International Immunopharmacology 58 (2018) 24–31

Contents lists available at ScienceDirect

International Immunopharmacology

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

Short chain fatty acid, acetate ameliorates sepsis-induced acute kidney injury by inhibition of NADPH oxidase signaling in T cells

Naif O. Al-Harbi[a], Ahmed Nadeem[a,*], Sheikh F. Ahmad[a], Moureq R. Alotaibi[a], Abdullah F. AlAsmari[a], Wael A. Alanazi[a], Mohammad M. Al-Harbi[a], Ahmad M. El-Sherbeeny[b], Khalid E. Ibrahim[c]

[a] Department of Pharmacology & Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[b] Industrial Engineering Department, College of Engineering, King Saud University, Riyadh, Saudi Arabia
[c] Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

ARTICLE INFO

Keywords:
Sepsis
NOX2
ROS
T cell
Antioxidants
Acute kidney injury

ABSTRACT

Sepsis affects millions of people worldwide and is associated with acute kidney injury (AKI). Innate and adaptive immune cells have been shown to play an important role in AKI through release of various inflammatory mediators which include reactive oxidant species (ROS). Acetate, a short chain fatty acid produced by gut bacteria has anti-inflammatory properties and has also been shown to modulate oxidative stress in different immune cells. Effects of acetate have been shown to be both GPR43 dependent and independent in different cells/tissues. However, the role of acetate on T cell NADPH oxidase (NOX2)/ROS signaling remains unexplored during sepsis-induced AKI. Therefore, the current study investigated the effect of acetate on sepsis-induced AKI parameters and T cell oxidant-antioxidant balance. Our results show that acetate ameliorates sepsis-induced AKI as reflected by a decrease in serum, creatinine/blood urea nitrogen and renal myeloperoxidase activity/lipid peroxides and restoration of kidney tubular structure. Moreover, acetate administration was associated with correction of oxidant-antioxidant imbalance in T cells during sepsis-induced AKI. Acetate produced its inhibitory effects on NOX2/ROS signaling via attenuation of histone deacetylase activity in T cells which was induced during AKI. Overall, the data suggest that acetate might be beneficial during sepsis-induced AKI by restoration of oxidant-antioxidant balance in T cells.

1. Introduction

Sepsis affects just under 1 million patients in the United States annually and affects many organs including the kidneys [1,2]. Acute kidney injury (AKI) is one of the most common complications of sepsis and is associated with long-term and short-term risk of mortality and morbidity [3–5]. More than half of the patients with septic shock develop AKI with the mortality rate over 75% in such patients [3,6].

Several mechanisms are involved in the development of sepsis related AKI. Systemic inflammatory response syndrome results in production of pro-inflammatory cytokines (such as IL-6, and TNF-α) and reactive oxygen species (ROS) which are thought to be key events in the development of endothelial damage and increased vascular permeability leading to organ injury and subsequent death in animals/patients with sepsis [4,7]. During septic shock, systemic release of inflammatory cytokines and oxidants can also affect renal vasculature leading to renal injury and inflammation thereby affecting fluid balance and toxic waste disposal of the body [4,8,9]. Therefore, antioxidants and anti-inflammatory therapies have been shown to have a protective effect on AKI in different models of endotoxaemia in humans/animals [10–12].

Commensal bacteria in the gut constantly ferment dietary fibers/proteins/undigested carbohydrates to produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate which have immune modulating properties [13,14]). Out of these SCFAs, acetate may travel to systemic circulation and has the potential to modify immune function of neutrophils, monocytes and T cells [14]. SCFA, acetate has been shown to affect oxidant-antioxidant balance and production of cytokines in different immune cells [15–18]. However, it is not known whether acetate affects oxidant-antioxidant balance in T cells during sepsis-induced AKI.

GPR43 has been shown to be involved in both pro- as well as anti-inflammatory activities in animal models of colitis, asthma, ureteritis, psoriasis, and rheumatoid arthritis [16,19,20]. Acetate modulates

* Corresponding author at: Department of Pharmacology & Toxicology, College of Pharmacy, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia.
E-mail address: anadeem@ksu.edu.sa (A. Nadeem).

https://doi.org/10.1016/j.intimp.2018.02.023
Received 1 December 2017; Received in revised form 7 February 2018; Accepted 28 February 2018

function of immune/non-immune cells through cell-surface G-protein-coupled receptor known as GPR43. Expression of GPR43 is reported to be high on tissues/cells such as colon, spleen, adipocytes, and monocytes/neutrophils [18,19]. On the other hand, T cells lack this cell surface receptor, however acetate can modulate T cell function through modification of histone deacetylases (HDAC) signaling [14,17,19,20]. HDAC signaling has been shown to be associated with transcriptional control of several inflammatory genes through epigenetic modifications [21,22]. In this study, we explored the effects of SCFA, acetate on sepsis-induced AKI and its association with HDAC signaling and oxidant-antioxidant balance in T cells as it has not been investigated previously.

ROS generation is critically important in the pathogenesis of AKI. ROS may be released from conventional sources such as neutrophils. This usually involves activation of membrane-bound NADPH oxidase (NOX2) along with cytosolic members leading to the production of ROS. Other sources which have been shown to release ROS have been rising steadily over the last few years [23,24]. T cells are known to express NOX2 either in steady state or under inflammatory conditions [16,25–28]. It has been shown that NOX2 in CD4 T cells is also involved in regulation of immune responses via ROS production [16,25,27,28]. This suggests that T cell oxidants may also contribute to the inflammatory burden under certain conditions. However, it is not known whether SCFA, acetate affects NOX2/ROS signaling in T cells during sepsis-induced AKI.

Several antioxidant defenses are available in T cells to protect them from ROS attack. They include both enzymatic and non-enzymatic antioxidants such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and glutathione [29–31]. Antioxidant-related protective mechanisms have been shown to be altered in sepsis/AKI [32–34]. However, effect of SCFA, acetate on T cell antioxidants needs to be investigated during sepsis-induced AKI.

Therefore, our study explored oxidant-antioxidant balance in T cells and its relationship with HDAC signaling during sepsis-induced AKI, and modulation of these pathways by SCFA, acetate. Our study shows that there is increased HDAC and NOX-2/ROS signaling in T cells during sepsis-induced AKI. Treatment with acetate attenuates sepsis-induced AKI by correcting oxidant-antioxidant imbalance through downregulation of HDAC signaling in T cells.

2. Materials and methods

2.1. Animals

Male BALB/c mice (25–30 g; 10–12 weeks old) were bred in-house and maintained under specific pathogen-free conditions. Total number of mice used in this for both in vivo and in vitro experiments was 45. Procedures were performed in accordance with the Experimental Animal Care and Use Committee, College of Pharmacy, King Saud University.

2.2. Sepsis-induced acute lung kidney (AKI)

Sepsis-induced AKI was induced by intraperitoneal injection of a freshly prepared solution of LPS (10 mg/kg, i.p.) dissolved in PBS. Mice were killed 24 h after treatment with LPS. Control mice received PBS only in a similar volume.

2.3. Drug treatments

To assess the role of acetate in sepsis-induced acute kidney injury, 200 mmol/l sodium acetate mixed in drinking water was provided to the mice for seven consecutive days [35–37]. During this period, bottles containing acetate and normal water were changed at every alternate day. We chose acetate over other SCFAs (such as butyrate and propionate) as acetate is the most abundant SCFA both in the intestine and blood where it contributes > 50% and > 80% respectively to the overall molar concentration of SCFAs both in humans and mice [35,38–40]. This method leads to acetate concentration of ~15 mmol/l and 1–2 mmol/l in intestine and blood respectively in mice [20,40].

Mice were divided into the following groups: Control group (Control): mice received only vehicle through i.p injection; LPS treated mice (LPS): mice received LPS through i.p injection as described above; Acetate and LPS treated mice (Acetate + LPS): mice were provided with acetate in drinking water for seven days before i.p. administration of LPS as described above; Acetate administered mice (Acetate): mice were provided with acetate in drinking water for seven days before i.p. administration of vehicle as described above. Mice were age-matched in different treatment groups to nullify any age-related effect on the study parameters. Spleen/kidney/blood samples were harvested at the end of the experiment for various analyses.

2.4. Determination of Renal injury biomarkers

Blood samples were taken from all groups at the end of the study, and serum BUN and creatinine levels were determined by autoanalyzer (Dimension® RxL Max®, Siemens, USA). Results were expressed in mmol/l or μmol/l for serum BUN and creatinine respectively.

2.5. Lipid peroxidation analysis

MDA, an end product of lipid peroxidation, was analyzed in renal tissue samples by measurement of malondialdehyde-thiobarbituric acid adducts by the method of [41] as described earlier [16]. Results were expressed in nmol/mg protein.

2.6. Measurement of myeloperoxidase activity

Myeloperoxidase (MPO) activity in the renal supernatants was estimated to assess neutrophil inflammation as reported earlier [16].

2.7. ROS measurement

T cell (1 million cells) ROS generation was measured by following oxidation of specific probe, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) using a multi-mode fluorescent microplate reader (FLUOstar Omega, BMG LabTech, USA). Fluorescence caused by oxidation of H_2DCFDA was recorded for 30 min at 485 nm (excitation) and 530 nm (emission) by the method of [42] as reported previously [16,24]. Data is expressed as fold mean fluorescence intensity (MFI) over control.

2.8. Flow cytometric analysis

Spleens were collected from different groups and prepared into single cell suspensions. After RBC lysis, cells were fluorescently labeled with cell surface antibodies against CD4/NOX2 (Biolegend, USA or Santa Cruz Biotech, USA). Cells were then stained intracellularly with specific SOD1 or nitrotyrosine monoclonal antibodies conjugated to PE/APC/FITC (Santa Cruz Biotech, USA) before acquisition of immunostained cells. The immunostained cells were analyzed for the proteins of interest by FC500 flow cytometer (Beckman Coulter, USA) using CXP software, based on the immunofluorescence characteristics as previously described [16].

2.9. Isolation of T cells

Spleens were collected after 24 h of LPS administration to create single cell suspensions. This suspension was utilized for isolation of T cells using Dynabeads® Untouched Mouse T Cell isolation negative selection kit (Invitrogen, USA). Isolated cells were used either for biochemical measurements or in vitro cell culture as described below.