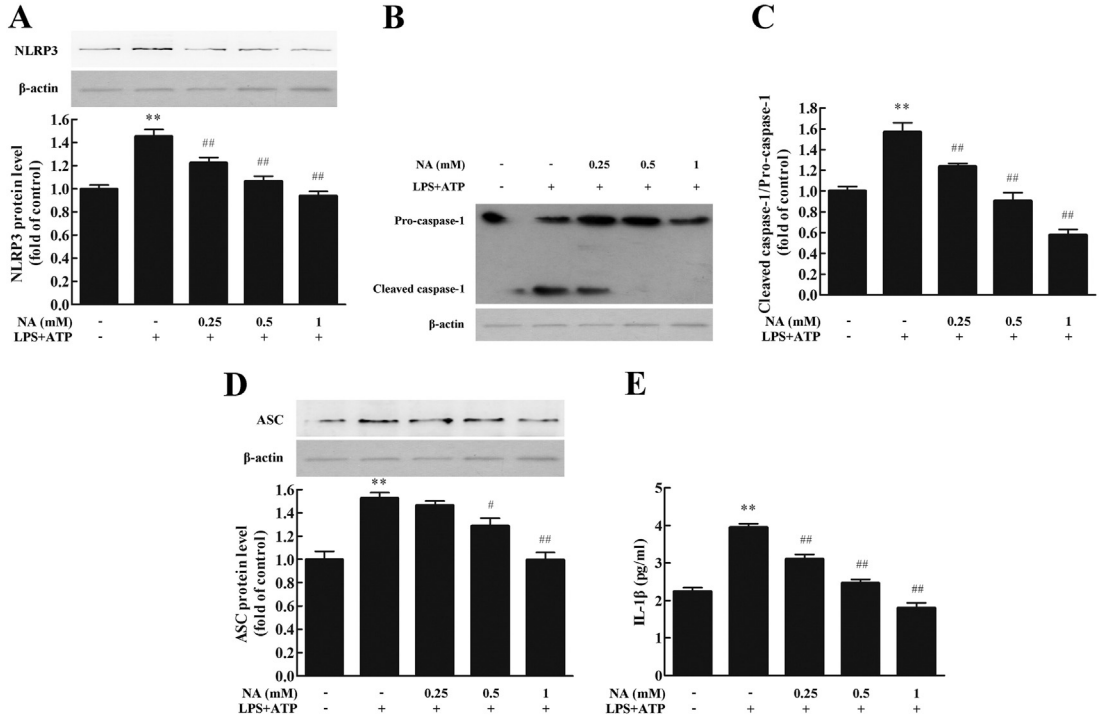

Fig. 1. NA inhibits LPS and ATP-induced the activation of NLRP3 inflammasome in HUVECs. The cells were pretreated with NA (0.25, 0.5, and 1 mM) for 1 h, and then stimulated with LPS (0.5 μg/ml) for 3.5 h, followed by incubation with ATP (5 mM) for 30 min. (A-D) The protein levels of NLRP3, pro-caspase-1, cleaved caspase-1 and ASC were determined by western blot. (E) The secretion of IL-1β in cell supernatants was detected by ELISA analysis. Data are expressed as mean ± SEM, n = 3. $^{**}P < 0.01$ vs. control group. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. LPS + ATP group.