Free Radical Biology and Medicine 108 (2017) 760–769

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Original article

Quercetin suppresses NLRP3 inflammasome activation in epithelial cells triggered by *Escherichia coli* O157:H7

Yansong Xue[a], Min Du[b], Mei-Jun Zhu[a,*]

[a] School of Food Science, Washington State University, Pullman, WA 99164, USA
[b] Department of Animal Science, Washington State University, Pullman, WA 99164, USA

ARTICLE INFO

Keywords:
Quercetin
Inflammasome
E. coli O157:H7
Polyphenol
Inflammation
Epithelial cells
Mitochondria

ABSTRACT

Inflammatory responses elicited by LRR and PYD domains-containing protein 3 (NLRP3) inflammasome is induced by a wide variety of stress signals including infectious agents and cellular disorders. *E. coli* O157:H7 causes serious gastrointestinal diseases that results in severe inflammation and oxidative stress, causing host cell damage. In this study, we found that *E. coli* O157:H7 infection induced NLRP3 assembly, caspase-1 activation and interleukin (IL)-1β and IL-18 release in Caco-2 cells. Infection also resulted in mitochondrial dysfunction with disrupted mitochondrial potential and mitochondrial complex-I activity, as well as the cytosolic release of cytochrome c and altered mitochondrial respiratory chain. The damage of mitochondria led to increased production of reactive oxygen species (ROS) and cytosolic release of mitochondrial DNA. Moreover, ROS was required for *E. coli* O157:H7 induced NLRP3 assembly as inhibiting mitochondrial ROS release by ROS scavengers Mito-TEMPO and N-acetylcysteine abrogated NLRP3 inflammasome activation in Caco-2 cells in response to *E. coli* O157:H7. Quercetin, one of the most important flavonoids in plant origin foods, had a protective role in inhibiting NLRP3 activation upon *E. coli* O157:H7 infection by protecting mitochondrial integrity and inhibiting mitochondrial ROS release. In addition, *E. coli* O157:H7 infection inhibited the host autophagy while quercetin treatment augmented autophagy activation, which further blocked ROS generation and IL-1β and IL-18 release. In summary, *E. coli* O157:H7 infection induced mitochondrial ROS release and NLRP3 assembly in host cells, while quercetin exerted a preventive role in host cells upon *E. coli* O157:H7 infection partially due to prevention of ROS production and activation of autophagy.

1. Introduction

E. coli O157:H7 is a major Shiga toxin (Stx)-producing pathogen that causes severe human diseases such as hemorrhagic colitis and hemolytic uremic syndrome (HUS) [1]. During infection, *E. coli* O157:H7 stimulates host to produce chemokines and proinflammatory mediators [2]. An excessive level of inflammatory cytokines was observed in the serum of HUS patients [3]. The increased secretion of cytokines by intestinal epithelial cells can subsequently provoke severe immune cell infiltration and intestinal tissue damage [4]. Additionally, the inflammatory cytokine interleukin-1β (IL-1β) enhances Stx binding to its receptor- glycosphingolipid globotriaosylceramide (Gb3) on endothelial cells, which promotes cell cytotoxicity [5].

Inflammasomes are multiple protein platforms integrated with inflammatory responses [6]. The NOD-like receptor (NLR) superfamily, pyrin domain containing 3 (NLRP3) inflammasome is well studied, which interacts with apoptosis-associated speck-like protein (ASC) [7,8], induces cleavage and activation of caspase-1, and further maturates the pro-inflammatory cytokines IL-1β and IL-18 [9]. NLRP3 is involved in inflammatory responses [10] and host defense against infections [11]. NLRP3 contributes to the recognition of *Salmonella* Typhimurium infection [12] and promotes caspase-1-dependent cell death and IL-1β processing in cultured macrophages. NLRP3 inflammasome-dependent IL-1β production is also observed in macrophages during *E. coli* O157:H7, *C. rodentium* [13], and *S. aureus* [14] infection. In addition, enterohemolysin contributes to NLRP3 inflammasome-dependent IL-1β production during *E. coli* O157:H7 macrophage infection [15]. These studies indicate that NLRP3 inflammasome activation is closely associated with host-bacteria interaction. There are various stimuli can trigger the assembly of inflammasome, among which, mitochondria damage was reported as an inducement of NLRP3 activation [16,17]. Although the mechanism remains elusive, mitochondria derived reactive oxygen species (ROS) is clearly involved in this processing [17].

There are intrinsic links between autophagy and inflammasomes [18]. Autophagy can negatively regulate inflammasome activation

* Corresponding author.
E-mail address: meijun.zhu@wsu.edu (M.-J. Zhu).

http://dx.doi.org/10.1016/j.freeradbiomed.2017.05.003
Received 12 February 2017; Received in revised form 22 April 2017; Accepted 1 May 2017
Available online 02 May 2017
0891-5849/

[19]. Deletion of the autophagic protein beclin-1 enhances the activity of caspase-1 and secretion of IL-1β and IL-18 via NLRP3 inflammasome [18,20]. Blockage of mitophagy results in the accumulation of damaged mitochondria that generate ROS, resulting in NLRP3 activation [21]. On the other hand, activation of autophagy inhibits IL-1β production through enhancing inflammasome degradation [22].

Phenolic compounds are well known for their anti-inflammatory and anti-oxidative effects. Epigallocatechin 3 gallate inhibits the expression of inflammatory protein-2 (MIP-2) and tumor necrosis factor-α (TNF-α) in LPS-induced macrophages [23]. Flavonoids such as genistein and kaempferol have suppressive effect on cyclooxygenase-2 (COX-2) expression in human colon DLD-1 cancer cells [24]. Quercetin is a flavonoid widely found in a variety of plants and fruits, which exhibits anti-hyperuricemia and anti-hyperlipidemia in human [25,26]. It also shows ROS scavenging and anti-inflammation activity in liver [27] and kidney [28], but its anti-inflammasome function upon pathogenic infection has not been reported. Thus, our hypothesis is that quercetin inhibits *E. coli* O157:H7-induced inflammasome activation through blocking ROS production and mediating autophagy activation.

2. Materials and methods

2.1. Reagents

The following materials were used: Mito-TEMPO (Enzo, New York, NY), N-acetylcysteine (NAC, Sigma, St. Louis, MO), rapamycin (Enzo), and quercetin (Sigma).

2.2. Cell line, media and, bacterial strains

The human colonic epithelial cell line Caco-2 cell was obtained from the American Type Culture Collection (Manassas, VA). Caco-2 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) (Sigma) supplemented with 10% fetal bovine serum (Sigma), 100 units/ml penicillin G, and 100 μg/ml of streptomycin (Sigma) at 37 °C with 5% CO_2. The *E. coli* O157:H7 EDL933 strain was obtained from the STEC center at Michigan State University, which were routinely grown in LB broth at 37 °C overnight with aeration.

2.3. Infection of E. coli O157:H7 to colonic epithelial cells

Bacterial infection assay was conducted as previously described [29]. Caco-2 cells (1×10^6 cells per well) were seeded in a 12-well plate for 12 h and pre-treated with 200 μM quercetin for another 12 h. Then cells were washed 3 times with PBS (pH 7.4), and challenged with 10^7 CFU/ml EDL933 (MOI 10:1). Caco-2 cells were collected for protein and mRNA extraction at 4 h post EDL933 infection at 37 °C with 5% CO_2.

2.4. Cell respiration measurement

Caco-2 cells were treated and infected as described above. After infection, the O_2 dissolution in medium was read using ORION 3 STAR portable dissolved oxygen meters (Thermo electron corporation, Madison, WI).

2.5. Quantitative reverse transcription PCR (qRT-PCR) analysis

Total mRNA was extracted from Caco-2 cells using RNeasy Mini Kit (Qiagen, Valencia, CA) and reverse transcribed using a iScript™ kit (Bio-Rad, Hercules, CA). cDNAs were used as a template for qRT-PCR analysis of selected genes using a CFX96™ Real-Time PCR Detection System (Bio-Rad). SYBR Green Master Mix (Bio-Rad) was used for all qRT-PCR reactions. Primers for qRT-PCR are listed in Supplementary Table 1. β-actin was used as the housekeeping gene. The amplification efficiency was 0.90–0.99 [29].

2.6. Immunoblotting

Immunoblotting analysis was conducted according to procedures previously described [29]. Antibodies against caspase-1 was from BioVision (Milpitas, CA). Anti-IL-1β, PARP, catalase, LC3B and HO-1were purchased from Cell Signaling Technology (Beverly, MA). Anti-NLRP3 antibody was purchased from Boster Biological Technology (Pleasanton, CA). Anti-PGC1-α and Cox4, cytochrome c and SOD1 were from Santa Cruz (Dallas, TX). Anti-β-actin antibody was purchased from DSHB (Iowa city, IA). Binding of antibodies was detected using HRP-coupled anti-rabbit or anti-mouse immunoglobulin, and visualized using chemiluminescence. Density of bands was quantified and then normalized with reference to the β-actin content.

2.7. MTT test

Caco-2 cells were cultured in a 96-well plate for 12 h and treated with 0 or 200 μM quercetin for another 12 h. Then, 10 μl of 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) was added to each well and incubated for additional 4 h. At the end of the incubation period the medium was removed and the converted dye was solubilized in DMSO. Absorbance of converted dye is measured at a wavelength of 540 nm [30].

2.8. Caspase-1 enzyme activity assay

Caspase-1 activity was analyzed using Caspase-1 Fluorometric Assay Kit from BioVision (Milpitas, CA) per manufacture's manual. Cell treatment and bacterial infection is described as above. Briefly, cells in each well were lysed with 200 μl lysis buffer and incubated on ice for 10 min. The lysates were then centrifuged at 12,000*g* for 10 min at 4 °C. The resulting supernatant of each sample was mixed with equal volume of 2× reaction buffer. Caspase-1 activity was measured using the YVAD-AFC substrate (50 μM final concentration) and absorbance was read using a fluorescence plate reader (BioTek Synergy H1, VT) at an excitation wavelength of 400 nm and an emission wavelength of 505 nm [31].

2.9. Intracellular ROS measurement

Intracellular ROS levels were measured using a cell-permeable fluorescent probe, 2,7-dichlorofluorescein diacetate (DCFH-DA) (Millipore, MA). Briefly, 200 μl of 1×10^5 cell/ml Caco-2 cells were seeded in a 96-well plate and cultured in DMEM complete media for 12 h. Then, cells were treated with 0 or 200 μM quercetin in DMEM complete media for another 12 h. Next, cells were infected with EDL933 (MOI: 10) for 4 h with or without quercetin supplementation. Then, cells were washed with PBS once and incubated with 100 μl of 10 μM fresh DCFH-DA in PBS for 30 min at 37 °C, followed with PBS wash. Fluorescence of each well was directly assessed using fluorescence plate reader (BioTek Synergy H1) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm [32]. All the values were normalized with control without any treatment. For microscopic analysis, the cell treatment and infection were the same as above. After infection, the cells were incubated with DCFH-DA. The fluorescence of H2DCFDA was visualized with EVOS FL fluorescence microscope (Life Technologies) [33].

For H_2O_2-induced ROS measurement, the cell monolayers were pretreated with quercetin for 12 h and washed with PBS once. Then they were incubated with 100 μl of 10 μM DCFH-DA in PBS for 30 min. After incubation, cells were washed with PBS once and then incubated with 100 μl of PBS or 0.5 mM H_2O_2 for 30 min. The H2DCFDA fluorescence was measured using fluorescence plate reader (BioTek Synergy H1) as described above [34].