Biomedicine & Pharmacotherapy 86 (2017) 48–56

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

13-Ethylberberine reduces HMGB1 release through AMPK activation in LPS-activated RAW264.7 cells and protects endotoxemic mice from organ damage

Dong Ung Lee[c,1], Young Shin Ko[a,1], Hye Jung Kim[a,b], Ki Churl Chang[a,b,*]

[a] Department of Pharmacology, School of Medicine Gyeongsang National University and Institute of Health Sciences, Jinju 52527, Republic of Korea
[b] Department of Convergence of Biomedical Sciences (BK21 PLUS Program), Gyeongsang National University and Institute of Health Sciences, Jinju 52527, Republic of Korea
[c] Division of Bioscience, Dongguk University, Gyeongju 780-714, Republic of Korea

ARTICLE INFO

Article history:
Received 25 July 2016
Received in revised form 14 November 2016
Accepted 24 November 2016

Keywords:
AMPK
Inflammation
Sepsis
HMGB1
p38MAPK
Vascular reactivity

ABSTRACT

High mobility group box 1 (HMGB1), a highly conserved non-histone DNA-binding protein, plays an important role in the pathogenesis of sepsis. Previously, the authors reported 13-ethylberberine (13-EBR) has anti-inflammatory and antibacterial effects. However, the effect of 13-EBR on HMGB1 release was not investigated. In the present study, it was hypothesized 13-EBR might reduce HMGB1 release by activating AMPK under septic conditions. The results obtained showed 13-EBR significantly reduced HMGB1 release from LPS-activated RAW264.7 cells, and that this reduction was reversed by silencing p38, or AMPK, or by co-treating cells with p38 MAPKinase inhibitor. 13-EBR increased the phosphorylations of p38 and AMPK, and the phosphorylation of p38 by 13-EBR was inhibited by AMPK-siRNA, indicating AMPK acted upstream of p38. In the lung tissues of LPS-treated mice, 13-EBR administration significantly increased p-AMPK but reduced inducible nitric oxide synthase (iNOS) protein levels. Hematoxylin and eosin staining revealed 13-EBR significantly reduced LPS-induced lung and liver damage. In addition, 13-EBR inhibited NF-kB in LPS-activated RAW264.7 cells, and in LPS-treated mice, 13-EBR administration significantly increased survival. Furthermore, co-administration of 13-EBR plus LPS prevented LPS-induced aortic rings hypocontractile response to phenylephrine *in vitro*. Taken together, these results indicate 13-EBR might offer a means of treating sepsis through AMPK activation.

1. Introduction

High-mobility group box 1 protein (HMGB1) is an ubiquitous 215-amino acid nuclear protein that binds to DNA, and thus, maintains genome stability and DNA processing and repair [1]. In addition to these intracellular homeostatic functions, HMGB1 can be released by necrotic or non-lethally damaged cells into cytoplasm where it behaves as a pro-inflammatory cytokine [1,2]. The passive liberation of HMGB1 appears to be a consequence of nucleorrhexis in a background of necrosis, whereas in the presence of infection, active secretion of HMGB1 by translocation across nuclear and plasma membranes has been reported in immune cells, including macrophages [1,2]. Sepsis is a systemic inflammatory response caused by excessive stimulation of the host immune system by pathogenic components [3]. It has been previously suggested the pathogenesis of sepsis involves inflammation, coagulation, and immune reactivity and that HMGB1 seems to play a critical role [4]. Thus, HMGB1 is being viewed as a late-phase mediator of sepsis, and as a therapeutic target in this context [5].

Berberine (BBR) is a naturally occurring isoquinoline alkaloid, and has been reported to modulate cytokine secretion, to increase

Abbreviations: BBR, berberine; CLP, cecal ligation and puncture; HMGB1, high mobility group box 1; HO-1, heme oxygenase-1; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NF-κB, Nuclear factor kappa B; NO, nitric oxide; siRNA, small interfering RNA; H&E, hematoxylin and eosin; PCNA, proliferating cell nuclear antigen; DMEM, dulbecco's modified eagle's medium; FBS, fatal bovine serum; AMPK, AMP-activated protein kinase; 13-EBR, 13-ethylberberine; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1beta; TLR, toll like receptor.

* Corresponding author at: Department of Pharmacology, School of Medicine Gyeongsan National University and Institute of Health Sciences, Jinju 52527, Republic of Korea.
E-mail address: kcchang@gnu.kr (K.C. Chang).

[1] Dong Ung Lee and Young Shin Ko contributed equally.

http://dx.doi.org/10.1016/j.biopha.2016.11.099
0753-3322/

endotoxemic mouse survival [6], and to inhibit HMGB1 release induced by heme oxygenase-1 (HO-1) upregulation in LPS-activated macrophages [7]. Furthermore, BBR was recently found to regulate the HMGB1-TLR4 axis and protect against myocardial ischemia [8]. These findings suggest BBR provides a sound basis for the development of drugs targeting HMGB1 for the treatment of diseases in which HMGB1 plays a key role. Previously, we reported 13-alkyl-substituted BBR derivatives have better activity than BBR against certain bacterial species [9] and in human cancer cell lines [10]. In particular, 13-ethylberberine (13-EBR) showed excellent inhibitory effects on inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production in LPS-activated RAW264.7 cells, and increased interleukin-12 (IL-12) production in LPS-treated splenic macrophages [11]. Furthermore, the inhibitions of proinflammatory cytokines by BBR are closely related to its ability to activate AMP-activated protein kinase (AMPK). In fact, we previously reported that metformin (an AMPK activator) inhibited HMGB1 release under LPS- or cecal ligation and puncture (CLP)-induced septic conditions [12]. It has also been shown persistent increases in plasma high mobility group box 1 (HMGB1) levels in patients with sepsis are correlated with degree of organ dysfunction and outcome [13,14]. Thus, the present study was designed to examine the hypothesis that 13-EBR reduces HMGB1 expression by activating AMPK in LPS-activated RAW264.7 cells and protects endotoxemic mice from organ damage.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and other tissue culture reagents were purchased from Gibco BRL (Rockville, MD). Primary antibodies for AMPK, HMGB1, proliferating cell nuclear antigen (PCNA), and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody for β-actin was obtained from Sigma-Aldrich (St. Louis, MO), and antibodies for p-ERK, p-JNK, and p-p38 were from Cell Signaling Technology (Beverly, MA). Small interfering RNAs for AMPK and p38 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Scrambled siRNA was purchased from Invitrogen (Carlsbad, CA), lipopolysaccharide (LPS, E coli, serotype 055:B5) from Sigma-Aldrich (St. Louis, MO). 13-Ethylberberine chloride was prepared as previously described [11].

2.2. Cell culture

RAW 264.7 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were grown in DMEM supplemented with 10% heat-inactivated FBS, penicillin (100 units/ml), streptomycin (100 mg/ml), L-glutamine (4.5 mg/ml), and

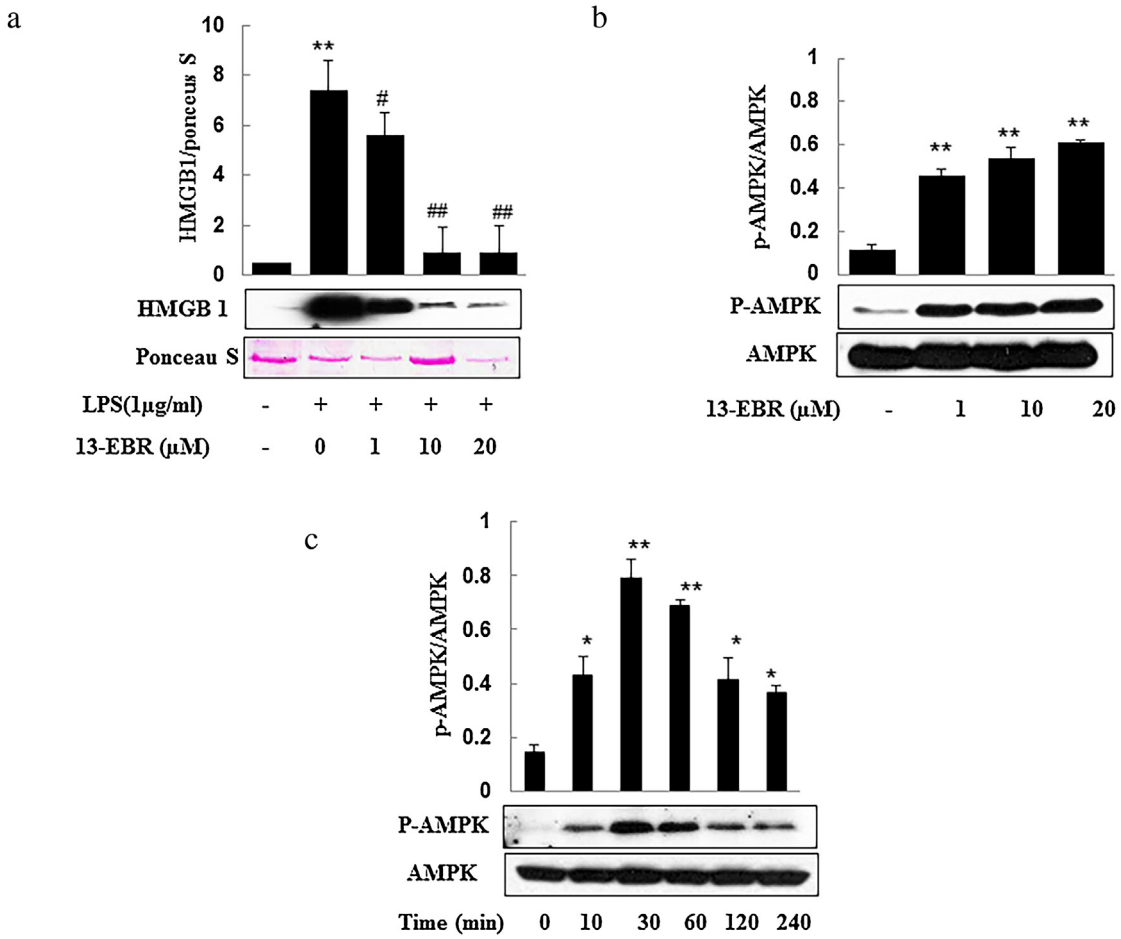

Fig. 1. 13-EBR reduced HMGB1 release from LPS-activated RAW264.7 cells by activating AMPK. Cells were treated with different concentrations (0–20 μM) of 13-EBR 1 h prior to LPS. After 16 h of incubation, HMGB1 release to media was detected by Western blotting (a). Phosphorylation of AMPK was detected by Western blotting after incubation with different concentrations (0–20 μM) of 13-EBR for 30 min (b) and after incubation with a fixed dose (10 μM) of 13-EBR for different times (0–240 min) (c). Results are expressed as the means ± SEMs of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, *vs.* untreated cells. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* LPS-treated cells.