European Journal of Pharmacology 705 (2013) 79–85

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

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

Immunopharmacology and inflammation

Emodin ameliorates lipopolysaccharide-induced mastitis in mice by inhibiting activation of NF-κB and MAPKs signal pathways

Depeng Li, Naisheng Zhang, Yongguo Cao, Wen Zhang, Gaoli Su, Yong Sun, Zhicheng Liu, Fengyang Li, Dejie Liang, Bo Liu, Mengyao Guo, Yunhe Fu, Xichen Zhang, Zhengtao Yang*

Department of Clinical Veterinary Medicine, College of Animal Science and Veterinary Medicine, Jilin University, Changchun, Jilin Province 130062, People's Republic of China

ARTICLE INFO

Article history:
Received 5 October 2012
Received in revised form
4 February 2013
Accepted 7 February 2013
Available online 13 March 2013

Keywords:
Emodin
LPS (Lipopolysaccharide)
Mastitis
Cytokine
Nuclear factor kappa-B (NF-κB)
MAPKs (Mitogen activated protein kinases)

ABSTRACT

Emodin is an anthraquinone derivative from the Chinese herb *Radix et Rhizoma Rhei*. It has been reported that emodin possesses a number of biological properties, such as anti-inflammatory, anti-virus, anti-bacteria, anti-tumor, and immunosuppressive properties. However, the effect of emodin on mastitis is not yet known. The aim of this study was to investigate whether emodin has protective effect against lipopolysaccharide (LPS)-induced mastitis in a mouse model. The mouse model of mastitis was induced by injection of LPS through the duct of mammary gland. Emodin was administered intraperitoneally with the dose of 1, 2, and 4 mg/kg respectively 1 h before and 12 h after induction of LPS. Emodin significantly reduced infiltration of neutrophilic granulocyte, activation of myeloperoxidase (MPO), concentration of tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), and interleukin-6 (IL-6), mRNA expression levels of TNF-α, IL-1β and IL-6, which were increased in LPS-induced mouse mastitis. In addition, emodin influenced nuclear factor kappa-B signal transduction pathway by inhibiting activation of nuclear transcription factor-kappaB (NF-κB) p65 and degradation inhibitor of NF-κB α (IκBα), and emodin also influenced mitogen activated protein kinases signal transduction pathway by depression activation of p38, extracellular signal-regulated kinase (ERK), and c-jun NH2-terminal kinase (JNK). In conclusion, these results indicated that emodin could exert beneficial effects on experimental mastitis induced by LPS and may represent a novel treatment strategy for mastitis.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Mastitis is reported in many species, although inflammatory changes of the mammary gland are most commonly identified in domestic dairy animals and humans (Barlow, 2011). Mastitis is described as the most expensive disease on dairy farms worldwide, due to the decrease of milk quantity and quality, and expenditure on the prevention and treatment strategy, such as extra use of antibiotic (Hogeveen et al., 2011). The process of this disease is consisted with a variety of reactions, such as edema and cytokines production in mammary gland.

LPS, a component of the outer membrane of Gram-negative bacteria, induced inflammatory models are recognized as valuable tools to study the effects of mastitis (Kauf et al., 2007; Lee et al., 2003). In acute mastitis caused by coliforms, recognition of LPS is fundamental to the MG immune response (Oviedo-Boyso et al., 2007). Nuclear factor-B (NF-κB), a primary regulator of inflammatory responses, plays a critical role in a variety of physiological and pathologic processes (Barnes and Karin, 1997). Activation of TLR4 by LPS eventually results in the activation of NF-κB, which is believed to play a central role in the regulation of genes encoding for the inflammatory cytokines, adhesion molecules, chemokines, and other growth factors involved in mammary inflammation (Asehnoune et al., 2005; Chen et al., 2003). MAPKs, which are a family of proteins, including p38, extracellular signal-regulated kinase (ERK), and c-jun NH2-terminal kinase (JNK), play a central role during inflammatory responses and in autoimmune diseases. (Herlaar and Brown, 1999; Takeda et al., 2003).

Emodin (1, 3, 8-trihydroxy-6-methyl-anthraquinone), as shown in Fig. 1, is an anthraquinone derivative from the Chinese herb *Radix et Rhizoma Rhei*. It has been reported that emodin possesses a number of biological properties, such as elimination of reactive oxygen species and anti-inflammatory (Huang et al., 2009). However, there have been few reports on the influence of emodin on mastitis. The aim of this study was to investigate the protective effects of intraperitoneal injection emodin against LPS-induced models of mastitis and the potential mechanism of these protective effects. The results of this study may be helpful in the discovery of new reagents to cure mastitis.

* Corresponding author. Tel.: +86 43187981688.
E-mail addresses: yzt@jlu.edu.cn, yangzhengtao01@sina.com (Z. Yang).

0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.02.021

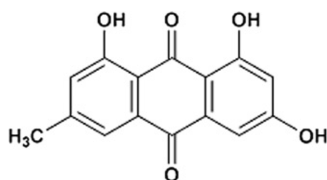

Fig. 1. Chemical structure of emodin.

2. Materials and methods

2.1. Animals

72 healthy female and 36 male BALB/c mice (68 weeks old) were purchased from the Center of Experimental Animals of Baiqiuen Medical College of Jilin University (Jilin, China). The mice were acclimated for 2–3 days prior to experimentation. Two females and one male rat were housed in one cage under pathogen-free conditions. Mice were maintained on standard rodent chow with water supplied ad libitum with a 12/12 h light/dark cycle. All experiments followed the guidelines for the care and use of laboratory animals published by the US National Institutes of Health.

2.2. Drugs and reagents

LPS (*Escherichia coli* 055:B5) was purchased from Sigma (St. Louis, MO, USA), diluted in sterile phosphate buffered saline (PBS) and adjusted to a concentration of 0.2 mg/ml. Emodin (purity $\geq$98% HPLC) was purchased from the National institute for the control of pharmaceutical and biological products. Mouse TNF-α, and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Biolegend (USA), IL-1β ELISA kit was obtained from eBioscience (USA). First strand cDNA synthesis kit was purchased from Fermentas (Burlington, Canada). Dexamethasone (DEX) Sodium Phosphate Injection (no. H41020055) was provided by Changle Pharmaceutical Co. (Xinxiang, Henan, China). The myeloperoxidase (MPO) Ab-1 was purchased from thermo (RB-373-A0). TRIzol reagent (14105) was purchased from invitrogen (USA). T-PER Tissue Protein Extraction Reagent (78510) was purchased from thermo. Rabbit monoclonal antibodies IκBα, p65, *p*-p65, ERK, *p*-ERK, p38, *p*-p38, JNK, *p*-JNK, and mouse monoclonal antibodies *p*-IκBα were purchased from Cell Signaling Technology Inc (Beverly, MA, USA). HRP-conjugated goat anti-rabbit, goat-mouse antibodies (GE Healthcare, Buckinghamshire, UK). All other chemicals were of reagent grade.

2.3. Experiment model

72 female mice were used 5–7 days after parturition. The pups were removed 1 h before induction of LPS. Both L4 (on the left) and R4 (on the right) abdominal mammary glands were infusion of LPS using a 100 μL syringe with a 30-gauge blunt needle. Lactating mice were anesthetized by ethylether, put on their back under a binocular. The teats and the surrounding area were disinfected with 70% ethanol. The very near end of the teats were cut. LPS (10 μg dissolved in 50 μL sterile phosphate buffered saline) was infused into mammary gland through the duct of mammary gland (Brouillette and Malouin, 2005; Li et al., 2013).

2.4. Experimental protocol

The lactating mice were randomly divided into six groups, including control group, LPS group, LPS+emodin group with the dose 1 mg/kg, 2 mg/kg, 4 mg/kg, respectively, LPS+DEX group with dose of 5 mg/kg DEX, and each group contained 12 mice. In the LPS+emodin and LPS+DEX groups, emodin and DEX were administered through intraperitoneal injection (i.p.) 1 h before and 12 h after LPS instillation respectively. The mice were killed using CO_2 inhalation 24 h after infusion of LPS, and the mammary gland was received and stored at −80 °C until used.

2.5. Histopathological analysis

At 24 h after instillation of LPS, the mammary tissues were harvested and fixed into 10% formaldehyde. The mammary tissues were dehydrated with graded alcohol and then embedded in paraffin. Hematoxylin and eosin staining was performed of the section for observation of pathological changes in the mammary tissues under a light microscopy.

2.6. Immunohistochemical evaluation of myeloperoxidase

Paraffin embedded mammary tissues sections were pretreated under the manufacturer's instructions of MPO Ab-1. The sections were incubated in 3% H_2O_2 for 10 min to block the activity of endogenous peroxidase, and then washed three times for 2 min each time using phosphate buffered saline (PBS). Following incubation with diluted (1:200) primary antibodies at 4 °C overnight, the sections were washed three times for 2 min, each time using PBS. Then the secondary antibody conjugated to HRP was added, followed by diaminobenzidine (DAB) staining. All the other treatments were referred to the general protocol instructions. The indicator of positive expression was brown staining.

2.7. Cytokines TNF-α, IL-1β and IL-6 measurements in mammary gland homogenates

Mammary gland homogenates were homogenized in PBS (1:9, w/v), and the lipid was removed from the supernatant. The supernatant was collected as described previously (Li et al., 2013). The supernatant was used to measure the levels of TNF-α, IL-1β and IL-6. All the operations were followed by the instruction of the enzyme-linked immunosorbent assay (ELISA) kits.

2.8. RT–PCR assay for TNF-α, IL-6, and IL-1β mRNA expression

Total RNA was extracted from mice mammary gland samples using TRIzol reagent. Integrity of RNA was confirmed by agarose gel electrophoresis, and then the RNA was reversed transcribed following the manufacturer's instructions of first strand cDNA synthesis kit from Fermentas (Burlington, Canada). The PCR mixture was prepared according to the manufacturer's instructions. The primers were: TNF-α, sense 5′-TGAGGTCAATCTGCCCAAGTA-3′ and antisense 5′-CAGGGAAGAATCTGGAAAGGT-3′; IL-1β, sense 5′-TTGTGGCTGTGGAGAAG-3′ and antisense 5′-CATCAGAGGCAAGGAGG-3′; IL-6, sense 5′-GGACTGATGCTGGTGAC-3′ and antisense 5′-AGGTTTGCCGAGTAGAT-3′. Each reaction was performed using 2 μg of total RNA. Parameters of PCR reactions were: the initial denaturation of the cDNA was accomplished at 94 °C for 5 min, and then 94 °C for 30 s of annealing (58 °C, 56 °C, and 55 °C for TNF-α, IL-1β, and IL-6, respectively), and 72 °C for 30 s for 30 cycles, and followed by extension at 72 °C for 5 min at last. The amplified PCR products were analyzed with 1% TAE agarose gel.

2.9. Western blot analysis for levels of activation of NF-KB and MAPKs signal pathways

Total proteins were extracted from the homogenate of mammary tissue using the T-PER Tissue Protein Extraction Reagent, and the concentrations of proteins were determined by Pierce BCA protein assay kit (23227, thermo, USA). The equal amounts of proteins (60 μg) were separated by 10% sodium dodecyl sulfate

Download English Version:

https://daneshyari.com/en/article/2532232

Download Persian Version:

https://daneshyari.com/article/2532232

Daneshyari.com