Archives of Biochemistry and Biophysics 654 (2018) 70–76

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Effects of uridine on plasma cytokines, nuclear factor-κB signaling, and heat-shock protein 72 expression in spleen lymphocytes from endotoxemic mice

Galina D. Mironova[a,b,*], Maxim O. Khrenov[c], Eugeny Yu. Talanov[a], Olga V. Glushkova[c], Svetlana B. Parfenyuk[c], Tatyana V. Novoselova[c], Sergey M. Lunin[c], Natalia V. Belosludtseva[a,b], Elena G. Novoselova[c], John J. Lemasters[d]

[a] Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Moscow Region, 142290, Russia
[b] Pushchino State Institute of Natural Sciences, Pushchino, Moscow Region, 142290, Russia
[c] Institute of Cell Biophysics RAS, Pushchino, Moscow Region, 142290, Russia
[d] Departments of Drug Discovery & Biomedical Sciences and Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC, SC 29425, USA

ARTICLE INFO

Keywords:
Endotoxin
Heat-shock protein 72
Inflammation
Mitochondrial ATP-Dependent potassium channel
Phosphorylated nuclear factor-κB
Uridine

ABSTRACT

In this study, we examined the effects of uridine on plasma cytokine levels, heat shock protein (HSP) 72 expression, and nuclear factor (NF)-κB signaling in spleen lymphocytes after exposure of male BALB/c mice to *Escherichia coli* lipopolysaccharide (LPS). Mice were treated with uridine (30 mg/kg body weight, intraperitoneal injection [i.p.]) or saline solution of LPS (2.5 mg/kg, i. p.). Endotoxin increased plasma levels of tumor necrosis factor-α, interferon-γ, interleukin (IL)-1, IL-2, and IL-6 by 2.1-, 1.9-, 1.7-, 1.6-, and 2.3-fold, respectively. Prior treatment with uridine prevented LPS-induced increases in all studied cytokines. In splenic lymphocytes, LPS treatment increased the expression of HSP 72 by 2.4-fold, whereas preliminary treatment with uridine completely prevented this effect. LPS also activated NF-κB signaling in splenic lymphocytes, and uridine decreased NF-κB pathway activity. Inhibitory analysis showed that the mechanism of uridine action was associated with the formation of the UDP-metabolic activator of the mitochondrial ATP-dependent potassium channel ($mitoK_{ATP}$) and the UTP-activator of glycogen synthesis in the tissues. A specific inhibitor of $mitoK_{ATP}$, 5-hydroxydecanoate (5 mg/kg), and an inhibitor of glycogen synthesis, galactosamine (110 mg/kg), prevented the effects of uridine. Thus, uridine itself or uridine phosphates, which increased after uridine treatment, appeared to inhibit pro-inflammatory responses induced by LPS application. Overall, these findings demonstrated that the mechanisms mediating the effects of uridine were regulated by activation of glycogen synthesis and opening of the $mitoK_{ATP}$, which in turn increased the energy potential of the cell and reduced oxidative stress.

1. Introduction

Increased production of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin (IL)-1α, is a major feature of inflammation caused by toxins from gram-negative bacteria [1]. TNF-α and IL-1α promote the formation of reactive oxygen species (ROS), which in turn activate the transcription factor nuclear factor (NF)-κB [2] and induce the translocation of NF-κB from the cytosol to the nucleus. H_2O_2 rapidly and effectively activates NF-κB signaling, and prolonged activation of NF-κB leads to the development of oxidative stress [3–6].

Oxidative stress occurs via increased production of reactive oxygen species (ROS) or impairment of antioxidant defenses that neutralize ROS [7]. Increased ROS production by the mitochondrial electron transport chain frequently contributes to oxidative stress [8–10]. Thus, main approaches to prevent or ameliorate oxidative stress include decreasing the formation of ROS, particularly in the mitochondria, and augmenting antioxidant defenses. Previously, we found that naturally occurring antioxidant nutrients decreased activation of NF-κB signaling after treatment of mice with lipopolysaccharide (LPS) [1].

Abbreviations used: Hsp72, heat shock protein 72; LPS, lipopolysaccharide; $mitoK_{ATP}$, mitochondrial ATP-dependent potassium channel; ROS, reactive oxygen species; SIRS, systemic inflammatory response syndrome; TNF-α, tumor necrosis factor-α; UDP, uridine 5′-diphosphate; 5-HD, 5-hydroxydecanoate; Gal, galactosamin

* Corresponding author. Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Institutskaya st. 3, Pushchino, Moscow region, 142290, Russia.
E-mail address: mironova40@mail.ru (G.D. Mironova).

https://doi.org/10.1016/j.abb.2018.07.006
Received 7 May 2018; Received in revised form 4 July 2018; Accepted 9 July 2018
0003-9861/

In sepsis, ROS production associated with inflammation and the development of oxidative stress is an important contributor to host cell and organ damage [11]. During high oxidative stress, the ability of cells to eliminate ROS becomes exhausted, possibly contributing to systemic inflammatory response syndrome (SIRS), which develops during bacterial sepsis [12–14]. SIRS advances to multiple organ dysfunction syndrome, which is frequently the cause of death from sepsis.

In our previous studies, we showed that uridine could effectively prevent oxidative stress after myocardial ischemia [15]. Uridine pretreatment decreases myocardial ROS production and downregulates myocardial antioxidant enzymes [16]. Protection by uridine may be related to activation of the mitochondrial ATP-dependent K^+ channel (mitoK$_{ATP}$), because uridine is a precursor of uridine diphosphate (UDP), which activates mitoK$_{ATP}$ [17], and specific inhibitors of the channel abrogate protection by uridine [15,16]. Notably, uridine is also a precursor of uridine triphosphate (UTP), which activates the synthesis of glycogen [18].

Here, we studied the effects of uridine on signaling systems under acute inflammation in mice induced by LPS from the gram-negative bacteria *Escherichia coli.* Our findings provided important insights into the functions of uridine and the mitoK$_{ATP}$, particularly with regard to cellular energy potential and oxidative stress.

2. Materials and methods

2.1. Animals and administration of drugs/LPS

All procedures used were in accordance with the regulations of the Ethics Committee of the Institute of Theoretical and Experimental Biophysics RAS. Male BALB/c mice (8–10 weeks old; 25–30 g) were housed under standard laboratory conditions (20–21 °C; 10–14-h light-dark cycle; 65% humidity, food and water available *ad libitum*). All reagents were injected intraperitoneally. Uridine (30 mg/kg body weight) was injected 1 h before injection of LPS (2.5 mg/kg) from *E. coli* (0.26. B6 serotype; Sigma, USA). Injection of LPS at a nonlethal dose was used to induce acute inflammation. The same dose of LPS has been shown to induce potent cytokine responses and sickness behavior [19], and the same dose of uridine has been used for cardioprotection [15,16].

In the first series of experiments, four experimental groups were used, as follows: (1) vehicle-treated mice (control); (2) mice injected with LPS; (3) mice treated with uridine 1 h prior to LPS injection; and (4) mice treated with uridine alone. For inhibitor analyses (the second series of experiments), we used the following experimental groups: (1) vehicle-treated mice (control); (2) mice treated with uridine alone; (3) mice treated with galactosamine (110 mg/kg) alone; (4) mice treated with 5-hydroxydecanoate (5-HD) (5 mg/kg), which was injected at 1, 3, and 4 h during the experiment; (5) mice treated with galactosamine at 1 h before uridine injection; (6) mice treated with 5-HD at 1 h before and 1 and 2 h after uridine injection; (7) mice injected with LPS; (8) mice treated with galactosamine 1 h prior to LPS injection; (9) mice treated with 5-HD at 1 h before and 1 and 2 h after LPS injection; (10) mice treated with uridine 1 h prior to LPS injection; (11) mice treated with uridine and galactosamine 1 h prior to LPS injection; and (12) mice treated with uridine 1 h before LPS injection and 5-HD at 1 h before and 1 and 2 h after LPS injection.

Mice were euthanized by decapitation 6 h after LPS or vehicle injection. Plasma, peritoneal macrophages, and spleen lymphocytes were obtained as described below.

There were four mice in each group, and six replicates per mouse. The mean of the average of these replicates is shown. All animals were analyzed individually and simultaneously.

2.2. Isolation of blood plasma and cells

Plasma was isolated from the blood collected at euthanasia from the carotid artery. Blood samples without anticoagulants were kept at 4 °C for 3–5 h and centrifuged at 200 × *g* (4°C), and the supernatants were then collected. Lymphocytes from the spleen were isolated by glass homogenization in Dulbecco's modified Eagle's medium (Sigma) containing 10 mM HEPES, 100 mg/mL streptomycin, and 10% bovine serum (pH 7.4). The homogenates were then centrifuged at 3000 × *g* at 4°C for 5 min. Erythrocytes were lysed in solution containing 9 mM Tris-HCl, 135 mM NaCl, 151 mM NH_4Cl (pH 7.2). After washing, 1.5×10^6 cells/well were cultured in RPMI 1640 with 10% fetal calf serum, 2 mM glutamine (Sigma), and 100 mg/mL streptomycin in 24-well plates for 24 h at 37 °C in humidified air with 5% CO_2. Cell-free supernatants after centrifugation were stored at −20 °C until used for analysis of cytokine levels.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Cytokines in serum were determined using ELISA Development Kits for mouse cytokines (Peprotech, USA). Assays were developed with 100 μL/well ABTS green dye (Sigma) diluted in 0.05 M citrate buffer (pH 4.0) supplemented with 0.01% H_2O_2. Absorbance was measured at 405 nm with a plate spectrophotometer (Multiscan EX, Thermo Electron Corporation).

2.4. Western blot analysis

To prepare specimens, 5×10^7 spleen lymphocytes were lysed for 2 min in an ultrasonic disintegrator under constant stirring. Protein concentrations were determined using the Bradford method (Sigma) following protein precipitation with acetone in an ice bath for 15 min. Thereafter, proteins were solubilized by 1:1 dilution in a solution containing 65.8 mM Tris-HCl (pH 6.8), 2.1% sodium dodecyl sulfate, 26.3% (w/v) glycerol, and 0.01% bromophenol blue; boiled for 5 min; and stored at 4 °C. Proteins (10 μg/lane) were separated by polyacrylamide gel electrophoresis on 10% gels, as previously described [20]. Proteins were then transferred to nitrocellulose membranes in a transblot chamber. After blocking with 5% w/v nonfat dry milk in TBS/Tween 20 (0.1%), membranes were exposed for 2 h to antibodies to the following mouse proteins: anti-Hsp70 antibody (1:1000 dilution, rabbit anti-mouse Hsp72, clone SPA-812, inducible form, StressGen), anti-phospho-NF-κB antibody (phospho-NF-κB p65 (Ser 536), Cell Signaling Technology, USA), rabbit anti-NF-κB antibody (NF-κB p65 Antibody, Cell Signaling Technology, USA), rabbit anti-phospho-IKKα/β Antibody II (Ser 176/180, Cell Signaling Technology, USA), rabbit anti-IKKβ antibody (IKKβ Antibody, Cell Signaling Technology, USA) For all primary antibodies, a 1:1000 dilution was used. After washing, nitrocellulose membranes were incubated for 1 h with anti-rabbit biotinylated antibodies (Jackson ImmunoResearch, USA; 1:100,000), and peroxidase-conjugated streptavidin (IMTEK, Russia; 1:10,000) was added for 1 h. The loading control was mouse monoclonal anti-human tubulin β (US Biological, Swampscott, MA, USA; 1:1000). An ECL-Plus chemiluminescent cocktail (Amersham/GE Healthcare, UK) was used to develop the blots following the manufacturer's instructions, with exposure to Kodak film. Images were copied using an HP ScanJet 300. Quantitative evaluation of protein bands in photographs was performed using the Qapa computer program.

2.5. High-performance liquid chromatography (HPLC) analysis of nucleoside phosphates

Mouse spleen tissues were excised and immediately frozen in liquid nitrogen. The tissues were pulverized with a cold mortar and pestle and then extracted with cold perchloric acid (tissue/perchloric acid ratio, 1:5). The mixture was then neutralized to pH 7 using 4 M K_2CO_3 and centrifuged at 14,000 × *g* (4 °C). Supernatants were stored for all subsequent procedures at 0–4°C.

Uridine nucleotides were assayed by HPLC (Knauer, Germany) using