Biomedicine & Pharmacotherapy 99 (2018) 313–318

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Dihydroartemisinin ameliorates sepsis-induced hyperpermeability of glomerular endothelium *via* up-regulation of occludin expression

Zuowang Cheng[a,b,1], Ruixia Qi[a,b,1], Liqun Li[b], Qiang Liu[b], Wenqian Zhang[c], Xia Zhou[d], Dongmei Xu[e], Thaddeus D. Allen[f], Silin Pan[g,h,*], Ju Liu[b,*]

[a] Taishan Medical College, Tai'an, Shandong, China
[b] Laboratory of Microvascular Medicine, Medical Research Center, Shandong Provincial Qianfoshan Hospital, Shandong University, Jinan, Shandong, China
[c] College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, Shandong, China
[d] Department of Rehabilitation Medicine, The Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong, China
[e] Department of Nephrology, Shandong Provincial Qianfoshan Hospital, Shandong University, Jinan, Shandong, China
[f] Tradewind BioScience, Daly City, CA, 94015 USA
[g] Qingdao Women and Children's Hospital, Qingdao University, Qingdao, Shandong, China
[h] Cardiovascular Center, Children's Hospital, Fudan University, Shanghai, China

ARTICLE INFO

Keywords:
Sepsis
Dihydroartemisinin
Glomerular endothelial cells
Permeability
Occludin

ABSTRACT

Sepsis, the systemic inflammatory responses after infection, remains a serious cause of morbidity and mortality in critically ill patients. The anti-malarial agent dihydroartemisinin (DHA) has been shown to be anti-inflammatory. In this study, we examined the effects of DHA on sepsis-induced acute kidney injury (AKI) and explored the mechanism underlying its mode of action in AKI. In a lipopolysaccharide (LPS)-induced mouse model, we observed that DHA treatment ameliorated glomerular injury, and relieved elevation of the urine albumin to creatinine ratio (UACR) and serum creatinine. At a concentration of 25 μM, DHA had no effect on overall cellular viability or apoptosis in assays with human renal glomerular endothelial cells (HRGECs), but significantly inhibited the tumor necrosis factor-α (TNF-α)-induced hyperpermeability of HRGEC monolayers. We found that TNF-α decreases the expression of the junctional protein occludin in HRGECs, which is reversed by DHA. Taken together, our results demonstrate that DHA decreases permeability of the glomerular endothelium by maintenance of occludin expression. This suggests DHA may have therapeutic utility in sepsis-induced AKI.

1. Introduction

Sepsis is the systemic inflammatory response to infection and a leading cause of death in the intensive care unit (ICU) patient population [1]. Elevated levels of bacterial endotoxin, for example, will induce a massive pro-inflammatory cytokine response, including elevation of TNF-α and interleukins (ILs) by monocytes and macrophages [2]. These circulating cytokines impair function in the kidney, lungs, brain, skin, and abdominal organs [3,4]. Acute kidney injury (AKI) is a frequent and serious complication of sepsis [5]. Sepsis-induced AKI occurs in between 15% and 20% of all ICU admissions, and mortality associated with sepsis ranges from 20% to 60% [6]. The pathophysiology of sepsis-induced AKI includes severe inflammation of the renal parenchyma with endothelial dysfunction, intra-glomerular thrombosis and tubular injury [5].

The impairment of the glomerular filtration barrier (GFB) is the most important clinical feature of AKI [7]. The GFB is composed of glomerular endothelium, the glomerular basement membrane (GBM) and the podocyte layer [8,9]. The glomerular endothelium is a semi-permeable membrane formed by glomerular endothelial cells (GECs) [10], which are a unique microvascular cell type with round shape and fenestrations [11,12]. During sepsis, GECs are exposed to circulating elements of the blood and are sensitive to various inflammatory factors [13]. With dysfunction of the GFB, glomerular capillaries become highly permeable to water, solutes and plasma proteins, resulting in edema and albuminuria [14]. GECs are connected by adherens, tight

Abbreviations: DHA, dihydroartemisinin; AKI, acute kidney injury; LPS, lipopolysaccharide; UACR, urine albumin creatinine ratio; HRGECs, human renal glomerular endothelial cells; TNF-α, tumor necrosis factor-α; VE-cadherin, vascular endothelial cadherin; TGF-β, transforming growth factor-β; ICU, intensive care unit; ILs, interleukins; GFB, glomerular filtration barrier; GBM, glomerular basement membrane; HUVECs, human umbilical vein endothelial cells; SBE, smad-binding element

* Corresponding authors.
E-mail addresses: silinpan@126.com (S. Pan), ju.liu@sdu.edu.cn (J. Liu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.biopha.2018.01.078
Received 2 November 2017; Received in revised form 31 December 2017; Accepted 12 January 2018

and gap junctions [15], which maintain cell to cell adhesion and control vascular permeability [16]. One of the primary targets for TNF-α is the tight junction [17]. Tight junctions, formed by the cytoplasmic adaptor proteins and integral membrane linker proteins such as occludin and claudins, provide highly polarized barriers for endothelial homeostasis. The junctional complex is highly dynamic in response to diverse physiological and pathological stimulations [18–20]. Occludin, a 65-kDa tetraspan integral membrane protein, is crucial for tight junction stabilization and barrier function [21,22].

Artemisinin is a sesquiterpene lactone endoperoxide extracted from the Artemisia annua plant [23]. It is widely used as an antimalarial drug due to its ability to inhibit the sarcoplasmic and endoplasmic reticulum calcium ATPase of Plasmodium falciparum [24]. Artemisinin and its derivatives also have anti-angiogenic [25,26] and anti-inflammatory effects [27,28]. Artemisinin effectively relieves the symptoms of lupus nephritis in mice [27]. In addition, an artemisinin derivative SM905 suppresses collagen-induced arthritis [28]. These findings suggest the potential of artemisinins for treating inflammatory-related diseases including sepsis.

DHA is a water-soluble derivative of artemisinin that produces few adverse side effects [24]. In this study, the effects of DHA treatment on the kidney injuries in a LPS induced sepsis mouse model were evaluated. *In vitro*, the effects of DHA on glomerular endothelial cell viability and permeability were examined. In addition, we detected the expression of the junctional protein occludin by DHA treatment. Our study explored the potential of DHA in treating sepsis-induced AKI and the underlying molecular mechanisms.

2. Materials and methods

2.1. Animals

The 6-week old C57BL/6J mice (weighted 22–25 g) were purchased from the Vital River Laboratory (Beijing, China). LPS (from *Escherichia coli* 055: B5) and DHA were purchased from purchased from Sigma Aldrich (St. Louis, MO, USA). To model sepsis, 20 mg/kg LPS were administered to mice by intraperitoneal injection [29,30]. One hour after injection, 50 mg/kg DHA suspended in PBS was administered to each mouse *via* intragastrical tube. After 24 h, urine was obtained by a 24-hr collection and the mice were sacrificed to isolate kidneys. All the procedures were carried out in accordance with guidelines prescribed by Animal Care Committee of Shandong University.

2.2. Creatinine measurements

The measurements of UACR were performed as previously described [31]. The urine samples were centrifuged at 2000 g for 5 min at 4 °C and the supernatant were collected for examination. Urine albumin was measured by the immunoturbidimetric method with a detection kit (Beijing Atom High-Tech, Beijing, China). Urine creatinine was measured by colorimetric analysis using the Jaffe's method (Roche Modular Diagnostics, Indianapolis, IN, USA). Serum creatinine was measured using a Creatinine Assay Kit (ab65340; Abcam, Cambridge, MA, USA) following the manufacturer's protocols.

2.3. Hematoxylin-eosin (HE) staining

Mouse kidneys were fixed with 4% paraformaldehyde, and embedded in tissue-Tek OCT (Sakura Finetek USA Inc, Torrance, CA, USA) over dry ice. The tissues were sectioned at 5 μm. The sections were stained with 1% hematoxylin (Yuanmu Biotechnology Co., Ltd., Shanghai, China) for 5 min. After washing, the sections were immersed in 1% hydrochloric acid-alcohol solution, washed in distilled water, and then stained with 0.5% eosin (Huihong Reagent Co., Ltd., Hunan, China) for 15 s. Following gradient ethanol dehydration, the sections were cleared in dimethylbenzene, and sealed with resinene (Boster Biological Engineering Co., Ltd., Wuhan, China). The morphological changes of the kidney tissues were observed under a light microscope and photographed using a BX-51 microscopic imaging system (Olympus Corporation, Tokyo, Japan)

2.4. Cell culture

HRGECs were purchased from ScienCell Research Laboratories (Carlsbad, CA, USA), and cultured in Dulbecco's modified Eagle's medium (DMEM) (Corning Inc., Corning, NY, USA), supplemented with 10% fetal bovine serum (Lonza, Basel, Switzerland), 100 IU/ml penicillin and 100 μg/ml streptomycin. The culture plates were incubated in humidified air at 37 °C with 5% CO_2. TNF-α was purchased from Miltenyi Biotec (Bergisch Gladbach, Germany), and applied to HRGEC cultures with a final concentration 20 ng/ml.

2.5. Fluorescein isothiocyanate–dextran transwell assay

HRGECs (1×10^5 cells) were seeded on top of the transwell insert in the 24-well Transwell chambers, and cultured for 24 h to reach confluence. After treatment with 25 μM DHA, 20 ng/ml TNF-α or the combination of both for 24 h, the chambers were washed with Hepes medium, and 1 mg of all-sized FITC-dextran (Invitrogen, Waltham, MA, USA) in 1ml PBS was added to the top chamber. After 4 h, samples were collected from the bottom chamber and read in a fluorometer (Molecular Devices) with excitation 485 nm and emission 520 nm.

2.6. Electric cell–substrate impedance sensing (ECIS) analysis

Intercellular resistance across a monolayer of HRGECs was measured using the ECIS technique (ECIS model 1600; Applied Bio Physics, Troy, NY, USA) [32]. Briefly, 8-well ECIS arrays (8W10E+) were first coated with fibronectin (Invitrogen Life Technologies, Carlsbad, CA, USA). Then, HRGECs were plated at a density that would allow formation of confluent monolayers directly on top of the electrodes. After treatment with media containing DHA and/or TNF-α, alternating current was applied across the electrodes and electrical resistance was recorded.

2.7. Annexin V-FITC/PI analysis and cell viability assay

Apoptosis of HRGECs treated with DHA was measured using an Annexin V-FITC and propidium iodide (PI) staining kit (Neobiosciences, Shenzhen, China) according to the manufacturer's protocol. Briefly, cells were pelleted and washed twice with PBS. Then, 1×10^6 cells were resuspended into a single cell suspension in binding buffer. The cells were stained with Annexin V-FITC (0.025%) for 3 min and PI (20 μg/mL) for 10 min in the dark. Detection of positive staining cells was performed using a FACSAriall flow cytometer (BD Biosciences, San Jose, CA, USA). The data were analyzed by the FACS Diva acquisition and analysis software. Cell viability was assessed by Trypan blue exclusion assay for the cells cultured on the 96-well plates. Cultures were washed and incubated in 0.05% trypsin for 2 min at 37 °C. After disaggregation, cell suspensions were diluted 1:1 in 0.4% Trypan blue (w/v in 0.9% NaCl; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and the percentage of dye-free cells was calculated.

2.8. Western blotting

Total cellular protein extract was prepared from HRGECs. Protein concentration was determined using the BCA assay (Bio-Rad, Hercules, CA, USA). Equal amounts of protein (40 μg) were separated in a 10% polyacrylamide gel by SDS-PAGE, and transferred onto a PVDF membrane (Sigma Aldrich, St. Louis, MO). Then the membrane was blocked with 2.5% BSA in Tris-buffered saline Tween (TBST) at room temperature for 2 h, and incubated overnight at 4 °C with a rabbit