International Immunopharmacology 61 (2018) 1–7

Contents lists available at ScienceDirect

International Immunopharmacology

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

Curcumin attenuates sepsis-induced acute organ dysfunction by preventing inflammation and enhancing the suppressive function of Tregs

Longwang Chen[a,b], Yang Lu[c], Linjun Zhao[a,b], Lili Hu[d], Qiaomeng Qiu[a,b], Zhuoling Zhang[a,b], Mengfang Li[a,b], Guangliang Hong[a,b], Bing Wu[a], Guangju Zhao[a,b,*], Zhongqiu Lu[a,b,e,*]

[a] Emergency Department, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China
[b] Wenzhou Key Laboratory of Emergency, Critical care, and Disaster Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China
[c] Department of Rheumatology, Wenzhou People's Hospital, Wenzhou 325000, China
[d] Central Blood Station of Wenzhou, Wenzhou 325000, China
[e] College of Nursing, Wenzhou Medical University, Wenzhou 325000, China

ARTICLE INFO

Keywords:
Regulatory T cells
Sepsis
Inflammation
Immunosuppression
Curcumin

ABSTRACT

Sepsis is characterized by the extensive release of cytokines and other mediators. It results in a dysregulated immune response and can lead to organ damage and death. Curcumin has anti-inflammatory properties and immunoregulation functions in various disorders such as sepsis, cancer, rheumatoid arthritis, cardiovascular diseases, lung fibrosis, gallstone formation, and diabetes. This paper investigates the effects of curcumin on immune status and inflammatory response in mice subjected to cecal ligation and puncture (CLP). Inflammatory tissue injury was evaluated by histological observation. Magnetic microbeads were used to isolate splenic $CD4^+CD25^+$ regulatory T cells (Tregs), and phenotypes were then analyzed by flow cytometry. The levels of Foxp3 were detected by Western blot and real-time PCR and cytokine levels were determined by enzyme-linked immunosorbent assay. We found that the administration of curcumin significantly alleviated inflammatory injury of the lung and kidney in septic mice. The suppressive function of Treg cells was enhanced and the plasma levels of IL-10 increased after treatment with curcumin. Furthermore, the secretion of plasma TNF-α and IL-6 was notably inhibited in septic mice treated with curcumin and administration with curcumin could improve survival after CLP. These data suggest that curcumin could be used as a potential therapeutic agent for sepsis.

1. Introduction

Sepsis is a complex and heterogeneous syndrome defined by a systemic inflammatory response to infection. At present, sepsis is a common disease in intensive care units (ICUs), with a 30–50% fatality rate [1]. Despite great advances in the pathogenesis of sepsis, there are no effective novel therapies that significantly reduce its mortality rate. Therefore, the treatment of sepsis has become a worldwide problem in modern critical-care medicine.

The immune response in sepsis can be characterized by a cytokine-mediated hyper-inflammation (cytokine storm) phase in which most patients survive, and a subsequent hypo-inflammation (immune paralysis or immunosuppression) phase [2]. A recent report has shown that $CD4^+CD25^+$ regulatory T cells (Tregs) play a pivotal role in the suppression of immune response in sepsis [3–5]. The enhanced suppressive function of $CD4^+CD25^+$ Tregs has been shown to be associated with fatal outcomes in patients with septic shock. FOXP3 (Forkhead/winged helix transcription factor p3) is specifically expressed in $CD25^+CD4^+$ Tregs and controls their development and function [6,7]. Mutations of the FOXP3 gene lead to deficiency or malfunction in natural Tregs and the consequent development of a similar autoimmune and/or inflammatory disorder in mice and humans [7]. Therefore, FOXP3 is a master control gene for Treg development and function. Many previous studies have demonstrated that the suppressive function of Treg cells restrains inflammatory responses in diverse diseases [8,9].

Curcumin, an orange-yellow polyphenol in curry spice, is a phytochemical obtained from the rhizome of the plant *Curcuma longa* [10]. Curcumin is used extensively worldwide due to its potent anti-inflammatory properties and antioxidant properties. Recent studies have provided strong evidence of the therapeutic potential of curcumin in inflammatory diseases, neoplastic disease, cardiovascular and neurodegenerative diseases, diabetes, and other disorders [11,12]. Several studies have also been conducted into the effects of curcumin on sepsis and have observed curcumin playing a pivotal role in several pathways of the pathophysiology of sepsis and exerting beneficial effects on the

* Corresponding authors at: Emergency Department, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China.
E-mail addresses: zgj_0523@126.com (G. Zhao), lzq640815@163.com (Z. Lu).

https://doi.org/10.1016/j.intimp.2018.04.041
Received 2 October 2017; Received in revised form 23 April 2018; Accepted 24 April 2018
1567-5769/ © 2018 Elsevier B.V. All rights reserved.

outcomes of sepsis in mice [13,14]. The present study explores the effects of curcumin on immune status and inflammatory response in an animal model of sepsis.

2. Materials and methods

2.1. Drugs

Curcumin (curcumin ≥ 80%; curcuminoid content ≥ 94%) was purchased from Sigma-Aldrich (St. Louis, MO). The content of bacterial endotoxin was < 0.1 EU/mg.

2.2. Reagents and kits

RPMI 1640, fetal calf serum (FCS), glutamine, penicillin, streptomycin, and HEPES were purchased from TianRunShanda Biotech Co. Ltd. (Beijing, China). Mouse $CD4^+CD25^+$ Treg MicroBeads were purchased from Miltenyi Biotec GmbH. (Bergisch Gladbach, Germany). Antibodies used for flow cytometric analysis, including fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD4, allophycocyanin (APC)-conjugated anti-mouse CD25, phycoerythrin (PE)-conjugated anti-mouse CD25 and PE-conjugated anti-mouse Foxp3, were purchased from eBioscience (San Diego, CA). Functional purified-grade anti-mouse CD3 and CD28 were purchased from eBioscience (San Diego, CA). The total RNA isolation and reverse transcription (RT) systems were purchased from Promega (Madison, WI). SYBR Green PCR Master MIX was purchased from Applied Biosystems (Foster City, CA). Enzyme-linked immunosorbent assay (ELISA) kits of murine IL-10, IL-6, and TNF-α were purchased from Biosource (Worcester, MA).

2.3. Mice

Male BALB/c mice were obtained from Shanghai SLAC Laboratory Animal Co. LTD (license:SCXK (HU) 2012-0002) and raised in the Laboratory Animal Center of Wenzhou Medical University (license:SYXK (ZHE) 2010-0150). The mice were 6–8 weeks in age, and weighed 20 ± 2 g. The mice were placed in cages and raised in a temperature-controlled room with 12 h of light and 12 h of darkness to acclimatize them for at least 7 days before use. All experimental manipulations were strictly in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Ethics Committee of Wenzhou Medical University.

2.4. Preparation of a mouse cecal ligation and puncture model

Cecal ligation and puncture (CLP) in mice is recognized as a reliable and clinically relevant animal model of human sepsis. Polymicrobial abdominal sepsis was induced using CLP as previously described [15]. The mice were anaesthetized with amobarbital sodium (0.05 g/kg) and a midline incision (1–2 cm) was made just caudal to the diaphragm, exposing the internal organs. The cecum was ligated and punctured with an 18-gauge needle in 2 places and a small amount of cecal content was expressed through the puncture wound to induce sepsis. The cecum was then returned to the peritoneal cavity, and the muscle and epidermal layers were sutured. A sterile saline solution (0.9%, 24 mL/kg of body weight) was intraperitoneally administered for fluid resuscitation. In the sham group, the cecum was exposed and the bowel massaged as described above, without ligation or puncturing.

2.5. Treatment protocol

All animals were allowed to acclimatize for 5 days prior to treatment. Next, they were randomly separated into the following 6 groups (n = 32/group). A sham cecal ligature puncture group (sham group), a cecal ligation and puncture group (CLP group), a CLP and corn oil treatment group (vehicle group), a CLP with curcumin (50 mg/kg) treatment group (L-cur group), a CLP with curcumin (100 mg/kg) treatment group (M-cur group), and a CLP with curcumin (200 mg/kg) treatment group (H-cur group). The curcumin dose was selected based on previous studies [16]. Twelve hours after CLP was induced, the mice in the curcumin groups were given curcumin daily via intragastric administration. The mice in the other groups received an equal volume of corn oil daily until the end of the experiment.

2.6. Magnetic isolation of $CD4^+$ T and Treg cells

Splenocytes were isolated aseptically from these animals using a cell mesh. Following hypotonic lysis of the residual erythrocytes, the splenocytes were washed twice and suspended in RPMI-1640 supplemented with 5% fetal bovine serum. After centrifugation, the precipitated cells were collected. The cells were isolated using anti-Treg (CD4/CD25) microbeads and a MiniMACSTM separator per the manufacturer's instructions. $CD4^+$ T cells were enriched via depletion of cells expressing CD8a, CD11b, CD45R, CD49b, and Ter-119 from splenocytes using a $CD4^+CD25^+$ Regulatory T Cell Isolation Kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). CD25 expression was used as the basis for further selection of $CD4^+CD25^+$ Tregs and $CD4^+CD25^-$ T cells. The purity of isolated $CD4^+CD25^+$ Tregs was verified using flow cytometric analysis with FITC-conjugated anti-CD4 and PE-conjugated anti-CD25 staining. The isolated Treg cells showed a purity level of approximately 90–95%.

2.7. Cytokine measurements

Blood samples were harvested at specified time points. Plasma IL-10, IL-6, and TNF-α were measured using ELISA. The plasma was aspirated and assayed in accordance per the manufacturer's instructions.

2.8. RT-qPCR

RT-PCR analysis of *Foxp3* was carried out using the TaqMan® Universal PCR Master Mix Protocol (Applied Biosystems, Grand Island, NY) with real-time PCR probes listed on the NCBI Probe Database. The amplification PCR consisted of a 1-min denaturation step at 95 °C followed by 40 cycles of 15 s at 95 °C and 40 s at 60 °C on a Sequence Detection System (Applied Biosystems, Foster City, CA). The primers sequences are shown below. *Foxp3* forward: 5′-GGTATTGAGGG TGGG TGTC AG-3′; reverse: 5′-CCACAGCATGGGTCTGTCTTC-3′, and GAPDH for-ward: 5′-AAGAGGGATGCTGCCCTTAC-3′; reverse: 5′-AAGAGGGAT GCTGCCCTTAC-3′. The mRNA levels were normalized with those of GAPDH, and the relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [17]. The relative mRNA levels in the sham group samples were assigned a value of 1 and served as a calibrator, while the corresponding mRNA levels in the experimental group were expressed as fold changes in comparison with those of the sham samples. All samples were analyzed in quadruplicate.

2.9. Western blotting

Total proteins were separated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were first blocked with 5% non-fat milk for 2 h at room temperature, followed by incubation with rabbit polyclonal antibody against murine *Foxp3* at 4 °C overnight. GAPDH was detected as a loading control. The blots were then developed using incubation with biotinylated anti-rabbit antibody (1:5000). Signals were detected using an ECL kit and X-ray films.

Download English Version:

https://daneshyari.com/en/article/8531029

Download Persian Version:

https://daneshyari.com/article/8531029

Daneshyari.com